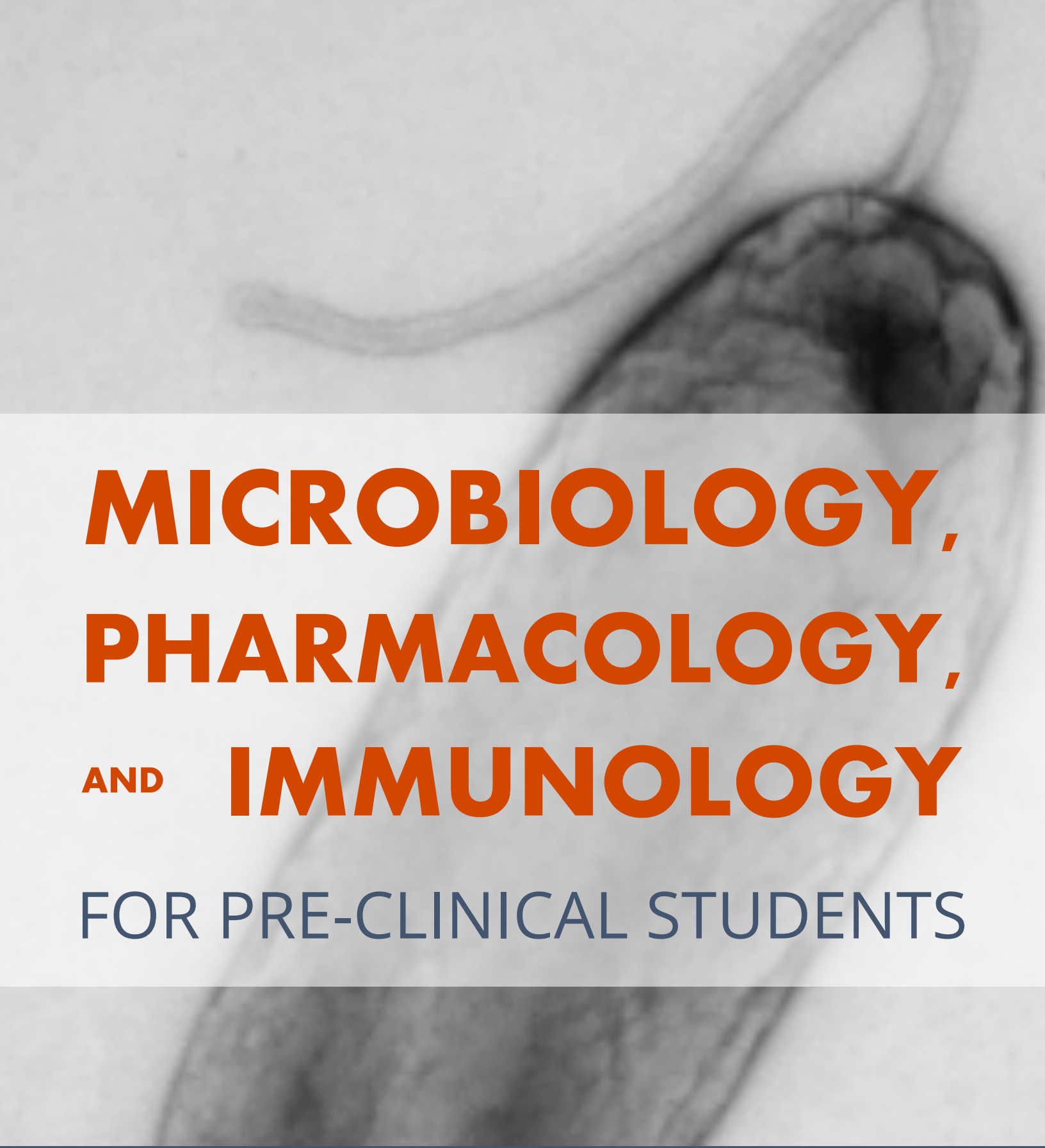

MICROBIOLOGY, PHARMACOLOGY, AND IMMUNOLOGY

FOR PRE-CLINICAL STUDENTS

ADAPTED BY

JENNIFER L. CLEVELAND | ANDREW P. BINKS | RENÉE J. LECLAIR

Microbiology, Pharmacology, and Immunology for Pre-Clinical Students is a peer-reviewed open textbook designed to fill a gap in undergraduate medical education (UME) and support medical school pre-clerkship education. It covers the areas of immunology, microbiology, systems-based infections, and global mechanisms of treatment. It is aligned to USMLE® (United States Medical Licensing Examination) and modified from OpenStax Microbiology.

The organization of this resource is driven by curricular structure to enhance integrated, multidisciplinary content delivery. This specific resource is intended to be used in various ways, mainly as a student quick-reference guide. The sections are not intended to be all-inclusive, but are primers for applied content delivery. The resource is organized into small chapters that can be used to support student preparation in any arrangement. Similarly, clinical context is only briefly discussed (or purposefully omitted) in order to allow the user to apply the basic content presented here in the clinical context used by their specific curricular structure. As cases and clinical correlates change regularly, it is beneficial to have flexible, short resources that can be applied to many scenarios.

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CONTENTS

INTRODUCTION

Microbiology, Pharmacology, and Immunology for Pre-Clinical Students is designed to fill a gap in undergraduate medical education (UME) and support pre-clerkship education in the content areas of immunology, microbiology, systems-based infections, and global mechanisms of treatment. Its content is aligned to USMLE® (United States Medical Licensing Examination) and modified from the well-established OpenStax Microbiology resource. Unlike traditional, discipline-based textbooks, the organization of this resource is driven by curricular structure to enhance integrated, multidisciplinary content delivery.

This specific resource is intended to be used in various ways, mainly as a quick reference guide, given that most of the content housed here is distributed across the Phase 1 curriculum. Therefore, the resource is organized into small chapters that can be used to support student preparation in any arrangement. The sections are not intended to be all-inclusive, but primers for applied content delivery. Similarly, clinical context is only briefly discussed (or purposefully omitted) allowing the user to apply the basic content (delivered here) in the clinical context used by their specific curricular structure. In our curriculum, these topic areas are interwoven into problem-based learning cases. The cases and clinical correlates change regularly and having the flexibility of short resources that can be applied to many scenarios across the Phase 1 curriculum is beneficial.

This resource is intended to provide learners with a high-level view of relevant topical areas that will be further elaborated on within the classroom or group setting. Unlike other traditional textbooks, it is not intended to include all content a learner would need about the relevant subject area but to function as a stepping stone towards mastery of the content.

As programs embrace the philosophy of student-directed learning embedded in adult learning theory, more simplified readily available resources will be essential to support this fast-paced learning of health professional educational programs. While many factors can contribute to a student's lack of preparation, lengthy textbook resources have a significant negative impact and often encourage learners to turn to outside material. So while an integrated curricular model enhances many aspects of learning, it can make preparation cumbersome and disjointed for students. This resource hopes to address this concern.

Finally, there is a wealth of "medical" content freely accessible online, and students can find themselves spending a significant amount of time trying to identify alternative resources that may—or may not—be appropriate. Faculty taking ownership to identify and adapt realistic materials for each session reduces the concern that students are finding misinformation through internet sources, and this project allows faculty to create a resource that harnesses the best attributes of many different formats into a product that best supports the learning environment. Otherwise, external online resources are also likely to contain extraneous content that is not aligned with the classroom learning objectives (akin to subject-based textbook chapters), so it can also reduce the perceived worth of preparation. If the integrated resource is generated correctly, concisely and accurately by the faculty, the students will gain trust, rely on the vetted resources and prepare for the active classroom.

— Renée LeClair

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Jennifer L. Cleveland is an assistant professor in the Department of Basic Science at Virginia Tech Carilion School of Medicine. She received a PharmD and MBA degree from Shenandoah University and has practiced in a variety of settings including hospitals, home healthcare, and independent pharmacy. She began her journey in clinical education at Jefferson College of Health Sciences, where she was instructor of clinical pharmacotherapeutics for physician assistant students for nine years. In 2017, she transitioned to Virginia Tech Carilion School of Medicine where her role includes teaching pharmacology to first- and second-year medical students, codirecting the problem-based learning curriculum, integrating pharmacology within clinical science, and facilitating small group sessions in the Health Systems Science and Interprofessional Practice program. With her background in clinical pharmacy and board-certification as a pharmacotherapy specialist, she brings a unique blend of expertise in her role as a medical educator.

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CHAPTER 1.

HOST DEFENSES, IMMUNODEFICIENCIES, AND AUTOIMMUNE DISORDERS

1.1 INTRODUCTION

Despite relatively constant exposure to pathogenic microbes in the environment, humans do not generally suffer from constant infection or disease. Under most circumstances, the body is able to defend itself from the threat of infection thanks to a complex immune system designed to repel, kill, and expel disease-causing invaders. Immunity as a whole can be described as two interrelated parts: nonspecific innate immunity and specific adaptive host defenses.

The nonspecific innate immune response provides a first line of defense that can often prevent infections from gaining a solid foothold in the body. These defenses are described as **nonspecific** because they do not target any specific pathogen; rather, they defend against a wide range of potential pathogens. They are called **innate** because they are built-in mechanisms of the human organism. Unlike the specific adaptive defenses, they are not acquired over time and they have no "memory" (they do not improve after repeated exposures to specific pathogens).

Broadly speaking, nonspecific innate defenses provide an immediate (or very rapid) response against potential pathogens. However, these responses are neither perfect nor impenetrable. They can be circumvented by pathogens on occasion, and sometimes they can even cause damage to the body, contributing to the signs and symptoms of infection (figure 1.1).

Nonspecific innate immunity can be characterized as a multifaceted system of defenses that targets invading pathogens in a nonspecific manner. In this chapter, we have divided the numerous defenses that make up this system into three categories: physical defenses, chemical defenses, and cellular defenses. However, it is important to keep in mind that these defenses do not function independently, and the categories often overlap. Table 1.1 provides an overview of the nonspecific defenses discussed in this chapter.

Physical defenses provide the body's most basic form of nonspecific defense. They include physical barriers to microbes, such as the skin and mucous membranes, as well as mechanical defenses that physically remove microbes and debris from areas of the body where they might cause harm or infection. In addition, the microbiome provides a measure of physical protection against disease, as microbes of the normal microbiota compete with pathogens for nutrients and cellular binding sites necessary to cause infection.

Overview of nonspecific innate immune defenses	
Physical defenses	Physical barriers
	Mechanical defenses
	Microbiome
Chemical defenses	Chemicals and enzymes in body fluids
	Antimicrobial peptides
	Plasma protein mediators
	Cytokines
	Inflammation-eliciting mediators
Cellular defenses	Granulocytes
	Agranulocytes

Table 1.1: Overview of nonspecific innate immune defenses

1.2 PHYSICAL DEFENSES

PHYSICAL BARRIERS

Physical barriers play an important role in preventing microbes from reaching tissues that are susceptible to infection. At the cellular level, barriers consist of cells that are tightly joined to prevent invaders from crossing through to deeper tissue. For example, the endothelial cells that line blood vessels have very tight cell-to-cell junctions, blocking microbes from gaining access to the bloodstream. Cell junctions are generally composed of cell membrane proteins that may connect with the extracellular matrix or with complementary proteins from neighboring cells. Tissues in various parts of the body have different types of cell junctions. These include tight junctions, desmosomes, and gap junctions, as illustrated in figure 1.1. Invading microorganisms may attempt to break down these substances chemically, using enzymes such as proteases that can cause structural damage to create a point of entry for pathogens.

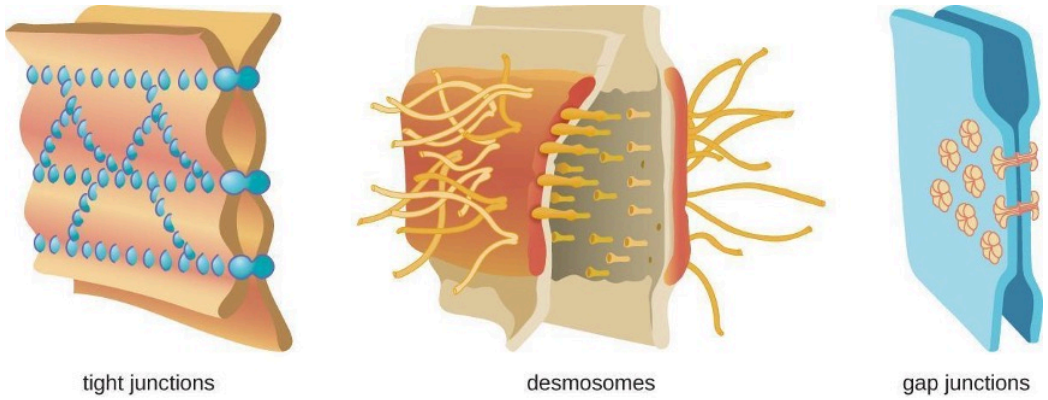


Figure 1.1: There are multiple types of cell junctions in human tissue, three of which are shown here. Tight junctions rivet two adjacent cells together, preventing or limiting material exchange through the spaces between them. Desmosomes have intermediate fibers that act like shoelaces, tying two cells together, allowing small materials to pass through the resulting spaces. Gap junctions are channels between two cells that permit their communication via signals. *Figure description available at the end of the chapter.*

The Skin Barrier

One of the body's most important physical barriers is the skin barrier, which is composed of three layers of closely packed cells. The thin upper layer is called the epidermis. A second, thicker layer is called the dermis. This layer contains hair follicles, sweat glands, nerves, and blood vessels. A layer of fatty tissue called the hypodermis lies beneath the dermis and contains blood and lymph vessels (figure 1.2).

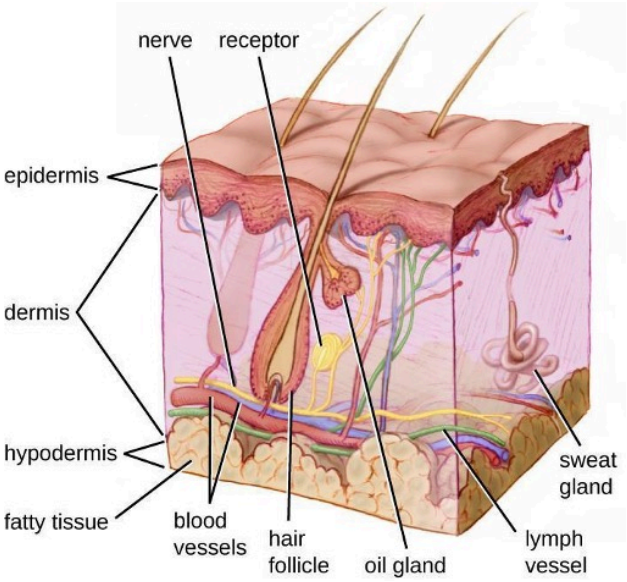


Figure 1.2: Human skin has three layers: the epidermis, the dermis, and the hypodermis. These provide a thick barrier between microbes outside the body and deeper tissues. Dead skin cells on the surface of the epidermis are continually shed, taking with them microbes on the skin's surface. *Figure description available at the end of the chapter.*

The topmost layer of skin, the epidermis, consists of cells that are packed with keratin. These dead cells remain as a tightly connected, dense layer of protein-filled cell husks on the surface of the skin. The keratin makes the skin's surface mechanically tough and resistant to degradation by bacterial enzymes. Fatty acids on the skin's surface create a dry, salty, and acidic environment that inhibits the growth of some microbes and is highly resistant to breakdown by bacterial enzymes. In addition, the dead cells of the epidermis are frequently shed, along with any microbes that may be clinging to them. Shed skin cells are continually replaced with new cells from below, providing a new barrier that will soon be shed in the same way.

Infections can occur when the skin barrier is compromised or broken. A wound can serve as a point of entry for opportunistic pathogens, which can infect the skin tissue surrounding the wound and possibly spread to deeper tissues.

Mucous Membranes

The mucous membranes lining the nose, mouth, lungs, and urinary and digestive tracts provide another nonspecific barrier against potential pathogens. Mucous membranes consist of a layer of epithelial cells bound by tight junctions. The epithelial cells secrete a moist, sticky substance called mucus, which covers and protects the more fragile cell layers beneath it as well as traps debris and particulate matter, including microbes. Mucus secretions also contain antimicrobial peptides.

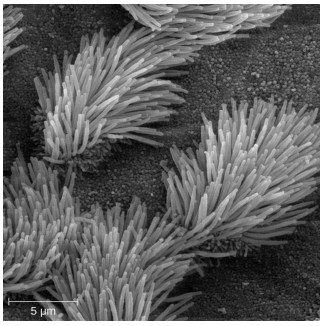


Figure 1.3: This scanning electron micrograph shows ciliated and nonciliated epithelial cells from the human trachea. The mucociliary escalator pushes mucus away from the lungs, along with any debris or microorganisms that may be trapped in the sticky mucus, and the mucus moves up to the esophagus where it can be removed by swallowing. Figure description available at the end of the chapter.

In many regions of the body, mechanical actions serve to flush mucus (along with trapped or dead microbes) out of the body or away from potential sites of infection. For example, in the respiratory system, inhalation can bring microbes, dust, mold spores, and other small airborne debris into the body. This debris becomes trapped in the mucus lining the respiratory tract, a layer known as the mucociliary blanket. The epithelial cells lining the upper parts of the respiratory tract are called ciliated epithelial cells because they have hair-like appendages known as cilia. Movement of the cilia propels debris-laden mucus out and away from the lungs. The expelled mucus is then swallowed and destroyed in the stomach, coughed up, or sneezed out (figure 1.3). This system of removal is often called the mucociliary escalator.

The mucociliary escalator is such an effective barrier to microbes that the lungs, the lowermost (and most sensitive) portion of the respiratory tract, were long considered to be a sterile environment in healthy individuals. Only recently has research suggested that healthy lungs may have a small normal microbiota. Disruption of the mucociliary escalator by the damaging effects of smoking or diseases such as cystic fibrosis can lead to increased colonization of bacteria in the lower respiratory tract and frequent infections. These effects highlight the importance of this physical barrier to host defenses.

Like the respiratory tract, the digestive tract is a portal of entry through which microbes enter the body, and the mucous membranes lining the digestive tract provide a nonspecific physical barrier against ingested microbes. The intestinal tract is lined with epithelial cells interspersed with mucus-secreting goblet cells (figure 1.4). This mucus mixes with material received from the stomach, trapping food-borne microbes and debris. The mechanical action of peristalsis, a series of muscular contractions in the digestive tract, moves the sloughed mucus and other material through the intestines, rectum, and anus, excreting the material in feces.

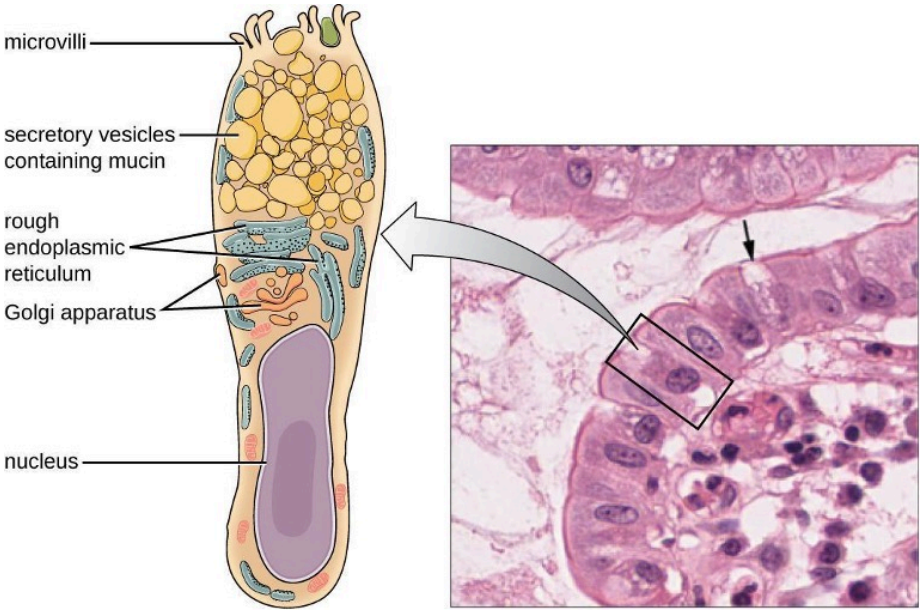


Figure 1.4: Goblet cells produce and secrete mucus. The arrows in this micrograph point to the mucus-secreting goblet cells (magnification 1600x) in the intestinal epithelium. Figure description available at the end of the chapter.

Endothelia

The epithelial cells lining the urogenital tract, blood vessels, lymphatic vessels, and certain other tissues are known as endothelia. These tightly packed cells provide a particularly effective frontline barrier against invaders. The endothelia of the blood-brain barrier, for example, protect the central nervous system (CNS), which consists of the brain and the spinal cord. The CNS is one of the most sensitive and important areas of the body, as microbial infection of the CNS can quickly lead to serious and often fatal inflammation. The cell junctions in the blood vessels traveling through the CNS are some of the tightest and toughest in the body, preventing any transient microbes in the bloodstream from entering the CNS. This keeps the cerebrospinal fluid that surrounds and bathes the brain and spinal cord sterile under normal conditions.

1.3 MECHANICAL DEFENSES

In addition to physical barriers that keep microbes out, the body has a number of mechanical defenses that physically remove pathogens from the body, preventing them from taking up residence. We have already discussed several examples of mechanical defenses, including the shedding of skin cells, the expulsion of mucus via the mucociliary escalator, and the excretion of feces through intestinal peristalsis. Other important examples of mechanical defenses include the flushing action of urine and tears, which both serve to carry microbes away from the body. The flushing action of urine is largely responsible for the normally sterile environment of the urinary tract, which includes the kidneys, ureters, and urinary bladder. Urine passing out of the body washes out transient microorganisms, preventing them from taking up residence. The eyes also have physical barriers and mechanical mechanisms for preventing infections. The eyelashes and eyelids prevent dust and airborne microorganisms from reaching the surface of the eye. Any microbes or debris that make it past these physical barriers may be flushed out by the mechanical action of blinking, which bathes the eye in tears, washing debris away (figure 1.5).

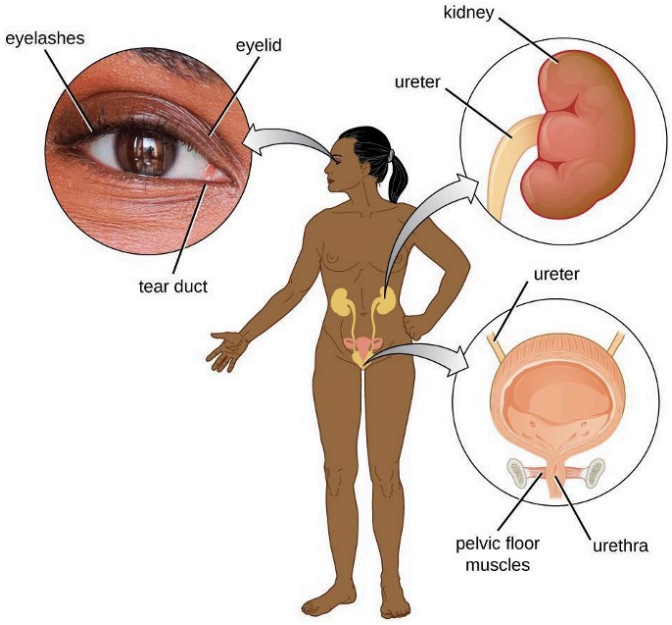


Figure 1.5: Tears flush microbes away from the surface of the eye. Urine washes microbes out of the urinary tract as it passes through; as a result, the urinary system is normally sterile. *Figure description available at the end of the chapter.*

MICROBIOME

In various regions of the body, resident microbiota serve as an important first-line defense against invading pathogens. Through their occupation of cellular binding sites and competition for available nutrients, the resident microbiota prevent the critical early steps of pathogen attachment and proliferation required for the establishment of an infection. For example, in the vagina, members of the resident microbiota compete with opportunistic pathogens like the yeast *Candida*. This competition prevents infections by limiting the availability of nutrients, thus inhibiting the growth of *Candida,* keeping its population in check. Similar competitions occur between the microbiota and potential pathogens on the skin, in the upper respiratory tract, and in the gastrointestinal tract. The resident microbiota also contribute to the chemical defenses of the innate nonspecific host defenses.

The importance of the normal microbiota in host defenses is highlighted by the increased susceptibility to infectious diseases when the microbiota is disrupted or eliminated. Treatment with antibiotics can significantly deplete the normal microbiota of the gastrointestinal tract, providing an advantage for pathogenic bacteria to colonize and cause diarrheal infection. In the case of diarrhea caused by *Clostridium difficile*, the infection can be severe and potentially lethal. One strategy for treating *C. difficile* infections is fecal transplantation, which involves the transfer of fecal material from a donor (screened for potential pathogens) into the intestines of the recipient patient as a method of restoring the normal microbiota and combating *C. difficile* infections.

Table 1.2 provides a summary of the physical defenses discussed in this section.

Defense	Examples	Function
Cellular barriers	Skin, mucous membranes, endothelial cells	Deny entry to pathogens
Mechanical barriers	Shedding of skin cells, mucociliary sweeping, peristalsis, flushing action of urine and tears	Remove pathogens from potential sites of infection
Microbiome	Resident bacteria of the skin, upper respiratory tract, gastrointestinal tract, and genitourinary tract	Compete with pathogens for cellular binding sites and nutrients

Table 1.2: Physical defenses of nonspecific innate immunity

1.4 CHEMICAL DEFENSES

In addition to physical defenses, the innate nonspecific immune system uses a number of chemical mediators that inhibit microbial invaders. The term **chemical mediators** encompasses a wide array of substances found in various body fluids and tissues throughout the body. Chemical mediators may work alone or in conjunction with each other to inhibit microbial colonization and infection.

Some chemical mediators are endogenously produced, meaning they are produced by human body cells; others are produced exogenously, meaning that they are produced by certain microbes that are part of the microbiome. Some mediators are produced continually, bathing the area in the antimicrobial substance; others are produced or activated primarily in response to some stimulus, such as the presence of microbes.

CHEMICAL AND ENZYMATIC MEDIATORS FOUND IN BODY FLUIDS

Fluids produced by the skin include examples of both endogenous and exogenous mediators. Sebaceous glands in the dermis secrete an oil called sebum that is released onto the skin surface through hair follicles. This sebum is an endogenous mediator, providing an additional layer of defense by helping seal off the pore of the hair follicle.This supplemental defense layer prevents bacteria on the skin's surface from invading sweat glands and surrounding tissue (figure 1.6). Certain members of the microbiome, such as the bacterium *Propionibacterium acnes* and the fungus *Malassezia*, among others, can use lipase enzymes to degrade sebum, using it as a food source. This produces oleic acid which creates a mildly acidic environment on the surface of the skin that is inhospitable to many pathogenic microbes. Oleic acid is an example of an exogenously produced mediator because it is produced by resident microbes and not directly by body cells.

Environmental factors that affect the microbiota of the skin can have a direct impact on the production of chemical mediators. Low humidity or decreased sebum production, for example, could make the skin less habitable for microbes that produce oleic acid, thus making the skin more susceptible to pathogens normally inhibited by the skin's low pH. Many skin moisturizers are formulated to counter such effects by restoring moisture and essential oils to the skin.

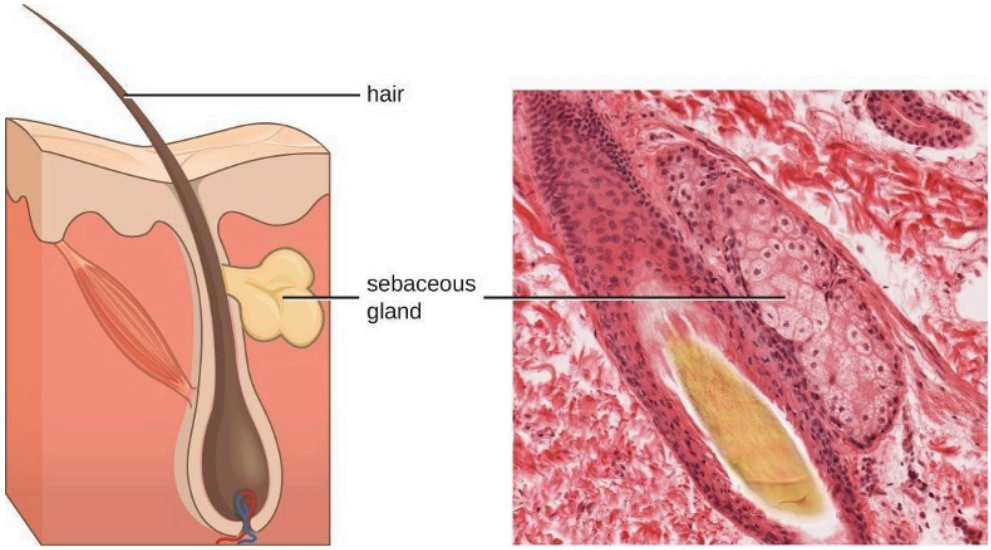


Figure 1.6: Sebaceous glands secrete sebum, a chemical mediator that lubricates and protects the skin from invading microbes. Sebum is also a food source for resident microbes that produce oleic acid, an exogenously produced mediator. *Figure description available at the end of the chapter.*

The digestive tract also produces a large number of chemical mediators that inhibit or kill microbes. In the oral cavity, saliva contains mediators such as lactoperoxidase enzymes, and mucus secreted by the esophagus contains the antibacterial enzyme lysozyme. In the stomach, highly acidic gastric fluid kills most microbes. In the lower digestive tract, the intestines have pancreatic and intestinal enzymes, antibacterial peptides (cryptins), bile produced from the liver, and specialized Paneth cells that produce lysozyme. Together, these mediators are able to eliminate most pathogens that manage to survive the acidic environment of the stomach.

In the urinary tract, urine flushes microbes out of the body during urination. Furthermore, the slight acidity of urine (the average pH is about 6) inhibits the growth of many microbes and potential pathogens in the urinary tract.

The female reproductive system employs lactate, an exogenously produced chemical mediator, to inhibit microbial growth. The cells and tissue layers composing the vagina produce glycogen, a branched and more complex polymer of glucose. Lactobacilli in the area ferment glycogen to produce lactate, lowering the pH in the vagina thereby inhibiting transient microbiota, opportunistic pathogens like *Candida* (a yeast associated with vaginal infections), and other pathogens responsible for sexually transmitted diseases.

In the eyes, tears contain the chemical mediators lysozyme and lactoferrin, both of which are capable of eliminating microbes that have found their way to the surface of the eyes. Lysozyme cleaves the bond between NAG and NAM in peptidoglycan, a component of the cell wall in bacteria. It is more effective against gram-positive bacteria, which lack the protective outer membrane associated with gram-negative bacteria. Lactoferrin inhibits microbial growth by chemically binding and sequestering iron. This effectively starves many microbes that require iron for growth.

In the ears, cerumen (earwax) exhibits antimicrobial properties due to the presence of fatty acids, which lower the pH to between 3 and 5.

The respiratory tract uses various chemical mediators in the nasal passages, trachea, and lungs. The mucus produced in the nasal passages contains a mix of antimicrobial molecules similar to those found in tears and saliva (e.g., lysozyme, lactoferrin, lactoperoxidase). Secretions in the trachea and lungs also contain lysozyme and lactoferrin, as well as a diverse group of additional chemical mediators, such as the lipoprotein complex called surfactant, which has antibacterial properties.

ANTIMICROBIAL PEPTIDES

The antimicrobial peptides (AMPs) are a special class of nonspecific cell-derived mediators with broad-spectrum antimicrobial properties. Some AMPs are produced routinely by the body, whereas others are primarily produced (or produced in greater quantities) in response to the presence of an invading pathogen. Research has begun exploring how AMPs can be used in the diagnosis and treatment of disease.

AMPs may induce cell damage in microorganisms in a variety of ways, including by inflicting damage to membranes, destroying DNA and RNA, or interfering with cell-wall synthesis. Depending on the specific antimicrobial mechanism, a particular AMP may inhibit only certain groups of microbes (e.g., gram-positive or gram-negative bacteria) or it may be more broadly effective against bacteria, fungi, protozoa, and viruses. Many AMPs are found on the skin, but they can also be found in other regions of the body.

A family of AMPs called defensins can be produced by epithelial cells throughout the body as well as by cellular defenses such as macrophages and neutrophils (see section 1.5). Defensins may be secreted or act inside host cells; they combat microorganisms by damaging their plasma membranes. AMPs called bacteriocins are produced exogenously by certain members of the resident microbiota within the gastrointestinal tract. The genes coding for these types of AMPs are often carried on plasmids and can be passed between different species within the resident microbiota through lateral or horizontal gene transfer.

There are numerous other AMPs throughout the body. The characteristics of a few of the more significant AMPs are summarized in table 1.3.

AMP	Secreted by	Body site	Pathogens inhibited	Mode of action
Bacteriocins	Resident microbiota	Gastrointestinal tract	Bacteria	Disrupt membrane
Cathelicidin	Epithelial cells, macrophages, and other cell types	Skin	Bacteria and fungi	Disrupt membrane
Defensins	Epithelial cells, macrophages, neutrophils	Throughout the body	Fungi, bacteria, and many viruses	Disrupt membrane
Dermcidin	Sweat glands	Skin	Bacteria and fungi	Disrupt membrane integrity and ion channels
Histatins	Salivary glands	Oral cavity	Fungi	Disrupt intracellular function

Table 1.3: Characteristics of selected antimicrobial peptides (AMPs)

PLASMA PROTEIN MEDIATORS

Many nonspecific innate immune factors are found in plasma, the fluid portion of blood. Plasma contains electrolytes, sugars, lipids, and proteins, each of which helps to maintain homeostasis (i.e., stable internal body functioning) and contains the proteins involved in the clotting of blood. Additional proteins found in blood plasma, such as acute-phase proteins, complement proteins, and cytokines, are involved in the nonspecific innate immune response.

Acute-Phase Proteins

The acute-phase proteins are another class of antimicrobial mediators. Acute-phase proteins are primarily produced in the liver and secreted into the blood in response to inflammatory molecules from the immune system. Examples of acute-phase proteins include C-reactive protein, serum amyloid A, ferritin, transferrin, fibrinogen, and mannose-binding lectin. Each of these proteins has a different chemical structure and inhibits or destroys microbes in some way (table 1.4).

Acute-phase protein	Function
C-reactive protein	Coats bacteria (opsonization), preparing them for ingestion by phagocytes
Serum amyloid A	Can stimulate the secretion of IL-8 from neutrophils
Ferritin	Bind and sequester iron, thereby inhibiting the growth of pathogens
Transferrin	Ferritin synthesis increases as a nonspecific response aspect of the general pattern of the systemic effects of inflammation ; transferrin is considered a negative acute phase protein
Fibrinogen	Involved in formation of blood clots that trap bacterial pathogens
Mannose-binding lectin	Activates complement cascade

Table 1.4: Some acute-phase proteins and their functions

The Complement System

The complement system is a group of plasma protein mediators that can act as an innate nonspecific defense while also serving to connect innate and adaptive immunity. The complement system is composed of more than 30 proteins (including C1 through C9) that normally circulate as precursor proteins in blood. These precursor proteins become activated when triggered by a variety of factors, including the presence of microorganisms. Complement proteins are considered part of innate nonspecific immunity because they are always present in the blood and tissue fluids, allowing them to be activated quickly. Also, when activated through the alternative pathway (described later in this section), complement proteins target pathogens in a nonspecific manner.

The process by which circulating complement precursors become functional is called **complement activation**. This process is a cascade that can be triggered by one of three different mechanisms, known as the alternative, classical, and lectin pathways.

The alternative pathway is initiated by the spontaneous activation of the complement protein C3. The hydrolysis of C3 produces two products, C3a and C3b. When no invader microbes are present, C3b is very quickly degraded in a hydrolysis reaction using the water in the blood. However, if invading microbes are present, C3b attaches to the surface of these microbes. Once attached, C3b will recruit other complement proteins in a cascade (figure 1.7).

The classical pathway provides a more efficient mechanism of activating the complement cascade, but it depends upon the production of antibodies by the specific adaptive immune defenses. To initiate the classical pathway, a specific antibody must first bind to the pathogen to form an antibody-antigen complex. This activates the first protein in the complement cascade, the C1 complex. The C1 complex is a multipart protein complex, and each component participates in the full activation of the overall complex. Following recruitment and activation of the C1 complex, the remaining classical pathway complement proteins are recruited and activated in a cascading sequence (figure 1.7).

The lectin activation pathway is similar to the classical pathway, but it is triggered by the binding of mannose-binding lectin, an acute-phase protein, to carbohydrates on the microbial surface. Like other acute-phase proteins, lectins are produced by liver cells and are commonly upregulated in response to inflammatory signals received by the body during an infection (figure 1.7).

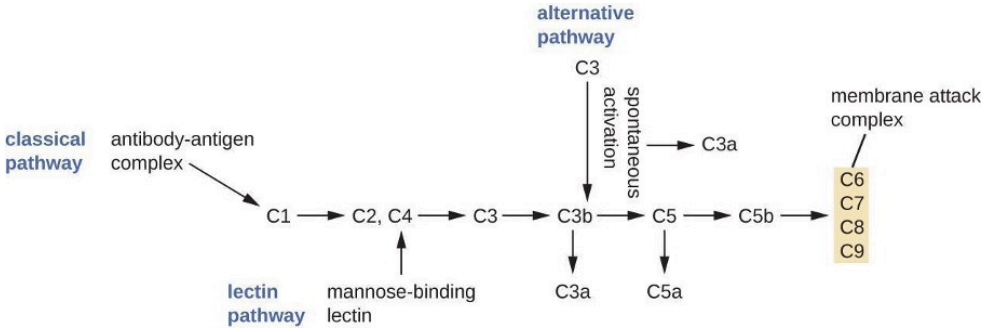


Figure 1.7: The three complement activation pathways have different triggers, as shown here, but all three result in the activation of the complement protein C3, which produces C3a and C3b. The latter binds to the surface of the target cell and then works with other complement proteins to cleave C5 into C5a and C5b. C5b also binds to the cell surface and then recruits C6 through C9; these molecules form a ring structure called the membrane attack complex (MAC), which punches through the cell membrane of the invading pathogen, causing it to swell and burst. *Figure description available at the end of the chapter.*

Although each complement activation pathway is initiated in a different way, they all provide the same protective outcomes: opsonization, inflammation, chemotaxis, and cytolysis. The term opsonization refers to the coating of a pathogen by a chemical substance (called an opsonin) that allows phagocytic cells to recognize, engulf, and destroy it more easily. Opsonins from the complement cascade include C1q, C3b, and C4b. Additional important opsonins include mannose-binding proteins and antibodies. The complement fragments C3a and C5a are well-characterized anaphylatoxins with potent proinflammatory functions. Anaphylatoxins activate mast cells, causing degranulation and the release of inflammatory chemical signals, including mediators that cause vasodilation and increased vascular permeability. C5a is also one of the most potent chemoattractants for neutrophils and other white blood cells, cellular defenses that will be discussed in the next section.

The complement proteins C6, C7, C8, and C9 assemble into a membrane attack complex (MAC), which allows C9 to polymerize into pores in the membranes of gram-negative bacteria. These pores allow water, ions, and other molecules to move freely in and out of the targeted cells, eventually leading to cell lysis and death of the pathogen (table 1.5). However, the MAC is only effective against gram-negative bacteria; it cannot penetrate the thick layer of peptidoglycan associated with cell walls of gram-positive bacteria. Since the MAC does not pose a lethal threat to gram-positive bacterial pathogens, complement-mediated opsonization is more important for their clearance.

Cytokines

Cytokines are soluble proteins that act as communication signals between cells. In a nonspecific innate immune response, various cytokines may be released to stimulate production of chemical mediators or other cell functions, such as cell proliferation, cell differentiation, inhibition of cell division, apoptosis, and chemotaxis.

When a cytokine binds to its target receptor, the effect can vary widely depending on the type of cytokine and the type of cell or receptor to which it has bound. The function of a particular cytokine can be described as autocrine, paracrine, or endocrine (table 1.5). In autocrine function, the same cell that releases the cytokine is the recipient of the signal; in other words, autocrine function is a form of self-stimulation by a cell. In contrast, paracrine function involves the release of cytokines from one cell to other nearby cells, stimulating some response from the recipient cells. Last, endocrine function occurs when cells release cytokines into the bloodstream to be carried to target cells much farther away.

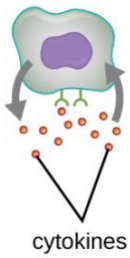
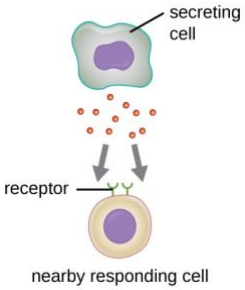
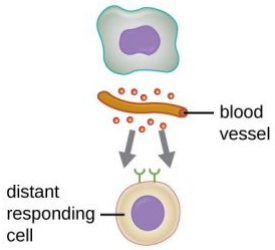
Autocrine	Paracrine	Endocrine
Same cell secretes and receives cytokine signal.	Cytokine signal secreted to a nearby cell.	Cytokine signal secreted to circulatory system; travels to distant cells.
		

Table 1.5: Autocrine, paracrine, and endocrine actions describe which cells are targeted by cytokines and how far the cytokines must travel to bind to their intended target cells' receptors.

Three important classes of cytokines are the interleukins, chemokines, and interferons. The interleukins were originally thought to be produced only by leukocytes (white blood cells) and to only stimulate leukocytes, thus the reasons for their name. Although interleukins are involved in modulating almost every function of the immune system, their role in the body is not restricted to immunity. Interleukins are also produced by and stimulate a variety of cells unrelated to immune defenses.

The chemokines are chemotactic factors that recruit leukocytes to sites of infection, tissue damage, and inflammation. In contrast to more general chemotactic factors, like complement factor C5a, chemokines are very specific in the subsets of leukocytes they recruit.

Interferons are a diverse group of immune signaling molecules and are especially important in our defense against viruses. Type I interferons (interferon-α and interferon-β) are produced and released by cells infected with a virus. These interferons stimulate nearby cells to stop production of mRNA, destroy RNA already produced, and reduce protein synthesis. These cellular changes inhibit viral replication and production of mature viruses, slowing the spread of the virus. Type I interferons also stimulate various immune cells involved in viral clearance to more aggressively attack virus-infected cells. Type II interferon (interferon-γ) is an important activator of immune cells (figure 1.8).

INFLAMMATION-ELICITING MEDIATORS

Many of the chemical mediators discussed in this section contribute in some way to inflammation and fever, which are nonspecific immune responses discussed in more detail in section 1.7. Cytokines stimulate the production of acute-phase proteins such as C-reactive protein and mannose-binding lectin in the liver. These acute-phase proteins act as opsonins, activating complement cascades through the lectin pathway.

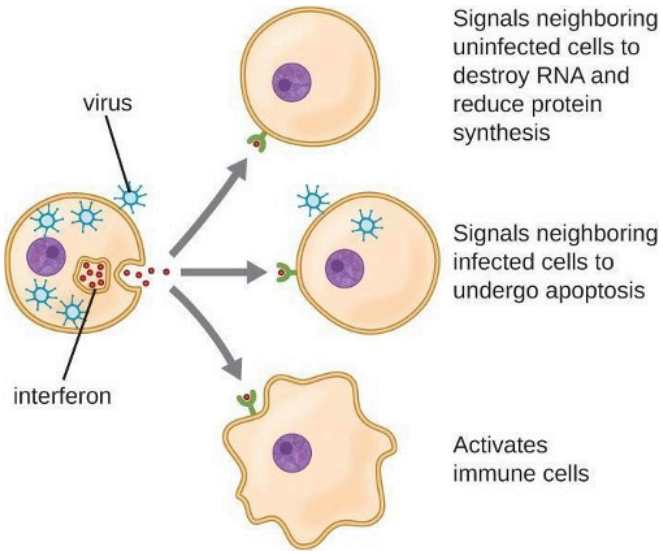


Figure 1.8: Interferons are cytokines released by a cell infected with a virus. Interferon-α and interferon-β signal uninfected neighboring cells to inhibit mRNA synthesis, destroy RNA, and reduce protein synthesis (top arrow). Interferon-α and interferon-β also promote apoptosis in cells infected with the virus (middle arrow). Interferon-γ alerts neighboring immune cells to an attack (bottom arrow). Although interferons do not cure the cell releasing them or other infected cells, which will soon die, their release may prevent additional cells from becoming infected, thus stemming the infection. *Figure description available at the end of the chapter.*

Some cytokines also bind mast cells and basophils, inducing them to release histamine, a proinflammatory compound. Histamine receptors are found on a variety of cells and mediate proinflammatory events, such as bronchoconstriction (tightening of the airways) and smooth muscle contraction.

In addition to histamine, mast cells may release other chemical mediators, such as leukotrienes. Leukotrienes are lipid-based, proinflammatory mediators that are produced from the metabolism of arachidonic acid in the cell membrane of leukocytes and tissue cells. Compared with the proinflammatory effects of histamine, those of leukotrienes are more potent and longer lasting. Together, these chemical mediators can induce coughing, vomiting, and diarrhea, which serve to expel pathogens from the body.

Certain cytokines also stimulate the production of prostaglandins, chemical mediators that promote the inflammatory effects of kinins and histamines. Prostaglandins can also help to set the body temperature higher, leading to fever, which promotes the activities of white blood cells and slightly inhibits the growth of pathogenic microbes (see section 1.7).

Another inflammatory mediator, bradykinin, contributes to edema, which occurs when fluids and leukocytes leak out of the bloodstream and into tissues. This mediator binds to receptors on cells in the capillary walls, causing the capillaries to dilate and become more permeable to fluids.

Table 1.6 provides a summary of the chemical defenses discussed in this section.

Defense	Examples	Function
Chemicals and enzymes in body fluids	Sebum from sebaceous glands	Provides oil barrier protecting hair follicle pores from pathogens
	Oleic acid from sebum and skin microbiota	Lowers pH to inhibit pathogens
	Lysozyme in secretions	Kills bacteria by attacking cell wall
	Acid in stomach, urine, and vagina	Inhibits or kills bacteria
	Digestive enzymes and bile	Kill bacteria
	Lactoferrin and transferrin	Bind and sequester iron, inhibiting bacterial growth
	Surfactant in lungs	Kills bacteria
Antimicrobial peptides	Defensins, bacteriocins, dermcidin, cathelicidin, histatins,	Kill bacteria by attacking membranes or interfering with cell functions
Plasma protein mediators	Acute-phase proteins (C-reactive protein, serum amyloid A, ferritin, fibrinogen, transferrin, and mannose-binding lectin)	Inhibit the growth of bacteria and assist in the trapping and killing of bacteria
	Complements C3b and C4b	Opsonization of pathogens to aid phagocytosis
	Complement C5a	Chemoattractant for phagocytes
	Complements C3a and C5a	Proinflammatory anaphylatoxins
Cytokines	Interleukins	Stimulate and modulate most functions of immune system
	Chemokines	Recruit white blood cells to infected area
	Interferons	Alert cells to viral infection, induce apoptosis of virus-infected cells, induce antiviral defenses in infected and nearby uninfected cells, stimulate immune cells to attack virus-infected cells
Inflammation-eliciting mediators	Histamine	Promotes vasodilation, bronchoconstriction, smooth muscle contraction, increased secretion and mucus production
	Leukotrienes	Promote inflammation; stronger and longer lasting than histamine

Defense	Examples	Function
	Prostaglandins	Promote inflammation and fever
	Bradykinin	Increases vasodilation and vascular permeability, leading to edema

Table 1.6: Chemical defenses of nonspecific innate immunity

1.5 CELLULAR DEFENSES

In the previous section, we discussed some of the chemical mediators found in plasma, the fluid portion of blood. The nonfluid portion of blood consists of various types of formed elements, so called because they are all formed from the same stem cells found in bone marrow. The three major categories of formed elements are: red blood cells (RBCs), also called erythrocytes; platelets, also called thrombocytes; and white blood cells (WBCs), also called leukocytes.

Red blood cells are primarily responsible for carrying oxygen to tissues. Platelets are cellular fragments that participate in blood clot formation and tissue repair. Several different types of WBCs participate in various nonspecific mechanisms of innate and adaptive immunity. In this section, we will focus primarily on the innate mechanisms of various types of WBCs.

HEMATOPOIESIS

All of the formed elements of blood are derived from pluripotent hematopoietic stem cells (HSCs) in the bone marrow. As the HSCs make copies of themselves in the bone marrow, individual cells receive different cues from the body that control how they develop and mature. As a result, the HSCs differentiate into different types of blood cells that, once mature, circulate in peripheral blood. This process of differentiation, called hematopoiesis, is shown in more detail in figure 1.9.

In terms of sheer numbers, the vast majority of HSCs become erythrocytes. Much smaller numbers become leukocytes and platelets. Leukocytes can be further subdivided into granulocytes, which are characterized by numerous granules visible in the cytoplasm, and agranulocytes, which lack granules. Table 1.7 provides an overview of the various types of formed elements, including their relative numbers, primary function, and lifespans.

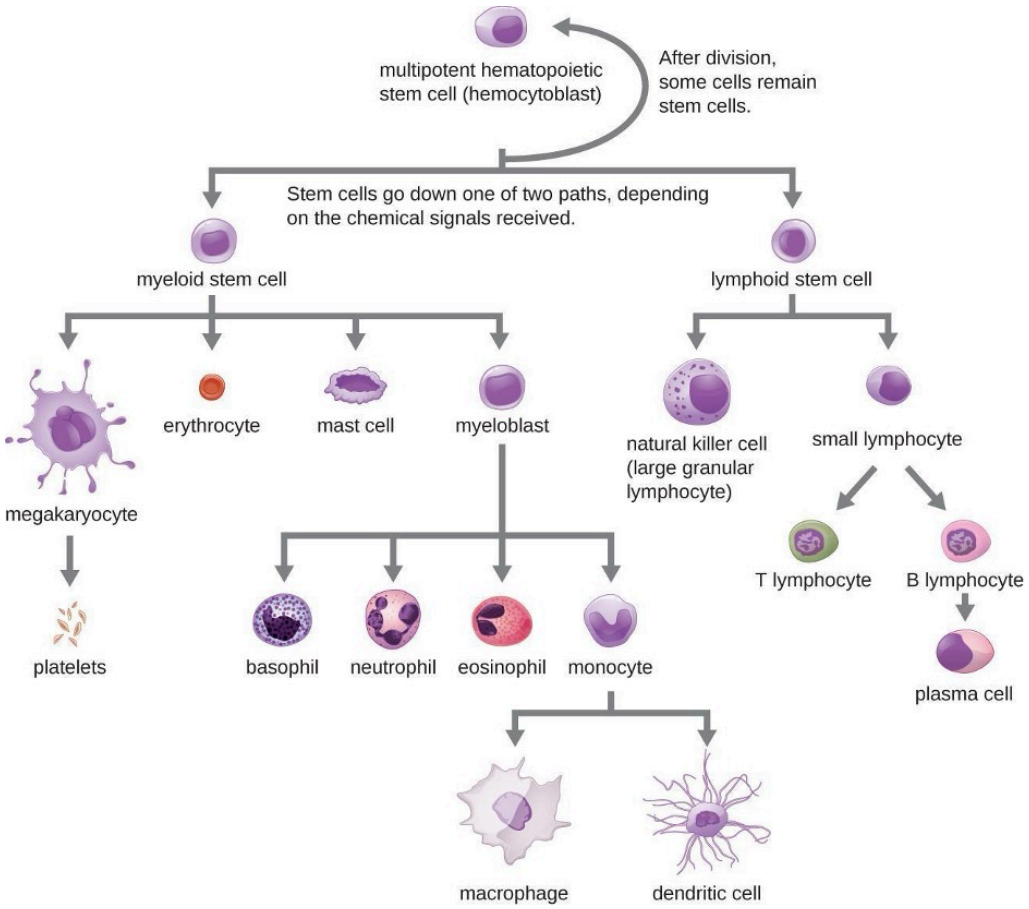
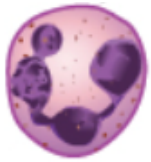
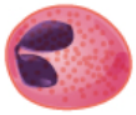
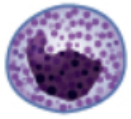


Figure 1.9: All the formed elements of the blood arise by differentiation of hematopoietic stem cells in the bone marrow. *Figure description available at the end of the chapter.*

Formed Element	Major Subtypes	Total leukocytes (%)	Numbers Present per Microliter(μL) and Mean (Range)	Appearance in Standard Blood Smear	Summary of Functions	Comments
Erythrocytes (red blood cells) 			5.2 million (4.4-6.0 million)	Flattened biconcave disk; no nucleus; pale red	Transport oxygen and some carbon dioxide between tissue and lungs	Lifespan of approximately 120 days
Leukocytes (white blood cells)			7000 (5000-10,000)	Obvious dark staining nucleus	All Function in body defenses	Exit capillaries and move into tissues; lifespan of usually a few hours or days
	Granulocytes including neutrophils, eosinophils, and basophils		4360 (1800-9950)	Abundant granules in cytoplasm, nucleus normally lobed	Nonspecific (innate) resistance to disease	Classified according to membrane-boun d granules in cytoplasm

Formed Element	Major Subtypes	Total leukocytes (%)	Numbers Present per Microliter(μL) and Mean (Range)	Appearance in Standard Blood Smear	Summary of Functions	Comments
	Neutrophils 	50-70	4150 (1800-7300)	Nucleus lobes increase with age; place lilac granules	Phagocytic; particularly effective against bacteria; release cytotoxic chemicals from granules	Most common leukocyte; lifespan of minutes to days
	Eosinphils 	1-3	165 (0-700)	Nucleus generally two-lobed; bright red-orange granules	Phagocytic cells; particularly active with antigen-antibody complexes; release degradative enzymes, toxic proteins and antihistamines; combat parasitic infections	lifespan of minutes to days
	Basophils 	<1	44 (0-150)	Nucleus generally two-lobed but difficult to see due to presence of heavy dense , dark purple granules	Pro-inflammatory	Least common leukocyte; lifespan unknown
	Agranulocytes including lymphocytes and monocytes		2640 (1700-4950)	Lack abundant granules in cytoplasm; have a simple-shaped nucleus that may be indented	Body defenses	Group consists of two major cell types from different linages

Formed Element	Major Subtypes	Total leukocytes (%)	Numbers Present per Microliter(μL) and Mean (Range)	Appearance in Standard Blood Smear	Summary of Functions	Comments
	Lymphocytes 	20-40	2185 (1500-4000)	Spherical cells with a single, often large, nucleus occupying much of the cell's volume; stains purple; seen in large (natural killer cells) and small (B and T cells) variants	Primarily specific (adaptive) immunity: T cells directly attack other cells (cellular immunity; B cells release antibodies (humoral immunity); natural killer cells are similar to T cells but nonspecific	Initial cells originate bone marrow, but secondary production occurs in lymphatic tissue; several distinct subtypes; memory cells form after exposure to a pathogen and rapidly increase in response to subsequent exposure; lifespan of many years
	Monocytes 	1-6	455 (200-950)	Largest leukocyte; has an indented or horseshoe-shaped nucleus	Very effective phagocytic cells engulfing pathogens or worn-out cells; also serve as antigen-presenting cells (APCs) or other components of the immune system	Produced in red bone marrow; referred to as macrophages and dendritic cells after leaving the circulation
Platelets 			350,000 (150,000-500,000)	Cellular fragments surrounded by a plasma membrane and containing granules; stains purple	Hemostasis; release growth factors for repair and healing of tissue	Formed from megakaryocytes that remain in the red bone marrow and shed platelets into circulation

Table 1.7: Formed elements of blood include erythrocytes (red blood cells), leukocytes (white blood cells), and platelets.

GRANULOCYTES

The various types of granulocytes can be distinguished from one another in a blood smear by the appearance of their nuclei and the contents of their granules, which confer different traits, functions, and staining properties. The neutrophils, also called polymorphonuclear neutrophils (PMNs), have a nucleus with three to five lobes and small, numerous, lilac-colored granules. Each lobe of the nucleus is connected by a thin strand of material to the other lobes. The eosinophils have fewer lobes in the nucleus (typically 2–3) and larger granules that stain reddish-orange. The basophils have a two-lobed nucleus and large granules that stain dark blue or purple (figure 1.10).

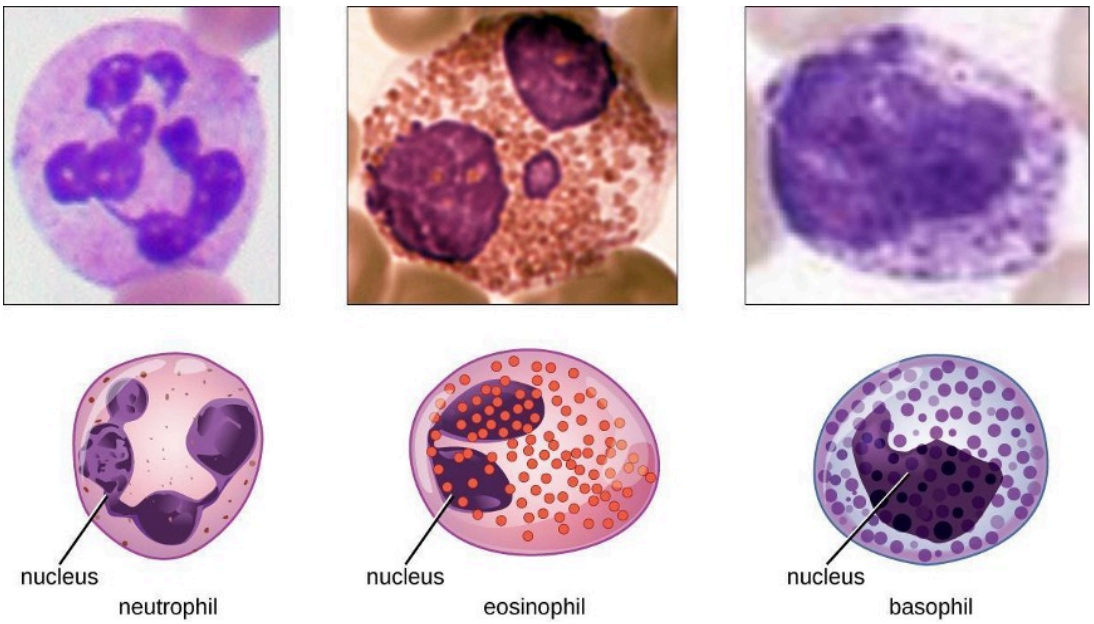


Figure 1.10: Granulocytes can be distinguished by the number of lobes in their nuclei and the staining properties of their granules. *Figure description available at the end of the chapter.*

Neutrophils (PMNs)

Neutrophils (PMNs) are frequently involved in the elimination and destruction of extracellular bacteria. They are capable of migrating through the walls of blood vessels to areas of bacterial infection and tissue damage, where they seek out and kill infectious bacteria. PMN granules contain a variety of defensins and hydrolytic enzymes that help them destroy bacteria through phagocytosis (described in more detail in section 1.6). In addition, when many neutrophils are brought into an infected area, they can be stimulated to release toxic molecules into the surrounding tissue to better clear infectious agents. This is called degranulation.

Another mechanism used by neutrophils is neutrophil extracellular traps (NETs), which are extruded meshes of chromatin that are closely associated with antimicrobial granule proteins and components. Chromatin is DNA with associated proteins (usually histone proteins, around which DNA wraps for organization and packing within a cell). By creating and releasing a mesh or lattice-like structure of chromatin that is coupled with antimicrobial proteins, the neutrophils can mount a highly concentrated and efficient attack against nearby pathogens. Proteins frequently associated with NETs include; lactoferrin, gelatinase, cathepsin G, and myeloperoxidase. Each has a different means of promoting antimicrobial activity, helping neutrophils eliminate pathogens. The toxic proteins in NETs may kill some of the body's own cells along with invading pathogens. However, this collateral damage can be repaired after the danger of the infection has been eliminated.

As neutrophils fight an infection, a visible accumulation of leukocytes, cellular debris, and bacteria at the site of infection can be observed. This buildup is what we call pus (also known as purulent or suppurative discharge or drainage). The presence of pus is a sign that the immune defenses have been activated against an infection; historically, some physicians believed that inducing pus formation could actually promote the healing of wounds. The practice of promoting **laudable pus** (by, for instance, wrapping a wound in greasy wool soaked in wine) dates back to the ancient physician Galen in the 2nd century AD, and was practiced in variant forms until the 17th century (though it was not universally accepted). Today, this method is no longer practiced because we now know that it is not effective. Although a small amount of pus formation can indicate a strong immune response, artificially inducing pus formation does not promote recovery.

Eosinophils

Eosinophils are granulocytes that protect against protozoa and helminths; they also play a role in allergic reactions. The granules of eosinophils, which readily absorb the acidic reddish dye eosin, contain histamine, degradative enzymes, and a compound known as major basic protein (MBP) (figure 1.10). MBP binds to the surface carbohydrates of parasites, and this binding is associated with disruption of the cell membrane and membrane permeability.

Basophils

Basophils have cytoplasmic granules of varied size and are named for their granules' ability to absorb the basic dye methylene blue (figure 1.10). Their stimulation and degranulation can result from multiple triggering events. Activated complement fragments C3a and C5a, produced in the activation cascades of complement proteins, act as anaphylatoxins by inducing degranulation of basophils and inflammatory responses. This cell type is important in allergic reactions and other responses that involve inflammation. One of the most abundant components of basophil granules is histamine, which is released along with other chemical factors when the basophil is stimulated. These chemicals can be chemotactic and can help to open the gaps between cells in the blood vessels. Other mechanisms for basophil triggering require the assistance of antibodies, as discussed in section 1.11.

Mast Cells

Hematopoiesis also gives rise to mast cells, which appear to be derived from the same common myeloid progenitor cell as neutrophils, eosinophils, and basophils. Functionally, mast cells are very similar to basophils, containing many of the same components in their granules (e.g., histamine) and playing a similar role in allergic responses and other inflammatory reactions. However, unlike basophils, mast cells leave the circulating blood and are most frequently found residing in tissues. They are often associated with blood vessels and nerves or found close to surfaces that interface with the external environment, such as the skin and mucous membranes in various regions of the body (figure 1.11).

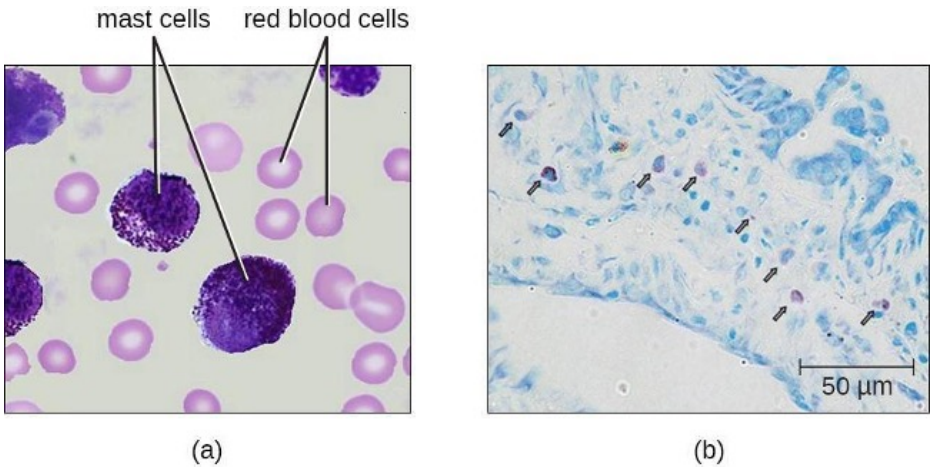


Figure 1.11: Mast cells function similarly to basophils by inducing and promoting inflammatory responses. (a) This figure shows mast cells in blood. In a blood smear, they are difficult to differentiate from basophils (b). Unlike basophils, mast cells migrate from the blood into various tissues. *Figure description available at the end of the chapter.*

Agranulocytes

As their name suggests, agranulocytes lack visible granules in the cytoplasm. Agranulocytes can be categorized as lymphocytes or monocytes (figure 1.9). Among the lymphocytes are natural killer cells, which play an important role in nonspecific innate immune defenses. Lymphocytes also include the B cells and T cells, and are central players in the specific adaptive immune defenses. The monocytes differentiate into macrophages and dendritic cells, which are collectively referred to as the mononuclear phagocyte system.

Natural Killer Cells

Most lymphocytes are primarily involved in the specific adaptive immune response. An exception is the natural killer cells (NK cells); these mononuclear lymphocytes use nonspecific mechanisms to recognize and destroy cells that are abnormal in some way. Cancer cells and cells infected with viruses are two examples of cellular abnormalities that are targeted by NK cells. Recognition of such cells involves a complex process of identifying inhibitory and activating molecular markers on the surface of the target cell. Molecular markers that make up the major histocompatibility complex (MHC) are expressed by healthy cells as an indication of "self." NK cells are able to recognize normal MHC markers on the surface of healthy cells, and these MHC markers serve as an inhibitory signal preventing NK cell activation. However, cancer cells and virus-infected cells actively diminish or eliminate expression of MHC markers on their surface. When these MHC markers are diminished or absent, the NK cell interprets this as an abnormality and as a cell in distress. This is one part of the NK cell activation process (figure 1.12). NK cells are also activated by binding to activating molecular molecules on the target cell. These activating molecular molecules include "altered self" or "nonself" molecules. When a NK cell recognizes a decrease in inhibitory normal MHC molecules and an increase in activating molecules on the surface of a cell, the NK cell will be activated to eliminate the cell in distress.

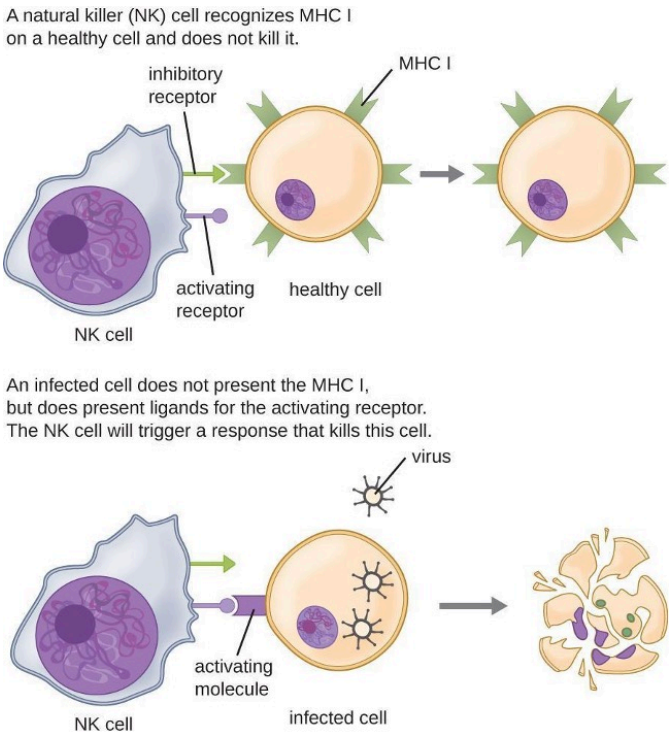


Figure 1.12: Natural killer (NK) cells are inhibited by the presence of the major histocompatibility cell (MHC) receptor on healthy cells. Cancer cells and virus-infected cells have reduced expression of MHC and increased expression of activating molecules. When a NK cell recognizes decreased MHC and increased activating molecules, it will kill the abnormal cell. *Figure description available at the end of the chapter.*

Once a cell has been recognized as a target, the NK cell can use several different mechanisms to kill its target. For example, it may express cytotoxic membrane proteins and cytokines that stimulate the target cell to undergo apoptosis, or controlled cell suicide. NK cells may also use perforin-mediated cytotoxicity to induce apoptosis in target cells. This mechanism relies on two toxins released from granules in the cytoplasm of the NK cell: perforin, a protein that creates pores in the target cell, and granzymes, proteases that enter through the pores into the target cell's cytoplasm, where they trigger a cascade of protein activation that leads to apoptosis. The NK cell binds to the abnormal target cell, releases its destructive payload, and detaches from the target cell. While the target cell undergoes apoptosis, the NK cell synthesizes more perforin and proteases to use on its next target.

NK cells contain these toxic compounds in granules in their cytoplasm. When stained, the granules are azurophilic and can be visualized under a light microscope (figure 1.13). Even though they have granules, NK cells are not considered granulocytes because their granules are far less numerous than those found in true granulocytes. Furthermore, NK cells have a different lineage than granulocytes, arising from lymphoid rather than myeloid stem cells (figure 1.10).

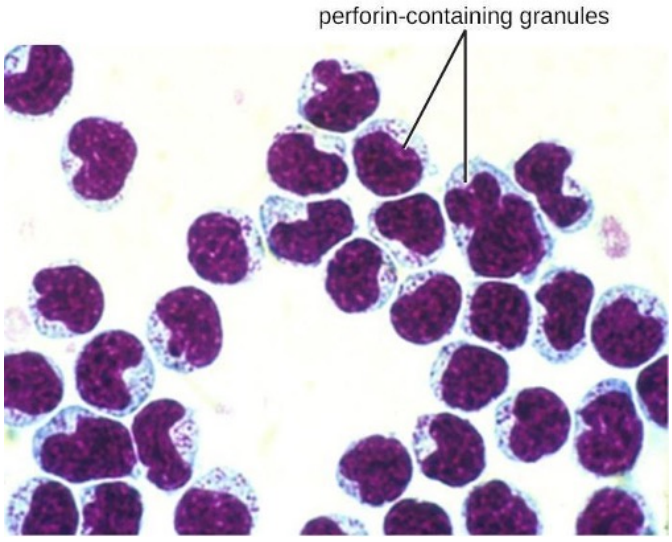


Figure 1.13: Natural killer cell with perforin-containing granules. *Figure description available at the end of the chapter.*

Monocytes

The largest of the white blood cells, monocytes have a nucleus that lacks lobes as well as granules in the cytoplasm (figure 1.14). Nevertheless, they are effective phagocytes, engulfing pathogens and apoptotic cells to help fight infection.

When monocytes leave the bloodstream and enter a specific body tissue, they differentiate into tissue-specific phagocytes called macrophages and dendritic cells. They are particularly important residents of lymphoid tissue, as well as nonlymphoid sites and organs. Macrophages and dendritic cells can reside in body tissues for significant lengths of time. Macrophages in specific body tissues develop characteristics suited to the particular tissue. Not only do they provide immune protection for the tissue in which they reside but they also support normal function of their neighboring tissue cells through the production of cytokines. Macrophages are given tissue-specific

names. A few examples of tissue-specific macrophages are listed in table 1.8. Dendritic cells are important sentinels residing in the skin and mucous membranes, which are portals of entry for many pathogens. Monocytes, macrophages, and dendritic cells are all highly phagocytic and are important promoters of the immune response because of their production and release of cytokines. These cells provide an essential bridge between innate and adaptive immune responses.

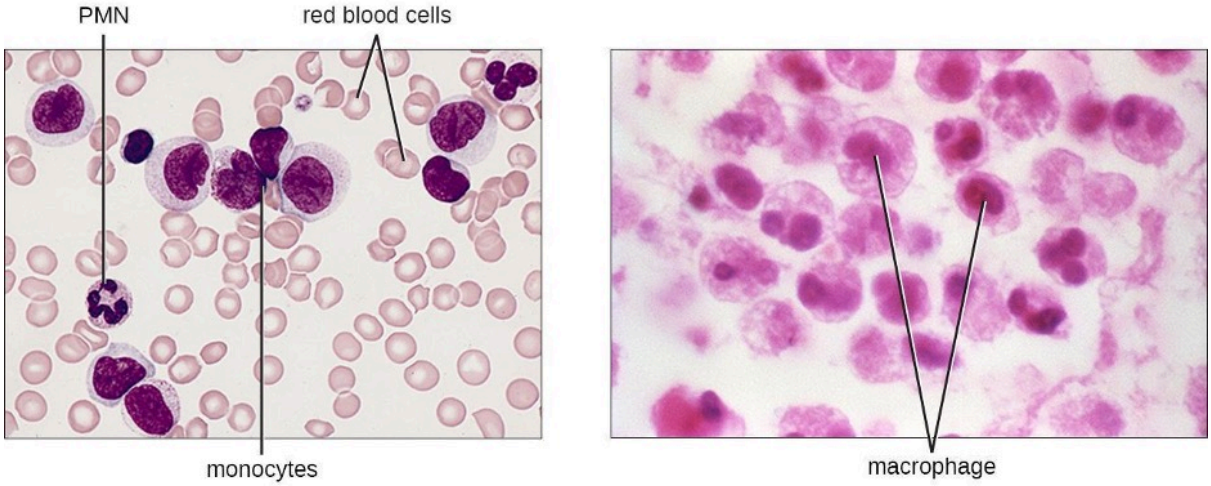


Figure 1.14: Monocytes are large, agranular white blood cells with a nucleus that lacks lobes. When monocytes leave the bloodstream, they differentiate and become macrophages with tissue-specific properties. *Figure description available at the end of the chapter.*

Tissue	Macrophage
Brain and central nervous system	Microglial cells
Liver	Kupffer Cells
Lungs	Alveolar macrophages (dust cells)
Peritoneal cavity	Peritoneal macrophages

Table 1.8: Macrophages found in various body tissues

1.6 PATHOGEN RECOGNITION AND PHAGOCYTOSIS

Several of the cell types discussed in the previous section can be described as phagocytes—cells whose main function is to seek, ingest, and kill pathogens. This process, called phagocytosis, was first observed in starfish in the 1880s by Nobel Prize-winning zoologist Ilya Metchnikoff (1845–1916), who made the connection to white blood cells (WBCs) in humans and other animals. At the time, Louis Pasteur (1822-1895) and other scientists believed that WBCs were spreading pathogens rather than killing them (which is true for some diseases, such as tuberculosis). But in most cases, phagocytes provide a strong, swift, and effective defense against a broad range of microbes, making them a critical component of innate nonspecific immunity. This section will focus on the mechanisms by which phagocytes are able to seek, recognize, and destroy pathogens.

EXTRAVASATION (DIAPEDESIS) OF LEUKOCYTES

Some phagocytes are leukocytes (WBCs) that normally circulate in the bloodstream. To reach pathogens located in infected tissue, leukocytes must pass through the walls of small capillary blood vessels within tissues. This process, called extravasation, or diapedesis, is initiated by complement factor C5a, as well as cytokines released into the immediate vicinity by resident macrophages and tissue cells responding to the presence of the infectious agent (figure 1.15). Similar to C5a, many of these cytokines are proinflammatory and chemotactic, and they bind to cells of small capillary blood vessels, initiating a response in the endothelial cells lining the inside of the blood vessel walls. This response involves the upregulation and expression of various cellular adhesion molecules and receptors. Leukocytes passing through will stick slightly to the adhesion molecules, slowing down and rolling along the blood vessel walls near the infected area. When they reach a cellular junction, they will bind to even more of these adhesion molecules, flattening out and squeezing through the cellular junction in a process known as transendothelial migration. This mechanism of **rolling adhesion** allows leukocytes to exit the bloodstream and enter the infected areas, where they can begin phagocytosing the invading pathogens.

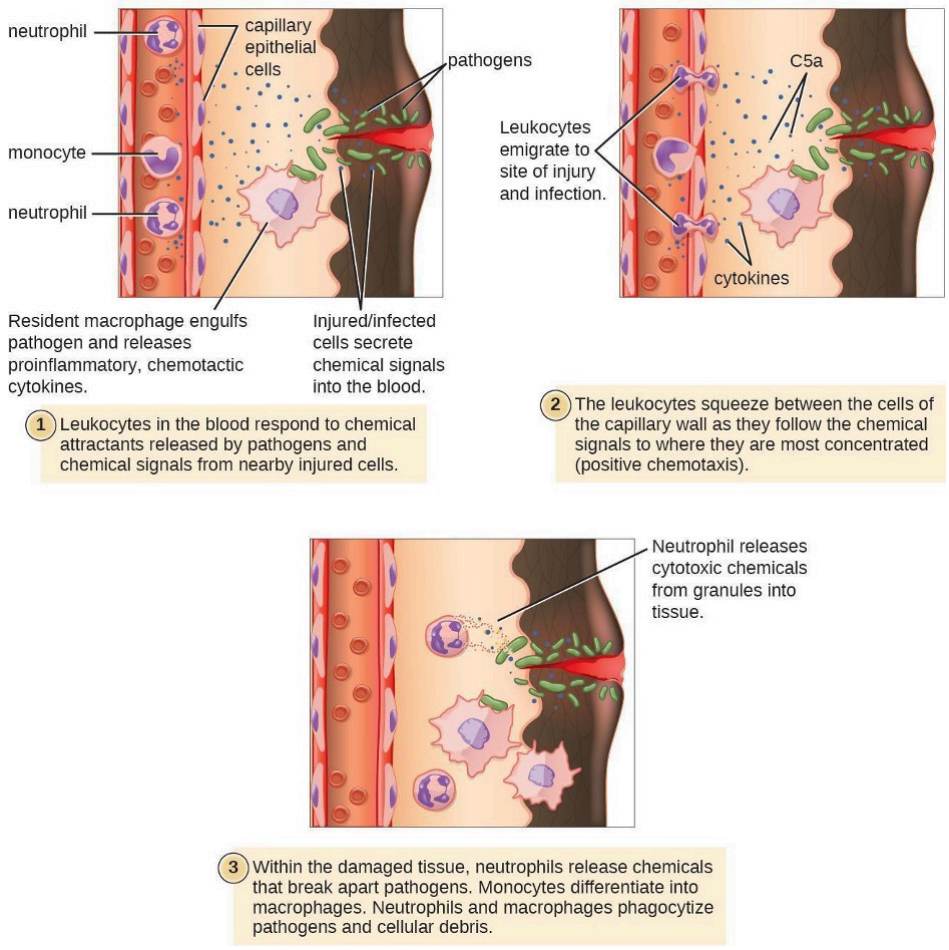


Figure 1.15: Damaged cells and macrophages that have ingested pathogens release cytokines that are proinflammatory and chemotactic for leukocytes. In addition, activation of complements at the site of infection results in production of the chemotactic and proinflammatory C5a. Leukocytes exit the blood vessel and follow the chemoattractant signal of cytokines and C5a to the site of infection. Granulocytes such as neutrophils release chemicals that destroy pathogens. They are also capable of phagocytosis and intracellular killing of bacterial pathogens. *Figure description available at the end of the chapter.*

Note that extravasation does not occur in arteries or veins. These blood vessels are surrounded by thicker, multilayer protective walls, in contrast to the thin single-cell-layer walls of capillaries. Furthermore, the blood flow in arteries is too turbulent to allow for rolling adhesion. Also, some leukocytes tend to respond to an infection more quickly than others. The first to arrive typically are neutrophils, often within hours of a bacterial infection. By contract, monocytes may take several days to leave the bloodstream and differentiate into macrophages.

PATHOGEN RECOGNITION

As described in the previous section, opsonization of pathogens by antibody; complement factors C1q, C3b, and C4b; and lectins can assist phagocytic cells in recognition of pathogens and attachment to initiate phagocytosis. However, not all pathogen recognition is opsonin dependent. Phagocytes can also recognize molecular structures that are common to many groups of pathogenic microbes. Such structures are called pathogen-associated molecular patterns (PAMPs). Common PAMPs include the following:

- peptidoglycan, found in bacterial cell walls;
- flagellin, a protein found in bacterial flagella;
- lipopolysaccharide (LPS) from the outer membrane of gram-negative bacteria;
- lipopeptides, molecules expressed by most bacteria; and
- nucleic acids such as viral DNA or RNA.

Like numerous other PAMPs, these substances are integral to the structure of broad classes of microbes.

The structures that allow phagocytic cells to detect PAMPs are called pattern recognition receptors (PRRs). One group of PRRs is the toll-like receptors (TLRs), which bind to various PAMPs and communicate with the nucleus of the phagocyte to elicit a response. Many TLRs (and other PRRs) are located on the surface of a phagocyte, but some can also be found embedded in the membranes of interior compartments and organelles (figure 1.16). These interior PRRs can be useful for the binding and recognition of intracellular pathogens that may have gained access to the inside of the cell before phagocytosis could take place. Viral nucleic acids, for example, might encounter an interior PRR, triggering production of the antiviral cytokine interferon.

In addition to providing the first step of pathogen recognition, the interaction between PAMPs and PRRs on macrophages provides an intracellular signal that activates the phagocyte, causing it to transition from a dormant state of readiness and slow proliferation to a state of hyperactivity, proliferation, production/secretion of cytokines, and enhanced intracellular killing. PRRs on macrophages also respond to chemical distress signals from damaged or stressed cells. This allows macrophages to extend their responses beyond protection from infectious diseases to a broader role in the inflammatory response initiated from injuries or other diseases.

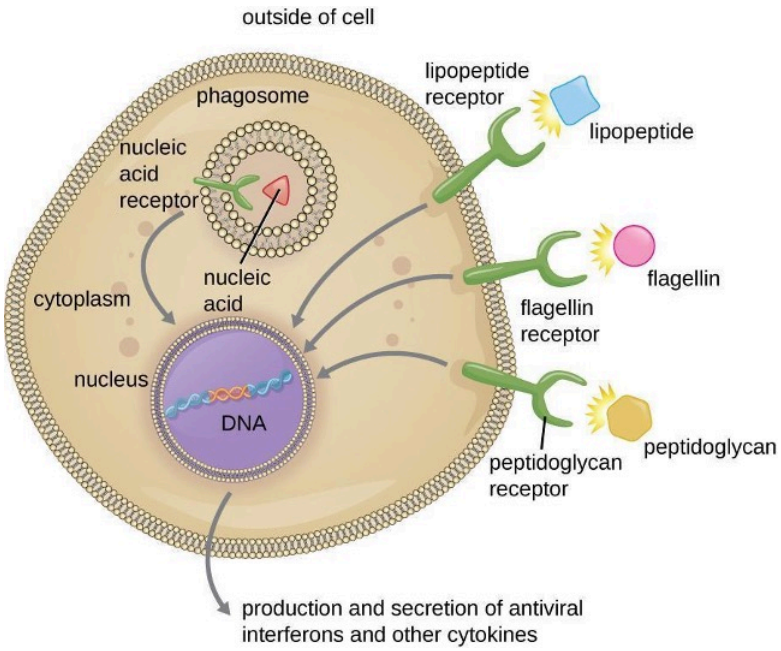


Figure 1.16: Phagocytic cells contain pattern recognition receptors (PRRs) capable of recognizing various pathogen-associated molecular patterns (PAMPs). These PRRs can be found on the plasma membrane or in internal phagosomes. When a PRR recognizes a PAMP, it sends a signal to the nucleus that activates genes involved in phagocytosis, cellular proliferation, production and secretion of antiviral interferons and proinflammatory cytokines, and enhanced intracellular killing. *Figure description available at the end of the chapter.*

PATHOGEN DEGRADATION

Once pathogen recognition and attachment occurs, the pathogen is engulfed in a vesicle and brought into the internal compartment of the phagocyte in a process called phagocytosis (figure 1.17). PRRs can aid in phagocytosis by first binding to the pathogen's surface, but phagocytes are also capable of engulfing nearby items even if they are not bound to specific receptors. To engulf the pathogen, the phagocyte forms a pseudopod that wraps around the pathogen and then pinches it off into a membrane vesicle called a phagosome. Acidification of the phagosome (pH decreases to the range of 4–5) provides an important early antibacterial mechanism. The phagosome containing the pathogen fuses with one or more lysosomes, forming a phagolysosome. Formation of the phagolysosome enhances the acidification, which is essential for activation of pH-dependent digestive lysosomal enzymes and production of hydrogen peroxide and toxic reactive oxygen species. Lysosomal enzymes such as lysozyme, phospholipase, and proteases digest the pathogen. Other enzymes are involved in a respiratory burst. During the respiratory burst, phagocytes will increase their uptake and consumption of oxygen, but not for energy production. The increased oxygen consumption is focused on the production of superoxide anion, hydrogen peroxide, hydroxyl radicals, and other reactive oxygen species that are antibacterial.

In addition to the reactive oxygen species produced by the respiratory burst, reactive nitrogen compounds with cytotoxic (cell-killing) potential can also form. For example, nitric oxide can react with superoxide to form peroxynitrite, a highly reactive nitrogen compound with degrading capabilities similar to those of the reactive oxygen species. Some phagocytes even contain an internal storehouse of microbicidal defensin proteins (e.g.,

neutrophil granules). These destructive forces can be released into the area around the cell to degrade microbes externally. Neutrophils, especially, can be quite efficient at this secondary antimicrobial mechanism.

Once degradation is complete, leftover waste products are excreted from the cell in an exocytic vesicle. However, it is important to note that not all remains of the pathogen are excreted as waste. Macrophages and dendritic cells are also antigen-presenting cells involved in the specific adaptive immune response. These cells further process the remains of the degraded pathogen and present key antigens (specific pathogen proteins) on their cellular surface. This is an important step for stimulation of some adaptive immune responses.

1.7 INFLAMMATION AND FEVER

The inflammatory response, or inflammation, is triggered by a cascade of chemical mediators and cellular responses that may occur when cells are damaged and stressed or when pathogens successfully breach the physical barriers of the innate immune system. Although inflammation is typically associated with negative consequences of injury or disease, it is a necessary process insofar as it allows for recruitment of the cellular defenses needed to eliminate pathogens, remove damaged and dead cells, and initiate repair mechanisms. Excessive inflammation, however, can result in local tissue damage and, in severe cases, may even become deadly.

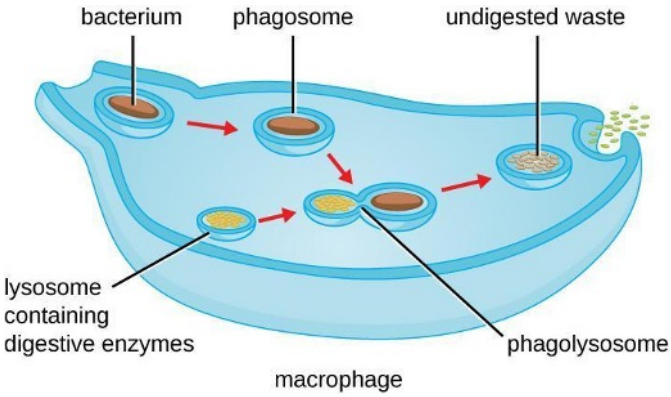


Figure 1.17: The stages of phagocytosis include the engulfment of a pathogen, the formation of a phagosome, the digestion of the pathogenic particle in the phagolysosome, and the expulsion of undigested materials from the cell. *Figure description available at the end of the chapter.*

ACUTE INFLAMMATION

An early, if not immediate, response to tissue injury is acute inflammation. Immediately following an injury, vasoconstriction of blood vessels will occur to minimize blood loss. The amount of vasoconstriction is related to the amount of vascular injury, but it is usually brief. Vasoconstriction is followed by vasodilation and increased vascular permeability, as a direct result of the release of histamine from resident mast cells. Increased blood flow and vascular permeability can dilute toxins and bacterial products at the site of injury or infection. They also contribute to the five observable signs associated with the inflammatory response: erythema (redness), edema (swelling), heat, pain, and altered function. Vasodilation and increased vascular permeability are also associated with an influx of phagocytes at the site of injury and/or infection. This can enhance the inflammatory response because phagocytes may release proinflammatory chemicals when they are activated by cellular distress signals released from damaged cells, by PAMPs, or by opsonins on the surface of pathogens. Activation of the complement system can further enhance the inflammatory response through the production of the anaphylatoxin C5a. Figure 1.18 illustrates a typical case of acute inflammation at the site of a skin wound.

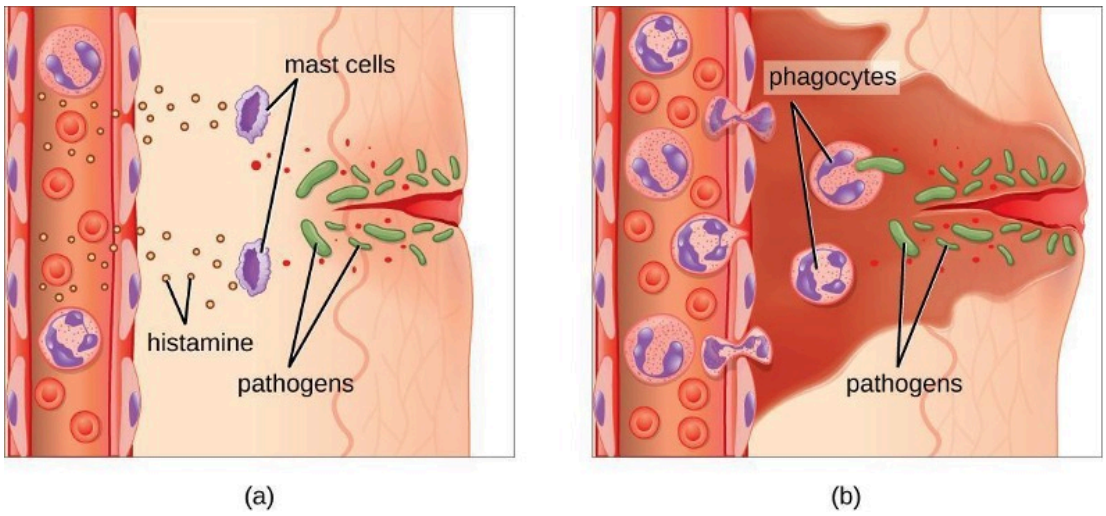


Figure 1.18: (a) Mast cells detect injury to nearby cells and release histamine, initiating an inflammatory response. (b) Histamine increases blood flow to the wound site, and increased vascular permeability allows fluid, proteins, phagocytes, and other immune cells to enter infected tissue. These events result in the swelling and reddening of the injured site, and the increased blood flow to the injured site causes it to feel warm. Inflammation is also associated with pain due to these events stimulating nerve pain receptors in the tissue. The interaction of phagocyte PRRs with cellular distress signals and PAMPs and opsonins on the surface of pathogens leads to the release of more proinflammatory chemicals, enhancing the inflammatory response. Figure description available at the end of the chapter.

During the period of inflammation, the release of bradykinin causes capillaries to remain dilated, flooding tissues with fluids and leading to edema. Increasing numbers of neutrophils are recruited to the area to fight pathogens. As the fight rages on, pus forms from the accumulation of neutrophils, dead cells, tissue fluids, and lymph. Typically, after a few days, macrophages will help to clear out this pus. Eventually, tissue repair can begin in the wounded area.

CHRONIC INFLAMMATION

When acute inflammation is unable to clear an infectious pathogen, chronic inflammation may occur. This often results in an ongoing (and sometimes futile) lower-level battle between the host organism and the pathogen. The wounded area may heal at a superficial level, but pathogens may still be present in deeper tissues, stimulating ongoing inflammation. Additionally, chronic inflammation may be involved in the progression of degenerative neurological diseases such as Alzheimer's, Parkinson's, heart disease, and metastatic cancer.

Chronic inflammation may lead to the formation of granulomas, pockets of infected tissue walled off and surrounded by WBCs. Macrophages and other phagocytes wage an unsuccessful battle to eliminate the pathogens and dead cellular materials within a granuloma. One example of a disease that produces chronic inflammation is tuberculosis, which results in the formation of granulomas in lung tissues. A tubercular granuloma is called a tubercle (figure 1.19). Tuberculosis will be covered in more detail in section 5.2.

Chronic inflammation is not just associated with bacterial infections. Chronic inflammation can be an important cause of tissue damage from viral infections. The extensive scarring observed with hepatitis C infections and liver cirrhosis is the result of chronic inflammation.

FEVER

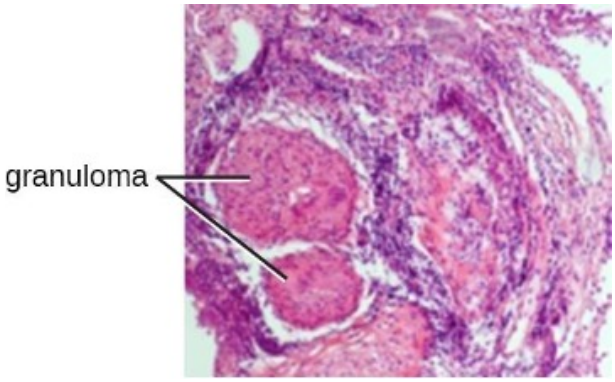


Figure 1.19: A tubercle is a granuloma in the lung tissue of a patient with tuberculosis. In this micrograph, white blood cells (stained purple) have walled off a pocket of tissue infected with Mycobacterium tuberculosis. Granulomas also occur in many other forms of disease. Used under fair use. Figure description available at the end of the chapter.

A fever is an inflammatory response that extends beyond the site of infection and affects the entire body, resulting in an overall increase in body temperature. Body temperature is normally regulated and maintained by the hypothalamus, an anatomical section of the brain that functions to maintain homeostasis in the body. However, certain bacterial or viral infections can result in the production of pyrogens, chemicals that effectively alter the "thermostat setting" of the hypothalamus to elevate body temperature and cause fever. Pyrogens may be exogenous or endogenous. For example, the endotoxin lipopolysaccharide (LPS), produced by gram-negative bacteria, is an exogenous pyrogen that may induce the leukocytes to release endogenous pyrogens such as interleukin-1 (IL-1), IL-6, interferon-γ (IFN-γ), and tumor necrosis factor (TNF). In a cascading effect, these molecules can then lead to the release of prostaglandin E2 (PGE2) from other cells, resetting the hypothalamus to initiate fever (figure 1.20).

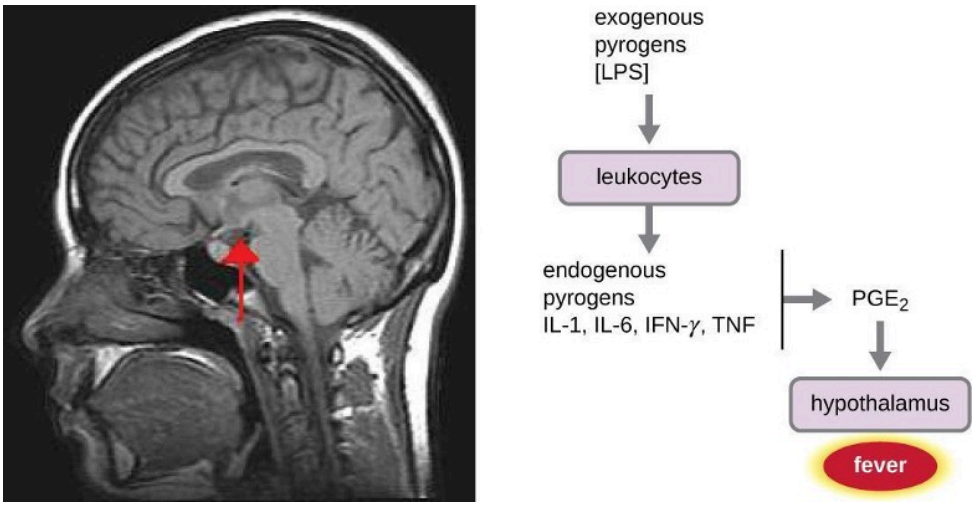


Figure 1.20: The role of the hypothalamus in the inflammatory response. Macrophages recognize pathogens in an area and release cytokines that trigger inflammation. The cytokines also send a signal up the vagus nerve to the hypothalamus. Figure description available at the end of the chapter.

Like other forms of inflammation, a fever enhances the innate immune defenses by stimulating leukocytes to kill pathogens. The rise in body temperature also may inhibit the growth of many pathogens since human pathogens are mesophiles with optimum growth occurring around 35 °C (95 °F). In addition, some studies suggest that fever may also stimulate release of iron-sequestering compounds from the liver, thereby starving out microbes that rely on iron for growth.[1]

During fever, the skin may appear pale due to vasoconstriction of the blood vessels in the skin, which is mediated by the hypothalamus to divert blood flow away from extremities, minimizing the loss of heat and raising the core temperature. The hypothalamus will also stimulate shivering of muscles, another effective mechanism of generating heat and raising the core temperature.

The crisis phase occurs when the fever breaks. The hypothalamus stimulates vasodilation, resulting in a return of blood flow to the skin and a subsequent release of heat from the body. The hypothalamus also stimulates sweating, which cools the skin as the sweat evaporates.

Although a low-level fever may help an individual overcome an illness, in some instances, this immune response can be too strong, causing tissue and organ damage and, in severe cases, even death. The inflammatory response to bacterial superantigens is one scenario in which a life-threatening fever may develop. Superantigens are bacterial or viral proteins that can cause an excessive activation of T cells from the specific adaptive immune defense, as well as an excessive release of cytokines that overstimulates the inflammatory response. For example, *Staphylococcus aureus* and *Streptococcus pyogenes* are capable of producing superantigens that cause toxic shock syndrome and scarlet fever, respectively. Both of these conditions can be associated with very high, life-threatening fevers in excess of 42 °C (108 °F).

1.8 OVERVIEW OF SPECIFIC ADAPTIVE IMMUNITY

Adaptive immunity is defined by two important characteristics: specificity and memory. Specificity refers to the adaptive immune system's ability to target specific pathogens, and memory refers to its ability to quickly respond to pathogens to which it has previously been exposed. For example, when an individual recovers from chickenpox, the body develops a *memory* of the infection that will *specifically* protect it from the causative agent, the varicella-zoster virus, if it is exposed to the virus again.

Specificity and memory are achieved by essentially programming certain cells involved in the immune response to respond rapidly to subsequent exposures of the pathogen. This programming occurs as a result of the first exposure to a pathogen or vaccine, which triggers a primary response. Subsequent exposures result in a secondary response that is faster and stronger as a result of the body's memory of the first exposure (figure 1.21). This secondary response, however, is specific to the pathogen in question. For example, exposure to one virus (e.g., varicella-zoster virus) will not provide protection against other viral diseases (e.g., measles, mumps, or polio).

Adaptive specific immunity involves the actions of two distinct cell types: B lymphocytes (B cells) and T lymphocytes (T cells). Although B cells and T cells arise from a common hematopoietic stem cell differentiation pathway (see figure 1.9), their sites of maturation and their roles in adaptive immunity are very different.

B cells mature in the bone marrow and are responsible for the production of glycoproteins called antibodies, or immunoglobulins. Antibodies are involved in the body's defense against pathogens and toxins in the extracellular environment. Mechanisms of adaptive specific immunity that involve B cells and antibody production are referred to as humoral immunity. The maturation of T cells occurs in the thymus. T cells function as the central orchestrator of both innate and adaptive immune responses. They are also responsible for destruction of cells infected with intracellular pathogens. The targeting and destruction of intracellular pathogens by T cells is called cell-mediated immunity, or cellular immunity.

ANTIGENS

Activation of the adaptive immune defenses is triggered by pathogen-specific molecular structures called antigens. Antigens are similar to the pathogen-associated molecular patterns (PAMPs) discussed in section 1.6; however, whereas PAMPs are molecular structures found on numerous pathogens, antigens are unique to a specific pathogen. The antigens that stimulate adaptive immunity to chickenpox, for example, are unique to the varicella-zoster virus but significantly different from the antigens associated with other viral pathogens.

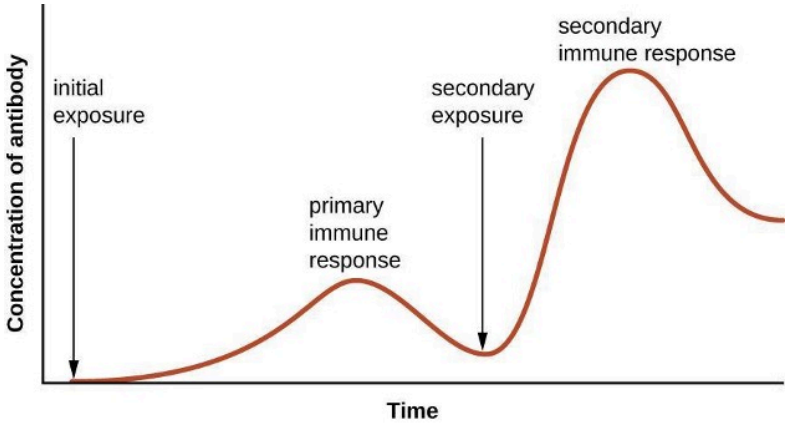


Figure 1.21: This graph illustrates the primary and secondary immune responses related to antibody production after an initial and secondary exposure to an antigen. Notice that the secondary response is faster and provides a much higher concentration of antibody. *Figure description available at the end of the chapter.*

The term *antigen* was initially used to describe molecules that stimulate the production of antibodies; in fact, the term comes from a combination of the words *anti*body and *gen*erator, and a molecule that stimulates antibody production is said to be **antigenic**. However, the role of antigens is not limited to humoral immunity and the production of antibodies; antigens also play an essential role in stimulating cellular immunity, and for this reason antigens are sometimes more accurately referred to as immunogens. In this text, however, we will typically refer to them as antigens.

Pathogens possess a variety of structures that may contain antigens. For example, antigens from bacterial cells may be associated with their capsules, cell walls, fimbriae, flagella, or pili. Bacterial antigens may also be associated with extracellular toxins and enzymes that they secrete. Viruses possess a variety of antigens associated with their capsids, envelopes, and the spike structures they use for attachment to cells.

Antigens may belong to any number of molecular classes, including carbohydrates, lipids, nucleic acids, proteins, and combinations of these molecules. Antigens of different classes vary in their ability to stimulate adaptive immune defenses as well as in the type of response they stimulate (humoral or cellular). The structural complexity of an antigenic molecule is an important factor in its antigenic potential. In general, more complex molecules are more effective as antigens. For example, the three-dimensional complex structure of proteins make them the most effective and potent antigens, capable of stimulating both humoral and cellular immunity. In comparison, carbohydrates are less complex in structure and therefore less effective as antigens; they can only stimulate humoral immune defenses. Lipids and nucleic acids are the least antigenic molecules. In some cases these structures may only become antigenic when combined with proteins or carbohydrates to form glycolipids, lipoproteins, or nucleoproteins.

One reason the three-dimensional complexity of antigens is so important is that antibodies and T cells do not recognize and interact with an entire antigen but with smaller exposed regions on the surface of antigens called epitopes. A single antigen may possess several different epitopes (figure 1.22), and different antibodies may bind to different epitopes on the same antigen (figure 1.23). For example, the bacterial flagellum is a large, complex protein structure that can possess hundreds or even thousands of epitopes with unique three-dimensional structures. Moreover, flagella from different bacterial species (or even strains of the same species) contain unique epitopes that can only be bound by specific antibodies.

An antigen's size is another important factor in its antigenic potential. Whereas large antigenic structures like flagella possess multiple epitopes, some molecules are too small to be antigenic by themselves. Such molecules, called haptens, are essentially free epitopes that are not part of the complex three-dimensional structure of a larger antigen. For a hapten to become antigenic, it must first attach to a larger carrier molecule (usually a protein) to produce a conjugate antigen. The hapten-specific antibodies produced in response to the conjugate antigen are then able to interact with unconjugated free hapten molecules. Haptens are not known to be associated with any specific pathogens, but they are responsible for some allergic responses. For example, the hapten urushiol, a molecule found in the oil of plants that cause poison ivy, causes an immune response that can result in a severe rash (called contact dermatitis). Similarly, the hapten penicillin can cause allergic reactions to drugs in the penicillin class.

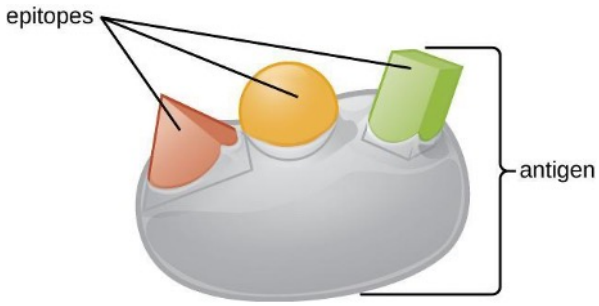


Figure 1.22: An antigen is a macromolecule that reacts with components of the immune system. A given antigen may contain several motifs that are recognized by immune cells. *Figure description available at the end of the chapter.*

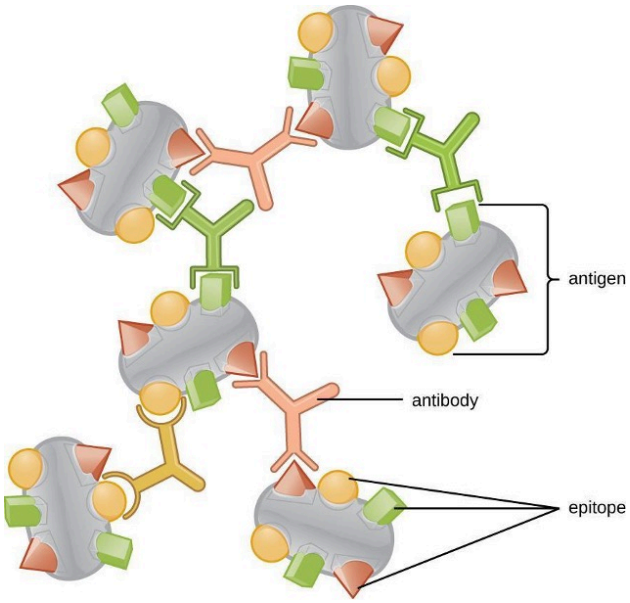


Figure 1.23: A typical protein antigen has multiple epitopes, shown by the ability of three different antibodies to bind to different epitopes of the same antigen. *Figure description available at the end of the chapter.*

ANTIBODIES

Antibodies (also called immunoglobulins) are glycoproteins that are present in both the blood and tissue fluids. The basic structure of an antibody monomer consists of four protein chains held together by disulfide bonds (figure 1.24). A disulfide bond is a covalent bond between the sulfhydryl *R* groups found on two cysteine amino acids. The two largest chains are identical to each other and are called the heavy chains. The two smaller chains are also identical to each other and are called the light chains. Joined together, the heavy and light chains form a basic Y-shaped structure.

The two "arms" of the Y-shaped antibody molecule are known as the Fab region (fragment of antigen binding). The far end of the Fab region is the variable region, which serves as the site of antigen binding. The amino acid sequence in the variable region dictates the three-dimensional structure, and thus the specific three-dimensional epitope to which the Fab region is capable of binding. Although the epitope specificity of the Fab regions is identical for each arm of a single antibody molecule, this region displays a high degree of variability between antibodies with different epitope specificities. Binding to the Fab region is necessary for neutralization of pathogens, agglutination or aggregation of pathogens, and antibody-dependent cell-mediated cytotoxicity.

The constant region of the antibody molecule includes the trunk of the Y and lower portion of each arm of the Y. The trunk of the Y is also called the Fc region (fragment of crystallization) and is the site of complement factor binding and binding to phagocytic cells during antibody-mediated opsonization.

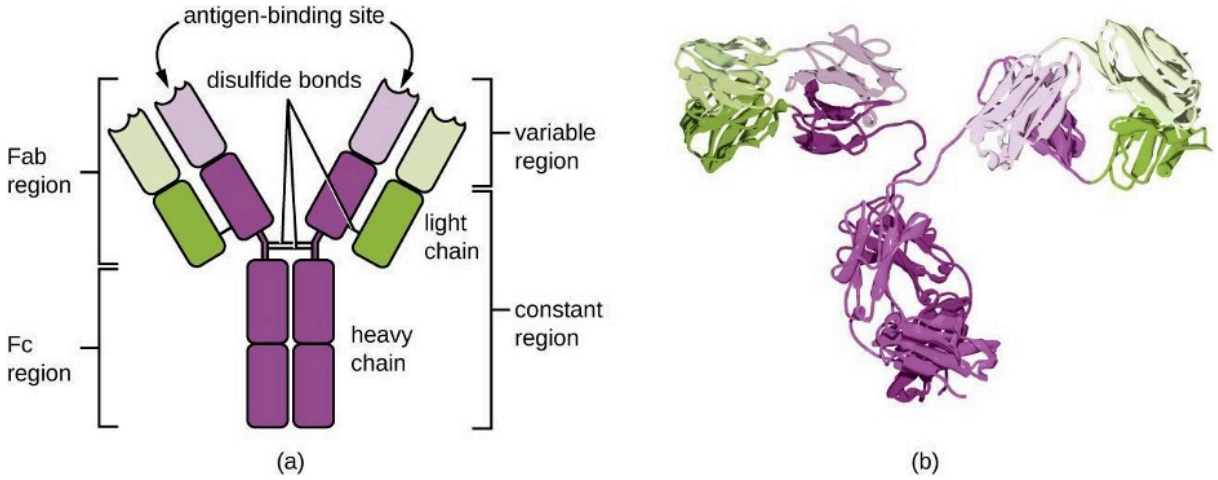


Figure 1.24: (a) The typical four-chain structure of a generic antibody monomer. (b) The corresponding three-dimensional structure of the antibody IgG. *Figure description available at the end of the chapter.*

Antibody Classes

The constant region of an antibody molecule determines its class, or isotype. The five classes of antibodies are IgG, IgM, IgA, IgD, and IgE. Each class possesses unique heavy chains designated by the Greek letters γ, μ, α, δ, and ε, respectively. Antibody classes also exhibit important differences in abundance in serum, arrangement, body sites of action, functional roles, and size (table 1.9).

IgG is a monomer that is by far the most abundant antibody in human blood, accounting for about 80% of total serum antibody. IgG penetrates efficiently into tissue spaces and is the only antibody class with the ability to cross the placental barrier, providing passive immunity to the developing fetus during pregnancy. IgG is also the most versatile antibody class in terms of its role in the body's defense against pathogens.

IgM is initially produced in a monomeric membrane-bound form that serves as an antigen-binding receptor on B cells. The secreted form of IgM assembles into a pentamer with five monomers of IgM bound together by a protein structure called the J chain. Although the location of the J chain relative to the Fc regions of the five monomers prevents IgM from performing some of the functions of IgG, the ten available Fab sites associated with a pentameric IgM make it an important antibody in the body's arsenal of defenses. IgM is the first antibody produced and secreted by B cells during the primary and secondary immune responses, making pathogen-specific IgM a valuable diagnostic marker during active or recent infections.

IgA accounts for about 13% of total serum antibody, and secretory IgA is the most common and abundant antibody class found in the mucus secretions that protect the mucous membranes. IgA can also be found in other secretions such as breast milk, tears, and saliva. Secretory IgA is assembled into a dimeric form with two monomers joined by a protein structure called the secretory component. One of the important functions of secretory IgA is to trap pathogens in mucus so that they can later be eliminated from the body.

Similar to IgM, **IgD** is a membrane-bound monomer found on the surface of B cells, where it serves as an antigen-binding receptor. However, IgD is not secreted by B cells, and only trace amounts are detected in serum. These trace amounts most likely come from the degradation of old B cells and the release of IgD molecules from their cytoplasmic membranes.

IgE is the least abundant antibody class in serum. Like IgG, it is secreted as a monomer, but its role in adaptive immunity is restricted to anti-parasitic defenses. The Fc region of IgE binds to basophils and mast cells. The Fab region of the bound IgE then interacts with specific antigen epitopes, causing the cells to release potent pro-inflammatory mediators. The inflammatory reaction resulting from the activation of mast cells and basophils aids in the defense against parasites, but this reaction is also central to allergic reactions.

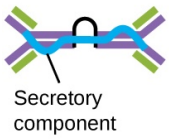
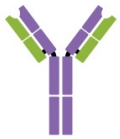
Properties	IgG monomer	IgM pentamer	Secretory IgA dimer	IgD monomer	IgE Monomer
Structure					
Heavy chains	γ	μ	α	δ	ε
Number of antigen-binding sites	2	10	4	2	2
Molecular weight (Daltons)	150,000	900,000	385,000	180,000	200,000
Percentage of total antibody in serum	80%	6%	13% (monomer)	<1%	<1%
Crosses placenta	yes	no	no	no	no
Fixes complement	yes	yes	no	no	no
Fc binds to	phagocytes				mast cells and basophils
Function	Neutralization, agglutination, complement activation, opsonization, and antibody-dependen t cell-mediated cytotoxicity.	Neutralization, agglutination, complement activation. The monomer serves as the B-cell receptor.	Neutralization and trapping of pathogens in mucus.	B-cell receptor.	Activation of basophils and mast cells against parasites and allergens.

Table 1.9: The five immunoglobulin (Ig) classes

ANTIGEN-ANTIBODY INTERACTIONS

Different classes of antibodies play important roles in the body's defense against pathogens. These functions include neutralization of pathogens, opsonization for phagocytosis, agglutination, complement activation, and antibody-dependent cell-mediated cytotoxicity. For most of these functions, antibodies also provide an important link between adaptive specific immunity and innate nonspecific immunity.

Neutralization involves the binding of certain antibodies (IgG, IgM, or IgA) to epitopes on the surface of pathogens or toxins, preventing their attachment to cells. For example, Secretory IgA can bind to specific pathogens and block initial attachment to intestinal mucosal cells. Similarly, specific antibodies can bind to certain toxins, blocking them from attaching to target cells and thus neutralizing their toxic effects. Viruses can be neutralized and prevented from infecting a cell by the same mechanism (figure 1.25).

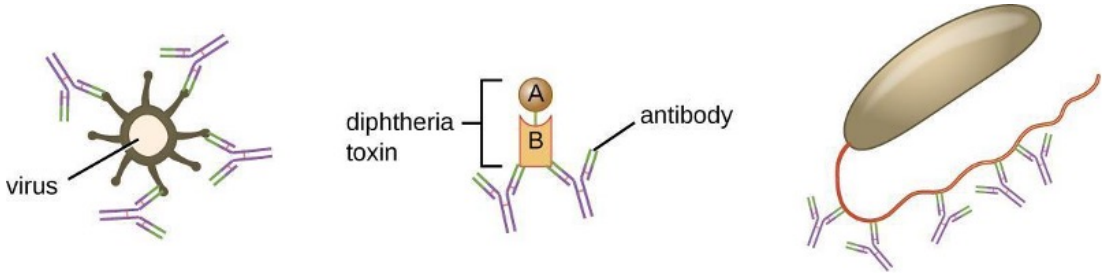


Figure 1.25: Neutralization involves the binding of specific antibodies to antigens found on bacteria, viruses, and toxins, preventing them from attaching to target cells. *Figure description available at the end of the chapter.*

As described in section 1.4, opsonization is the coating of a pathogen with molecules, such as complement factors, C-reactive protein, and serum amyloid A, to assist in phagocyte binding to facilitate phagocytosis. IgG antibodies also serve as excellent opsonins, binding their Fab sites to specific epitopes on the surface of pathogens. Phagocytic cells such as macrophages, dendritic cells, and neutrophils have receptors on their surfaces that recognize and bind to the Fc portion of the IgG molecules; thus, IgG helps such phagocytes attach to and engulf the pathogens they have bound (figure 1.26).

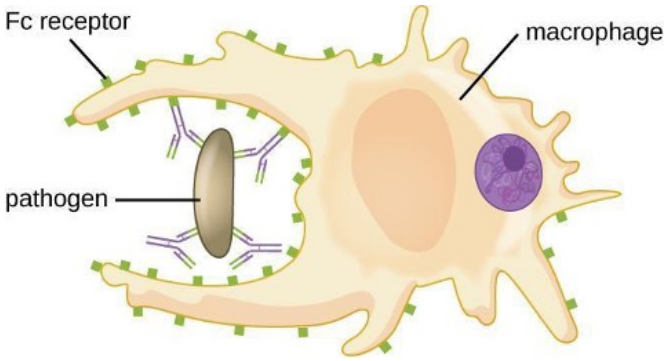


Figure 1.26: Antibodies serve as opsonins and inhibit infection by tagging pathogens for destruction by macrophages, dendritic cells, and neutrophils. These phagocytic cells use Fc receptors to bind to IgG-opsonized pathogens and initiate the first step of attachment before phagocytosis. *Figure description available at the end of the chapter.*

Agglutination or aggregation involves the cross-linking of pathogens by antibodies to create large aggregates (figure 1.27). IgG has two Fab antigen-binding sites, which can bind to two separate pathogen cells, clumping them together. When multiple IgG antibodies are involved, large aggregates can develop; these aggregates are easier for the kidneys and spleen to filter from the blood and easier for phagocytes to ingest for destruction. The pentameric structure of IgM provides ten Fab binding sites per molecule, making it the most efficient antibody for agglutination.

Another important function of antibodies is activation of the complement cascade. The complement system is an important component of the innate defenses, promoting the inflammatory response, recruiting phagocytes to site of infection, enhancing phagocytosis by opsonization, and killing gram-negative bacterial pathogens with the membrane attack complex (MAC). Complement activation can occur through three different pathways (see figure 1.7), but the most efficient is the classical pathway, which requires the initial binding of IgG or IgM antibodies to the surface of a pathogen cell, allowing for recruitment and activation of the C1 complex.

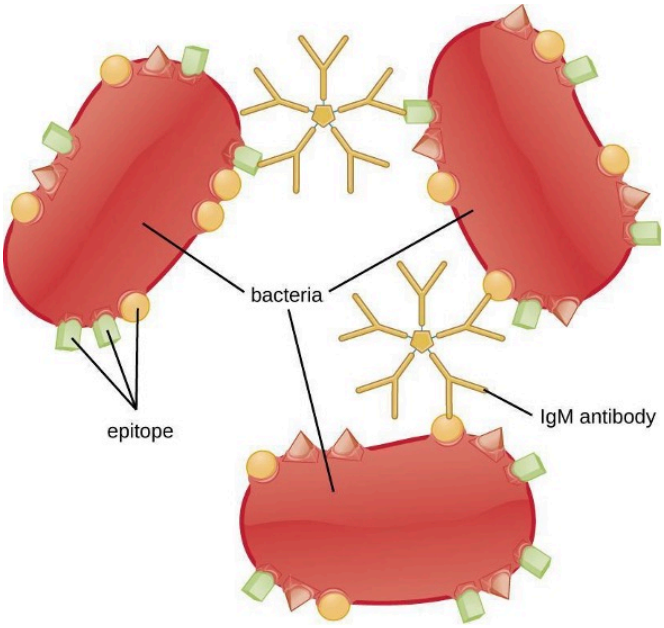


Figure 1.27: Antibodies, especially IgM antibodies, agglutinate bacteria by binding to epitopes on two or more bacteria simultaneously. When multiple pathogens and antibodies are present, aggregates form when the binding sites of antibodies bind with separate pathogens. *Figure description available at the end of the chapter.*

Yet another important function of antibodies is antibody-dependent cell-mediated cytotoxicity (ADCC), which enhances killing of pathogens that are too large to be phagocytosed. This process is best characterized for natural killer cells (NK cells), as shown in figure 1.28, but it can also involve macrophages and eosinophils. ADCC occurs when the Fab region of an IgG antibody binds to a large pathogen; Fc receptors on effector cells (e.g., NK cells) then bind to the Fc region of the antibody, bringing them into close proximity with the target pathogen. The effector cell then secretes powerful cytotoxins (e.g., perforin and granzymes) that kill the pathogen.

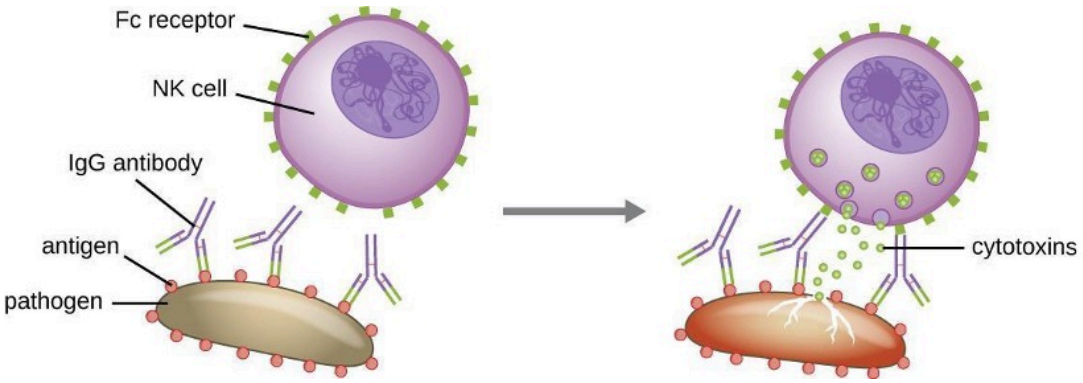


Figure 1.28: In this example of ADCC, antibodies bind to a large pathogenic cell that is too big for phagocytosis and then bind to Fc receptors on the membrane of a natural killer cell. This interaction brings the NK cell into close proximity, where it can kill the pathogen through release of lethal extracellular cytotoxins. *Figure description available at the end of the chapter.*

1.9 MAJOR HISTOCOMPATIBILITY COMPLEXES AND ANTIGEN-PRESENTING CELLS

As discussed in section 1.5, major histocompatibility complex (MHC) molecules are expressed on the surface of healthy cells, identifying them as normal and "self" to natural killer (NK) cells. MHC molecules also play an important role in the presentation of foreign antigens, which is a critical step in the activation of T cells and thus an important mechanism of the adaptive immune system.

MAJOR HISTOCOMPATIBILITY COMPLEX MOLECULES

The major histocompatibility complex (MHC) is a collection of genes coding for MHC molecules found on the surface of all nucleated cells of the body. In humans, the MHC genes are also referred to as human leukocyte antigen (HLA) genes. Mature red blood cells, which lack a nucleus, are the only cells that do not express MHC molecules on their surface.

There are two classes of MHC molecules involved in adaptive immunity, MHC I and MHC II (figure 1.29). MHC I molecules are found on all nucleated cells; they present normal self-antigens as well as abnormal or nonself pathogens to the effector T cells involved in cellular immunity. In contrast, MHC II molecules are only found on macrophages, dendritic cells, and B cells; they present abnormal or nonself pathogen antigens for the initial activation of T cells.

Both types of MHC molecules are transmembrane glycoproteins that assemble as dimers in the cytoplasmic membrane of cells, but their structures are quite different. MHC I molecules are composed of a longer α protein chain coupled with a smaller β2 microglobulin protein, and only the α chain spans the cytoplasmic membrane. The α chain of the MHC I molecule folds into three separate domains: α1, α2 and α3. MHC II molecules are composed of two protein chains (an α and a β chain) that are approximately similar in length. Both chains of the MHC II molecule possess portions that span the plasma membrane, and each chain folds into two separate domains: α1 and α2, and β1, and β2. In order to present abnormal or non-self-antigens to T cells, MHC molecules have a cleft that serves as the antigen-binding site near the top (or outermost) portion of the MHC I or MHC II dimer. For MHC I, the antigen-binding cleft is formed by the α1 and α2 domains, whereas for MHC II, the cleft is formed by the α1 and β1 domains (figure 1.32).

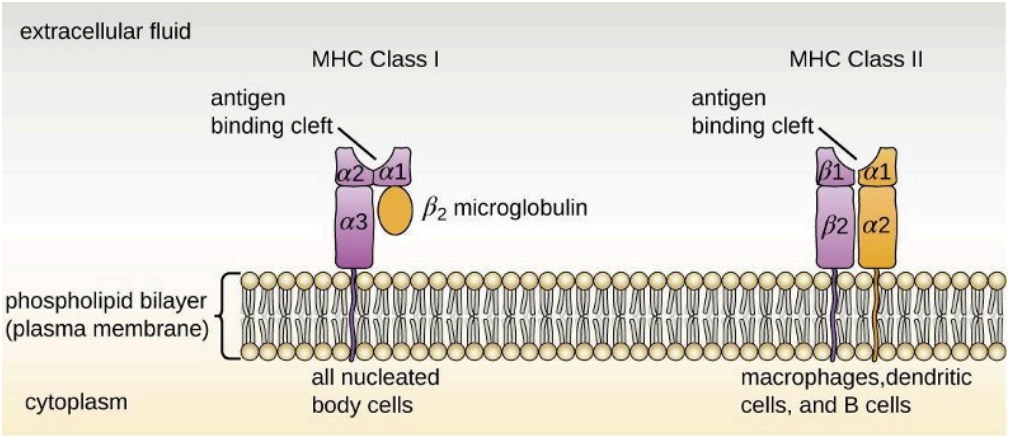


Figure 1.29: MHC I are found on all nucleated body cells, and MHC II are found on macrophages, dendritic cells, and B cells (along with MHC I). The antigen-binding cleft of MHC I is formed by domains α1 and α2. The antigen-binding cleft of MHC II is formed by domains α1 and β1. Figure description available at the end of the chapter.

ANTIGEN-PRESENTING CELLS (APCS)

All nucleated cells in the body have mechanisms for processing and presenting antigens in association with MHC molecules. This signals the immune system, indicating whether the cell is normal and healthy or infected with an intracellular pathogen. However, only macrophages, dendritic cells, and B cells have the ability to present antigens specifically for the purpose of activating T cells; for this reason, these types of cells are sometimes referred to as antigen-presenting cells (APCs).

While all APCs play a similar role in adaptive immunity, there are some important differences to consider. Macrophages and dendritic cells are phagocytes that ingest and kill pathogens that penetrate the first-line barriers (i.e., skin and mucous membranes). B cells, on the other hand, do not function as phagocytes but play a primary role in the production and secretion of antibodies. In addition, whereas macrophages and dendritic cells recognize pathogens through nonspecific receptor interactions (e.g., PAMPs, toll-like receptors, and receptors for opsonizing complement or antibody), B cells interact with foreign pathogens or their free antigens using antigen-specific immunoglobulin as receptors (monomeric IgD and IgM). When the immunoglobulin receptors bind to an antigen, the B cell internalizes the antigen by endocytosis before processing and presenting the antigen to T cells.

Antigen Presentation with MHC II Molecules

MHC II molecules are only found on the surface of APCs. Macrophages and dendritic cells use similar mechanisms for processing and presentation of antigens and their epitopes in association with MHC II; B cells use somewhat different mechanisms that will be described further in section 1.11. For now, we will focus on the steps of the process as they pertain to dendritic cells.

After a dendritic cell recognizes and attaches to a pathogen cell, the pathogen is internalized by phagocytosis and is initially contained within a phagosome. Lysosomes containing antimicrobial enzymes and chemicals fuse with the phagosome to create a phagolysosome, where degradation of the pathogen for antigen processing begins. Proteases (protein-degrading) are especially important in antigen processing because only protein antigen epitopes are presented to T cells by MHC II (figure 1.30).

APCs do not present all possible epitopes to T cells; only a selection of the most antigenic or immunodominant epitopes are presented. The mechanism by which epitopes are selected for processing and presentation by an APC is complicated and not well understood; however, once the most antigenic, immunodominant epitopes have been processed, they associate within the antigen-binding cleft of MHC II molecules and are translocated to the cell surface of the dendritic cell for presentation to T cells.

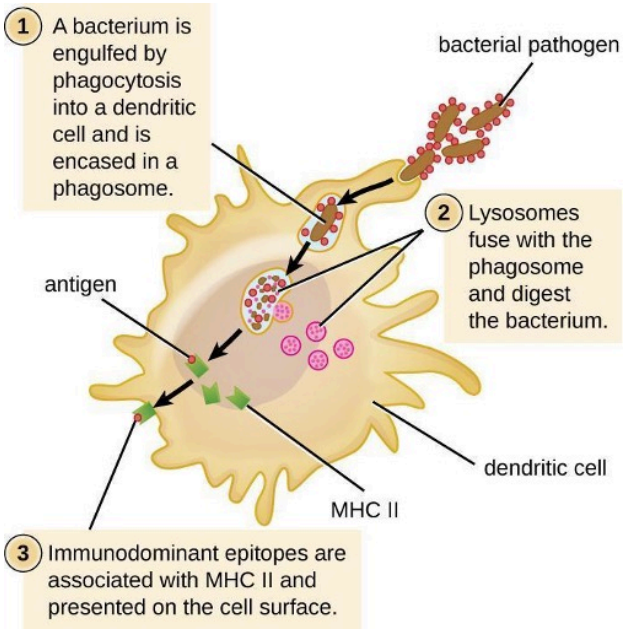


Figure 1.30: A dendritic cell phagocytoses a bacterial cell and brings it into a phagosome. Lysosomes fuse with the phagosome to create a phagolysosome, where antimicrobial chemicals and enzymes degrade the bacterial cell. Proteases process bacterial antigens, and the most antigenic epitopes are selected and presented on the cell's surface in conjunction with MHC II molecules. T cells recognize the presented antigens and are thus activated. *Figure description available at the end of the chapter.*

Antigen Presentation with MHC I Molecules

MHC I molecules, found on all normal, healthy, nucleated cells, signal to the immune system that the cell is a normal "self" cell. In a healthy cell, proteins normally found in the cytoplasm are degraded by proteasomes (enzyme complexes responsible for degradation and processing of proteins) and processed into self-antigen epitopes; these self-antigen epitopes bind within the MHC I antigen-binding cleft and are then presented on the cell surface. Immune cells, such as NK cells, recognize these self-antigens and do not target the cell for destruction. However, if a cell becomes infected with an intracellular pathogen (e.g., a virus), protein antigens specific to the pathogen are processed in the proteasomes and bind with MHC I molecules for presentation on the cell surface. This presentation of pathogen-specific antigens with MHC I signals that the infected cell must be targeted for destruction along with the pathogen.

Before elimination of infected cells can begin, APCs must first activate the T cells involved in cellular immunity. If an intracellular pathogen directly infects the cytoplasm of an APC, then the processing and presentation of antigens can occur as described (in proteasomes and on the cell surface with MHC I). However, if the intracellular pathogen does not directly infect APCs, an alternative strategy called cross-presentation is utilized. In cross-presentation, antigens are brought into the APC by mechanisms normally leading to presentation with MHC II (i.e., through phagocytosis), but the antigen is presented on an MHC I molecule for CD8 T cells. The exact mechanisms by which cross-presentation occurs are not yet well understood, but it appears that cross-presentation is primarily a function of dendritic cells and not macrophages or B cells.

1.10 T LYMPHOCYTES AND CELLULAR IMMUNITY

As explained in section 1.8, the antibodies involved in humoral immunity often bind pathogens and toxins before they can attach to and invade host cells. Thus, humoral immunity is primarily concerned with fighting pathogens in extracellular spaces. However, pathogens that have already gained entry to host cells are largely protected from the humoral antibody-mediated defenses. Cellular immunity, on the other hand, targets and eliminates intracellular pathogens through the actions of T lymphocytes, or T cells (figure 1.31). T cells also play a more central role in orchestrating the overall adaptive immune response (humoral as well as cellular) along with the cellular defenses of innate immunity.

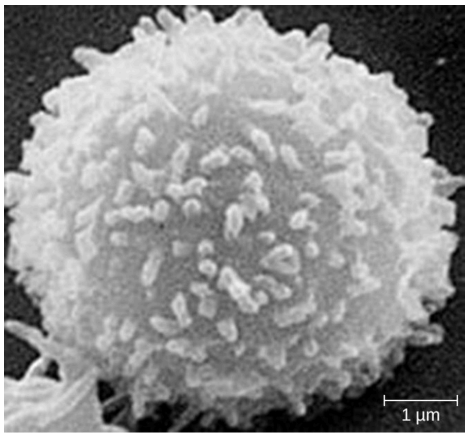


Figure 1.31: This scanning electron micrograph shows a T lymphocyte, which is responsible for the cell-mediated immune response. The spike-like membrane structures increase surface area, allowing for greater interaction with other cell types and their signals. *Figure description available at the end of the chapter.*

T CELL PRODUCTION AND MATURATION

T cells, like all other white blood cells involved in innate and adaptive immunity, are formed from multipotent hematopoietic stem cells (HSCs) in the bone marrow (see figure 1.9). However, unlike the white blood cells of innate immunity, eventual T cells differentiate first into lymphoid stem cells that then become small, immature lymphocytes, sometimes called lymphoblasts. The first steps of differentiation occur in the red marrow of bones (figure 1.32), after which immature T lymphocytes enter the bloodstream and travel to the thymus for the final steps of maturation (figure 1.33). Once in the thymus, the immature T lymphocytes are referred to as thymocytes.

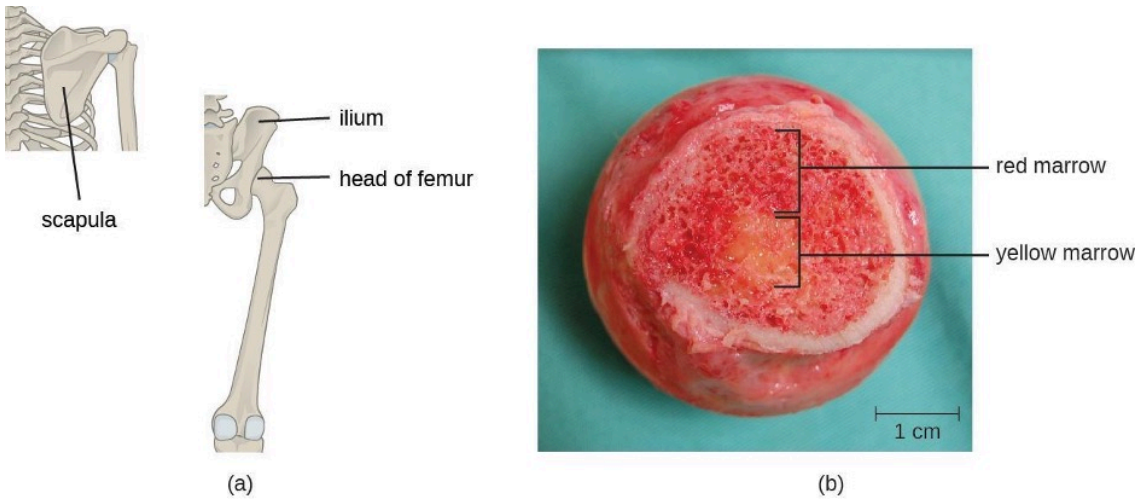


Figure 1.32: (a) Red bone marrow can be found in the head of the femur (thighbone) and is also present in the flat bones of the body, such as the ilium and the scapula. (b) Red bone marrow is the site of production and differentiation of many formed elements of blood, including erythrocytes, leukocytes, and platelets. The yellow bone marrow is populated primarily with adipose cells. *Figure description available at the end of the chapter.*

The maturation of thymocytes within the thymus can be divided into three critical steps of positive and negative selection, collectively referred to as thymic selection. The first step of thymic selection occurs in the cortex of the thymus and involves the development of a functional T-cell receptor (TCR) that is required for activation by APCs. Thymocytes with defective TCRs are removed by negative selection through the induction of apoptosis (programmed controlled cell death). The second step of thymic selection also occurs in the cortex and involves the positive selection of thymocytes that will interact appropriately with MHC molecules. Thymocytes that can interact appropriately with MHC molecules receive a positive stimulation that moves them further through the process of maturation, whereas thymocytes that do not interact appropriately are not stimulated and are eliminated by apoptosis. The third and final step of thymic selection occurs in both the cortex and medulla and involves negative selection to remove self-reacting thymocytes, those that react to self-antigens, by apoptosis. This final step is sometimes referred to as **central tolerance** because it prevents self-reacting T cells from reaching the bloodstream and potentially causing autoimmune disease, which occurs when the immune system attacks healthy "self" cells.

Despite central tolerance, some self-reactive T cells generally escape the thymus and enter the peripheral bloodstream. Therefore, a second line of defense called **peripheral tolerance** is needed to protect against autoimmune disease. Peripheral tolerance involves mechanisms of anergy and inhibition of self-reactive T cells by regulatory T cells. Anergy refers to a state of nonresponsiveness to antigen stimulation. In the case of self-reactive T cells that escape the thymus, lack of an essential co-stimulatory signal required for activation causes anergy and prevents autoimmune activation. Regulatory T cells participate in peripheral tolerance by inhibiting the activation and function of self-reactive T cells and by secreting anti-inflammatory cytokines.

It is not completely understood what events specifically direct maturation of thymocytes into regulatory T cells. Current theories suggest the critical events may occur during the third step of thymic selection, when most self-reactive T cells are eliminated. Regulatory T cells may receive a unique signal that is below the threshold required to target them for negative selection and apoptosis. Consequently, these cells continue to mature and then exit the thymus, armed to inhibit the activation of self-reactive T cells.

It has been estimated that the three steps of thymic selection eliminate 98% of thymocytes. The remaining 2% that exit the thymus migrate through the bloodstream and lymphatic system to sites of secondary lymphoid organs/tissues, such as the lymph nodes, spleen, and tonsils (figure 1.33), where they await activation through the presentation of specific antigens by APCs. Until they are activated, they are known as mature naïve T cells.

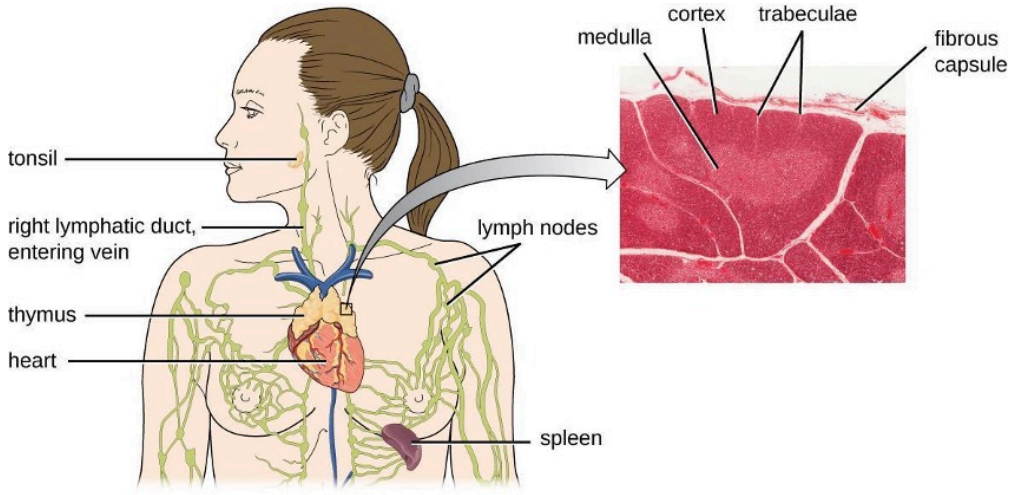


Figure 1.33: The thymus is a bi-lobed, H-shaped glandular organ that is located just above the heart. It is surrounded by a fibrous capsule of connective tissue. The darkly staining cortex and the lighter staining medulla of individual lobules are clearly visible in the light micrograph of the thymus of a newborn (top right, LM × 100). *Figure description available at the end of the chapter.*

CLASSES OF T CELLS

T cells can be categorized into three distinct classes: helper T cells, regulatory T cells, and cytotoxic T cells. These classes are differentiated based on their expression of certain surface molecules, their mode of activation, and their functional roles in adaptive immunity (table 1.10).

All T cells produce a cluster of differentiation (CD) molecules, cell surface glycoproteins that can be used to identify and distinguish between the various types of white blood cells. Although T cells can produce a variety of CD molecules, CD4 and CD8 are the two most important used for differentiation of the classes. Helper T cells and regulatory T cells are characterized by the expression of CD4 on their surface, whereas cytotoxic T cells are characterized by the expression of CD8.

Classes of T cells can also be distinguished by the specific MHC molecules and APCs with which they interact for activation. Helper T cells and regulatory T cells can only be activated by APCs presenting antigens associated with MHC II. In contrast, cytotoxic T cells recognize antigens presented in association with MHC I, either by APCs or by nucleated cells infected with an intracellular pathogen.

The different classes of T cells also play different functional roles in the immune system. Helper T cells serve as the central orchestrators that help activate and direct functions of humoral and cellular immunity. In addition, helper T cells enhance the pathogen-killing functions of macrophages and NK cells of innate immunity. In contrast, the primary role of regulatory T cells is to prevent undesirable and potentially damaging immune responses. Their role in peripheral tolerance, for example, protects against autoimmune disorders, as discussed earlier. Finally, cytotoxic T cells are the primary effector cells for cellular immunity. They recognize and target cells that have been infected by intracellular pathogens, destroying infected cells along with the pathogens inside.

Class	Surface CD Molecules	Activation	Functions
Helper T cells	CD4	APCs presenting antigens associated with MHC II	Orchestrate humoral and cellular immunity
			Involved in the activation of macrophages and NK cells
Regulatory T cells	CD4	APCs presenting antigens associated with MHC II	Involved in peripheral tolerance and prevention of autoimmune responses
Cytotoxic T cells	CD8	APCs or infected nucleated cells presenting antigens associated with MHC I	Destroy cells infected with intracellular pathogens

Table 1.10: Classes of T cells

T-CELL RECEPTORS

For both helper T cells and cytotoxic T cells, activation is a complex process that requires the interactions of multiple molecules and exposure to cytokines. The T-cell receptor (TCR) is involved in the first step of pathogen epitope recognition during the activation process.

The TCR comes from the same receptor family as the antibodies IgD and IgM, the antigen receptors on the B cell membrane surface, and thus shares common structural elements. Similar to antibodies, the TCR has a variable region and a constant region, and the variable region provides the antigen-binding site (figure 1.34). However, the structure of TCR is smaller and less complex than the immunoglobulin molecules (figure 1.24). Whereas immunoglobulins have four peptide chains and Y-shaped structures, the TCR consists of just two peptide chains (α and β chains), both of which span the cytoplasmic membrane of the T cell.

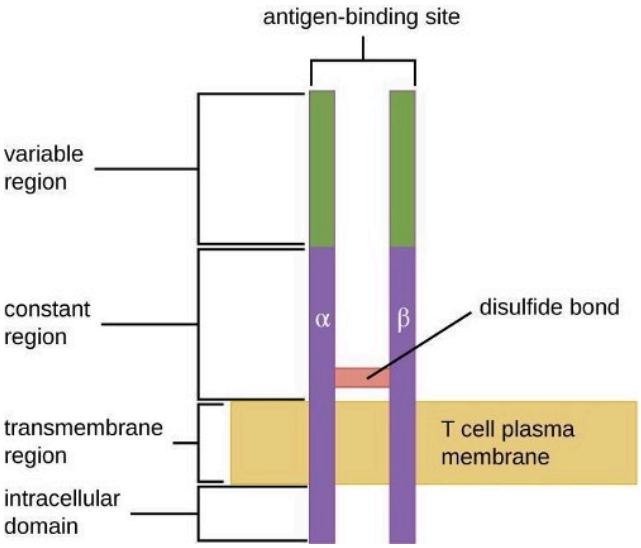


Figure 1.34: A T-cell receptor spans the cytoplasmic membrane and projects variable binding regions into the extracellular space to bind processed antigens associated with MHC I or MHC II molecules. Figure description available at the end of the chapter.

TCRs are epitope-specific, and it has been estimated that 25 million T cells with unique epitope-binding TCRs are required to protect an individual against a wide range of microbial pathogens. Because the human genome only contains about 25,000 genes, we know that each specific TCR cannot be encoded by its own set of genes. This raises the question of how such a vast population of T cells with millions of specific TCRs can be achieved. The answer is a process called genetic rearrangement, which occurs in the thymus during the first step of thymic selection.

The genes that code for the variable regions of the TCR are divided into distinct gene segments called variable (V), diversity (D), and joining (J) segments. The genes segments associated with the α chain of the TCR consist of 70 or more different Vα segments and 61 different Jα segments. The gene segments associated with the β chain of the TCR consist of 52 different Vβ segments, two different Dβ segments, and 13 different Jβ segments. Dur-

ing the development of the functional TCR in the thymus, genetic rearrangement in a T cell brings together one Vα segment and one Jα segment to code for the variable region of the α chain. Similarly, genetic rearrangement brings one of the Vβ segments together with one of the Dβ segments and one of the Jβ segments to code for the variable region of the β chain. All the possible combinations of rearrangements between different segments of V, D, and J provide the genetic diversity required to produce millions of TCRs with unique epitope-specific variable regions.

ACTIVATION AND DIFFERENTIATION OF HELPER T CELLS

Helper T cells can only be activated by APCs presenting processed foreign epitopes in association with MHC II. The first step in the activation process is TCR recognition of the specific foreign epitope presented within the MHC II antigen-binding cleft. The second step involves the interaction of CD4 on the helper T cell with a region of the MHC II molecule separate from the antigen-binding cleft. This second interaction anchors the MHC II-TCR complex and ensures that the helper T cell is recognizing both the foreign ("nonself") epitope and "self" antigen of the APC; both recognitions are required for activation of the cell. In the third step, the APC and T cell secrete cytokines that activate the helper T cell. The activated helper T cell then proliferates, dividing by mitosis to produce clonal naïve helper T cells that differentiate into subtypes with different functions (figure 1.35).

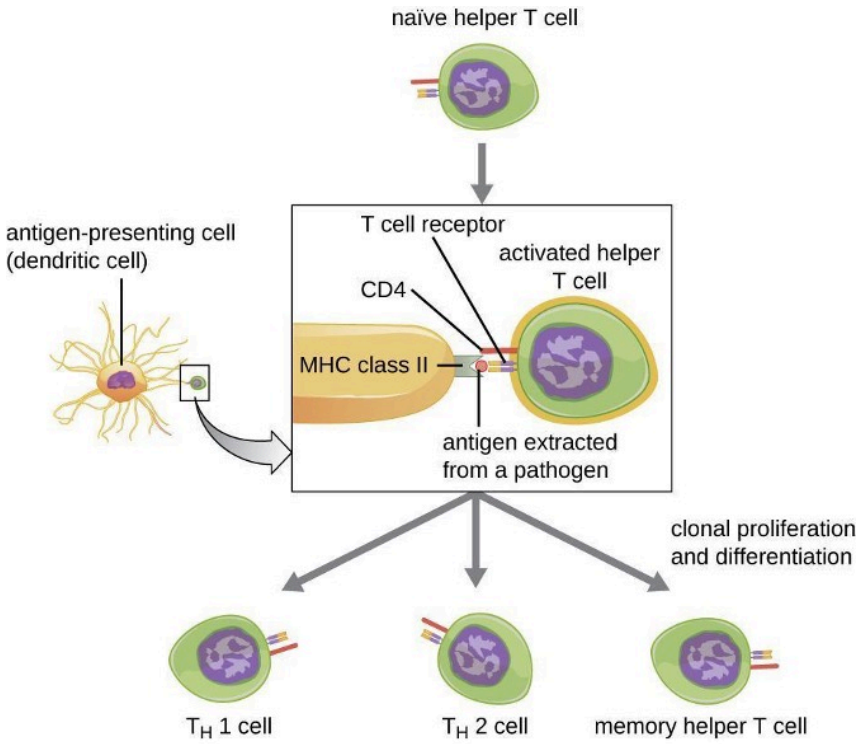


Figure 1.35: This illustration depicts the activation of a naïve (unactivated) helper T cell by an antigen-presenting cell and the subsequent proliferation and differentiation of the activated T cell into different subtypes. *Figure description available at the end of the chapter.*

Activated helper T cells can differentiate into one of four distinct subtypes, summarized in table 1.11. The differentiation process is directed by APC-secreted cytokines. Depending on which APC-secreted cytokines interact with an activated helper T cell, the cell may differentiate into a T helper 1 (TH1) cell, a T helper 2 (TH2) cell, or a memory helper T cell. The two types of helper T cells are relatively short-lived effector cells, meaning that

they perform various functions of the immediate immune response. In contrast, memory helper T cells are relatively long lived; they are programmed to "remember" a specific antigen or epitope in order to mount a rapid, strong, secondary response to subsequent exposures.

TH1 cells secrete their own cytokines that are involved in stimulating and orchestrating other cells involved in adaptive and innate immunity. For example, they stimulate cytotoxic T cells, enhancing their killing of infected cells and promoting differentiation into memory cytotoxic T cells. TH1 cells also stimulate macrophages and neutrophils to become more effective in their killing of intracellular bacteria. They can also stimulate NK cells to become more effective at killing target cells.

TH2 cells play an important role in orchestrating the humoral immune response through their secretion of cytokines that activate B cells and direct B cell differentiation and antibody production. Various cytokines produced by TH2 cells orchestrate antibody class switching, which allows B cells to switch between the production of IgM, IgG, IgA, and IgE as needed to carry out specific antibody functions and to provide pathogen-specific humoral immune responses.

A third subtype of helper T cells called TH17 cells was discovered through observations that immunity to some infections is not associated with TH1 or TH2 cells. TH17 cells and the cytokines they produce appear to be specifically responsible for the body's defense against chronic mucocutaneous infections. Patients who lack sufficient TH17 cells in the mucosa (e.g., HIV patients) may be more susceptible to bacteremia and gastrointestinal infections.[2]

Subtype	Functions
T_H1 cells	Stimulate cytotoxic T cells and produce memory cytotoxic T cells
	Stimulate macrophages and neutrophils (PMNs) for more effective intracellular killing of pathogens
	Stimulate NK cells to kill more effectively
T_H2 cells	Stimulate B cell activation and differentiation into plasma cells and memory B cells
	Direct antibody class switching in B cells
T_H17 cells	Stimulate immunity to specific infections such as chronic mucocutaneous infections
Memory helper T cells	"Remember" a specific pathogen and mount a strong, rapid secondary response upon re-exposure

Table 1.11: Subtypes of helper T cells

ACTIVATION AND DIFFERENTIATION OF CYTOTOXIC T CELLS

Cytotoxic T cells (also referred to as cytotoxic T lymphocytes, or CTLs) are activated by APCs in a three-step process similar to that of helper T cells. The key difference is that the activation of cytotoxic T cells involves recognition of an antigen presented with MHC I (as opposed to MHC II) and interaction of CD8 (as opposed to CD4) with the receptor complex. After the successful co-recognition of foreign epitope and self-antigen, the production of cytokines by the APC and the cytotoxic T cell activates clonal proliferation and differentiation. Activated cytotoxic T cells can differentiate into effector cytotoxic T cells that target pathogens for destruction or memory cells that are ready to respond to subsequent exposures.

As noted, proliferation and differentiation of cytotoxic T cells is also stimulated by cytokines secreted from TH1 cells activated by the same foreign epitope. The co-stimulation that comes from these TH1 cells is provided by secreted cytokines. Although it is possible for activation of cytotoxic T cells to occur without stimulation from TH1 cells, the activation is not as effective or long-lasting.

Once activated, cytotoxic T cells serve as the effector cells of cellular immunity, recognizing and killing cells infected with intracellular pathogens through a mechanism very similar to that of NK cells. However, whereas NK cells recognize nonspecific signals of cell stress or abnormality, cytotoxic T cells recognize infected cells through antigen presentation of pathogen-specific epitopes associated with MHC I. Once an infected cell is recognized, the TCR of the cytotoxic T cell binds to the epitope and releases perforin and granzymes that destroy the infected cell (figure 1.36). Perforin is a protein that creates pores in the target cell, and granzymes are proteases that enter the pores and induce apoptosis. This mechanism of programmed cell death is a controlled and efficient means of destroying and removing infected cells without releasing the pathogens inside to infect neighboring cells, as might occur if the infected cells were simply lysed.

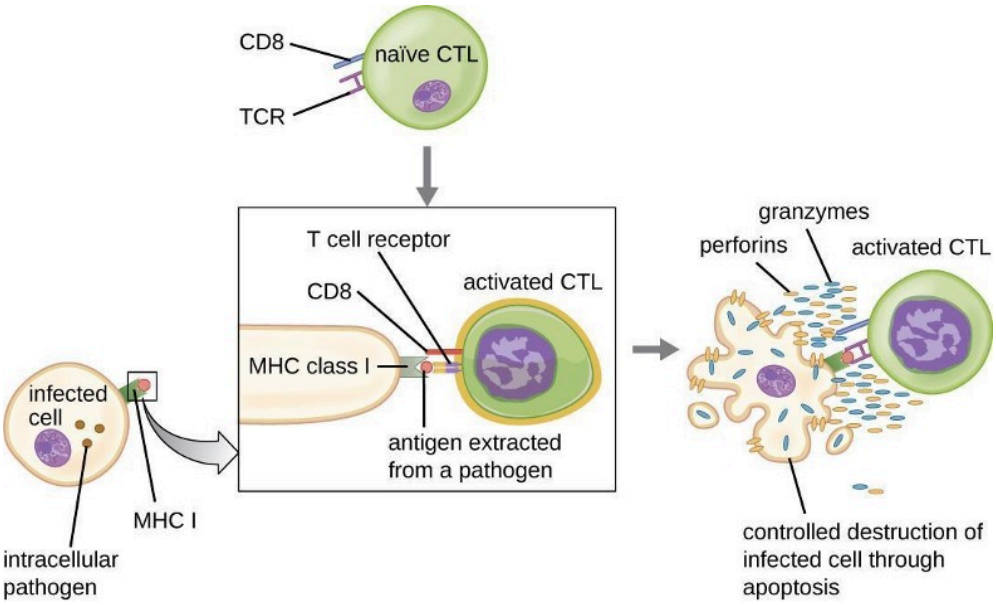


Figure 1.36: This figure illustrates the activation of a naïve (unactivated) cytotoxic T cell (CTL) by an antigen-presenting MHC I molecule on an infected body cell. Once activated, the CTL releases perforin and granzymes that invade the infected cell and induce controlled cell death, or apoptosis. *Figure description available at the end of the chapter.*

SUPERANTIGENS AND UNREGULATED ACTIVATION OF T CELLS

When T cell activation is controlled and regulated, the result is a protective response that is effective in combating infections. However, if T cell activation is unregulated and excessive, the result can be life-threatening. Certain bacterial and viral pathogens produce toxins known as superantigens (see section 2.14) that can trigger such an unregulated response. Known bacterial superantigens include toxic shock syndrome toxin (TSST), staphylococcal enterotoxins, streptococcal pyrogenic toxins, streptococcal superantigen, and the streptococcal mitogenic exotoxin. Viruses known to produce superantigens include Epstein-Barr virus (human herpesvirus 4), cytomegalovirus (human herpesvirus 5), among others.

The mechanism of T cell activation by superantigens involves their simultaneous binding to MHC II molecules of APCs and the variable region of the TCR β chain. This binding occurs outside of the antigen-binding cleft of MHC II, so the superantigen will bridge together and activate MHC II and TCR without specific foreign epitope recognition (figure 1.37). The result is an excessive, uncontrolled release of cytokines, often called a cytokine storm, which stimulates an excessive inflammatory response. This can lead to a dangerous decrease in blood pressure, shock, multi-organ failure, and potentially, death.

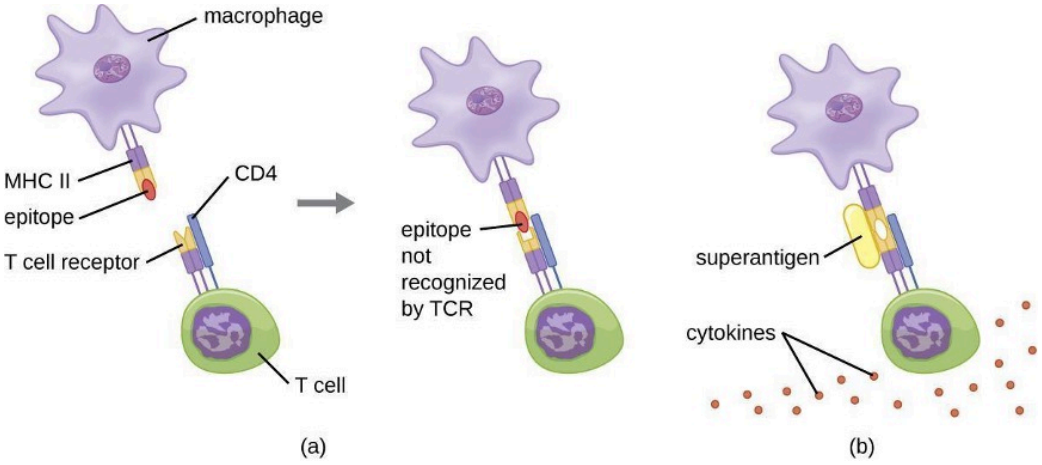


Figure 1.37: (a) The macrophage in this figure is presenting a foreign epitope that does not match the TCR of the T cell. Because the T cell does not recognize the epitope, it is not activated. (b) The macrophage in this figure is presenting a superantigen that is not recognized by the TCR of the T cell, yet the superantigen still is able to bridge and bind the MHC II and TCR molecules. This nonspecific, uncontrolled activation of the T cell results in an excessive release of cytokines that activate other T cells and cause excessive inflammation. *Figure description available at the end of the chapter.*

1.11 B LYMPHOCYTES AND HUMORAL IMMUNITY

Humoral immunity refers to mechanisms of the adaptive immune defenses that are mediated by antibodies secreted by B lymphocytes, or B cells. This section will focus on B cells and discuss their production and maturation, receptors, and mechanisms of activation.

B CELL PRODUCTION AND MATURATION

Like T cells, B cells are formed from multipotent hematopoietic stem cells (HSCs) in the bone marrow and follow a pathway through lymphoid stem cells and lymphoblast (see figure 1.9). Unlike T cells, however, lymphoblasts destined to become B cells do not leave the bone marrow and travel to the thymus for maturation. Rather, eventual B cells continue to mature in the bone marrow.

The first step of B cell maturation is an assessment of the functionality of their antigen-binding receptors. This occurs through positive selection for B cells with normal functional receptors. A mechanism of negative selection is then used to eliminate self-reacting B cells and minimize the risk of autoimmunity. Negative selection of self-reacting B cells can involve elimination by apoptosis, editing or modification of the receptors so they are no longer self-reactive, or induction of anergy in the B cell. Immature B cells that pass the selection in the bone marrow then travel to the spleen for their final stages of maturation. There they become naïve mature B cells, i.e., mature B cells that have not yet been activated.

B-CELL RECEPTORS

Like T cells, B cells possess antigen-specific receptors with diverse specificities. Although they rely on T cells for optimum function, B cells can be activated without help from T cells. B-cell receptors (BCRs) for naïve mature B cells are membrane-bound monomeric forms of IgD and IgM. They have two identical heavy chains and two identical light chains connected by disulfide bonds into a basic Y shape (figure 1.38). The trunk of the Y-shaped molecule, the constant region of the two heavy chains, spans the B cell membrane. The two antigen-binding sites exposed to the exterior of the B cell are involved in the binding of specific pathogen epitopes to initiate the activation process. It is estimated that each naïve mature B cell has upwards of 100,000 BCRs on its membrane, and each of these BCRs has an identical epitope-binding specificity.

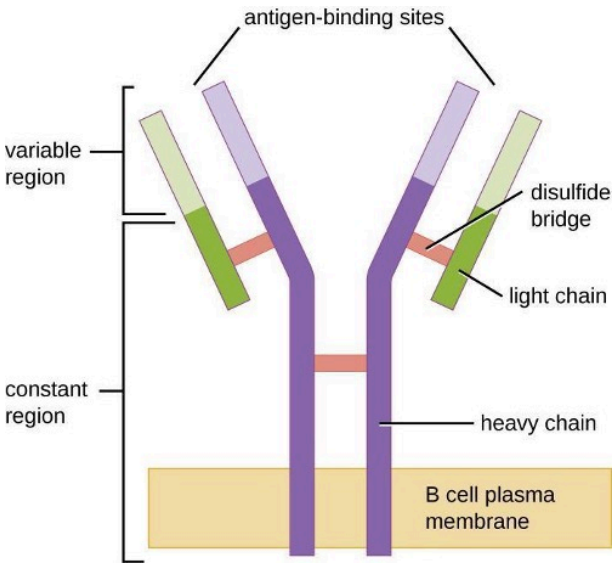


Figure 1.38: B-cell receptors are embedded in the membranes of B cells. The variable regions of all of the receptors on a single cell bind the same specific antigen. *Figure description available at the end of the chapter.*

In order to be prepared to react to a wide range of microbial epitopes, B cells, like T cells, use genetic rearrangement of hundreds of gene segments to provide the necessary diversity of receptor specificities. The variable region of the BCR heavy chain is made up of V, D, and J segments, similar to the β chain of the TCR. The variable region of the BCR light chain is made up of V and J segments, similar to the α chain of the TCR. Genetic rearrangement of all possible combinations of V-J-D (heavy chain) and V-J (light chain) provides for millions of unique antigen-binding sites for the BCR and for the antibodies secreted after activation.

One important difference between BCRs and TCRs is the way they can interact with antigenic epitopes. Whereas TCRs can only interact with antigenic epitopes that are presented within the antigen-binding cleft of MHC I or MHC II, BCRs do not require antigen presentation with MHC; they can interact with epitopes on free antigens or with epitopes displayed on the surface of intact pathogens. Another important difference is that TCRs only recognize protein epitopes, whereas BCRs can recognize epitopes associated with different molecular classes (e.g., proteins, polysaccharides, lipopolysaccharides).

Activation of B cells occurs through different mechanisms depending on the molecular class of the antigen. Activation of a B cell by a protein antigen requires the B cell to function as an APC, presenting the protein epitopes with MHC II to helper T cells. Because of their dependence on T cells for activation of B cells, protein antigens are classified as T-dependent antigens. In contrast, polysaccharides, lipopolysaccharides, and other nonprotein antigens are considered T-independent antigens because they can activate B cells without antigen processing and presentation to T cells.

T Cell-Independent Activation of B cells

Activation of B cells without the cooperation of helper T cells is referred to as T cell-independent activation and occurs when BCRs interact with T-independent antigens. T-independent antigens (e.g., polysaccharide capsules, lipopolysaccharide) have repetitive epitope units within their structure, and this repetition allows for the cross-linkage of multiple BCRs, providing the first signal for activation (figure 1.39). Because T cells are not involved,

the second signal has to come from other sources, such as interactions of toll-like receptors with PAMPs or interactions with factors from the complement system.

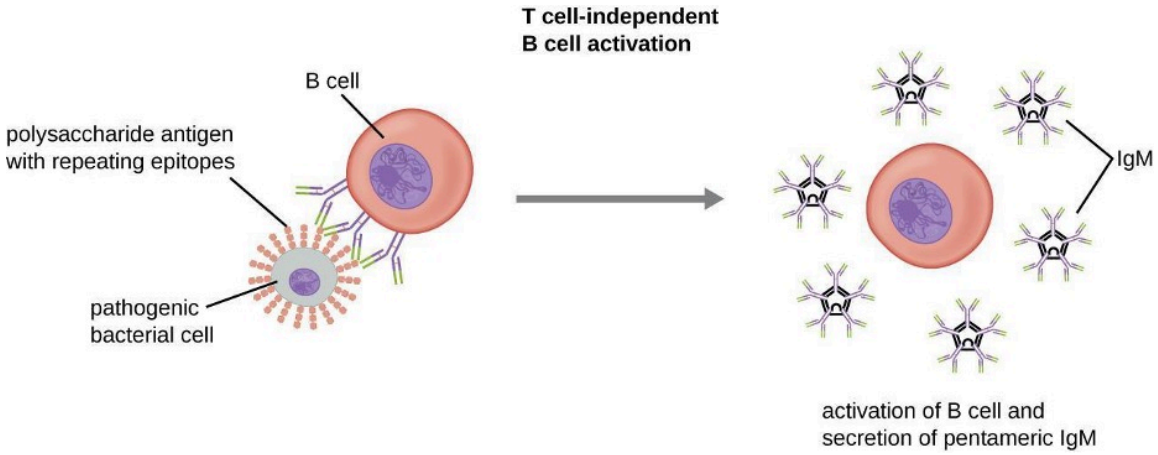


Figure 1.39: T-independent antigens have repeating epitopes that can induce B cell recognition and activation without involvement from T cells. A second signal, such as interaction of TLRs with PAMPs (not shown), is also required for activation of the B cell. Once activated, the B cell proliferates and differentiates into antibody-secreting plasma cells. *Figure description available at the end of the chapter.*

Once a B cell is activated, it undergoes clonal proliferation and daughter cells differentiate into plasma cells. Plasma cells are antibody factories that secrete large quantities of antibodies. After differentiation, the surface BCRs disappear and the plasma cell secretes pentameric IgM molecules that have the same antigen specificity as the BCRs (figure 1.39).

The T cell-independent response is short-lived and does not result in the production of memory B cells. Thus it will not result in a secondary response to subsequent exposures to T-independent antigens.

T Cell-Dependent Activation of B cells

T cell-dependent activation of B cells is more complex than T cell-independent activation, but the resulting immune response is stronger and develops memory. T cell-dependent activation can occur either in response to free protein antigens or to protein antigens associated with an intact pathogen. Interaction between the BCRs on a naïve mature B cell and a free protein antigen stimulates internalization of the antigen, whereas interaction with antigens associated with an intact pathogen initiates the extraction of the antigen from the pathogen before internalization. Once internalized inside the B cell, the protein antigen is processed and presented with MHC II. The presented antigen is then recognized by helper T cells specific to the same antigen. The TCR of the helper T cell recognizes the foreign antigen, and the T cell's CD4 molecule interacts with MHC II on the B cell. The coordination between B cells and helper T cells that are specific to the same antigen is referred to as linked recognition.

Once activated by linked recognition, TH2 cells produce and secrete cytokines that activate the B cell and cause proliferation into clonal daughter cells. After several rounds of proliferation, additional cytokines provided by the TH2 cells stimulate the differentiation of activated B cell clones into memory B cells, which will quickly respond to subsequent exposures to the same protein epitope, and plasma cells that lose their membrane BCRs and initially secrete pentameric IgM (figure 1.40).

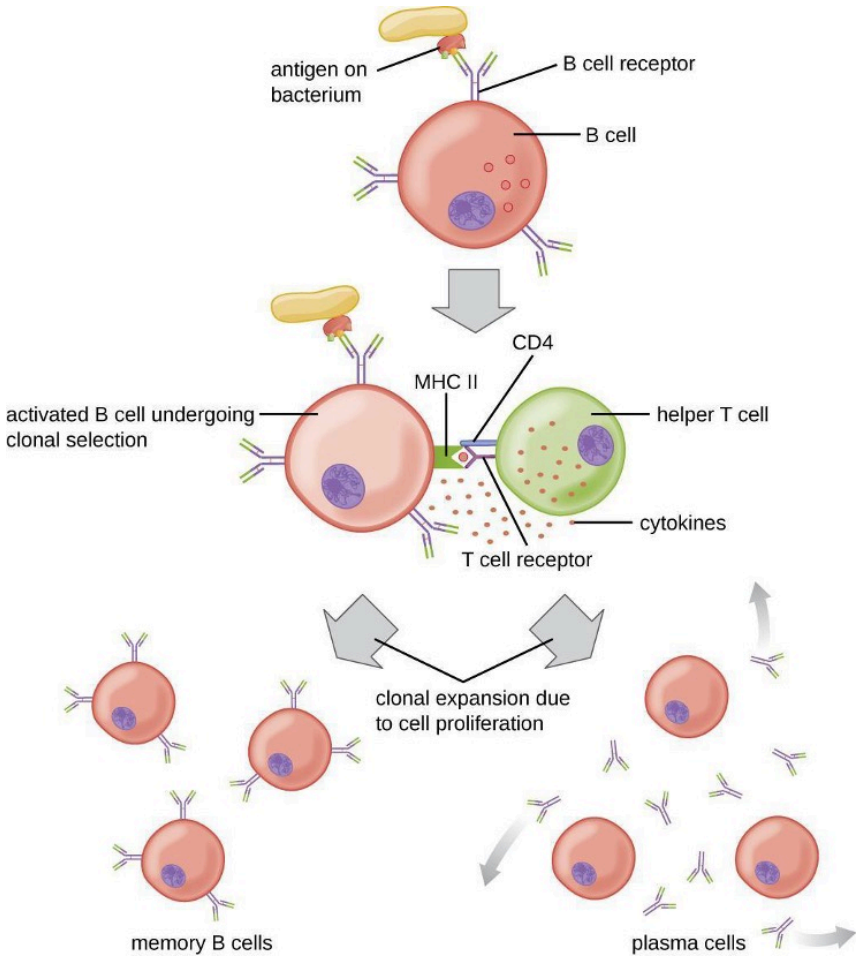


Figure 1.40: In T cell-dependent activation of B cells, the B cell recognizes and internalizes an antigen and presents it to a helper T cell that is specific to the same antigen. The helper T cell interacts with the antigen presented by the B cell, which activates the T cell and stimulates the release of cytokines that then activate the B cell. Activation of the B cell triggers proliferation and differentiation into B cells and plasma cells. *Figure description available at the end of the chapter.*

After initial secretion of IgM, cytokines secreted by TH2 cells stimulate the plasma cells to switch from IgM production to production of IgG, IgA, or IgE. This process, called class switching or isotype switching, allows plasma cells cloned from the same activated B cell to produce a variety of antibody classes with the same epitope specificity. Class switching is accomplished by genetic rearrangement of gene segments encoding the constant region, which determines an antibody's class. The variable region is not changed, so the new class of antibody retains the original epitope specificity.

PRIMARY AND SECONDARY RESPONSES

T cell-dependent activation of B cells plays an important role in both the primary and secondary responses associated with adaptive immunity. With the first exposure to a protein antigen, a T cell-dependent primary antibody response occurs. The initial stage of the primary response is a lag period, or latent period, of approximately 10 days, during which no antibody can be detected in serum. This lag period is the time required for all of the steps of the primary response, including naïve mature B cells' antigens binding with BCRs, antigen processing and presentation, helper T cell activation, B cell activation, and clonal proliferation. The end of the lag period

is characterized by a rise in IgM levels in the serum, as TH2 cells stimulate B cell differentiation into plasma cells. IgM levels reach their peak around 14 days after primary antigen exposure; at about this same time, TH2 stimulates antibody class switching, and IgM levels in serum begin to decline. Meanwhile, levels of IgG increase until they reach a peak about three weeks into the primary response (figure 1.41).

During the primary response, some of the cloned B cells are differentiated into memory B cells programmed to respond to subsequent exposures. This secondary response occurs more quickly and forcefully than the primary response. The lag period is decreased to only a few days and the production of IgG is significantly higher than observed for the primary response (figure 1.41). In addition, the antibodies produced during the secondary response are more effective and bind with higher affinity to the targeted epitopes. Plasma cells produced during secondary responses live longer than those produced during the primary response, so levels of specific antibodies remain elevated for a longer period of time.

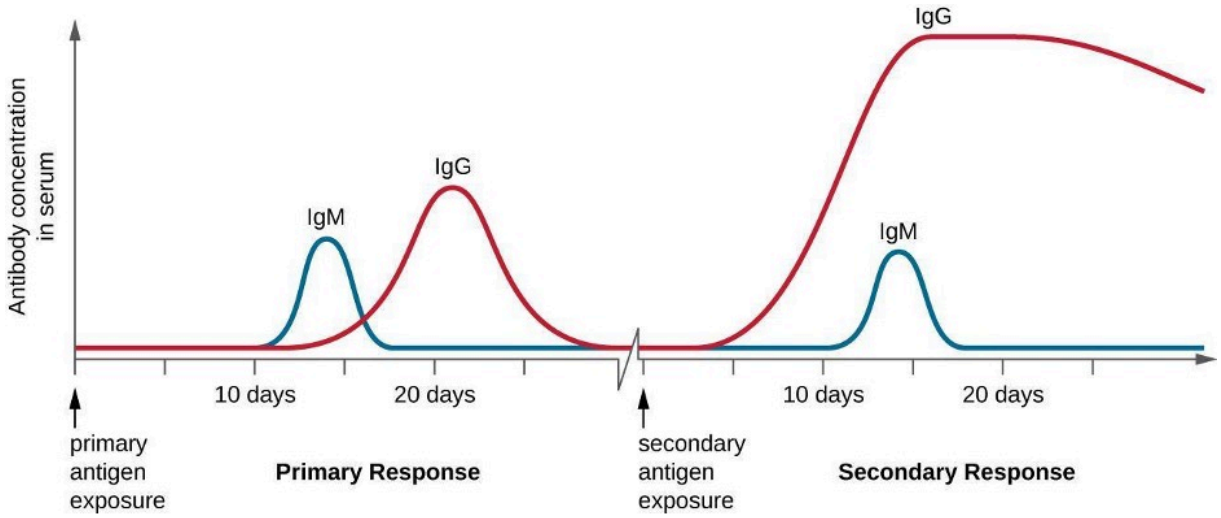


Figure 1.41: Compared to the primary response, the secondary antibody response occurs more quickly and produces antibody levels that are higher and more sustained. The secondary response mostly involves IgG. Figure description available at the end of the chapter.

1.12 VACCINES

For many diseases, prevention is the best form of treatment, and few strategies for disease prevention are as effective as vaccination. Vaccination is a form of artificial immunity. By artificially stimulating the adaptive immune defenses, a vaccine triggers memory cell production similar to that which would occur during a primary response. In so doing, the patient is able to mount a strong secondary response upon exposure to the pathogen—but without having to first suffer through an initial infection. In this section, we will explore several different kinds of artificial immunity along with various types of vaccines and the mechanisms by which they induce artificial immunity.

CLASSIFICATIONS OF ADAPTIVE IMMUNITY

All forms of adaptive immunity can be described as either active or passive. Active immunity refers to the activation of an individual's own adaptive immune defenses, whereas passive immunity refers to the transfer of adaptive immune defenses from another individual or animal. Active and passive immunity can be further subdivided based on whether the protection is acquired naturally or artificially.

Natural active immunity is adaptive immunity that develops after natural exposure to a pathogen (table 1.12). Examples would include the lifelong immunity that develops after recovery from a chickenpox or measles infection (although an acute infection is not always necessary to activate adaptive immunity). The length of time that an individual is protected can vary substantially depending upon the pathogen and antigens involved. For example, activation of adaptive immunity by protein spike structures during an intracellular viral infection can activate lifelong immunity, whereas activation by carbohydrate capsule antigens during an extracellular bacterial infection may activate shorter-term immunity.

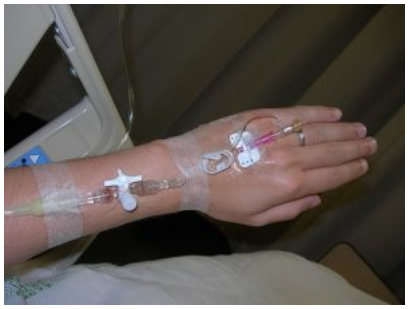
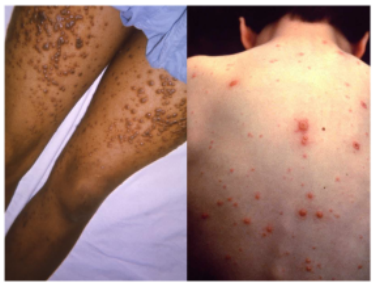
	Natural acquired	Artificial required
Passive	Immunity acquired from antibodies passed in breast milk or through placenta 	Immunity gained through antibodies harvested from another person or an animal 
Active	Immunity gained through illness and recovery 	Immunity acquired through a vaccine 

Table 1.12: The four mechanisms of acquisition of immunity.

Natural passive immunity involves the natural passage of antibodies from a mother to her child before and after birth. IgG is the only antibody class that can cross the placenta from mother's blood to the fetal blood supply. Placental transfer of IgG is an important passive immune defense for the infant, lasting up to six months after birth. Secretory IgA can also be transferred from mother to infant through breast milk.

Artificial passive immunity refers to the transfer of antibodies produced by a donor (human or animal) to another individual. This transfer of antibodies may be done as a prophylactic measure (i.e., to prevent disease after exposure to a pathogen) or as a strategy for treating an active infection. For example, artificial passive immunity is commonly used for post-exposure prophylaxis against rabies, hepatitis A, hepatitis B, and chickenpox (in high-risk individuals). Active infections treated by artificial passive immunity include cytomegalovirus infections in immunocompromised patients and Ebola virus infections. In 1995, eight patients in the Democratic Republic of the Congo with active Ebola infections were treated with blood transfusions from patients who were recovering from Ebola. Only one of the eight patients died (a 12.5% mortality rate), which was much lower than the expected 80% mortality rate for Ebola in untreated patients.[3]Artificial passive immunity is also used for the treatment of diseases caused by bacterial toxins, including tetanus, botulism, and diphtheria.

Artificial active immunity is the foundation for vaccination. It involves the activation of adaptive immunity through the deliberate exposure of an individual to weakened or inactivated pathogens, or preparations consisting of key pathogen antigens.

Herd Immunity

The four kinds of immunity just described result from an individual's adaptive immune system. For any given disease, an individual may be considered immune or susceptible depending on his or her ability to mount an effective immune response upon exposure. Thus, any given population is likely to have some individuals who are immune and other individuals who are susceptible. If a population has very few susceptible individuals, even those susceptible individuals will be protected by a phenomenon called herd immunity. Herd immunity has nothing to do with an individual's ability to mount an effective immune response; rather, it occurs because there are too few susceptible individuals in a population for the disease to spread effectively.

Vaccination programs create herd immunity by greatly reducing the number of susceptible individuals in a population. Even if some individuals in the population are not vaccinated, as long as a certain percentage is immune (either naturally or artificially), the few susceptible individuals are unlikely to be exposed to the pathogen. However, because new individuals are constantly entering populations (for example, through birth or relocation), vaccination programs are necessary to maintain herd immunity.

VARIOLATION AND VACCINATION

Thousands of years ago, it was first recognized that individuals who survived a smallpox infection were immune to subsequent infections. Variolation refers to the deliberate inoculation of individuals with infectious material from scabs or pustules of smallpox victims. Infectious materials were either injected into the skin or introduced through the nasal route. The infection that developed was usually milder than naturally acquired smallpox, and recovery from the milder infection provided protection against the more serious disease.

Although the majority of individuals treated by variolation developed only mild infections, the practice was not without risks. More serious and sometimes fatal infections did occur, and because smallpox was contagious, infections resulting from variolation could lead to epidemics. Even so, the practice of variolation for smallpox prevention spread to other regions, including India, Africa, and Europe.

Although variolation had been practiced for centuries, the English physician Edward Jenner (1749–1823) is generally credited with developing the modern process of vaccination. Jenner observed that milkmaids who developed cowpox, a disease similar to smallpox but milder, were immune to the more serious smallpox. This led Jenner to hypothesize that exposure to a less virulent pathogen could provide immune protection against a more virulent pathogen, providing a safer alternative to variolation. In 1796, Jenner tested his hypothesis by obtaining infectious samples from a milkmaid's active cowpox lesion and injecting the materials into a young boy (figure 1.42). The boy developed a mild infection that included a low-grade fever, discomfort in his axillae (armpit), and loss of appetite. When the boy was later exposed to infectious samples from smallpox lesions, he did not contract smallpox.[4] This new approach was termed vaccination, a name deriving from the use of cowpox (Latin *vacca* meaning "cow") to protect against smallpox. Today, we know that Jenner's vaccine worked because the cowpox virus is genetically and antigenically related to the *Variola* viruses that caused smallpox. Exposure to cowpox antigens resulted in a primary response and the production of memory cells that had identical or related epitopes of Variola virus upon a later exposure to smallpox.

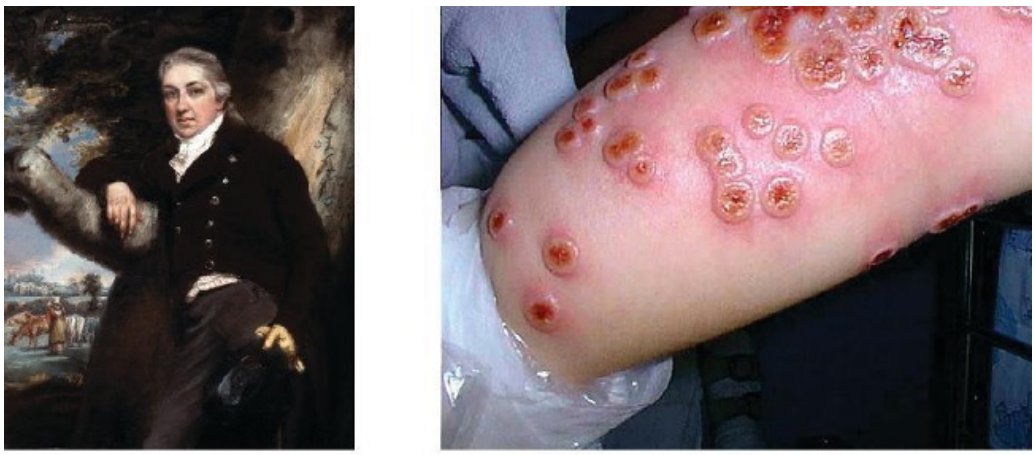


Figure 1.42: (a) A painting of Edward Jenner depicts a cow and a milkmaid in the background. (b) Lesions on a patient infected with cowpox, a zoonotic disease caused by a virus closely related to the one that causes smallpox. *Figure description available at the end of the chapter.*

The success of Jenner's smallpox vaccination led other scientists to develop vaccines for other diseases. Perhaps the most notable was Louis Pasteur, who developed vaccines for rabies, cholera, and anthrax. During the 20th and 21st centuries, effective vaccines were developed to prevent a wide range of diseases caused by viruses (e.g., chickenpox and shingles, hepatitis, measles, mumps, polio, and yellow fever) and bacteria (e.g., diphtheria, pneumococcal pneumonia, tetanus, and whooping cough).

CLASSES OF VACCINES

For a vaccine to provide protection against a disease, it must expose an individual to pathogen-specific antigens that will stimulate a protective adaptive immune response. By its very nature, this entails some risk. As with any pharmaceutical drug, vaccines have the potential to cause adverse effects. However, the ideal vaccine causes no severe adverse effects and poses no risk of contracting the disease that it is intended to prevent. Various types of vaccines have been developed with these goals in mind. These different classes of vaccines are described in the next section and summarized in table 1.13.

Live Attenuated Vaccines

Live attenuated vaccines expose an individual to a weakened strain of a pathogen with the goal of establishing a subclinical infection that will activate the adaptive immune defenses. Pathogens are attenuated to decrease their virulence using methods such as genetic manipulation (to eliminate key virulence factors) or long-term culturing in an unnatural host or environment (to promote mutations and decrease virulence).

By establishing an active infection, live attenuated vaccines stimulate a more comprehensive immune response than some other types of vaccines. Live attenuated vaccines activate both cellular and humoral immunity and stimulate the development of memory for long-lasting immunity. In some cases, vaccination of one individual with a live attenuated pathogen can even lead to natural transmission of the attenuated pathogen to other individuals. This can cause the other individuals to also develop an active, subclinical infection that activates their adaptive immune defenses.

Disadvantages associated with live attenuated vaccines include the challenges associated with long-term storage and transport as well as the potential for a patient to develop signs and symptoms of disease during the active infection (particularly in immunocompromised patients). There is also a risk of the attenuated pathogen reverting back to full virulence. Table 1.13 lists examples of live attenuated vaccines.

Inactivated Vaccines

Inactivated vaccines contain whole pathogens that have been killed or inactivated with heat, chemicals, or radiation. For inactivated vaccines to be effective, the inactivation process must not affect the structure of key antigens on the pathogen.

Because the pathogen is killed or inactive, inactivated vaccines do not produce an active infection, and the resulting immune response is weaker and less comprehensive than that provoked by a live attenuated vaccine. Typically the response involves only humoral immunity, and the pathogen cannot be transmitted to other individuals. In addition, inactivated vaccines usually require higher doses and multiple boosters, possibly causing inflammatory reactions at the site of injection.

Despite these disadvantages, inactivated vaccines do have the advantages of long-term storage stability and ease of transport. Also, there is no risk of causing severe active infections. However, inactivated vaccines are not without their side effects. Table 1.13 lists examples of inactivated vaccines.

Subunit Vaccines

Whereas live attenuated and inactive vaccines expose an individual to a weakened or dead pathogen, subunit vaccines only expose the patient to the key antigens of a pathogen—not whole cells or viruses. Subunit vaccines can be produced either by chemically degrading a pathogen and isolating its key antigens or by producing the antigens through genetic engineering. Because these vaccines contain only the essential antigens of a pathogen, the risk of side effects is relatively low. Table 1.13 lists examples of subunit vaccines.

Toxoid Vaccines

Like subunit vaccines, toxoid vaccines do not introduce a whole pathogen to the patient; they contain inactivated bacterial toxins, called toxoids. Toxoid vaccines are used to prevent diseases in which bacterial toxins play an important role in pathogenesis. These vaccines activate humoral immunity that neutralizes the toxins. Table 1.13 lists examples of toxoid vaccines.

Conjugate Vaccines

A conjugate vaccine is a type of subunit vaccine that consists of a protein conjugated to a capsule polysaccharide. Conjugate vaccines have been developed to enhance the efficacy of subunit vaccines against pathogens that have protective polysaccharide capsules that help them evade phagocytosis, causing invasive infections that can lead to meningitis and other serious conditions. The subunit vaccines against these pathogens introduce T-independent capsular polysaccharide antigens that result in the production of antibodies that can opsonize the capsule and thus combat the infection; however, children under the age of two years do not respond effectively to these vaccines. Children do respond effectively when vaccinated with the conjugate vaccine, in which a protein with T-dependent antigens is conjugated to the capsule polysaccharide. The conjugated protein-polysaccharide antigen stimulates production of antibodies against both the protein and the capsule polysaccharide. Table 1.13 lists examples of conjugate vaccines.

Class	Description	Advantages	Disadvantages	Examples
Live attenuated	Weakened strain of whole pathogen	Cellular and humoral immunity	Difficult to store and transport	Chickenpox, German measles, measles, mumps, tuberculosis, typhoid fever, yellow fever
		Long-lasting immunity	Risk of infection in immunocompromised patients	
		Transmission to contacts	Risk of reversion	
Inactivated	Whole pathogen killed or inactivated with heat, chemicals, or radiation	Ease of storage and transport	Weaker immunity (humoral only)	Cholera, hepatitis A, influenza, plague, rabies
		No risk of severe active infection	Higher doses and more boosters required	
Subunit	Immunogenic antigens	Lower risk of side effects	Limited longevity	Anthrax, hepatitis B, influenza, meningitis, papillomavirus, pneumococcal pneumonia, whooping cough
			Multiple doses required	
			No protection against antigenic variation	
Toxoid	Inactivated bacterial toxin	Humoral immunity to neutralize toxin	Does not prevent infection	Botulism, diphtheria, pertussis, tetanus
Conjugate	Capsule polysaccharide conjugated to protein	T-dependent response to capsule	Costly to produce	Meningitis (Haemophilus influenzae, Streptococcus pneumoniae, Neisseria meningitides)
		Better response in young children	No protection against antigenic variation	
			May interfere with other vaccines	

Table 1.13: Classes of vaccines

1.13 HYPERSENSITIVITIES

There are mechanisms by which adaptive immune defenses, both humoral and cellular, protect us from infectious diseases. However, these same protective immune defenses can also be responsible for undesirable reactions called hypersensitivity reactions. Hypersensitivity reactions are classified by their immune mechanism.

- **Type I hypersensitivity** reactions involve immunoglobulin E (IgE) antibody against soluble antigen, triggering mast cell degranulation.
- **Type II hypersensitivity** reactions involve IgG and IgM antibodies directed against cellular antigens, leading to cell damage mediated by other immune system effectors.
- **Type III hypersensitivity** reactions involve the interactions of IgG, IgM, and, occasionally, IgA[5] antibodies with antigen to form immune complexes. Accumulation of immune complexes in tissue leads to tissue damage mediated by other immune system effectors.
- **Type IV hypersensitivity** reactions are T-cell–mediated reactions that can involve tissue damage mediated by activated macrophages and cytotoxic T cells.

TYPE 1 HYPERSENSITIVITIES

When a presensitized individual is exposed to an allergen, it can lead to a rapid immune response that occurs almost immediately. Such a response is called an allergy and is classified as a type I hypersensitivity. Allergens may be seemingly harmless substances such as animal dander, molds, or pollen. Allergens may also be substances considered innately more hazardous, such as insect venom or therapeutic drugs. Food intolerances can also yield allergic reactions as individuals become sensitized to foods such as peanuts or shellfish (figure 1.43). Regardless of the allergen, the first exposure activates a primary IgE antibody response that sensitizes an individual to type I hypersensitivity reaction upon subsequent exposure.

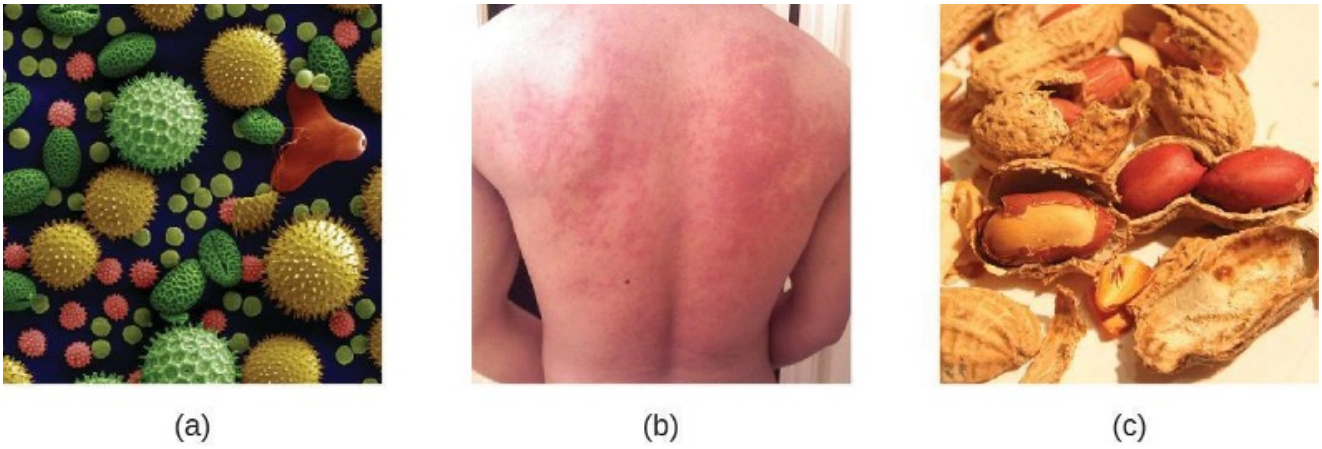


Figure 1.43: (a) Allergens in plant pollen, shown here in a colorized electron micrograph, may trigger allergic rhinitis or hay fever in sensitive individuals. (b) Skin rashes are often associated with allergic reactions. (c) Peanuts can be eaten safely by most people but can provoke severe allergic reactions in sensitive individuals. *Figure description available at the end of the chapter.*

For susceptible individuals, a first exposure to an allergen activates a strong TH2 cell response (figure 1.44). Cytokines interleukin (IL)-4 and IL-13 from the TH2 cells activate B cells specific to the same allergen, resulting in clonal proliferation, differentiation into plasma cells, and antibody-class switching from production of IgM to production of IgE. The fragment crystallizable (Fc) regions of the IgE antibodies bind to specific receptors on the surface of mast cells throughout the body. It is estimated that each mast cell can bind up to 500,000 IgE molecules, with each IgE molecule having two allergen-specific fragment antigen-binding (Fab) sites available for binding allergen on subsequent exposures. By the time this occurs, the allergen is often no longer present and there is no allergic reaction, but the mast cells are primed for a subsequent exposure and the individual is sensitized to the allergen.

On subsequent exposure, allergens bind to multiple IgE molecules on mast cells, cross-linking the IgE molecules. Within minutes, this cross-linking of IgE activates the mast cells and triggers degranulation, a reaction in which the contents of the granules in the mast cell are released into the extracellular environment. Preformed components that are released from granules include histamine, serotonin, and bradykinin (table 1.14). The activated mast cells also release newly formed lipid mediators (leukotrienes and prostaglandins from membrane arachidonic acid metabolism) and cytokines such as tumor necrosis factor (table 1.15).

The chemical mediators released by mast cells collectively cause the inflammation and signs and symptoms associated with type I hypersensitivity reactions. Histamine stimulates mucus secretion in nasal passages and tear formation from lacrimal glands, promoting the runny nose and watery eyes of allergies. Interaction of histamine with nerve endings causes itching and sneezing. The vasodilation caused by several of the mediators can result in hives, headaches, angioedema (swelling that often affects the lips, throat, and tongue), and hypotension (low blood pressure). Bronchoconstriction caused by some of the chemical mediators leads to wheezing, dys-

pnea (difficulty breathing), coughing, and, in more severe cases, cyanosis (bluish color to the skin or mucous membranes). Vomiting can result from stimulation of the vomiting center in the cerebellum by histamine and serotonin. Histamine can also cause relaxation of intestinal smooth muscles and diarrhea.

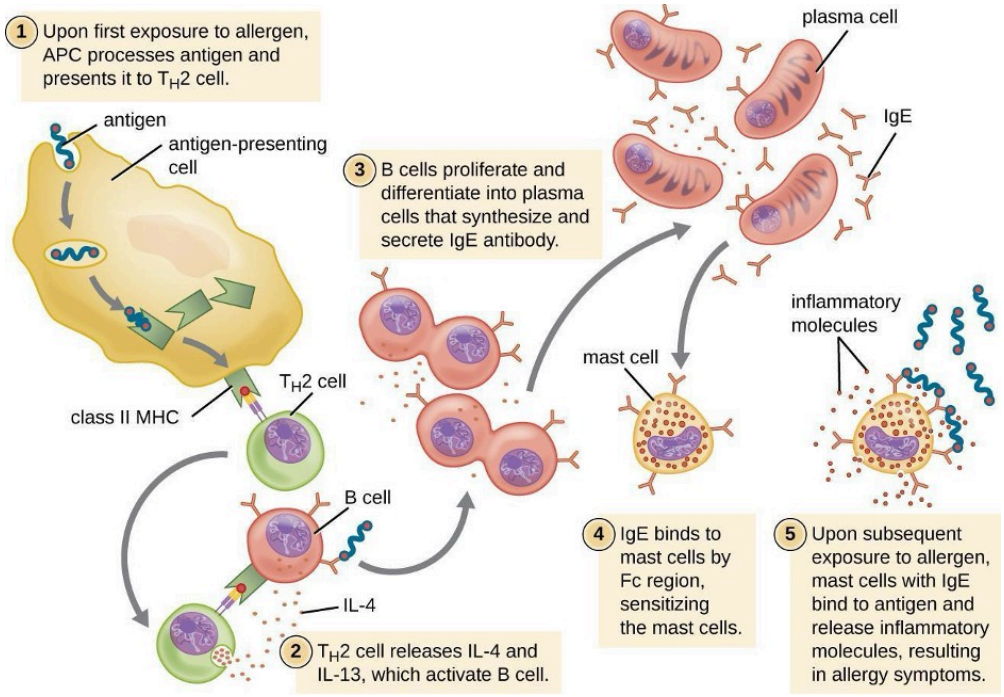


Figure 1.44: On first exposure to an allergen in a susceptible individual, antigen-presenting cells process and present allergen epitopes with major histocompatibility complex (MHC) II to T helper cells. B cells also process and present the same allergen epitope to TH2 cells, which release cytokines IL-4 and IL-13 to stimulate proliferation and differentiation into IgE-secreting plasma cells. The IgE molecules bind to mast cells with their Fc region, sensitizing the mast cells for activation with subsequent exposure to the allergen. With each subsequent exposure, the allergen cross-links IgE molecules on the mast cells, activating the mast cells and causing the release of preformed chemical mediators from granules (degranulation), as well as newly formed chemical mediators that collectively cause the signs and symptoms of type I hypersensitivity reactions. *Figure description available at the end of the chapter.*

Granule Component	Activity
Heparin	Stimulates the generation of bradykinin, which causes increased vascular permeability, vasodilation, bronchoconstriction, and increased mucus secretion
Histamine	Causes smooth-muscle contraction, increases vascular permeability, increases mucus and tear formation
Seratonin	Increases vascular permeability, causes vasodilation and smooth-muscle contraction

Table 1.14: Selected preformed components of mast cell granules

Chemical Mediator	Activity
Leukotriene	Causes smooth-muscle contraction and mucus secretion, increases vascular permeability
Prostaglandin	Causes smooth-muscle contraction and vasodilation
TNF-α (cytokine)	Causes inflammation and stimulates cytokine production by other cell types

Table 1.15: Selected newly formed chemical mediators of inflammation and allergic response

Type I hypersensitivity reactions can be either localized or systemic. Localized type I hypersensitivity reactions include hay fever rhinitis, hives, and asthma (table 1.16). Systemic type I hypersensitivity reactions are referred to as anaphylaxis or anaphylactic shock. Although anaphylaxis shares many symptoms common with the localized type I hypersensitivity reactions, the swelling of the tongue and trachea, blockage of airways, dangerous drop in blood pressure, and development of shock can make anaphylaxis especially severe and life-threatening. In fact, death can occur within minutes of onset of signs and symptoms.

Late-phase reactions in type I hypersensitivities may develop 4–12 hours after the early phase and are mediated by eosinophils, neutrophils, and lymphocytes that have been recruited by chemotactic factors released from mast cells. Activation of these recruited cells leads to the release of more chemical mediators that cause tissue damage and late-phase symptoms of swelling and redness of the skin, coughing, wheezing, and nasal discharge.

Individuals who possess genes for maladaptive traits, such as intense type I hypersensitivity reactions to otherwise harmless components of the environment, would be expected to suffer reduced reproductive success. With this kind of evolutionary selective pressure, such traits would not be expected to persist in a population. This suggests that type I hypersensitivities may have an adaptive function. There is evidence that the IgE produced during type I hypersensitivity reactions is actually meant to counter helminth infections.[6] Helminths are one of few organisms that possess proteins that are targeted by IgE. In addition, there is evidence that helminth infections at a young age reduce the likelihood of type I hypersensitivities to innocuous substances later in life. Thus it may be that allergies are an unfortunate consequence of strong selection in the mammalian lineage or earlier for a defense against parasitic worms.

Common Name	Cause	Signs and Symptoms
Allergy-induced asthma	Inhalation of allergens	Constriction of bronchi, labored breathing, coughing, chills, body aches
Anaphylaxis	Systemic reaction to allergens	Hives, itching, swelling of tongue and throat, nausea, vomiting, low blood pressure, shock
Hay fever	Inhalation of mold or pollen	Runny nose, watery eyes, sneezing
Hives (urticaria)	Food or drug allergens, insect stings	Raised, bumpy skin rash with itching; bumps may converge into large raised areas

Table 1.16: Type I hypersensitivities

TYPE II (CYTOTOXIC) HYPERSENSITIVITIES

Immune reactions categorized as type II hypersensitivities, or cytotoxic hypersensitivities, are mediated by IgG and IgM antibodies binding to cell-surface antigens or matrix-associated antigens on basement membranes. These antibodies can either activate complement, resulting in an inflammatory response and lysis of the targeted cells, or they can be involved in antibody-dependent cell-mediated cytotoxicity (ADCC) with cytotoxic T cells.

In some cases, the antigen may be a self-antigen, in which case the reaction would also be described as an autoimmune disease (for more information on autoimmune disorders, see section 1.14). In other cases, antibodies may bind to naturally occurring, but exogenous, cell-surface molecules such as antigens associated with blood typing found on red blood cells (RBCs). This leads to the coating of the RBCs by antibodies, activation of the complement cascade, and complement-mediated lysis of RBCs, as well as opsonization of RBCs for phagocytosis. Two examples of type II hypersensitivity reactions involving RBCs are hemolytic transfusion reaction (HTR) and hemolytic disease of the newborn (HDN). These type II hypersensitivity reactions, which will be discussed in greater detail, are summarized in table 1.17.

Common Name	Cause	Signs and Symptoms
Hemolytic disease of the newborn (HDN)	IgG from mother crosses the placenta, targeting the fetus' RBCs for destruction	Anemia, edema, enlarged liver or spleen, hydrops (fluid in body cavity), leading to death of newborn in severe cases
Hemolytic transfusion reactions (HTR)	IgG and IgM bind to antigens on transfused RBCs, targeting donor RBCs for destruction	Fever, jaundice, hypotension, disseminated intravascular coagulation, possibly leading to kidney failure and death

Table 1.17: Common type II hypersensitivities

Immunohematology is the study of blood and blood-forming tissue in relation to the immune response. Antibody-initiated responses against blood cells are type II hypersensitivities, thus falling into the field of immunohematology. For students first learning about immunohematology, understanding the immunological mechanisms involved is made even more challenging by the complex nomenclature system used to identify different blood-group antigens, often called blood types. The first blood-group antigens either used alphabetical names or were named for the first person known to produce antibodies to the red blood cell antigen (e.g., Kell, Duffy, or Diego). However, in 1980, the International Society of Blood Transfusion (ISBT) Working Party on Terminology created a standard for blood-group terminology in an attempt to more consistently identify newly discovered blood group antigens. New antigens are now given a number and assigned to a blood-group system, collection, or series. However, even with this effort, blood-group nomenclature is still inconsistent.

ABO Blood Group Incompatibility

The recognition that individuals have different blood types was first described by Karl Landsteiner (1868–1943) in the early 1900s, based on his observation that serum from one person could cause a clumping of RBCs from another. These studies led Landsteiner to the identification of four distinct blood types. Subsequent research by other scientists determined that the four blood types were based on the presence or absence of surface carbohydrates A and B, and this provided the foundation for the ABO blood group system that is still in use today (figure 1.45). The functions of these antigens are unknown, but some have been associated with normal biochemical functions of the cell. Furthermore, ABO blood types are inherited as alleles (one from each parent), and they display patterns of dominant and codominant inheritance. The alleles for A and B blood types are codominant

to each other, and both are dominant over blood type O. Therefore, individuals with genotypes of AA or AO have type A blood and express the A carbohydrate antigen on the surface of their RBCs. People with genotypes of BB or BO have type B blood and express the B carbohydrate antigen on the surface of their RBCs. Those with a genotype of AB have type AB blood and express both A and B carbohydrate antigens on the surface of their RBCs. Finally, individuals with a genotype of OO have type O blood and lack A and B carbohydrates on the surface of their RBCs.

It is important to note that the RBCs of all four ABO blood types share a common protein receptor molecule, and it is the addition of specific carbohydrates to the protein receptors that determines A, B, and AB blood types. The genes that are inherited for the A, B, and AB blood types encode enzymes that add the carbohydrate component to the protein receptor. Individuals with O blood type still have the protein receptor but lack the enzymes that would add carbohydrates that would make their red blood cell type A, B, or AB.

IgM antibodies in plasma that cross-react with blood group antigens not present on an individual's own RBCs are called isohemagglutinins (figure 1.45). Isohemagglutinins are produced within the first few weeks after birth and persist throughout life. These antibodies are produced in response to exposure to environmental antigens from food and microorganisms. A person with type A blood has A antigens on the surface of their RBCs and will produce anti-B antibodies to environmental antigens that resemble the carbohydrate component of B antigens. Those with type B blood have B antigens on the surface of their RBCs and will produce anti-A antibodies to environmental antigens that are similar to the carbohydrate component of A antigens. People with blood type O lack both A and B antigens on their RBCs and, therefore, produce both anti-A and anti-B antibodies. Conversely, people with AB blood type have both A and B antigens on their RBCs and, therefore, lack anti-A and anti-B antibodies.

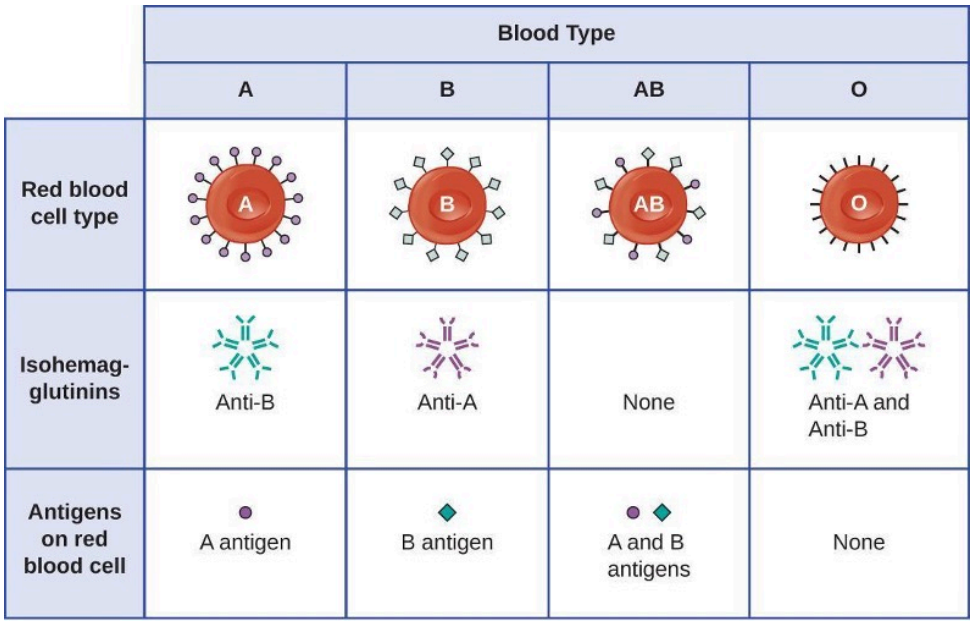


Figure 1.45: Blood types. *Figure description available at the end of the chapter.*

A patient may require a blood transfusion because they lack sufficient RBCs (anemia) or because they have experienced significant loss of blood volume through trauma or disease. Although the blood transfusion is given to help the patient, it is essential that the patient receive a transfusion with matching ABO blood type. A transfusion with an incompatible ABO blood type may lead to a strong, potentially lethal type II hypersensitivity cytotoxic response called hemolytic transfusion reaction (HTR) (figure 1.46).

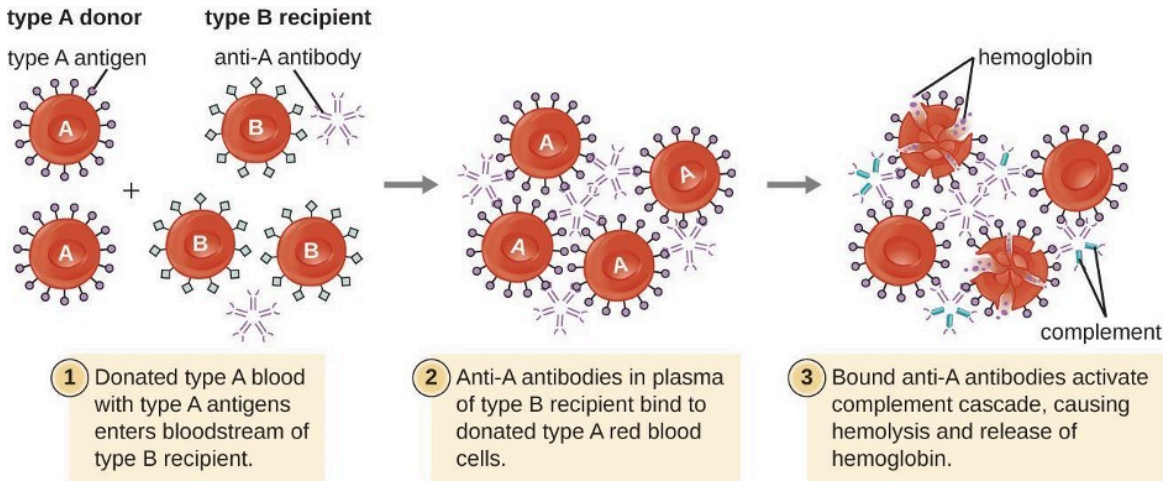


Figure 1.46: (1) A type II hypersensitivity hemolytic transfusion reaction (HTR) leading to hemolytic anemia. Blood from a type A donor is administered to a patient with type B blood. (2) The anti-A isohemagglutinin IgM antibodies in the recipient bind to and agglutinate the incoming donor type A red blood cells. (3) The bound anti-A antibodies activate the classical complement cascade, resulting in destruction of the donor red blood cells. Figure description available at the end of the chapter.

For instance, if a person with type B blood receives a transfusion of type A blood, their anti-A antibodies will bind to and agglutinate the transfused RBCs. In addition, activation of the classical complement cascade will lead to a strong inflammatory response, and the complement membrane attack complex (MAC) will mediate massive hemolysis of the transfused RBCs. The debris from damaged and destroyed RBCs can occlude blood vessels in the alveoli of the lungs and the glomeruli of the kidneys. Within 1 to 24 hours of an incompatible transfusion, the patient experiences fever, chills, pruritus (itching), urticaria (hives), dyspnea, hemoglobinuria (hemoglobin in the urine), and hypotension (low blood pressure). In the most serious reactions, dangerously low blood pressure can lead to shock, multi-organ failure, and death of the patient.

Hospitals, medical centers, and associated clinical laboratories typically use hemovigilance systems to minimize the risk of HTRs due to clerical error. Hemovigilance systems are procedures that track transfusion information from the donor source and blood products obtained to the follow-up of recipient patients. Hemovigilance systems used in many countries identify HTRs and their outcomes through mandatory reporting (e.g., to the Food and Drug Administration in the United States), and this information is valuable to help prevent such occurrences in the future. For example, if an HTR is found to be the result of laboratory or clerical error, additional blood products collected from the donor at that time can be located and labeled correctly to avoid additional HTRs. As a result of these measures, HTR-associated deaths in the United States occur in about one per 2 million transfused units.[7]

Rh Factors

Many different types of erythrocyte antigens have been discovered since the description of the ABO red cell antigens. The second most frequently described RBC antigens are Rh factors, named after the rhesus macaque (*Macaca mulatta*) factors identified by Karl Landsteiner and Alexander Weiner in 1940. The Rh system of RBC antigens is the most complex and immunogenic blood group system, with more than 50 specificities identified to date. Of all the Rh antigens, the one designated Rho (Weiner) or D (Fisher-Race) is the most immunogenic. Cells are classified as Rh positive (Rh+) if the Rho/D antigen is present or as Rh negative (Rh–) if the Rho/D antigen is absent. In contrast to the carbohydrate molecules that distinguish the ABO blood groups and are the targets of IgM isohemagglutinins in HTRs, the Rh factor antigens are proteins. As discussed in section 1.11,

protein antigens activate B cells and antibody production through a T-cell–dependent mechanism, and the TH2 cells stimulate class switching from IgM to other antibody classes. In the case of Rh factor antigens, TH2 cells stimulate class switching to IgG, and this has important implications for the mechanism of HDN.

Like ABO incompatibilities, blood transfusions from a donor with the wrong Rh factor antigens can cause a type II hypersensitivity HTR. However, in contrast to the IgM isohemagglutinins produced early in life through exposure to environmental antigens, production of anti-Rh factor antibodies requires the exposure of an individual with Rh– blood to Rh+ positive RBCs and activation of a primary antibody response. Although this primary antibody response can cause an HTR in the transfusion patient, the hemolytic reaction would be delayed up to 2 weeks during the extended lag period of a primary antibody response (section 1.11). However, if the patient receives a subsequent transfusion with Rh+ RBCs, a more rapid HTR would occur with anti-Rh factor antibodies already present in the blood. Furthermore, the rapid secondary antibody response would provide even more anti-Rh factor antibodies for the HTR.

Rh factor incompatibility between mother and fetus can also cause a type II hypersensitivity hemolytic reaction, referred to as hemolytic disease of the newborn (HDN) (figure 1.47). If an Rh– woman carries an Rh+ baby to term, the mother's immune system can be exposed to Rh+ fetal red blood cells. This exposure will usually occur during the last trimester of pregnancy and during the delivery process. If this exposure occurs, the Rh+ fetal RBCs will activate a primary adaptive immune response in the mother, and anti-Rh factor IgG antibodies will be produced. IgG antibodies are the only class of antibody that can cross the placenta from mother to fetus; however, in most cases, the first Rh+ baby is unaffected by these antibodies because the first exposure typically occurs late enough in the pregnancy that the mother does not have time to mount a sufficient primary antibody response before the baby is born.

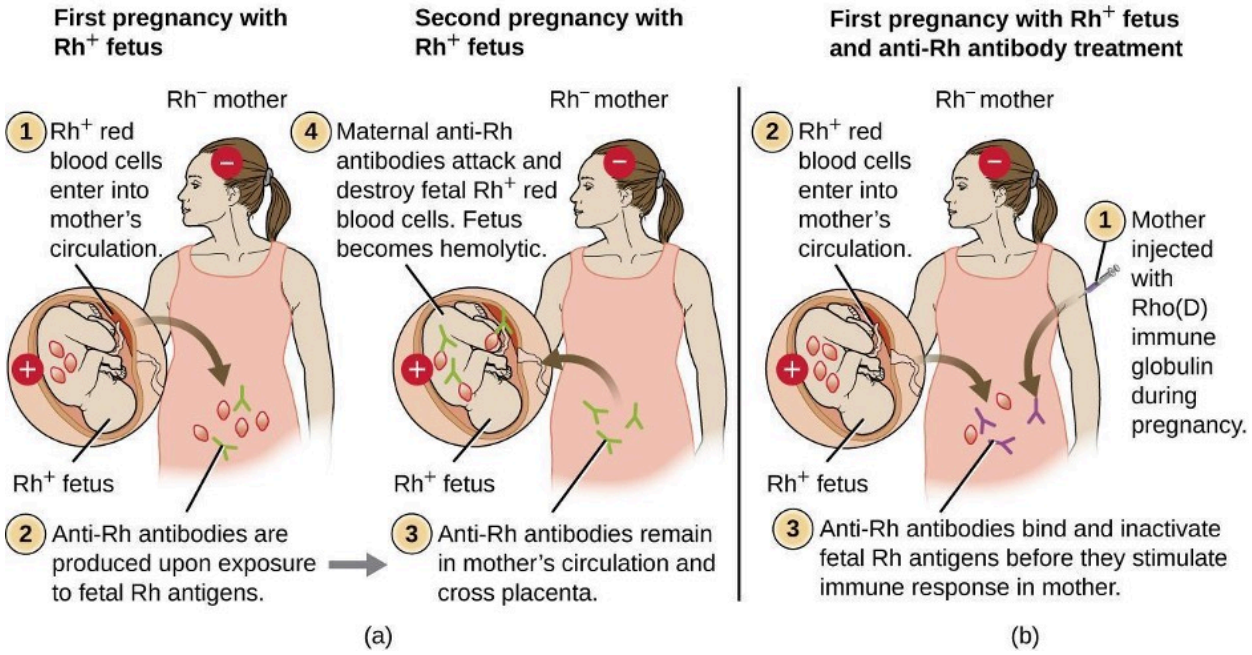


Figure 1.47: (a) When an Rh− mother has an Rh+ fetus, fetal erythrocytes are introduced into the mother's circulatory system before or during birth, leading to production of anti-Rh IgG antibodies. These antibodies remain in the mother and, if she becomes pregnant with a second Rh+ baby, they can cross the placenta and attach to fetal Rh+ erythrocytes. Complement-mediated hemolysis of fetal erythrocytes results in a lack of sufficient cells for proper oxygenation of the fetus. (b) HDN can be prevented by administering Rho(D) immune globulin during and after each pregnancy with an Rh+ fetus. The immune globulin binds fetal Rh+ RBCs that gain access to the mother's bloodstream, preventing activation of her primary immune response. Figure description available at the end of the chapter.

If a subsequent pregnancy with an Rh+ fetus occurs, however, the mother's second exposure to the Rh factor antigens causes a strong secondary antibody response that produces larger quantities of anti-Rh factor IgG. These antibodies can cross the placenta from mother to fetus and cause HDN, a potentially lethal condition for the baby (figure 1.48).

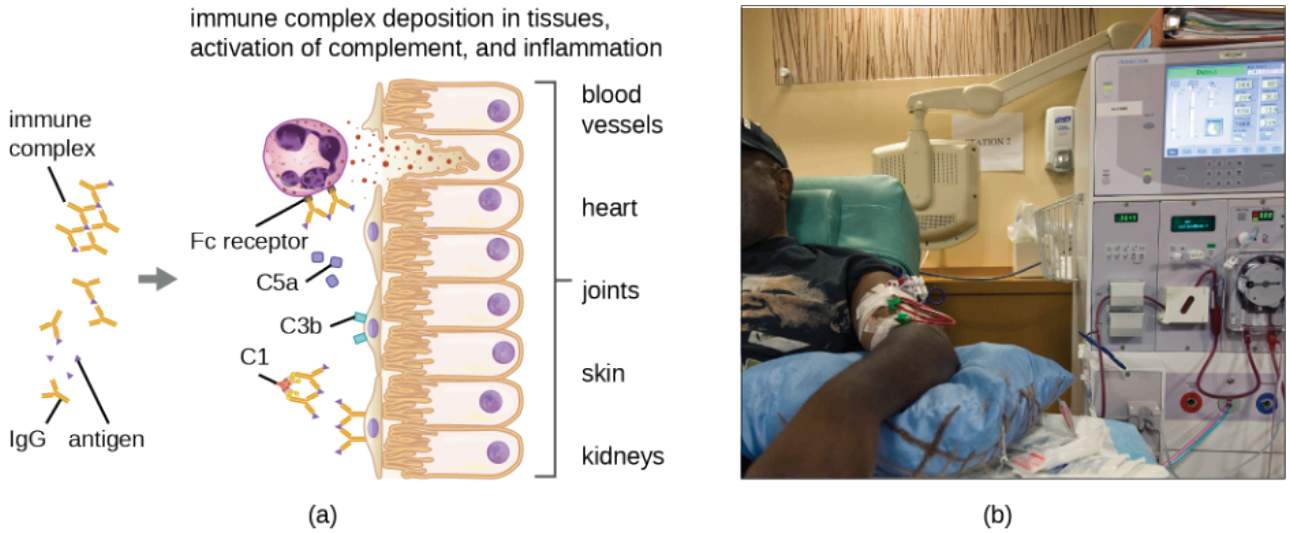


Figure 1.48: Type III hypersensitivities and the systems they affect. (a) Immune complexes form and deposit in tissue. Complement activation, stimulation of an inflammatory response, and recruitment and activation of neutrophils result in damage to blood vessels, heart tissue, joints, skin, and/or kidneys. (b) If the kidneys are damaged by a type III hypersensitivity reaction, dialysis may be required. *Figure description available at the end of the chapter.*

Prior to the development of techniques for diagnosis and prevention, Rh factor incompatibility was the most common cause of HDN, resulting in thousands of infant deaths each year worldwide.[8] For this reason, the Rh factors of prospective parents are regularly screened, and treatments have been developed to prevent HDN caused by Rh incompatibility. To prevent Rh factor-mediated HDN, human Rho(D) immune globulin (e.g., RhoGAM) is injected intravenously or intramuscularly into the mother during the 28th week of pregnancy and within 72 hours after delivery. Additional doses may be administered after events that may result in transplacental hemorrhage (e.g., umbilical blood sampling, chorionic villus sampling, abdominal trauma, amniocentesis). This treatment is initiated during the first pregnancy with an Rh+ fetus. The anti-Rh antibodies in Rho(D) immune globulin will bind to the Rh factor of any fetal RBCs that gain access to the mother's bloodstream, preventing these Rh+ cells from activating the mother's primary antibody response. Without a primary anti-Rh factor antibody response, the next pregnancy with an Rh+ will have minimal risk of HDN. However, the mother will need to be retreated with Rho(D) immune globulin during that pregnancy to prevent a primary anti-Rh antibody response that could threaten subsequent pregnancies.

TYPE III HYPERSENSITIVITIES

Type III hypersensitivities are immune-complex reactions that were first characterized by Nicolas Maurice Arthus (1862–1945) in 1903. To produce antibodies for experimental procedures, Arthus immunized rabbits by injecting them with serum from horses. However, while immunizing rabbits repeatedly with horse serum, Arthus noticed a previously unreported and unexpected localized subcutaneous hemorrhage with edema at the site of injection. This reaction developed within 3 to 10 hours after injection. This localized reaction to non-self serum proteins was called an **Arthus reaction**. An Arthus reaction occurs when soluble antigens bind with IgG in a ratio that results in the accumulation of antigen-antibody aggregates called immune complexes.

A unique characteristic of type III hypersensitivity is antibody excess (primarily IgG), coupled with a relatively low concentration of antigen, resulting in the formation of small immune complexes that deposit on the surface of the epithelial cells lining the inner lumen of small blood vessels or on the surfaces of tissues (figure 1.48). This immune complex accumulation leads to a cascade of inflammatory events that include the following:

1. IgG binding to antibody receptors on localized mast cells, resulting in mast-cell degranulation
2. Complement activation with production of pro-inflammatory C3a and C5a (see section 1.4)
3. Increased blood-vessel permeability with chemotactic recruitment of neutrophils and macrophages

Because these immune complexes are not an optimal size and are deposited on cell surfaces, they cannot be phagocytosed in the usual way by neutrophils and macrophages, which, in turn, are often described as "frustrated." Although phagocytosis does not occur, neutrophil degranulation results in the release of lysosomal enzymes that cause extracellular destruction of the immune complex, damaging localized cells in the process. Activation of coagulation pathways also occurs, resulting in thrombi (blood clots) that occlude blood vessels and cause ischemia that can lead to vascular necrosis and localized hemorrhage.

Systemic type III hypersensitivity (serum sickness) occurs when immune complexes deposit in various body sites, resulting in a more generalized systemic inflammatory response. These immune complexes involve non-self proteins such as antibodies produced in animals for artificial passive immunity (see section 1.12 for more information on vaccines), certain drugs, or microbial antigens that are continuously released over time during chronic infections (e.g., subacute bacterial endocarditis, chronic viral hepatitis). The mechanisms of serum sickness are similar to those described in localized type III hypersensitivity but involve widespread activation of mast cells, complement, neutrophils, and macrophages, which causes tissue destruction in areas such as the kidneys, joints, and blood vessels. As a result of tissue destruction, symptoms of serum sickness include chills, fever, rash, vasculitis, and arthritis. Development of glomerulonephritis or hepatitis is also possible.

Autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis can also involve damaging type III hypersensitivity reactions when auto-antibodies form immune complexes with self antigens. These conditions are discussed in section 1.14.

TYPE IV HYPERSENSITIVITIES

Type IV hypersensitivities are not mediated by antibodies like the other three types of hypersensitivities. Rather, type IV hypersensitivities are regulated by T cells and involve the action of effector cells. These types of hypersensitivities can be organized into three subcategories based on T-cell subtype, type of antigen, and the resulting effector mechanism (table 1.18).

In the first type IV subcategory, CD4 TH1-mediated reactions are described as delayed-type hypersensitivities (DTH). The sensitization step involves the introduction of antigen into the skin and phagocytosis by local antigen presenting cells (APCs). The APCs activate helper T cells, stimulating clonal proliferation and differentiation into memory TH1 cells. Upon subsequent exposure to the antigen, these sensitized memory TH1 cells release cytokines that activate macrophages, and activated macrophages are responsible for much of the tissue damage. Examples of this TH1-mediated hypersensitivity are observed in the Mantoux tuberculin skin test and contact dermatitis.

In the second type IV subcategory, CD4 TH2-mediated reactions result in chronic asthma or chronic allergic rhinitis. In these cases, the soluble antigen is first inhaled, resulting in eosinophil recruitment and activation with the release of cytokines and inflammatory mediators.

In the third type IV subcategory, CD8 cytotoxic T lymphocyte (CTL)-mediated reactions are associated with tissue transplant rejection and contact dermatitis (figure 1.49). For this form of cell-mediated hypersensitivity, APCs process and present the antigen with MHC I to naïve CD8 T cells. When these naïve CD8 T cells are activated, they proliferate and differentiate into CTLs. Activated TH1 cells can also enhance the activation of the CTLs. The activated CTLs then target and induce granzyme-mediated apoptosis in cells presenting the same antigen with MHC I. These target cells could be "self" cells that have absorbed the foreign antigen (such as with contact dermatitis due to poison ivy), or they could be transplanted tissue cells displaying foreign antigen from the donor.

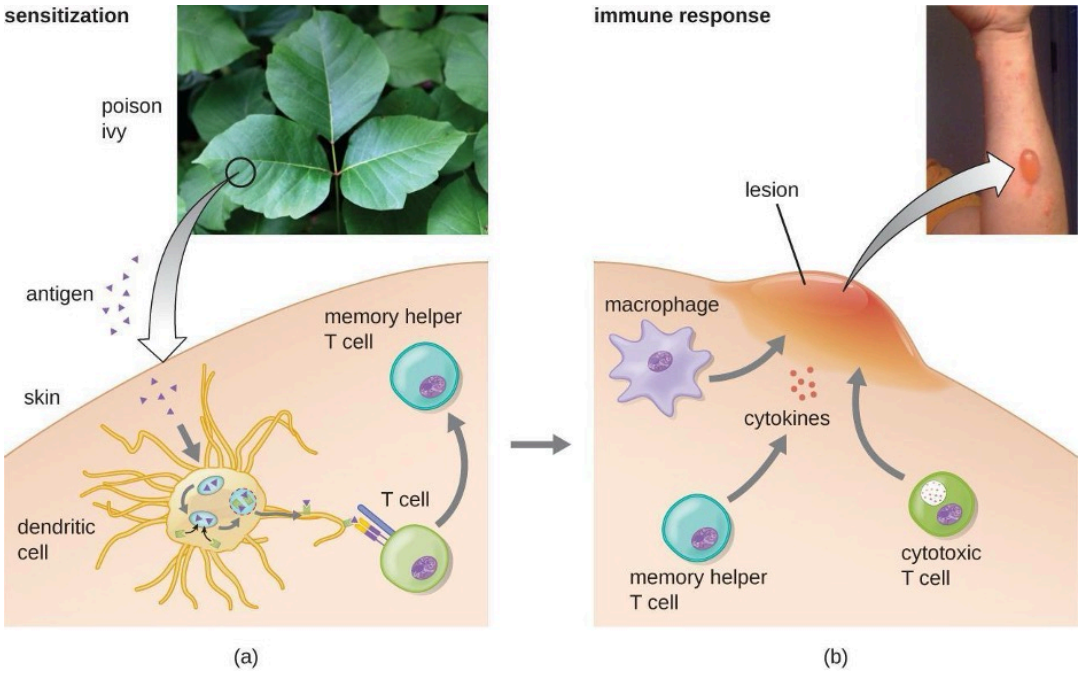


Figure 1.49: Exposure to hapten antigens in poison ivy can cause contact dermatitis, a type IV hypersensitivity. (a) The first exposure to poison ivy does not result in a reaction. However, sensitization stimulates helper T cells, leading to production of memory helper T cells that can become reactivated on future exposures. (b) Upon secondary exposure, the memory helper T cells become reactivated, producing inflammatory cytokines that stimulate macrophages and cytotoxic T cells to induce an inflammatory lesion at the exposed site. This lesion, which will persist until the allergen is removed, can inflict significant tissue damage if it continues long enough. *Figure description available at the end of the chapter.*

Subcategory	Antigen	Effector Mechanism	Examples
1	Soluble antigen	Activated macrophages damage tissue and promote inflammatory response	Contact dermatitis (e.g., exposure to latex) and delayed-type hypersensitivity (e.g., tuberculin reaction)
2	Soluble antigen	Eosinophil recruitment and activation release cytokines and pro-inflammatory chemicals	Chronic asthma and chronic allergic rhinitis
3	Cell-associated antigen	CTL-mediated cytotoxicity	Contact dermatitis (e.g., contact with poison ivy) and tissue-transplant rejection

Table 1.18: Type IV hypersensitivities

HYPERSENSITIVITY PNEUMONITIS

Some diseases caused by hypersensitivities are not caused exclusively by one type. For example, hypersensitivity pneumonitis (HP), which is often an occupational or environmental disease, occurs when the lungs become inflamed due to an allergic reaction to inhaled dust, endospores, bird feathers, bird droppings, molds, or chemicals. HP goes by many different names associated with various forms of exposure (figure 1.50). HP associated with bird droppings is sometimes called pigeon fancier's lung or poultry worker's lung—both common in bird breeders and handlers. Cheese handler's disease, farmer's lung, sauna takers' disease, and hot-tub lung are other names for HP associated with exposure to molds in various environments.

Figure 1.50: Occupational exposure to dust, mold, and other allergens can result in hypersensitivity pneumonitis. (a) People exposed daily to large numbers of birds may be susceptible to poultry worker's lung. (b) Workers in a cheese factory may become sensitized to different types of molds and develop cheese handler's disease. *Figure description available at the end of the chapter.*

Pathology associated with HP can be due to both type III (mediated by immune complexes) and type IV (mediated by TH1 cells and macrophages) hypersensitivities. Repeated exposure to allergens can cause alveolitis due to the formation of immune complexes in the alveolar wall of the lung accompanied by fluid accumulation, and the formation of granulomas and other lesions in the lung as a result of TH1-mediated macrophage activation. Alveolitis with fluid and granuloma formation results in poor oxygen perfusion in the alveoli, which, in turn, can cause symptoms such as coughing, dyspnea, chills, fever, sweating, myalgias, headache, and nausea. Symptoms may occur as quickly as 2 hours after exposure and can persist for weeks if left untreated.

Table 1.19 summarizes the mechanisms and effects of each type of hypersensitivity discussed in this section.

	Type I	Type II	Type III	Type IV
Immune Reactant	IgE	IgG or IgM	IgG and IgM	T cells
Antigen form	Soluble antigen	Cell-bound antigen	Soluble antigen	Soluble or cell-bound antigen
Mechanism of activation	Allergen-specific IgE antibodies bind to mast cells via their Fc receptor. When the specific allergen binds to the IgE, cross-linking of IgE induces degranulation of mast cells.	IgG or IgM antibody binds to cellular antigen, leading to complement activation and cell lysis. IgG can also mediate ADCC with cytotoxic T cells, natural killer cells, macrophages, and neutrophils.	Antigen-antibody complexes are deposited in tissues. Complement activation provides inflammatory mediators and recruits neutrophils. Enzymes released from neutrophils damage tissue.	TH1 cells secrete cytokines, which activate macrophages and cytotoxic T cells.
Examples of hypersensitivity reactions	Local and systemic anaphylaxis, seasonal hay fever, food allergies, and drug allergies	Red blood cell destruction after transfusion with mismatched blood types or during hemolytic disease of the newborn.	Local and systemic Post-streptococcal glomerulonephritis, rheumatoid arthritis, and systemic lupus erythematosus	Contact dermatitis, type I diabetes mellitus, and multiple sclerosis

Table 1.19: Components of the immune system cause four types of hypersensitivities. Notice that types I–III are B-cell/antibody-mediated hypersensitivities, whereas type IV hypersensitivity is exclusively a T-cell phenomenon.

DIAGNOSIS OF HYPERSENSITIVITIES

Diagnosis of type I hypersensitivities is a complex process requiring several diagnostic tests in addition to a well-documented patient history. Serum IgE levels can be measured, but elevated IgE alone does not confirm allergic disease. As part of the process to identify the antigens responsible for a type I reaction allergy, testing through a prick puncture skin test (PPST) or an intradermal test can be performed. PPST is carried out with the introduction of allergens in a series of superficial skin pricks on the patient's back or arms (figure 1.51). PPSTs are considered to be the most convenient and least expensive way to diagnose allergies, according to the US Joint Council of Allergy and the European Academy of Allergy and Immunology. The second type of testing, the intradermal test, requires injection into the dermis with a small needle. This needle, also known as a tuberculin needle, is attached to a syringe containing a small amount of allergen. Both the PPST and the intradermal tests are observed for 15–20 minutes for a wheal-flare reaction to the allergens. Measurement of any wheal (a raised, itchy bump) and flare (redness) within minutes indicates a type I hypersensitivity, and the larger the wheal-flare reaction, the greater the patient's sensitivity to the allergen.

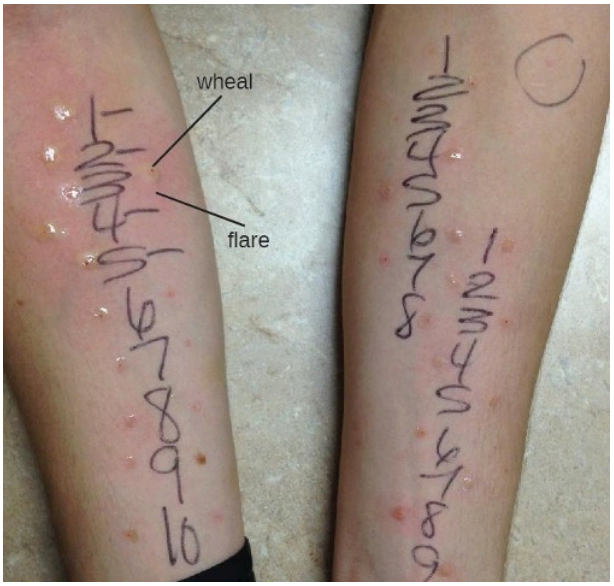


Figure 1.51: Results of an allergy skin-prick test to test for type I hypersensitivity to a group of potential allergens. A positive result is indicated by a raised area (wheal) and surrounding redness (flare). *Figure description available at the end of the chapter.*

Type III hypersensitivities can often be misdiagnosed because of their nonspecific inflammatory nature. The symptoms are easily visible, but they may be associated with any of a number of other diseases. A strong, comprehensive patient history is crucial to proper and accurate diagnosis. Tests used to establish the diagnosis of hypersensitivity pneumonitis (resulting from type III hypersensitivity) include bronchoalveolar lavage (BAL), pulmonary function tests, and high-resolution computed tomography (HRCT).

TREATMENTS OF HYPERSENSITIVITIES

Allergic reactions can be treated in various ways. Prevention of allergic reactions can be achieved by desensitization (hyposensitization) therapy, which can be used to reduce the hypersensitivity reaction through repeated injections of allergens. Extremely dilute concentrations of known allergens (determined from the allergen tests) are injected into the patient at prescribed intervals (e.g., weekly). The quantity of allergen delivered by the shots is slowly increased over a buildup period until an effective dose is determined and that dose is maintained for the duration of treatment, which can last years. Patients are usually encouraged to remain in the doctor's office for 30 minutes after receiving the injection in case the allergens administered cause a severe systemic reaction. Doctors' offices that administer desensitization therapy must be prepared to provide resuscitation and drug treatment in the case of such an event.

Desensitization therapy is used for insect sting allergies and environmental allergies. The allergy shots elicit the production of different interleukins and IgG antibody responses instead of IgE. When excess allergen-specific IgG antibodies are produced and bind to the allergen, they can act as blocking antibodies to neutralize the allergen before it can bind IgE on mast cells. There are early studies using oral therapy for desensitization of food allergies that are promising.[910] These studies involve feeding children who have allergies tiny amounts of the allergen (e.g., peanut flour) or related proteins over time. Many of the subjects show reduced severity of reaction to the food allergen after the therapy.

There are also therapies designed to treat severe allergic reactions. Emergency systemic anaphylaxis is treated initially with an epinephrine injection, which can counteract the drop in blood pressure. Individuals with known severe allergies often carry a self-administering auto-injector that can be used in case of exposure to the allergen (e.g., an insect sting or accidental ingestion of a food that causes a severe reaction). By self-administering an epinephrine shot (or sometimes two), the patient can stem the reaction long enough to seek medical attention. Follow-up treatment generally involves giving the patient antihistamines and slow-acting corticosteroids for several days after the reaction to prevent potential late-phase reactions. However, the effects of antihistamine and corticosteroid treatment are not well studied and are used based on theoretical considerations.

Treatment of milder allergic reactions typically involves antihistamines and other anti-inflammatory drugs. A variety of antihistamine drugs are available, in both prescription and over-the-counter strengths. There are also antileukotriene and antiprostaglandin drugs that can be used in tandem with antihistamine drugs in a combined (and more effective) therapy regime.

Treatments of type III hypersensitivities include preventing further exposure to the antigen and the use of anti-inflammatory drugs. Some conditions can be resolved when exposure to the antigen is prevented. Anti-inflammatory corticosteroid inhalers can also be used to diminish inflammation to allow lung lesions to heal. Systemic corticosteroid treatment, oral or intravenous, is also common for type III hypersensitivities affecting body systems. Treatment of hypersensitivity pneumonitis includes avoiding the allergen, along with the possible addition of prescription steroids such as prednisone to reduce inflammation.

Treatment of type IV hypersensitivities includes antihistamines, anti-inflammatory drugs, analgesics, and, if possible, eliminating further exposure to the antigen.

1.14 AUTOIMMUNE DISORDERS

In 1970, artist Walt Kelly developed a poster promoting Earth Day, featuring a character from *Pogo*, his daily newspaper comic strip. In the poster, Pogo looks out across a litter-strewn forest and says wryly, "We have met the enemy and he is us." Pogo was not talking about the human immune system, but he very well could have been. Although the immune system protects the body by attacking invading "enemies" (pathogens), in some cases, the immune system can mistakenly identify the body's own cells as the enemy, resulting in autoimmune disease.

Autoimmune diseases are those in which the body is attacked by its own specific adaptive immune response. In normal, healthy states, the immune system induces tolerance, which is a lack of an anti-self immune response. However, with autoimmunity, there is a loss of immune tolerance, and the mechanisms responsible for autoimmune diseases include type II, III, and IV hypersensitivity reactions. Autoimmune diseases can have a variety of mixed symptoms that flare up and disappear, making diagnosis difficult.

The causes of autoimmune disease are a combination of the individual's genetic makeup and the effect of environmental influences, such as sunlight, infections, medications, and environmental chemicals. However, the vagueness of this list reflects our poor understanding of the etiology of these diseases. Except in a very few specific diseases, the initiation event(s) of most autoimmune states has not been fully characterized.

There are several possible causes for the origin of autoimmune diseases and autoimmunity is likely due to several factors. Evidence now suggests that regulatory T and B cells play an essential role in the maintenance of tolerance and prevention of autoimmune responses. The regulatory T cells are especially important for inhibiting autoreactive T cells that are not eliminated during thymic selection and escape the thymus (see section 1.10). In addition, antigen mimicry between pathogen antigens and our own self antigens can lead to cross-reactivity and autoimmunity. Hidden self-antigens may become exposed because of trauma, drug interactions, or disease states, and trigger an autoimmune response. All of these factors could contribute to autoimmunity. Ultimately, damage to tissues and organs in the autoimmune disease state comes as a result of inflammatory responses that are inappropriate; therefore, treatment often includes immunosuppressive drugs and corticosteroids.

ORGAN-SPECIFIC AUTOIMMUNE DISEASES

Some autoimmune diseases are considered organ specific, meaning that the immune system targets specific organs or tissues. Examples of organ-specific autoimmune diseases include celiac disease, Graves disease, Hashimoto thyroiditis, type I diabetes mellitus, and Addison disease.

Celiac Disease

Celiac disease is largely a disease of the small intestine, although other organs may be affected. People in their 30s and 40s, and children are most commonly affected, but celiac disease can begin at any age. It results from a reaction to proteins, commonly called gluten, found mainly in wheat, barley, rye, and some other grains. The disease has several genetic causes (predispositions) and poorly understood environmental influences. On exposure to gluten, the body produces various autoantibodies and an inflammatory response. The inflammatory response in the small intestine leads to a reduction in the depth of the microvilli of the mucosa, which hinders absorption and can lead to weight loss and anemia. The disease is also characterized by diarrhea and abdominal pain, symptoms that are often misdiagnosed as irritable bowel syndrome.

Diagnosis of celiac disease is accomplished from serological tests for the presence of primarily IgA antibodies to components of gluten, the transglutaminase enzyme, and autoantibodies to endomysium, a connective tissue surrounding muscle fibers. Serological tests are typically followed up with endoscopy and biopsy of the duodenal mucosa. Serological screening surveys have found about 1% of individuals in the United Kingdom are positive even though they do not all display symptoms.[11] This early recognition allows for more careful monitoring and prevention of severe disease.

Celiac disease is treated with complete removal of gluten-containing foods from the diet, which results in improved symptoms and reduced risk of complications. Other theoretical approaches include breeding grains that do not contain the immunologically reactive components or developing dietary supplements that contain enzymes that break down the protein components that cause the immune response.[12]

Disorders of the Thyroid

Graves disease is the most common cause of hyperthyroidism in the United States. Symptoms of Graves disease result from the production of thyroid-stimulating immunoglobulin (TSI), also called TSH-receptor antibody. TSI targets and binds to the receptor for thyroid stimulating hormone (TSH), which is naturally produced by the pituitary gland. TSI may cause conflicting symptoms because it may stimulate the thyroid to make too much thyroid hormone or block thyroid hormone production entirely, making diagnosis more difficult. Signs and symptoms of Graves disease include heat intolerance, rapid and irregular heartbeat, weight loss, goiter (a swollen thyroid gland, protruding under the skin of the throat [figure 1.52]) and exophthalmia (bulging eyes) often referred to as Graves ophthalmopathy (figure 1.53).

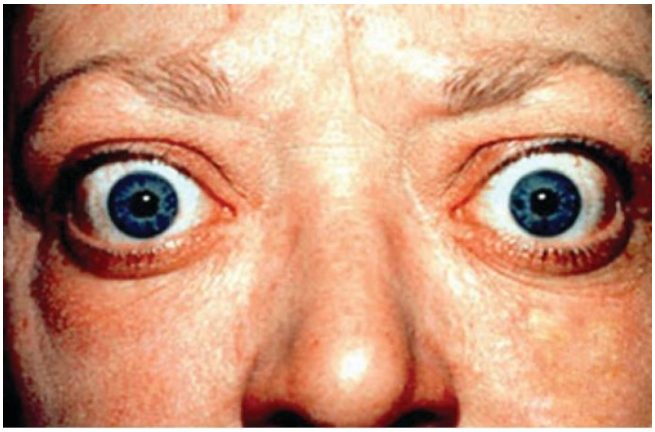


Figure 1.53: Exophthalmia, or Graves ophthalmopathy, is a sign of Graves disease. Figure description available at the end of the chapter.

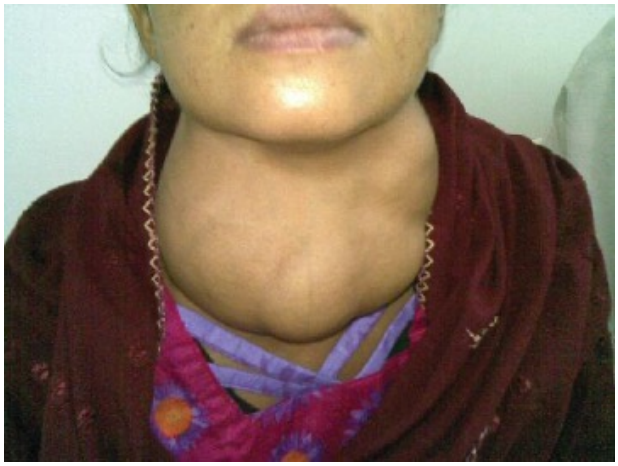


Figure 1.52: Goiter, a hypertrophy of the thyroid, is a symptom of Graves disease and Hashimoto thyroiditis. Figure description available at the end of the chapter.

The most common cause of hypothyroidism in the United States is Hashimoto thyroiditis, also called chronic lymphocytic thyroiditis. Patients with Hashimoto thyroiditis often develop a spectrum of different diseases because they are more likely to develop additional autoimmune diseases such as Addison disease (discussed later in this section), type 1 diabetes, rheumatoid arthritis, and celiac disease. Hashimoto thyroiditis is a TH1 cell-mediated disease that occurs when the thyroid gland is attacked by cytotoxic lymphocytes, macrophages, and autoantibodies. This autoimmune response leads to numerous symptoms that include goiter (figure 1.52), cold intolerance, muscle weakness, painful and stiff joints, depression, and memory loss.

Type 1 Diabetes

Juvenile diabetes, or type 1 diabetes mellitus, is usually diagnosed in children and young adults. It is a T-cell-dependent autoimmune disease characterized by the selective destruction of the β cells of the islets of Langerhans in the pancreas by CD4 TH1-mediated CD8 T cells, anti-β-cell antibodies, and macrophage activity. There is also evidence that viral infections can have either a potentiating or inhibitory role in the development of type 1 diabetes (T1D) mellitus. The destruction of the β cells causes a lack of insulin production by the pancreas. In T1D, β-cell destruction may take place over several years, but symptoms of hyperglycemia, extreme increase in thirst and urination, weight loss, and extreme fatigue usually have a sudden onset, and diagnosis usually does not occur until most β cells have already been destroyed.

Autoimmune Addison Disease

Destruction of the adrenal glands (the glands lying above the kidneys that produce glucocorticoids, mineralocorticoids, and sex steroids) is the cause of Addison disease, also called primary adrenal insufficiency (PAI). Today, up to 80% of Addison disease cases are diagnosed as autoimmune Addison disease (AAD), which is caused by an autoimmune response to adrenal tissues disrupting adrenal function. Disruption of adrenal function causes impaired metabolic processes that require normal steroid hormone levels, causing signs and symptoms throughout the body. There is evidence that both humoral and CD4 TH1-driven CD8 T-cell–mediated immune mechanisms are directed at the adrenal cortex in AAD. There is also evidence that the autoimmune response is associated with autoimmune destruction of other endocrine glands as well, such as the pancreas and thyroid, conditions collectively referred to as autoimmune polyendocrine syndromes (APS). In up to 80% of patients with AAD, antibodies are produced to three enzymes involved in steroid synthesis: 21-hydroxylase (21-OH), 17α-hydroxylase, and cholesterol side-chain–cleaving enzyme.[13] The most common autoantibody found in AAD is to 21-OH, and antibodies to any of the key enzymes for steroid production are diagnostic for AAD. The adrenal cortex cells are targeted, destroyed, and replaced with fibrous tissue by immune-mediated inflammation. In some patients, at least 90% of the adrenal cortex is destroyed before symptoms become diagnostic.

Symptoms of AAD include weakness, nausea, decreased appetite, weight loss, hyperpigmentation (figure 1.54), hyperkalemia (elevated blood potassium levels), hyponatremia (decreased blood sodium levels), hypoglycemia (decreased levels of blood sugar), hypotension (decreased blood pressure), anemia, lymphocytosis (decreased levels of white blood cells), and fatigue. Under extreme stress, such as surgery, accidental trauma, or infection, patients with AAD may experience an adrenal crisis that causes the patient to vomit, experience abdominal pain, back or leg cramps, and even severe hypotension leading to shock.

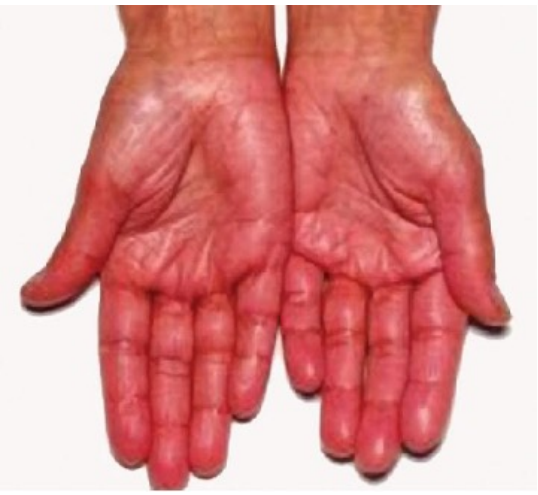


Figure 1.54: Hyperpigmentation is a sign of Addison disease. *Figure description available at the end of the chapter.*

SYSTEMIC AUTOIMMUNE DISEASES

Whereas organ-specific autoimmune diseases target specific organs or tissues, systemic autoimmune diseases are more generalized, targeting multiple organs or tissues throughout the body. Examples of systemic autoimmune diseases include multiple sclerosis, myasthenia gravis, psoriasis, rheumatoid arthritis, and systemic lupus erythematosus.

Multiple Sclerosis

Multiple sclerosis (MS) is an autoimmune central nervous system disease that affects the brain and spinal cord. Lesions in multiple locations within the central nervous system are a hallmark of multiple sclerosis and are caused by infiltration of immune cells across the blood-brain barrier. The immune cells include T cells that promote inflammation, demyelination, and neuron degeneration, all of which disrupt neuronal signaling. Symptoms of MS include visual disturbances; muscle weakness; difficulty with coordination and balance; sensations such as numbness, prickling, or "pins and needles"; and cognitive and memory problems.

Myasthenia Gravis

Autoantibodies directed against acetylcholine receptors (AChRs) in the synaptic cleft of neuromuscular junctions lead to myasthenia gravis (figure 1.55). Anti-AChR antibodies are high-affinity IgGs and their synthesis requires activated CD4 T cells to interact with and stimulate B cells. Once produced, the anti-AChR antibodies affect neuromuscular transmission by at least three mechanisms:

- Complement binding and activation at the neuromuscular junction
- Accelerated AChR endocytosis of molecules cross-linked by antibodies
- Functional AChR blocking, which prevents normal acetylcholine attachment to, and activation of, AChR

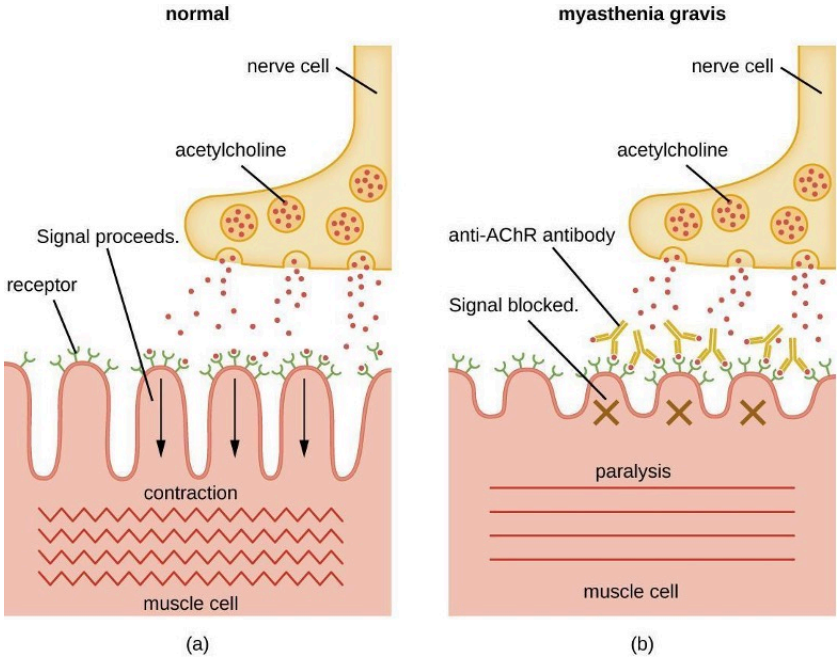


Figure 1.55: Myasthenia gravis and impaired muscle contraction. (a) Normal release of the neurotransmitter acetylcholine stimulates muscle contraction. (b) In myasthenia gravis, autoantibodies block the receptors for acetylcholine (AChr) on muscle cells, resulting in paralysis. *Figure description available at the end of the chapter.*

Regardless of the mechanism, the effect of anti-AChR is extreme muscle weakness and potentially death through respiratory arrest in severe cases.

Psoriasis

Psoriasis is a skin disease that causes itchy or sore patches of thick, red skin with silvery scales on the elbows, knees, scalp, back, face, palms, feet, and sometimes other areas. Some individuals with psoriasis also get a form of arthritis called psoriatic arthritis, in which the joints can become inflamed. Psoriasis results from the complex interplay between keratinocytes, dendritic cells, and T cells, and the cytokines produced by these various cells. In a process called cell turnover, skin cells that grow deep in the skin rise to the surface. Normally, this process takes a month. In psoriasis, as a result of cytokine activation, cell turnover happens in just a few days. The thick inflamed patches of skin that are characteristic of psoriasis develop because the skin cells rise too fast.

Rheumatoid Arthritis

The most common chronic inflammatory joint disease is rheumatoid arthritis (RA) (figure 1.56) and it is still a major medical challenge because of unsolved questions related to the environmental and genetic causes of the disease. RA involves type III hypersensitivity reactions and the activation of CD4 T cells, resulting in chronic release of the inflammatory cytokines IL-1, IL-6, and tumor necrosis factor-α (TNF-α). The activated CD4 T cells also stimulate the production of rheumatoid factor (RF) antibodies and anticyclic citrullinated peptide antibodies (anti-CCP) that form immune complexes. Increased levels of acute-phase proteins, such as C-reactive protein (CRP), are also produced as part of the inflammatory process and participate in complement fixation with the antibodies on the immune complexes. The formation of immune complexes and reaction to the immune factors cause an inflammatory process in joints, particularly in the hands, feet, and legs. Diagnosis of RA is based on elevated levels of RF, anti-CCP, quantitative CRP, and the erythrocyte sedimentation rate (ESR) (modified Westergren). In addition, radiographs, ultrasound, or magnetic resonance imaging scans can identify joint damage, such as erosions, a loss of bone within the joint, and narrowing of joint space.

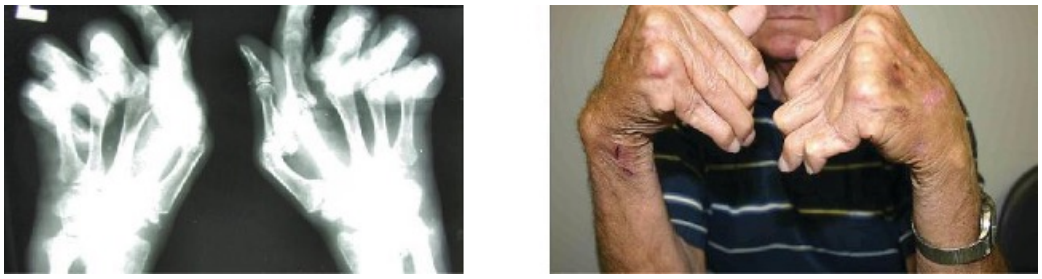


Figure 1.56: The radiograph (left) and photograph (right) show damage to the hands typical of rheumatoid arthritis. *Figure description available at the end of the chapter.*

Systemic Lupus Erythematosus

The damage and pathology of systemic lupus erythematosus (SLE) is caused by type III hypersensitivity reactions. Autoantibodies produced in SLE are directed against nuclear and cytoplasmic proteins. Anti-nuclear antibodies (ANAs) are present in more than 95% of patients with SLE,[14] with additional autoantibodies including anti-double–stranded DNA (ds-DNA) and anti-Sm antibodies (antibodies to small nuclear ribonucleoprotein). Anti-ds-DNA and anti-Sm antibodies are unique to patients with SLE; thus, their presence is included in the classification criteria of SLE. Cellular interaction with autoantibodies leads to nuclear and cellular destruction, with components released after cell death leading to the formation of immune complexes.

Because autoantibodies in SLE can target a wide variety of cells, symptoms of SLE can occur in many body locations. However, the most common symptoms include fatigue, fever with no other cause, hair loss, and a sunlight-sensitive "butterfly" or wolf-mask (lupus) rash that is found in about 50% of people with SLE (figure 1.57). The rash is most often seen over the cheeks and bridge of the nose, but can be widespread. Other symptoms may appear depending on affected areas. The joints may be affected, leading to arthritis of the fingers, hands, wrists, and knees. Effects on the brain and nervous system can lead to headaches, numbness, tingling, seizures, vision problems, and personality changes. There may also be abdominal pain, nausea, vomiting, arrhythmias, shortness of breath, and blood in the sputum. Effects on the skin can lead to additional areas of skin lesions, and vasoconstriction can cause color changes in the fingers when they are cold (Raynaud phenomenon). Effects on the kidneys can lead to edema in the legs and weight gain. A diagnosis of SLE depends on identification of four of 11 of the most common symptoms and confirmed production of an array of autoantibodies unique to SLE. A positive test for ANAs alone is not diagnostic.

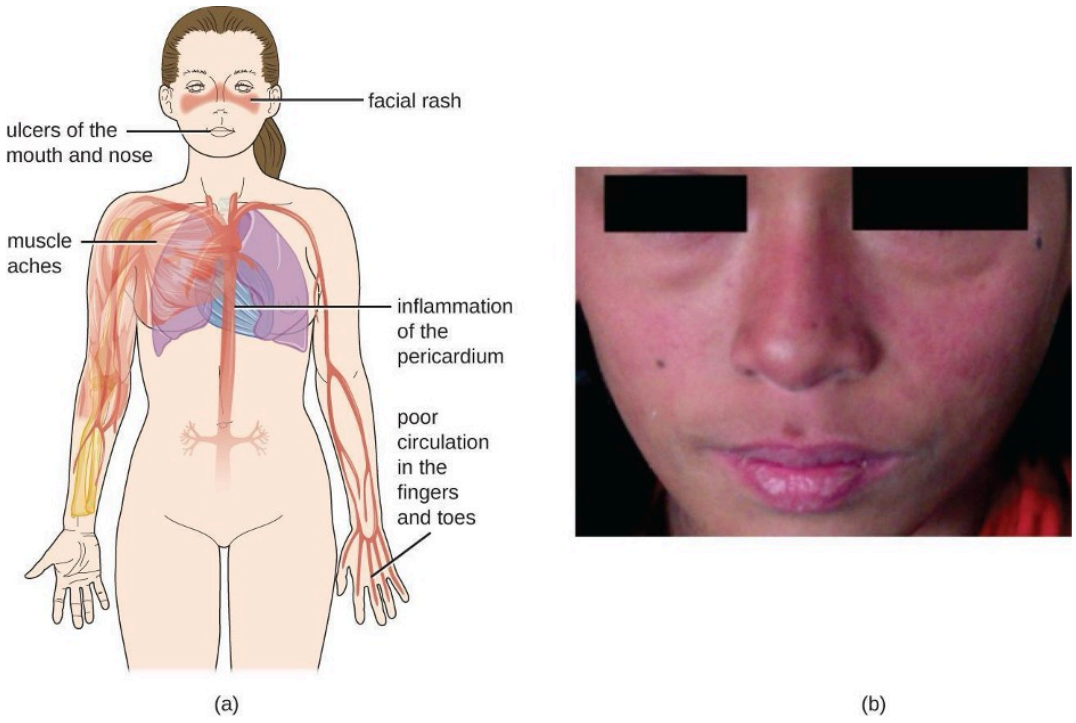


Figure 1.57: (a) Systemic lupus erythematosus is characterized by autoimmunity to the individual's own DNA and/or proteins. (b) This patient is presenting with a butterfly rash, one of the characteristic signs of lupus. *Figure description available at the end of the chapter.*

Table 1.20 summarizes the causes, signs, and symptoms of select autoimmune diseases.

Disease	Cause	Signs and Symptoms
Addison disease	Destruction of adrenal gland cells by cytotoxic T cells	Weakness, nausea, hypotension, fatigue; adrenal crisis with severe pain in abdomen, lower back, and legs; circulatory system collapse, kidney failure
Celiac disease	Antibodies to gluten become autoantibodies that target cells of the small intestine	Severe diarrhea, abdominal pain, anemia, malnutrition
Diabetes mellitus (type I)	Cytotoxic T-cell destruction of the insulin-producing β cells of the pancreas	Hyperglycemia, extreme increase in thirst and urination, weight loss, extreme fatigue
Graves disease	Autoantibodies target thyroid-stimulating hormone receptors, resulting in overstimulation of the thyroid	Hyperthyroidism with rapid and irregular heartbeat, heat intolerance, weight loss, goiter, exophthalmia
Hashimoto thyroiditis	Thyroid gland is attacked by cytotoxic T cells, lymphocytes, macrophages, and autoantibodies	Thyroiditis with goiter, cold intolerance, muscle weakness, painful and stiff joints, depression, memory loss
Multiple sclerosis (MS)	Cytotoxic T-cell destruction of the myelin sheath surrounding nerve axons in the central nervous system	Visual disturbances, muscle weakness, impaired coordination and balance, numbness, prickling or "pins and needles" sensations, impaired cognitive function and memory

Disease	Cause	Signs and Symptoms
Myasthenia gravis	Autoantibodies directed against acetylcholine receptors within the neuromuscular junction	Extreme muscle weakness eventually leading to fatal respiratory arrest
Psoriasis	Cytokine activation of keratinocytes causes rapid and excessive epidermal cell turnover	Itchy or sore patches of thick, red skin with silvery scales; commonly affects elbows, knees, scalp, back, face, palms, feet
Rheumatoid arthritis	Autoantibodies, immune complexes, complement activation, phagocytes, and T cells damage membranes and bone in joints	Joint inflammation, pain and disfigurement, chronic systemic inflammation
Systemic lupus erythematosus (SLE)	Autoantibodies directed against nuclear and cytoplasmic molecules form immune complexes that deposit in tissues. Phagocytic cells and complement activation cause tissue damage and inflammation	Fatigue, fever, joint pain and swelling, hair loss, anemia, clotting, a sunlight-sensitive "butterfly" rash, skin lesions, photosensitivity, decreased kidney function, memory loss, confusion, depression

Table 1.20: Select autoimmune diseases

1.15 ORGAN TRANSPLANTATION AND REJECTION

A graft is the transplantation of an organ or tissue to a different location, with the goal of replacing a missing or damaged organ or tissue. Grafts are typically relocated without their attachments to the circulatory system and must reestablish these, in addition to the other connections and interactions with their new surrounding tissues. There are different types of grafts depending on the source of the new tissue or organ. Tissues that are transplanted from one genetically distinct individual to another within the same species are called allografts. An interesting variant of the allograft is an isograft, in which tissue from one twin is transplanted to another. As long as the twins are monozygotic (therefore, essentially genetically identical), the transplanted tissue is virtually never rejected. If tissues are transplanted from one area on an individual to another area on the same individual (e.g., a skin graft on a burn patient), it is known as an autograft. If tissues from an animal are transplanted into a human, this is called a xenograft.

TRANSPLANT REJECTION

The different types of grafts described above have varying risks for rejection (table 1.21). Rejection occurs when the recipient's immune system recognizes the donor tissue as foreign (non-self), triggering an immune response. The major histocompatibility complex markers MHC I and MHC II, more specifically identified as human leukocyte antigens (HLAs), play a role in transplant rejection. The HLAs expressed in tissue transplanted from a genetically different individual or species may be recognized as non-self molecules by the host's dendritic cells. If this occurs, the dendritic cells will process and present the foreign HLAs to the host's helper T cells and cytotoxic T cells, thereby activating them. Cytotoxic T cells then target and kill the grafted cells through the same mechanism they use to kill virus-infected cells; helper T cells may also release cytokines that activate macrophages to kill graft cells.

Graft	Procedure	Complications
Autograft	From self to self	No rejection concerns
Isograft	from identical twin to twin	Little concern of rejection
Allograft	from relative or nonrelative to individual	Rejection possible
Xenograft	From animal to human	Rejection possible

Table 1.21: Types of tissue and organ grafts and their complications

With the three highly polymorphic MHC I genes in humans (*HLA-A*, *HLA-B*, and *HLA-C*) determining compatibility, each with many alleles segregating in a population, odds are extremely low that a randomly chosen donor will match a recipient's six-allele genotype (the two alleles at each locus are expressed codominantly). This is why a parent or a sibling may be the best donor in many situations—a genetic match between the MHC genes is much more likely and the organ is much less likely to be rejected.

Although matching all of the MHC genes can lower the risk for rejection, there are a number of additional gene products that also play a role in stimulating responses against grafted tissue. Because of this, no non-self grafted tissue is likely to completely avoid rejection. However, the more similar the MHC gene match, the more likely the graft is to be tolerated for a longer time. Most transplant recipients, even those with tissues well matched to their MHC genes, require treatment with immunosuppressant drugs for the rest of their lives. This can make them more vulnerable than the general population to complications from infectious diseases. It can also result in transplant-related malignancies because the body's normal defenses against cancer cells are being suppressed.

GRAFT-VERSUS-HOST DISEASE

A form of rejection called graft-versus-host disease (GVHD) primarily occurs in recipients of bone marrow transplants and peripheral blood stem cells. GHVD presents a unique situation because the transplanted tissue is capable of producing immune cells; APCs in the donated bone marrow may recognize the host cells as non-self, leading to activation of the donor cytotoxic T cells. Once activated, the donor's T cells attack the recipient cells, causing acute GVHD.

Acute GVHD typically develops within weeks after a bone marrow transplant, causing tissue damage affecting the skin, gastrointestinal tract, liver, and eyes. In addition, acute GVHD may also lead to a cytokine storm, an unregulated secretion of cytokines that may be fatal. In addition to acute GVHD, there is also the risk for chronic GVHD developing months after the bone marrow transplant. The mechanisms responsible for chronic GVHD are not well understood.

To minimize the risk of GVHD, it is critically important to match the HLAs of the host and donor as closely as possible in bone marrow transplants. In addition, the donated bone marrow is processed before grafting to remove as many donor APCs and T cells as possible, leaving mostly hematopoietic stem cells.

THE FUTURE OF TRANSPLANTATION

Historically speaking, the practice of transplanting tissues—and the complications that can accompany such procedures—is a relatively recent development. It was not until 1954 that the first successful organ transplantation between two humans was achieved. Yet the field of organ transplantation has progressed rapidly since that time.

Nonetheless, the practice of transplanting non-self tissues may soon become obsolete. Scientists are now attempting to develop methods by which new organs may be grown *in vitro* from an individual's own harvested cells to replace damaged or abnormal ones. Because organs produced in this way would contain the individual's own cells, they could be transplanted into the individual without risk for rejection.

An alternative approach that is gaining renewed research interest is genetic modification of donor animals, such as pigs, to provide transplantable organs that do not elicit an immune response in the recipient. The approach involves excising the genes in the pig (in the embryo) that are most responsible for the rejection reaction after transplantation. Finding these genes and effectively removing them is a challenge, however. So too is identifying and neutralizing risks from viral sequences that might be embedded in the pig genome, posing a risk for infection in the human recipient.

1.16 IMMUNODEFICIENCY

Immunodeficiencies are inherited (primary) or acquired (secondary) disorders in which elements of host immune defenses are either absent or functionally defective. In developed countries, most immunodeficiencies are inherited, and they are usually first seen in clinics as recurrent or overwhelming infections in infants. However, on a global scale, malnutrition is the most common cause of immunodeficiency and would be categorized as an acquired immunodeficiency. Acquired immunodeficiencies are more likely to develop later in life, and the pathogenic mechanisms of many remain obscure.

PRIMARY IMMUNODEFICIENCY

Primary immunodeficiencies, which number more than 250, are caused by inherited defects of either nonspecific innate or specific adaptive immune defenses. In general, patients born with primary immunodeficiency (PI) commonly have an increased susceptibility to infection. This susceptibility can become apparent shortly after birth or in early childhood for some individuals, whereas other patients develop symptoms later in life. Some primary immunodeficiencies are due to a defect of a single cellular or humoral component of the immune system; others may result from defects of more than one component. Examples of primary immunodeficiencies include chronic granulomatous disease, X-linked agammaglobulinemia, selective IgA deficiency, and severe combined immunodeficiency disease.

Chronic Granulomatous Disease

The causes of chronic granulomatous disease (CGD) are defects in the NADPH oxidase system of phagocytic cells, including neutrophils and macrophages, that prevent the production of superoxide radicals in phagolysosomes. The inability to produce superoxide radicals impairs the antibacterial activity of phagocytes. As a result, infections in patients with CGD persist longer, leading to a chronic local inflammation called a granuloma. Microorganisms that are the most common causes of infections in patients with CGD include *Aspergillus* spp., *Staphylococcus aureus*, *Chromobacterium violaceum*, *Serratia marcescens*, and *Salmonella typhimurium*.

X-Linked Agammaglobulinemia

Deficiencies in B cells due to defective differentiation lead to a lack of specific antibody production known as X-linked agammaglobulinemia. In 1952, Ogden C. Bruton (1908–2003) described the first immunodeficiency in a boy whose immune system failed to produce antibodies. This defect is inherited on the X chromosome and is characterized by the absence of immunoglobulin in the serum; it is called Bruton X-linked agammaglobulinemia (XLA). The defective gene, *BTK,* in XLA is now known to encode a tyrosine kinase called Bruton tyrosine kinase

(Btk). In patients whose B cells are unable to produce sufficient amounts of Btk, the B-cell maturation and differentiation halts at the pre-B-cell stage of growth. B-cell maturation and differentiation beyond the pre-B-cell stage of growth is required for immunoglobulin production. Patients who lack antibody production suffer from recurrent infections almost exclusively due to extracellular pathogens that cause pyogenic infections including: *Haemophilus influenzae*, *Streptococcus pneumoniae*, *S. pyogenes*, and *S. aureus*. Because cell-mediated immunity is not impaired, these patients are not particularly vulnerable to infections caused by viruses or intracellular pathogens.

Selective IgA Deficiency

The most common inherited form of immunoglobulin deficiency is selective IgA deficiency, affecting about one in 800 people. Individuals with selective IgA deficiency produce normal levels of IgG and IgM, but are not able to produce secretory IgA. IgA deficiency predisposes these individuals to lung and gastrointestinal infections for which secretory IgA is normally an important defense mechanism. Infections in the lungs and gastrointestinal tract can involve a variety of pathogens, including: *H. influenzae*, *S. pneumoniae*, *Moraxella catarrhalis*, *S. aureus*, *Giardia lamblia*, or pathogenic strains of *Escherichia coli*.

Severe Combined Immunodeficiency

Patients who suffer from severe combined immunodeficiency (SCID) have B-cell and T-cell defects that impair T-cell dependent antibody responses as well as cell-mediated immune responses. Patients with SCID also cannot develop immunological memory, so vaccines provide them no protection, and live attenuated vaccines (e.g., for varicella-zoster, measles virus, rotavirus, poliovirus) can actually cause the infection they are intended to prevent. The most common form is X-linked SCID, which accounts for nearly 50% of all cases and occurs primarily in males. Patients with SCID are typically diagnosed within the first few months of life after developing severe, often life-threatening, opportunistic infection by *Candida* spp., *Pneumocystis jirovecii*, or pathogenic strains of *E. coli*.

Without treatment, babies with SCID do not typically survive infancy. In some cases, a bone marrow transplant may successfully correct the defects in lymphocyte development that lead to the SCID phenotype, by replacing the defective component. However, this treatment approach is not without risks, as demonstrated by the famous case of David Vetter (1971–1984), better known as "Bubble Boy" (figure 1.58). Vetter, a patient with SCID who lived in a protective plastic bubble to prevent exposure to opportunistic microbes, received a bone marrow transplant from his sister. Because of a latent Epstein-Barr virus infection in her bone marrow, however, he developed mononucleosis and died of Burkitt lymphoma at the age of 12 years.

Figure 1.58: David Vetter, popularly known as "The Bubble Boy," was born with SCID and lived most of his life isolated inside a plastic bubble. Here he is shown outside the bubble in a suit specially built for him by NASA. *Figure description available at the end of the chapter.*

SECONDARY IMMUNODEFICIENCY

A secondary immunodeficiency occurs as a result of an acquired impairment in the function of B cells, T cells, or both. Secondary immunodeficiencies can be caused by:

- Systemic disorders such as diabetes mellitus, malnutrition, hepatitis, or HIV infection
- Immunosuppressive treatments such as cytotoxic chemotherapy, bone marrow ablation before transplantation, or radiation therapy
- Prolonged critical illness due to infection, surgery, or trauma in the very young, elderly, or hospitalized patients

Unlike primary immunodeficiencies, which have a genetic basis, secondary immunodeficiencies are often reversible if the underlying cause is resolved. Patients with secondary immunodeficiencies develop an increased susceptibility to an otherwise benign infection by opportunistic pathogens such as *Candida* spp., *P. jirovecii*, and *Cryptosporidium*.

HIV infection and the associated acquired immunodeficiency syndrome (AIDS) are the best-known secondary immunodeficiencies. AIDS is characterized by profound CD4 T-cell lymphopenia (decrease in lymphocytes). The decrease in CD4 T cells is the result of various mechanisms, including HIV-induced pyroptosis (a type of apoptosis that stimulates an inflammatory response), viral cytopathic effect, and cytotoxicity to HIV-infected cells.

The most common cause of secondary immunodeficiency worldwide is severe malnutrition, which affects both innate and adaptive immunity. More research and information are needed for the more common causes of secondary immunodeficiency; however, the number of new discoveries in AIDS research far exceeds that of any other single cause of secondary immunodeficiency. AIDS research has paid off extremely well in terms of discoveries and treatments; increased research into the most common cause of immunodeficiency, malnutrition, would likely be as beneficial.

Table 1.22 summarizes primary and secondary immunodeficiencies, their effects on immune function, and typical outcomes.

Disease	Effect on Immune Function	Outcomes
Primary immunodeficiencies		
Chronic granulomatous disease	Impaired killing of bacteria within the phagolysosome of neutrophils and macrophages	Chronic infections and granulomas
Selective IgA deficiency	Inability to produce secretory IgA	Predisposition to lung and gastrointestinal infections
Severe combined immunodeficiency disease (SCID)	Deficient humoral and cell-mediated immune responses	Early development of severe and life-threatening opportunistic infections
X-linked agammaglobulinemia	Flawed differentiation of B cells and absence of specific antibodies	Recurrent infections almost exclusively due to pathogens that cause pyogenic infections
Secondary immunodeficiencies		
Immunosuppressive therapies (e.g., chemotherapy, radiotherapy)	Impaired humoral and/or cell-mediated immune responses	Opportunistic infections, rare cancers

Disease	Effect on Immune Function	Outcomes
Malnutrition	Impaired humoral and/or cell-mediated immune responses	Opportunistic infections, rare cancers
Viral infection (e.g., HIV)	Impaired cell-mediated immune responses due to CD4 T-cell lymphopenia	Opportunistic infections, rare cancers

Table 1.22: Primary and Secondary Immunodeficiencies

1.17 CANCER IMMUNOBIOLOGY AND IMMUNOTHERAPY

Cancer involves a loss of the ability of cells to control their cell cycle, the stages each eukaryotic cell goes through as it grows and then divides. When this control is lost, the affected cells rapidly divide and often lose the ability to differentiate into the cell type appropriate for their location in the body. In addition, they lose contact inhibition and can start to grow on top of each other. This can result in formation of a tumor. It is important to make a distinction here: The term **cancer** is used to describe the diseases resulting from loss of cell-cycle regulation and subsequent cell proliferation. But the term **tumor** is more general. A tumor is an abnormal mass of cells, and a tumor can be benign (not cancerous) or malignant (cancerous).

Traditional cancer treatment uses radiation and/or chemotherapy to destroy cancer cells; however, these treatments can have unwanted side effects because they harm normal cells as well as cancer cells. Newer, promising therapies attempt to enlist the patient's immune system to target cancer cells specifically. It is known that the immune system can recognize and destroy cancerous cells, and some researchers and immunologists also believe, based on the results of their experiments, that many cancers are eliminated by the body's own defenses before they can become a health problem. This idea is not universally accepted by researchers, however, and needs further investigation for verification.

CELL-MEDIATED RESPONSE TO TUMORS

Cell-mediated immune responses can be directed against cancer cells, many of which do not have the normal complement of self-proteins, making them a target for elimination. Abnormal cancer cells may also present tumor antigens. These tumor antigens are not a part of the screening process used to eliminate lymphocytes during development; thus, even though they are self-antigens, they can stimulate and drive adaptive immune responses against abnormal cells.

Presentation of tumor antigens can stimulate naïve helper T cells to become activated by cytokines such as IL-12 and differentiate into TH1 cells. TH1 cells release cytokines that can activate natural killer (NK) cells and enhance the killing of activated cytotoxic T cells. Both NK cells and cytotoxic T cells can recognize and target cancer cells as well as induce apoptosis through the action of perforins and granzymes. In addition, activated cytotoxic T cells can bind to cell-surface proteins on abnormal cells and induce apoptosis by a second killing mechanism called the CD95 (Fas) cytotoxic pathway.

Despite these mechanisms for removing cancerous cells from the body, cancer remains a common cause of death. Unfortunately, malignant tumors tend to actively suppress the immune response in various ways. In some cancers, the immune cells themselves are cancerous. In leukemia, lymphocytes that would normally facilitate the immune response become abnormal. In other cancers, the cancerous cells can become resistant to induction of apoptosis. This may occur through the expression of membrane proteins that shut off cytotoxic T cells or that induce regulatory T cells that can shut down immune responses.

The mechanisms by which cancer cells alter immune responses are still not fully understood, and this is a very active area of research. As scientists' understanding of adaptive immunity improves, cancer therapies that harness the body's immune defenses may someday be more successful in treating and eliminating cancer.

CANCER VACCINES

There are two types of cancer vaccines: preventive and therapeutic. Preventive vaccines are used to prevent cancer from occurring, whereas therapeutic vaccines are used to treat patients with cancer. Most preventive cancer vaccines target viral infections that are known to lead to cancer. These include vaccines against human papillomavirus (HPV) and hepatitis B, which help prevent cervical and liver cancer, respectively.

Most therapeutic cancer vaccines are in the experimental stage. They exploit tumor-specific antigens to stimulate the immune system to selectively attack cancer cells. Specifically, they aim to enhance TH1 function and interaction with cytotoxic T cells, which, in turn, results in more effective attack on abnormal tumor cells. In some cases, researchers have used genetic engineering to develop antitumor vaccines in an approach similar to that used for DNA vaccines. The vaccine contains a recombinant plasmid with genes for tumor antigens; theoretically, the tumor gene would not induce new cancer because it is not functional, but it could trick the immune system into targeting the tumor gene product as a foreign invader.

The first FDA-approved therapeutic cancer vaccine was sipuleucel-T (Provenge), approved in 2010 to treat certain cases of prostate cancer.[15] This unconventional vaccine is custom-designed using the patient's own cells. APCs are removed from the patient and cultured with a tumor-specific molecule; the cells are then returned to the patient. This approach appears to enhance the patient's immune response against the cancer cells. Another therapeutic cancer vaccine (talimogene laherparepvec, also called T-VEC or Imlygic) was approved by the FDA in 2015 for treatment of melanoma, a form of skin cancer. This vaccine contains a virus that is injected into tumors, where it infects and lyses the tumor cells. The virus also induces a response in lesions or tumors besides those into which the vaccine is injected, indicating that it is stimulating a more general (as opposed to local) antitumor immune response in the patient.

Figure Descriptions

Figure 1.1: Tight junctions – two membranes connected with many spot welds in multiple lines. Desmosomes – two membranes with long strands weaving them together. Gap junctions – two membranes with a few spot welds each of which has a pore in the center.

Figure 1.2: A diagram of a section of skin. The bottom layer is the hypodermis and is mostly made up of large circular cells (fatty tissue). The next layer up, and the thickest layer is the dermis. At the bottom of the dermis are blood vessels, lymph vessels, and nerves, all of which run throughout the dermis. Sweat glands are coiled tubes that lead to the surface. Hair follicles are thick vase-shaped structures containing a hair; an oil gland is attached to the hair follicle. The top layer is the epidermis and is made of many layers of flat cells.

Figure 1.3: A spongy-looking surface with tufts of long hairs. Each hair is about 5 μm long; each tuft is about 10 μm in diameter.

Figure 1.4: Figure a is a diagram of a single goblet cell. The cell is tall and slightly hourglass shaped. The bottom of the cell is filled with a nucleus. The top shows the Golgi apparatus (folds of membranes), rough endoplasmic reticulum (folds of membranes with dots), secretory vesicles containing mucin (large bubbles), and microvilli (finger-like projections at the top). Figure b is a micrograph of two goblet cells within a row of epithelial cells. The epithelial cells are rectangular with a large nucleus visible. The goblet cells are thinner and have a clear (uncolored) top.

Figure 1.5: A diagram of a person. An arrow from the eye points to a larger image that shows eyelashes, the eyelid, and tear ducts. An arrow from the abdominal region shows a larger kidney are ureter. An arrow from the groin region shows a larger bladder, including ureter pelvic floor muscles, and urethra.

Figure 1.6: A micrograph and diagram both show a large hair follicle (a vase-shaped pocket) with a hair projecting out past the epidermis. On the side of the hair follicle is the sebaceous gland, which is a lumpy structure.

Figure 1.7: A diagram outlining the three complement pathways. At the top is the invading pathogen. Two antibodies bind to an antigen on the surface of the pathogen. C1 binds to the antigen-antibody complex. This is labeled the classic pathway. C1 causes C2 and C4 to be cut into two pieces. Parts of C2 and C4 bind together to form C3 convertase. The alternate pathway also leads to C3 convertase but does so directly. C3 convertase then cuts C3 in two and one of these binds to C3 convertase. The resulting enzyme is called C5 convertase. C5 convertase lyses C5 into two pieces. One of the C5 pieces joins other complement proteins (C6, C7, C8 and C9) to create a pore through the membrane of the invading cell. This pore kills the cell. Endogenous proteins on the host cell protect the host membrane from the complement proteins.

Figure 1.8: A cell with viruses inside it releases signals labeled interferons. The interferons travel to 3 different cells. The interferon signals neighboring uninfected cells to destroy RNA and reduce protein synthesis. The interferon signals neighboring infected cells to undergo apoptosis. The interferon also activates immune cells.

Figure 1.9: A flowchart showing progression of development for formed elements of blood. At the top is a multipotent hematopoietic stem cell (hemocytoblast). This cell divides and after division some of the new cells remain stem cells. Others go down one of two paths depending on the chemical signals received. One path begins with lymphoid stem cells which can either become natural killer cells (large granular lymphocytes) or small lymphocytes. The natural killer cell is a medium-large purple cell. Small lymphocytes can either become T lymphocytes or B lymphocytes. The T and B lymphocytes are medium-sized cells with a large nucleus. B lymphocytes become plasma cells, which are medium-sized cells with a large nucleus. The other option for the stem cell is to become a myeloid stem cell. Myeloid stem cells follow one of four paths. One path leads to megakaryocyte which leads to platelets. Platelets are small flecks. The second path leads to erythrocyte. Erythrocytes are small donut-shaped

red cells. The third path leads to mast cells. The fourth path leads to basophil, neutrophil, eosinophil, or monocyte. Basophils are medium cells with many dark purple spots. Neutrophils are medium pink cells with a multilobed nucleus. Eosinophils are medium size cells with many pink spots. Monocytes lead to macrophages or dendritic cells. Macrophages are large irregularly shaped cells. Dendritic cells have longer tendons branching off of them.

Figure 1.10: Neutrophils have a multi-lobed nucleus. Eosinophils have a two-lobed nucleus and distinct pink spots when stained. Basophils have a two-lobed nucleus and distinct purple spots when stained. Each type of granulocyte is illustrated with a micrograph above it.

Figure 1.11: a) Mast cells in blood. Mast cells are large purple cells, red blood cells are small pink cells with a clear center. b) mast cell outside of blood.

Figure 1.12: NK cells have both inhibitory and activating receptors. Normal cells have signals on their MHC molecules that bind to the inhibitory receptors; so the NK cell does not kill them. Cells that are infected with virus have ligands that bind to the activating receptor; this causes the NK cell to kill them.

Figure 1.13: Many red blood cells with a single larger cell. The larger cell is pink with a purple region that fills nearly the entire cell. The purple region is labeled perforin-containing granules.

Figure 1.14: Monocytes are large cells with a large purple nucleus. There is a cluster of them in a field of smaller red blood cells. A PMN is also visible with a dark, multi-lobed nucleus. Macrophages are large cells with a defined nucleus.

Figure 1.15: A diagram with 3 steps. The first step states: leukocytes in the blood respond to chemical attractants released by pathogens and chemical signals from nearby injured cells. An injury to the surface of the skin is labeled: injured/infected cells secrete chemical signals into the blood. Pathogens are present in the wound. Neutrophils and monocytes are in the bloodstream, and the outside of the vessel is labeled capillary epithelial cells. A resident macrophage engulfs the pathogens and releases proinflammatory chemotactic cytokines. The second step states: the leukocytes squeeze between the cells of the capillary wall as they follow the chemical signals to where they are most concentrated (positive chemotaxis). The leukocytes emigrate to the site of injury and infection. The chemical signals present include C5a and cytokines. The third panel states: Within the damaged tissue, neutrophils release chemicals that break apart pathogens. Monocytes differentiate into macrophages. Neutrophils and macrophages phagocytize pathogens and cellular debris. Neutrophils release cytotoxic chemicals from granules into tissue.

Figure 1.16: A cell with three receptors. The lipopeptide receptor binds lipopeptides, the flagellin receptor binds flagellin, and the peptidoglycan receptor binds peptidoglycans. A fourth receptor (the nucleic acid receptor which binds to nucleic acids) is found on the membrane of the phagosome. All four receptors have an arrow pointing to the nucleus which contains DNA. An arrow pointing out reads:production and secretion of antiviral interferons and other cytokines.

Figure 1.17: Pseudopods of the larger cell engulf a smaller cell labeled infectious bacterium. The resulting vesicle containing the bacterium is labeled phagosome. This fuses with a lysosome which contains digestive enzymes. The resulting vesicle is labeled phagolysosome. Exocytosis removes the remaining debris.

Figure 1.18: a) a diagram of a wound in the skin that has let pathogens enter. Mast cells release histamines, which signal to cells in the bloodstream. B) The cells have left the bloodstream; these phagocytes are engulfing the pathogens.

Figure 1.19: A micrograph of a tubercle which consists of many darkly staining cells that form a circular structure.

Figure 1.20: A diagram with exogenous pyrogen at the top. These activate leukocytes which in turn release IL-6. The leukocytes also produce pyrogenic cytokines (IL-1, TNF-α, IFN-γ) which lead to the production of IL-6. IL-6 signals the circumventricular organs of the brain to produce PGE2 which results in fever. The temperature-dependent feedback on cytokine expression decreases the production of IL-6 in a negative feedback loop.

Figure 1.21: A graph with time on the X axis and concentration of antibody on the Y axis. The concentration is near 0 at the initial exposure and increases during the primary immune response. The concentration then drops back down but remains above the level at initial exposure. The secondary exposure increases the concentration of antibody to higher levels than the primary response. And even after dropping back down this count remains relatively high.

Figure 1.22: A drawing of an antigen as a large sphere with different shapes on the surface labeled epitopes.

Figure 1.23: Many antigens (shown as large spheres) each with multiple shapes on the surface labeled epitopes. Different antibodies are shown each with a binding site specific to one of the epitopes.

Figure 1.24: a) An antibody is a Y shape made of four strands. The two inner strands form the actual Y shape and are the heavy chains. The two light chains sit on the outsides of the top regions of the Y. The bottom portion of the Y (made of only heavy chains) is called the Fc Region. The Fc region along with half of the top portion of the Y (made of both light and heavy chains) is the constant regions. The variable region is the very tips of the Y and is made of both light and heavy chains. The antigen binding site is in the variable region. Disulfide bridges hold the antigen's shape. B) a space filling model of the antigen.

Figure 1.25: A virus is drawn as a circle with knobs on it. Antigens bind to the knobs, thereby surrounding the virus. Next image shows antibody binding to diphtheria toxin. Next image shows antibody binding to a bacterial cell.

Figure 1.26: A macrophage with projections that are engulfing a pathogen with antibodies attached to it.

Figure 1.27: Bacterial cells with various epitopes (shown as different shapes). IgM antibodies are bound to multiple bacteria; all attached to the same shaped epitope which matches their binding sites.

Figure 1.28: Fc receptors on an NK cell bind to the Fc region of the IgG bound to the antigen on the surface of a pathogen. This causes the NK cell to release toxins that kill the pathogen.

Figure 1.29: Drawing of a phospholipid bilayer (plasma membrane). An MHC Class I protein molecule is found in all nucleated body cells. It has a linear portion in the membrane and four portions on the outer side of the cell. One of these portions connects to the membrane-spanning portion; two form the antigen binding site; and the fourth is labeled the Beta-2 microglobulin. MHC Class II protein molecules are found in lymphocytes and macrophages. This has two membrane-spanning portions (each attached to a portion on the outside of the cell). The two portions attached to these form the antigen-binding site.

Figure 1.30: The process of phagocytosis. 1: A bacterium is engulfed by phagocytosis into a dendritic cell and is encased in a phagosome. 2: Lysosomes fuse with the phagosome and digest the bacterium. 3: Immunodominant epitopes are associated with MHC II and presented on the cell surface.

Figure 1.31: A micrograph of a round cell approximately 7 micrometers in diameter. The cell has a studded surface.

Figure 1.32: a) A drawing of a femur; a long bone with a round head. B) A cross-section of the head of the femur.

Figure 1.33: A drawing of the thymus (a structure sitting on the surface of the heart); the spleen (a kidney-shaped structure in the upper left abdomen; the right lymphatic duct entering vein (a tube in the neck); lymph nodes (enlarged regions of lymph ducts); and a tonsil in the cheek. A callout shows a micrograph of the thymus which has a surface layer labeled fibrous capsule, a central tissue labeled medulla, out tissue labeled cortex, and lighter branches in the cortex labeled trabeculae.

Figure 1.34: Drawing of two bars spanning the T cell plasma membrane. On one side of the membrane is the intracellular domain. The transmembrane region spans the membrane. The constant region is outside the membrane; a disulfide bond holds these two bars together in the constant region. The variable region is at the top and contains the antigen-binding sites.

Figure 1.35: A native helper T cell binds to an antigen extracted from a pathogen sitting on the MCH Class II protein of an antigen-presenting cell (dendritic cell). The portion of the helper T cell that binds is the T cell receptor and is stabilized by CD4. After this binding the helper T cell is activated and can become TH1 cell; TH2 cell or memory helper T cell.

Figure 1.36: A naïve Cytotoxid T cell has a T cell receptor and CD8. The T cell receptor binds to an antigen extracted from a pathogen on the MHC class I of an infected pathogen. The CD8 stabilizes this reaction. This causes the activated CTL to release granzymes and perforins, which result in the controlled destruction of the infected cell through apoptosis.

Figure 1.37: a) the T cell receptor on the T cell recognizes the epitope on the MHC II on the macrophage and binds. B) The T cell receptor binds even though it does not recognize the epitope because the superantigen is bound. Many dots labeled cytokines are present.

Figure 1.38: A B cell plasma membrane has two long rectangles spanning it; these form a Y shape. Two shorter rectangles sit on the outside of the upper portion of the Y. The region spanning the membrane and halfway through the bars of the Y is the constant region. The upper region is the variable region which has the antigen binding sites. The long rectangles are the heavy chains. The shorter rectangles are the light chains. Multiple disulfide bridges hold the constant region together.

Figure 1.39: A circle with small chains of hexagons projecting from the surface is a pathogenic bacterial cell. The chains are polysaccharide antigens with repeating epitopes. Antibodies on the B cell bind to these epitopes. This causes the activation of the B cell and secretion of pentameric IgM.

Figure 1.40: 1: BCR interaction with antigen on intact pathogen. An antigen on the surface of a bacterium binds to the B cell receptor on the B cell. s: Antigen processing and presentation with MHC II. The antigen is on the MHC II. 3: Antigen presentation and activation of helper T cell. T cell receptor of helper T cell binds to antigen on MHCII. This is stabilized by CD4. Helper T releases cytokines. 4: Cytokines stimulate clonal proliferation and differentiation into memory B cells and antibody-secreting plasma cells.

Figure 1.41: A graph with time on the X-axis and antibody concentration in serum. At first there is very little antibody (near 0). The lag period does not see a significant increase. In the primary response, IgM peaks for about 5 days and drops. At the same time IgG increases and then drops. This creates an increase in antibody count with a plateau of about 5 days as both antibody types are present. The secondary response sees a prolonged peak of IgG with a peak of IgM for about 1 to 2 days at about the same time as the peak of IgG. The total antibody is also higher but isn't at its plateau for as long as it is in the primary response.

Figure 1.42: a) Painting of Edward Jenner. B) Photo of many red lumps on the skin.

Figure 1.43: A) micrograph of pollen granules in different shapes and with different surface features. B) photo of a rash on a person's back. C) Photo of peanuts.

Figure 1.44: Drawing of TH2 cell response. 1: Upon first exposure to allergen, antigen-presenting cell processes antigen and presents it to TH2 Cell. A large antigen-presenting cell is shown engulfing an antigen which is attached to a Class II MHC inside the cell. This class II MHC is then placed on the surface with the antigen on the end of the MHC. The TH2 cell has a receptor that binds to the antigen on the MHC. 2: TH2 cell releases IL-4 and IL-13 which activates B cell. The TH2 cell has unbound from the antigen-presenting cell and binds to a B cell with the antigen on it's MHC and antibodies. The TH2 cell then releases small dots. 3: B cells proliferate and differentiate into plasma cells that synthesize and secrete IgE antibody. B cell is shown dividing. These cells then become plasma cells which are larger and are producing many IgE 4: IgE binds to mast cells by Fc region, sensitizing the mast cells. Mast cell is shown with IgE bound to it. 5: Upon subsequent exposure to allergen, mast cells with IgE bind to antigen and release inflammatory molecules, resulting in allergy symptoms. Antigen is shown bound to mast cell and the mast cell is releasing little dots labeled inflammatory molecules.

Figure 1.45: Table of Blood Types. Type A blood has red blood cells with A antigens as surface markers. It produces anti-B isohemagglutinins. Type B blood has red blood cells with B antigens as surface markers. It produces anti-A isohemagglutinins. Type AB blood has red blood cells with both A and B antigens as surface markers. It produces neither isohemagglutinins. Type O blood has red blood cells with neither A nor B antigens as surface markers. It produces both anti-A and anti-B isohemagglutinins.

Figure 1.46: Diagram of a Type A donor giving blood to a Type B recipient. 1: Donated type A blood with type A antigens enters bloodstream of type B recipient. Red blood cells with Type A antigens and red blood cells with Type B antigens are shown; anti-A antibody is also present. 2: Anti-A antibodies in plasma of Type B recipient bind to donated type A red blood cells. 3: Bound anti-A antibodies activate complement cascade, releasing hemoglobulin and destroying red blood cells. Small dots are shown destroying type A cells.

Figure 1.47: First pregnancy with Rh+ fetus resulting in healthy newborn. The diagram shows an Rh- person and an Rh+ fetus. Rh+ red blood cells cross the placenta into pregnant person's circulation. This causes anti-Rh antibodies to be produced in the person upon exposure to fetal Rh antigens. The second pregnancy with Rh+ fetus results in hemolytic newborn. The diagram shows an Rh- person with an Rh+ fetus. Anti-Rh antibodies remain in pregnant person's circulation from the first pregnancy and cross the placenta. Maternal anti-Rh antibodies attack and destroy fetal Rh+ red blood cells. b) First pregnancy with Rh+ fetus and anti-Rh antibody treatment resulting in healthy newborn The diagram shows an Rh- person and an Rh+ fetus. Rh+ red blood cells from the fetus cross placenta into pregnant person's circulation. Anti-Rh antibodies (Rhogam) bind and inactivate fetal Rh antigens before they stimulate immune response in the pregnant person.

Figure 1.48: IgG binds to antigens and forms an immune complex made of multiple IgG and antigens. Immune complex deposition in tissues, activation of complement and inflammation. Image shows Immune complex binding to tissues, binding to C1, C3b and C5a binding to tissues. And IgG binding to neutrophils. Tissue damage is shown. B) Person on a dialysis machine.

Figure 1.49: a) Sensitization. Antigens from poison ivy enter dendritic cells in skin. The dendritic cell activates a T-cell which becomes a memory helper T cell. b) Immune response. Macrophages, memory helper T cells, and cytotoxic T cells produce a large lesion on the skin due to cytokine activation.

Figure 1.50: A) Photo of chickens in a coop. b) Photo of a worker in a warehouse.

Figure 1.51: Photo of a person with many dots in a row on their skin. The dots are numbered and marks are next to those that are swollen.

Figure 1.52: A person with a large swollen neck.

Figure 1.53: Photo of a person with large bulging eyes.

Figure 1.54: Two hands, palm up are shown. They appear to be more red than is normal.

Figure 1.55: a) Diagram of a normal nerve cell releasing acetylcholine which binds to receptors on the muscle cell. This signal is processed and the muscle cell contracts. B) Diagram of myasthenia gravis. The nerve cell releases acetylcholine but anti-AChR antibodies bind to the acetylcholine so it cannot bind to the receptors on the muscle cells. Because the signal is blocked the muscle is paralyzed and does not contract.

Figure 1.56: X-ray and photo of hands with joints bent at unusual angles.

Figure 1.57: a) Diagram of symptoms include: a rash on the phase, ulcers of the nose and mouth, muscle aches, inflammation of the pericardium (heart region), poor circulation in the fingers and toes. B) photo of a butterfly rash on the face.

Figure 1.58: Photo of a boy in a suit similar to a space suit.

Figure References

Figure 1.1: There are multiple types of cell junctions in human tissue, three of which are shown here. Modification of work by (c) Mariana Ruiz Villareal. CC BY 4.0.

Figure 1.2: Human skin has three layers: the epidermis, the dermis, and the hypodermis. Modification of work by National Institutes of Health. Public Domain. https://commons.wikimedia.org/wiki/File:Anatomy_The_Skin_-_NCI_Visuals_Online.jpg

Figure 1.3: This scanning electron micrograph shows ciliated and nonciliated epithelial cells from the human trachea. Charles Daghlian. Public Domain. https://commons.wikimedia.org/wiki/File:Bronchiolar_epithelium_3_-_SEM.jpg

Figure 1.4: Goblet cells produce and secrete mucus. (c) 2012. Regents of University of Michigan Medical School. Redistribution authorized with attribution.

Figure 1.5: Tears flush microbes away from the surface of the eye. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.6: Sebaceous glands secrete sebum, a chemical mediator that lubricates and protects the skin from invading microbes. (c) 2012. Regents of University of Michigan Medical School. Redistribution authorized with attribution.

Figure 1.7: The three complement activation pathways have different triggers, as shown here, but all three result in the activation of the complement protein C3, which produces C3a and C3b. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Table 1.5: Autocrine, paracrine, and endocrine actions describe which cells are targeted by cytokines and how far the cytokines must travel to bind to their intended target cells' receptors. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.8: Interferons are cytokines released by a cell infected with a virus. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.9: All the formed elements of the blood arise by differentiation of hematopoietic stem cells in the bone marrow. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.10: Granulocytes can be distinguished by the number of lobes in their nuclei and the staining properties of their granules. Top Left: "neutrophil" micrograph: modification of work by (c) Ed Uthman. CC BY 4.0. Top Middle and Right: Public Domain. Bottom: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.11: Mast cells function similarly to basophils by inducing and promoting inflammatory responses. (a) Credit: Left: Copyright unknown. (c) Right: Modification of Figure 1C. in Greenland, J.R., Xu, X., Sayah, D.M. et al. Mast cells in a murine lung ischemia-reperfusion model of primary graft dysfunction. Respir Res 15, 95 (2014). https://doi.org/10.1186/s12931-014-0095-0 CC BY 4.0.

Figure 1.12: Natural killer (NK) cells are inhibited by the presence of the major histocompatibility cell (MHC) receptor on healthy cells. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.13: Natural killer cell with perforin-containing granules. Modification of Figure 1 in Rolstad B (2014) The early days of NK cells: an example of how a phenomenon led to detection of a novel immune receptor system – lessons from a rat model. Front. Immunol. 5:283. https://doi.org/10.3389/fimmu.2014.00283 CC BY 4.0

Figure 1.14: Monocytes are large, agranular white blood cells with a nucleus that lacks lobes. Left: Modification of work by Armed Forces Institute of Pathology. Public Domain. https://commons.wikimedia.org/wiki/File:AML-M4.jpg. Right: Modification of work by Centers for Disease Control and Prevention/Martin D. Hicklin. Public Domain. https://phil.cdc.gov/Details.aspx?pid=6625

Figure 1.15: Damaged cells and macrophages that have ingested pathogens release cytokines that are proinflammatory and chemotactic for leukocytes. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.16: Phagocytic cells contain pattern recognition receptors (PRRs) capable of recognizing various pathogen-associated molecular patterns (PAMPs). (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.17: The stages of phagocytosis include the engulfment of a pathogen, the formation of a phagosome, the digestion of the pathogenic particle in the phagolysosome, and the expulsion of undigested materials from the cell. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.18: Mast cells detect injury to nearby cells and release histamine, initiating an inflammatory response. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.19: A tubercle is a granuloma in the lung tissue of a patient with tuberculosis. Modified figure by 1C in (c) Piotrowski WJ, Górski P, Duda-Szymańska J, Kwiatkowska S. Mycobacterium tuberculosis as a sarcoid factor? A case report of family sarcoidosis. Am J Case Rep. 2014 May 16;15:216-20. CC BY-NC-ND 3.0 Unported. https://pmc.ncbi.nlm.nih.gov/articles/PMC4026149. Reproduced under Fair Use.

Figure 1.20: The role of the hypothalamus in the inflammatory response. Left: Public Domain. https://commons.wikimedia.org/wiki/File:Hypothalamus.jpg. Right: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.21: This graph illustrates the primary and secondary immune responses related to antibody production after an initial and secondary exposure to an antigen. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.22: An antigen is a macromolecule that reacts with components of the immune system. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.23: A typical protein antigen has multiple epitopes, shown by the ability of three different antibodies to bind to different epitopes of the same antigen. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.24: The typical four-chain structure of a generic antibody monomer. Credit a: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology. Credit b: modification of work by Tim Vickers. Public Domain. https://commons.wikimedia.org/wiki/File:Antibody_IgG2.png

Table 1.9: The five immunoglobulin (Ig) classes. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.25: Neutralization involves the binding of specific antibodies to antigens found on bacteria, viruses, and toxins, preventing them from attaching to target cells. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.26: Antibodies serve as opsonins and inhibit infection by tagging pathogens for destruction by macrophages, dendritic cells, and neutrophils. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.27: Antibodies, especially IgM antibodies, agglutinate bacteria by binding to epitopes on two or more bacteria simultaneously. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.28: In this example of ADCC, antibodies bind to a large pathogenic cell that is too big for phagocytosis and then bind to Fc receptors on the membrane of a natural killer cell. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.29: MHC I are found on all nucleated body cells, and MHC II are found on macrophages, dendritic cells, and B cells (along with MHC I). (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.30: A dendritic cell phagocytoses a bacterial cell and brings it into a phagosome. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.31: This scanning electron micrograph shows a T lymphocyte, which is responsible for the cell-mediated immune response. Modification of work by National Cancer Institute. Public Domain. https://commons.wikimedia.org/wiki/File:Red_White_Blood_cells.jpg

Figure 1.32: (a) Red bone marrow can be found in the head of the femur (thighbone) and is also present in the flat bones of the body, such as the ilium and the scapula. Left: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology. Right: Stevenfruitsmaak. Public Domain. https://commons.wikimedia.org/wiki/File:Caput_femoris_cortex_medulla.jpg

Figure 1.33: The thymus is a bi-lobed, H-shaped glandular organ that is located just above the heart. Left: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology. Right: (c) 2012. Regents of University of Michigan Medical School. Redistribution authorized with attribution.

Figure 1.34: A T-cell receptor spans the cytoplasmic membrane and projects variable binding regions into the extracellular space to bind processed antigens associated with MHC I or MHC II molecules. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.35: This illustration depicts the activation of a naïve (unactivated) helper T cell by an antigen-presenting cell and the subsequent proliferation and differentiation of the activated T cell into different subtypes. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.36: This figure illustrates the activation of a naïve (unactivated) cytotoxic T cell (CTL) by an antigen-presenting MHC I molecule on an infected body cell. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.37: (a) The macrophage in this figure is presenting a foreign epitope that does not match the TCR of the T cell. Because the T cell does not recognize the epitope, it is not activated. Modification of work by (c) "Microbiotic"/YouTube. CC BY 4.0.

Figure 1.38: B-cell receptors are embedded in the membranes of B cells. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.39: T-independent antigens have repeating epitopes that can induce B cell recognition and activation without involvement from T cells. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.40: In T cell-dependent activation of B cells, the B cell recognizes and internalizes an antigen and presents it to a helper T cell that is specific to the same antigen. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.41: Compared to the primary response, the secondary antibody response occurs more quickly and produces antibody levels that are higher and more sustained. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Table 1.12: The four mechanisms of acquisition of immunity. Top left: by USDA/Ken Hammond. Public Domain. https://commons.wikimedia.org/wiki/File:Breastfeeding_infant.jpg; credit top right photo: by Michaelberry https://commons.wikimedia.org/wiki/File:Intravenous_therapy_2007-SEP-13-Singapore.JPG; bottom left photo: Centers for Disease Control and Prevention. Public Domain. https://commons.wikimedia.org/wiki/File:Varicella.jpg; credit bottom right photo: Airman 1st Class Destinee Doughert / U.S. Air Force. Public Domain. https://nara.getarchive.net/media/us-air-force-senior-airman-tamika-bradley-20th-medical-feec7e

Figure 1.42: A painting of Edward Jenner depicts a cow and a milkmaid in the background. Public Domain. https://commons.wikimedia.org/wiki/File:Edward_Jenner.jpg; Right: Figure 1 from Palkonen P. et al. 2003. "Cowpox with Severe Generalized Eruption, Finland" Emerging Infections Diseases. 9 (11): 1458-1461. https://wwwnc.cdc.gov/eid/past-issues/volume-9. Public Domain.

Figure 1.43: a. Allergens in plant pollen. Dartmouth Electron Microscope Facility. Public Domain. https://commons.wikimedia.org/wiki/File:Misc_pollen_colorized.jpg. b. Hives. Modification of work of DLdoubleE. Public Domain. https://commons.wikimedia.org/wiki/File:Hives_on_back.jpg. c. Peanuts. Public Domain.

Figure 1.44: On first exposure to an allergen in a susceptible individual, antigen-presenting cells process and present allergen epitopes with major histocompatibility complex (MHC) II to T helper cells. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.45: Blood types. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.46: A type II hypersensitivity hemolytic transfusion reaction (HTR) leading to hemolytic anemia. Blood from a type A donor is administered to a patient with type B blood. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.47: When an Rh– mother has an Rh+ fetus, fetal erythrocytes are introduced into the mother's circulatory system before or during birth, leading to production of anti-Rh IgG antibodies. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.48: Type III hypersensitivities and the systems they affect. Left: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology. Right: Tech. Sgt. Bennie J. Davis III / US Air Force; Public Domain. https://www.af.mil/News/Photos/igphoto/2000316503

Figure 1.49: Exposure to hapten antigens in poison ivy can cause contact dermatitis, a type IV hypersensitivity. Illustrations: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology. Left: Adapted under Fair Use http://blogimages.serenataassets.com/pollennation/wp-content/uploads/2014/09/poison-ivy-160×160.jpg. Right: modification of work by Larsonja. Public Domain. https://commons.wikimedia.org/wiki/File:Urushiol_induced_contact_dermatitis.jpg

Figure 1.50: Occupational exposure to dust, mold, and other allergens can result in hypersensitivity pneumonitis. Left: Modification of work (c) The Global Orphan Project. CC BY 4.0. Right: kytrangho. Public Domain. https://pixabay.com/photos/gruyere-cheese-factory-switzerland-272462

Figure 1.51: Results of an allergy skin-prick test to test for type I hypersensitivity to a group of potential allergens. Modification of work (c) OakleyOriginals. CC BY 4.0. https://flic.kr/p/wx3wWU

Figure 1.52: Goiter, a hypertrophy of the thyroid, is a symptom of Graves disease and Hashimoto thyroiditis. By Almazi. Public Domain. https://commons.wikimedia.org/wiki/File:Goitre.jpg

Figure 1.53: Exophthalmia, or Graves ophthalmopathy, is a sign of Graves disease. Modification of work (c) Jonathan Trobe, University of Michigan Kellogg Eye Center. CC BY 3.0 Unported. https://commons.wikimedia.org/wiki/File:Proptosis_and_lid_retraction_from_Graves%27_Disease.jpg

Figure 1.54: Hyperpigmentation is a sign of Addison disease. Modification of work (c) Petros Perros. CC BY 2.5 Generic. https://commons.wikimedia.org/wiki/File:A_69-Year-Old_Female_with_Tiredness_and_a_Persistent_Tan_02.png

Figure 1.55: Myasthenia gravis and impaired muscle contraction. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 1.56: The radiograph (left) and photograph (right) show damage to the hands typical of rheumatoid arthritis. Left: Jojo. Public Domain. https://commons.wikimedia.org/wiki/File:Reumatoidalne_zapalenie_stawow_01.jpg. Right: (c) modification of work (c) handarmdoc. CC BY 2.0. https://flic.kr/p/f35DuE

Figure 1.57: Systemic lupus erythematosus. Left: Modification of work by Mikael Häggström. https://commons.wikimedia.org/wiki/File:Symptoms_of_SLE.svg. Public Domain. Right: Figure 4 from Shrestha D, Dhakal AK, Shiva RK, Shakya A, Shah SC, Shakya H. Systemic lupus erythematosus and granulomatous lymphadenopathy. BMC Pediatr. 2013 Nov 5;13:179. doi: 10.1186/1471-2431-13-179. CC BY 2.0.

Figure 1.58: David Vetter, popularly known as "The Bubble Boy," was born with SCID and lived most of his life isolated inside a plastic bubble. NASA Johnson Space Center. Public domain.

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CHAPTER 2.

BASIC MICROBIOLOGY

2.1 INTRODUCTION TO PROKARYOTIC CELLS

All plant cells and animal cells are eukaryotic. Some microorganisms are composed of prokaryotic cells, whereas others are composed of eukaryotic cells. Prokaryotic microorganisms are classified within the domains Archaea and Bacteria, whereas eukaryotic organisms are classified within the domain Eukarya.

The structures inside a cell are analogous to the organs inside a human body, with unique structures suited to specific functions. Some of the structures found in prokaryotic cells are similar to those found in some eukaryotic cells; others are unique to prokaryotes. Although there are some exceptions, eukaryotic cells tend to be larger than prokaryotic cells. The comparatively larger size of eukaryotic cells dictates the need to compartmentalize various chemical processes within different areas of the cell, using complex membrane-bound organelles. In contrast, prokaryotic cells generally lack membrane-bound organelles; however, they often contain inclusions that compartmentalize their cytoplasm. Figure 2.1 illustrates structures typically associated with prokaryotic cells. These structures are described in more detail in the next section.

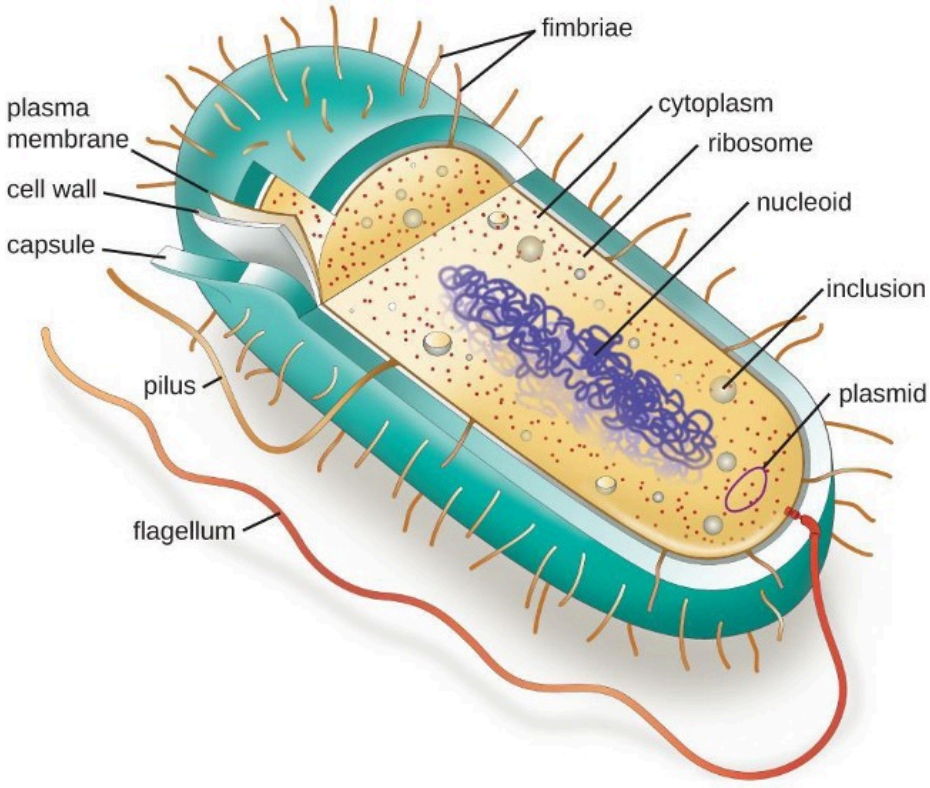


Figure 2.1: A typical prokaryotic cell contains a cell membrane, chromosomal DNA that is concentrated in a nucleoid, ribosomes, and a cell wall. Some prokaryotic cells may also possess flagella, pili, fimbriae, and capsules. *Figure description available at the end of the chapter.*

COMMON CELL MORPHOLOGIES AND ARRANGEMENTS

Individual cells of a particular prokaryotic organism are typically similar in shape, or cell morphology. Although thousands of prokaryotic organisms have been identified, only a handful of cell morphologies are commonly seen microscopically. Table 2.1 names and illustrates cell morphologies commonly found in prokaryotic cells. In addition to cellular shape, prokaryotic cells of the same species may group together in certain distinctive arrangements depending on the plane of cell division. Some common arrangements are shown in table 2.2.

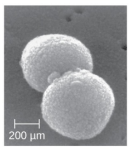
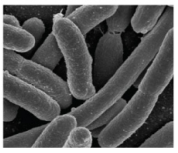
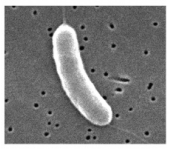
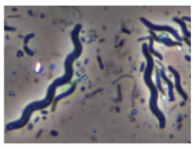
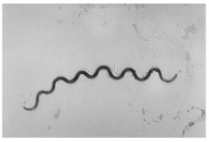
Name	Description	Illustration	Image
Coccus (pl. cocci)	Round		
Bacillus (pl. bacilli)	Rod		
Vibrio (pl. vibrios)	Curved rod		
Coccobacillus (pl. coccobacilli)	Short rod		
Spirillum (pl. spirilla)	Spiral		
Spirochete (pl. spirochetes)	Long, loose, helical spiral		

Table 2.1: Common prokaryotic cell shapes

Name	Description	Illustration
Coccus (pl. cocci)	Single coccus	
Diplococcus (pl. diplococci)	Pair of two cocci	
Tetrad (pl. tetrads)	Grouping of four cells arranged in a square	
Streptococcus (pl. streptococci)	Chain of cocci	
Staphylococcus (pl. staphylococci)	Cluster of cocci	
Bacillus (pl. bacilli)	Single rod	
Streptobacillus (pl. streptobacilli)	Chain of rods	

Table 2.2: Common prokaryotic cell arrangements

THE NUCLEOID

Prokaryotic DNA and DNA-associated proteins are concentrated within the nucleoid region of the cell (figure 2.2). In general, prokaryotic DNA interacts with nucleoid-associated proteins (NAPs) that assist in the organization and packaging of the chromosome. In bacteria, NAPs function similar to histones, which are the DNA-organizing proteins found in eukaryotic cells. In archaea, the nucleoid is organized by either NAPs or histone-like DNA organizing proteins.

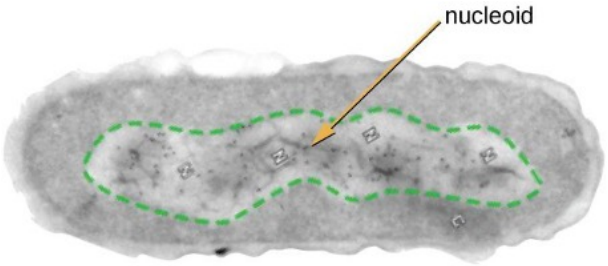


Figure 2.2: The nucleoid region (the area enclosed by the green dashed line) is a condensed area of DNA found within prokaryotic cells. Because of the density of the area, it does not readily stain and appears lighter in color when viewed with a transmission electron microscope. *Figure description available at the end of the chapter.*

PLASMIDS

Prokaryotic cells may also contain extrachromosomal DNA, or DNA that is not part of the chromosome. This extrachromosomal DNA is found in plasmids, which are small, circular, double-stranded DNA molecules. Plasmids are more commonly found in bacteria; however, plasmids have been found in archaea and eukaryotic organisms. Plasmids often carry genes that confer advantageous traits such as antibiotic resistance. They are, therefore, important to the survival of the organism.

In prokaryotes, horizontal gene transfer (HGT), the introduction of genetic material from one organism to another organism within the same generation, is an important way to introduce genetic diversity. HGT allows even distantly related species to share genes, influencing their phenotypes. It is thought that HGT is more prevalent in prokaryotes but that only a small fraction of the prokaryotic genome may be transferred by this type of transfer at any one time. As the phenomenon is investigated more thoroughly, it may be revealed to be even more common. Many scientists believe that HGT and mutation are significant sources of genetic variation, the raw material for the process of natural selection, in prokaryotes.

HGT in prokaryotes is known to occur by the three primary mechanisms that are illustrated in figure 2.3 and summarized in table 2.3:

1. Transformation: naked DNA is taken up from the environment
2. Transduction: genes are transferred between cells in a virus (see section 2.11)
3. Conjugation: use of a hollow tube called a conjugation pilus to transfer genes between cells

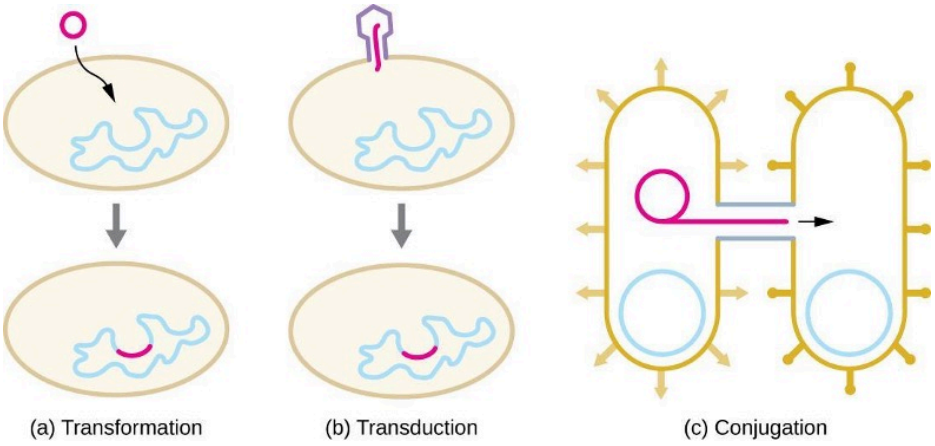


Figure 2.3: There are three prokaryote-specific mechanisms leading to horizontal gene transfer in prokaryotes. a) In transformation, the cell takes up DNA directly from the environment. The DNA may remain separate as a plasmid or be incorporated into the host genome. b) In transduction, a bacteriophage injects DNA that is a hybrid of viral DNA and DNA from a previously infected bacterial cell. c) In conjugation, DNA is transferred between cells through a cytoplasmic bridge after a conjugation pilus draws the two cells close enough to form the bridge. *Figure description available at the end of the chapter.*

Term	Definition
Conjugation	Transfer of DNA through direct contact using a conjugation pilus
Transduction	Mechanism of horizontal gene transfer in bacteria in which genes are transferred through viral infection
Transformation	Mechanism of horizontal gene transfer in which naked environmental DNA is taken up by a bacterial cell
Transposition	Process whereby DNA independently excises from one location in a DNA molecule and integrates elsewhere

Table 2.3: Summary of mechanisms of genetic diversity in prokaryotes

RIBOSOMES

Ribosomes, themselves, are constructed from proteins, along with ribosomal RNA (rRNA). Prokaryotic ribosomes are found in the cytoplasm. They are called 70S ribosomes because they have a size of 70S (figure 2.4), whereas eukaryotic cytoplasmic ribosomes have a size of 80S. Although they are the same size, bacterial and archaeal ribosomes have different proteins and rRNA molecules, and the archaeal versions are more similar to their eukaryotic counterparts than to those found in bacteria.

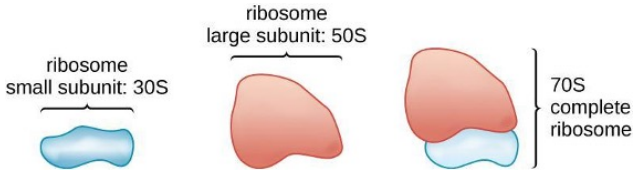


Figure 2.4: Prokaryotic ribosomes (70S) are composed of two subunits: the 30S (small subunit) and the 50S (large subunit), each of which are composed of protein and rRNA components. *Figure description available at the end of the chapter.*

INCLUSIONS

As single-celled organisms living in unstable environments, some prokaryotic cells have the ability to store excess nutrients within cytoplasmic structures called inclusions. Storing nutrients in a polymerized form is advantageous because it reduces the buildup of osmotic pressure that occurs as a cell accumulates solutes. Various types of inclusions store glycogen and starches, which contain carbon that cells can access for energy. Volutin granules, also called metachromatic granules because of their staining characteristics, are inclusions that store polymerized inorganic phosphate that can be used in metabolism and assist in the formation of biofilms. Microbes known to contain volutin granules include the archaea *Methanosarcina*, the bacterium *Corynebacterium diphtheriae*, and the unicellular eukaryotic alga *Chlamydomonas*. Sulfur granules, another type of inclusion, are found in sulfur bacteria of the genus *Thiobacillus*; these granules store elemental sulfur, which the bacteria use for metabolism.

Occasionally, certain types of inclusions are surrounded by a phospholipid monolayer embedded with protein. Polyhydroxybutyrate (PHB), which can be produced by species of *Bacillus* and *Pseudomonas*, is an example of an inclusion that displays this type of monolayer structure. Industrially, PHB has also been used as a source of biodegradable polymers for bioplastics. Several different types of inclusions are shown in figure 2.5.

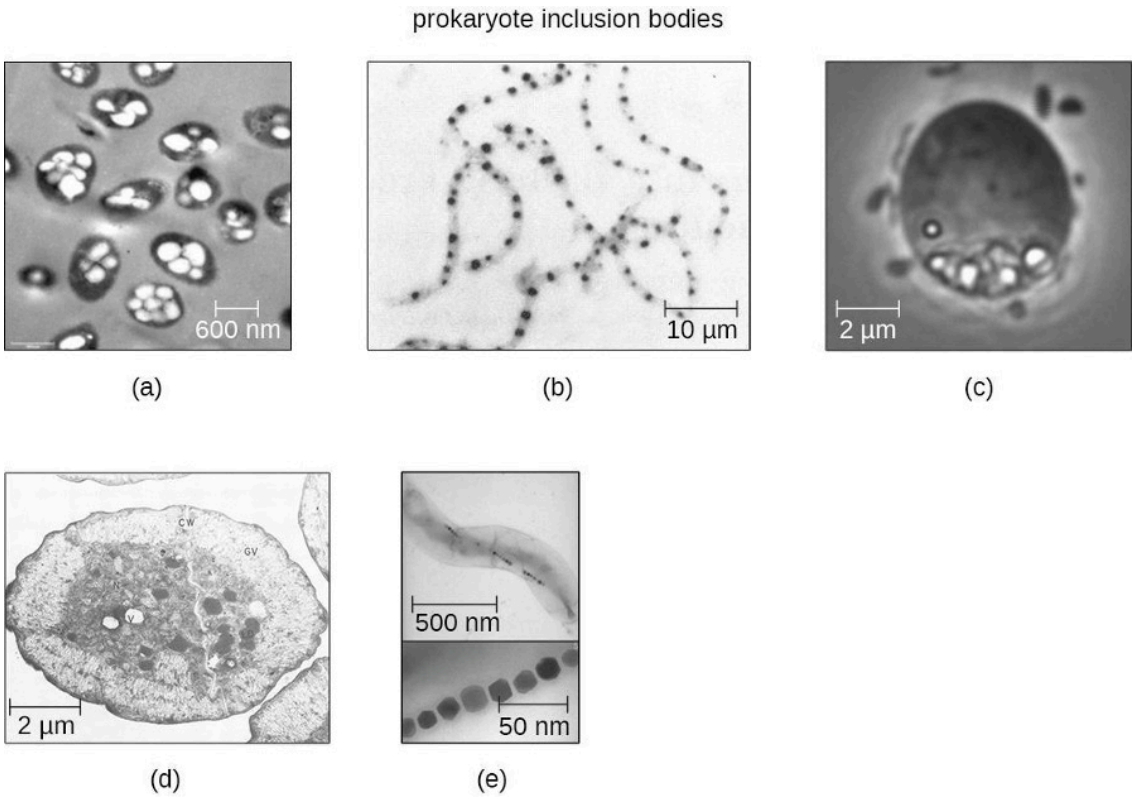


Figure 2.5: Prokaryotic cells may have various types of inclusions. (a) A transmission electron micrograph of polyhydroxybutryrate lipid droplets. (b) A light micrograph of volutin granules. (c) A phase-contrast micrograph of sulfur granules. (d) A transmission electron micrograph of magnetosomes. (e) A transmission electron micrograph of gas vacuoles. *Figure description available at the end of the chapter.*

ENDOSPORES

Bacterial cells are generally observed as vegetative cells, but some genera of bacteria have the ability to form endospores, structures that essentially protect the bacterial genome in a dormant state when environmental conditions are unfavorable. Endospores (not to be confused with the reproductive spores formed by fungi) allow some bacterial cells to survive long periods without food or water, as well as exposure to chemicals, extreme temperatures, and even radiation. Table 2.4 compares the characteristics of vegetative cells and endospores.

Vegetative Cells	Endospores
Sensitive to extreme temperatures and radiation	Resistant to extreme temperatures and radiation
Gram-positive	Do not absorb Gram stain, only special endospore stains (see section 2.2)
Normal water content and enzymatic activity	Dehydrated; no metabolic activity
Capable of active growth and metabolism	Dormant; no growth or metabolic activity

Table 2.4: Characteristics of vegetative cells versus endospores

The process by which vegetative cells transform into endospores is called sporulation, and it generally begins when nutrients become depleted or environmental conditions become otherwise unfavorable (figure 2.6). The process begins with the formation of a septum in the vegetative bacterial cell. The septum divides the cell asymmetrically, separating a DNA forespore from the mother cell. The forespore, which will form the core of the endospore, is essentially a copy of the cell's chromosomes and is separated from the mother cell by a second membrane. A cortex gradually forms around the forespore by laying down layers of calcium and dipicolinic acid between membranes. A protein spore coat then forms around the cortex while the DNA of the mother cell disintegrates. Further maturation of the endospore occurs with the formation of an outermost exosporium. The endospore is released upon disintegration of the mother cell, completing sporulation.

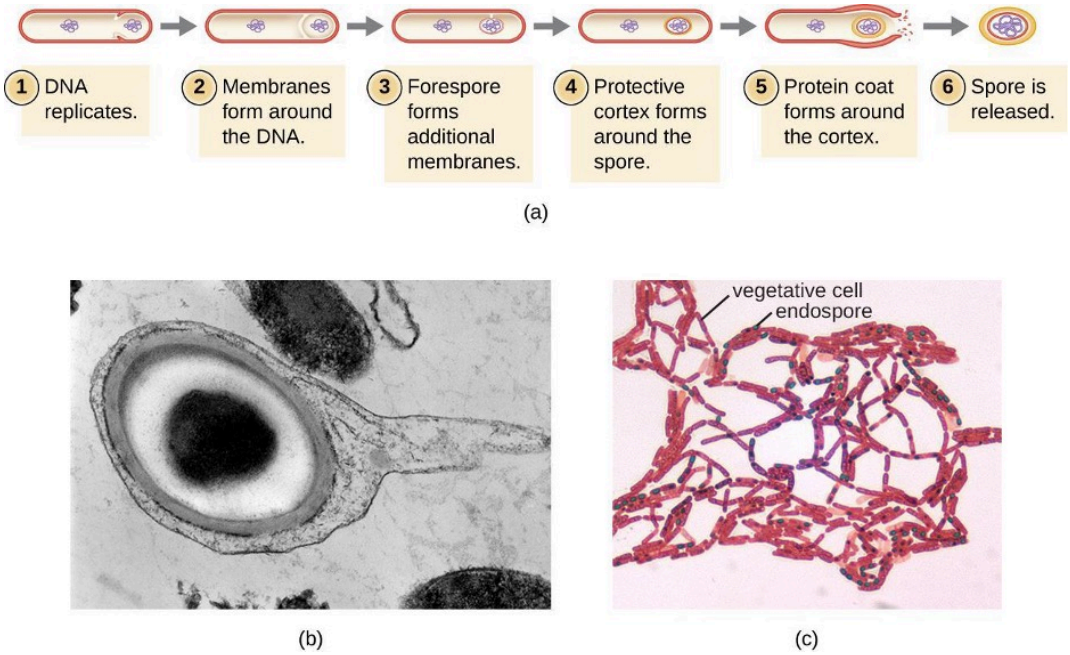


Figure 2.6: (a) Sporulation begins following asymmetric cell division. The forespore becomes surrounded by a double layer of membrane, a cortex, and a protein spore coat, before being released as a mature endospore upon disintegration of the mother cell. (b) An electron micrograph of a Carboxydothermus hydrogenoformans endospore. (c) These Bacillus spp. cells are undergoing sporulation. The endospores have been visualized using Malachite Green spore stain. *Figure description available at the end of the chapter.*

Not all bacteria have the ability to form endospores; however, there are a number of clinically significant endospore-forming, gram-positive bacteria of the genera *Bacillus* and *Clostridium*. These include *B. anthracis*, the causative agent of anthrax, which produces endospores capable of surviving for many decades1; *C. tetani* (causes tetanus); *C. difficile* (causes pseudomembranous colitis); *C. perfringens* (causes gas gangrene); and *C. botulinum* (causes botulism). Pathogens such as these are particularly difficult to combat because their endospores are so hard to kill.[1]

CELL WALL

The major component of bacterial cell walls is called peptidoglycan (or murein); it is only found in bacteria. Structurally, peptidoglycan resembles a layer of meshwork or fabric (figure 2.7). Each layer is composed of long chains of alternating molecules of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM). The structure of the long chains has significant two-dimensional tensile strength due to the formation of peptide bridges that connect NAG and NAM within each peptidoglycan layer. In gram-negative bacteria, tetrapeptide chains extending from each NAM unit are directly cross-linked, whereas in gram-positive bacteria, these tetrapeptide

chains are linked by pentaglycine cross-bridges. Peptidoglycan subunits are made inside of the bacterial cell and then exported and assembled in layers, giving the cell its shape.

Since peptidoglycan is unique to bacteria, many antibiotic drugs are designed to interfere with peptidoglycan synthesis, weakening the cell wall and making bacterial cells more susceptible to the effects of osmotic pressure (see section 9.2). In addition, certain cells of the human immune system are able to "recognize" bacterial pathogens by detecting peptidoglycan on the surface of a bacterial cell; these cells then engulf and destroy the bacterial cell, using enzymes such as lysozyme, which breaks down and digests the peptidoglycan in their cell walls (see section 1.6).

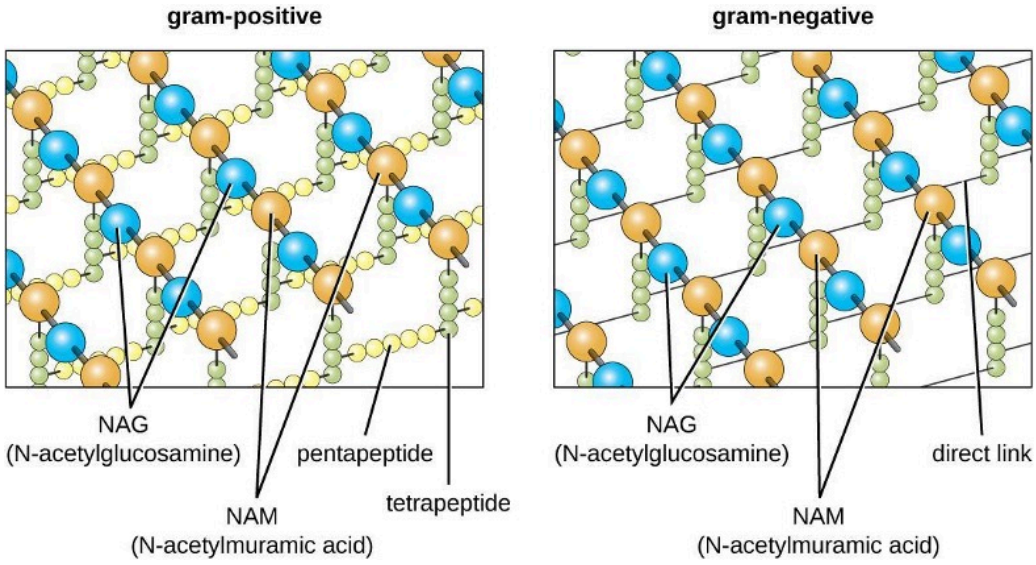


Figure 2.7: Peptidoglycan is composed of polymers of alternating NAM and NAG subunits, which are cross-linked by peptide bridges linking NAM subunits from various glycan chains. This provides the cell wall with tensile strength in two dimensions. Figure description available at the end of the chapter.

The Gram staining protocol (see section 2.2) is used to differentiate two common types of cell wall structures (figure 2.8). Gram-positive cells have a cell wall consisting of many layers of peptidoglycan totaling 30–100 nm in thickness. These peptidoglycan layers are commonly embedded with teichoic acids (TAs), carbohydrate chains that extend through and beyond the peptidoglycan layer.[2] TA is thought to stabilize peptidoglycan by increasing its rigidity. TA also plays a role in the ability of pathogenic gram-positive bacteria such as *Streptococcus* to bind to certain proteins on the surface of host cells, enhancing their ability to cause infection. In addition to peptidoglycan and TAs, bacteria of the family *Mycobacteriaceae* have an external layer of waxy mycolic acids in their cell wall; as described in section 2.2, these bacteria are referred to as acid-fast, since acid-fast stains must be used to penetrate the mycolic acid layer for purposes of microscopy (figure 2.9).

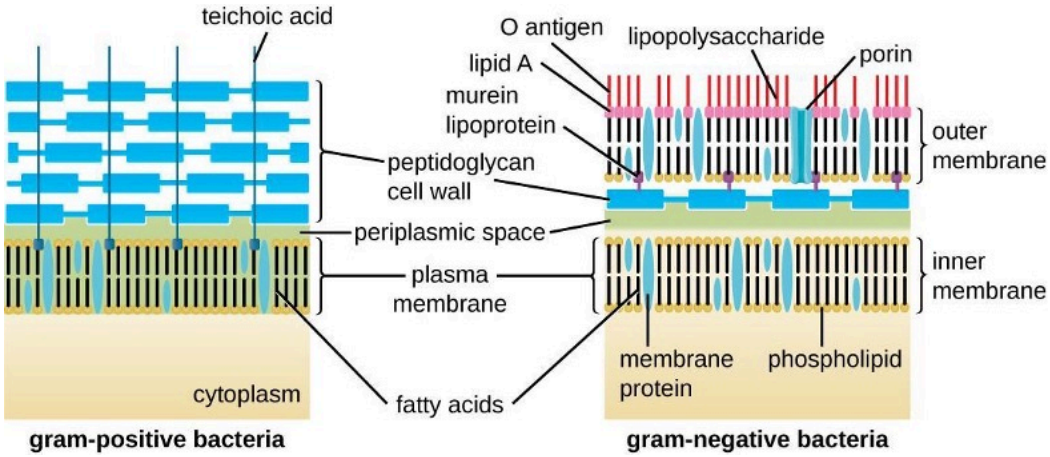


Figure 2.8: Bacteria contain two common cell wall structural types. Gram-positive cell walls are structurally simple, containing a thick layer of peptidoglycan with embedded teichoic acid external to the plasma membrane.[footnote]B. Zuber et al. "Granular Layer in the Periplasmic Space of Gram-Positive Bacteria and Fine Structures of Enterococcus gallinarum and Streptococcus gordonii Septa Revealed by Cryo-Electron Microscopy of Vitreous Sections." Journal of Bacteriology 188 no. 18 (2006):6652–6660[/footnote] Gram-negative cell walls are structurally more complex, containing a thin layer of peptidoglycan and an outer membrane containing lipopolysaccharide. Figure description available at the end of the chapter.

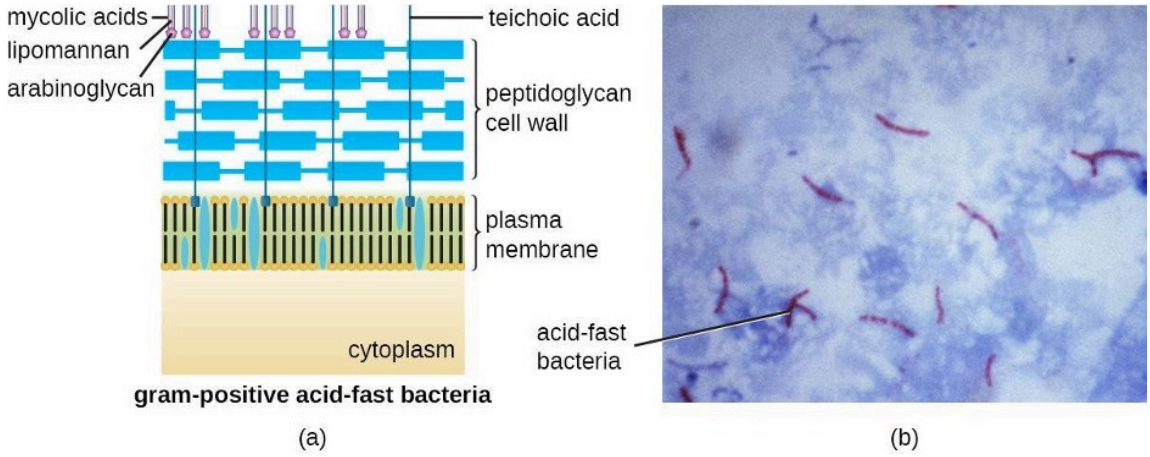


Figure 2.9: (a) Some gram-positive bacteria, including members of the Mycobacteriaceae, produce waxy mycolic acids found exterior to their structurally-distinct peptidoglycan. (b) The acid-fast staining protocol detects the presence of cell walls that are rich in mycolic acid. Acid-fast cells are stained red by carbolfuchsin. Figure description available at the end of the chapter.

Gram-negative cells have a much thinner layer of peptidoglycan (no more than about 4 nm thick[3]) than gram-positive cells, and the overall structure of their cell envelope is more complex. In gram-negative cells, a gel-like matrix occupies the periplasmic space between the cell wall and the plasma membrane. Additionally, there is a second lipid bilayer called the outer membrane, which is external to the peptidoglycan layer (figure 2.8). This outer membrane is attached to the peptidoglycan by murein lipoprotein. The outer leaflet of the outer membrane contains the molecule lipopolysaccharide (LPS), which functions as an endotoxin in infections involving gram-negative bacteria, contributing to symptoms such as fever, hemorrhaging, and septic shock. Each LPS molecule is composed of Lipid A, a core polysaccharide, and an O-side chain that is composed of sugar-like molecules that comprise the external face of the LPS (figure 2.10). The composition of the O-side chain varies

between different species and strains of bacteria. Parts of the O-side chain called antigens can be detected using serological or immunological tests to identify specific pathogenic strains like *Escherichia coli* O157:H7, a deadly strain of bacteria that causes bloody diarrhea and kidney failure.

Archaeal cell wall structure differs from that of bacteria in several significant ways. First, archaeal cell walls do not contain peptidoglycan; instead, they contain a similar polymer called pseudopeptidoglycan (pseudomurein) in which NAM is replaced with a different subunit. Other archaea may have a layer of glycoproteins or polysaccharides that serves as the cell wall instead of pseudopeptidoglycan. Last, as is the case with some bacterial species, there are a few archaea that appear to lack cell walls entirely.

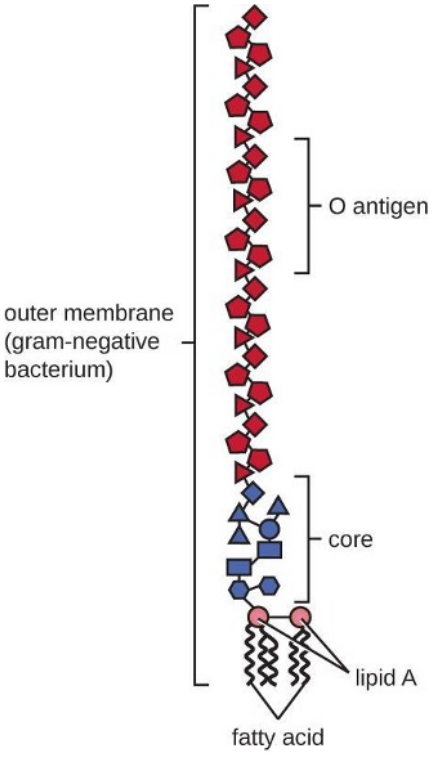


Figure 2.10: The outer membrane of a gram-negative bacterial cell contains lipopolysaccharide (LPS), a toxin composed of Lipid A embedded in the outer membrane, a core polysaccharide, and the O-side chain. Figure description available at the end of the chapter.

GLYCOCALYCES AND S-LAYERS

Although most prokaryotic cells have cell walls, some may have additional cell envelope structures exterior to the cell wall, such as glycocalyces and S-layers. A glycocalyx is a sugar coat, of which there are two important types: capsules and slime layers. A capsule is an organized layer located outside of the cell wall and usually composed of polysaccharides or proteins (figure 2.11). A slime layer is a less tightly organized layer that is only loosely attached to the cell wall and can be more easily washed off. Slime layers may be composed of polysaccharides, glycoproteins, or glycolipids.

Glycocalyces allow cells to adhere to surfaces, aiding in the formation of biofilms (colonies of microbes that form in layers on surfaces). In nature, most microbes live in mixed communities within biofilms, partly because the biofilm affords them some level of protection. Biofilms generally hold water like a sponge, preventing desiccation. They also protect cells from predation and hinder the action of antibiotics and disinfectants. All of these properties are advantageous to the microbes living in a biofilm, but they present challenges in a clinical setting, where the goal is often to eliminate microbes.

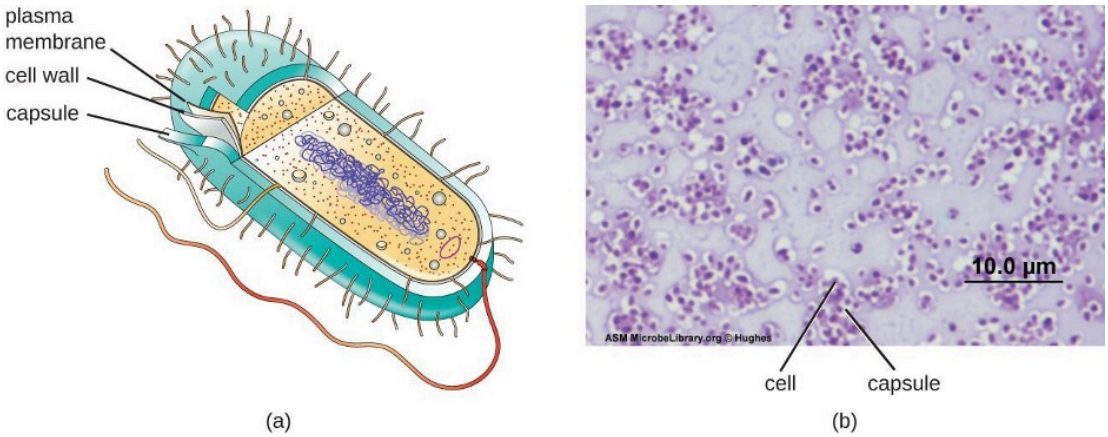


Figure 2.11: (a) Capsules are a type of glycocalyx composed of an organized layer of polysaccharides. (b) A capsule stain of Pseudomonas aeruginosa, a bacterial pathogen capable of causing many different types of infections in humans. Figure description available at the end of the chapter.

The ability to produce a capsule can contribute to a microbe's pathogenicity (ability to cause disease) because the capsule can make it more difficult for phagocytic cells (such as white blood cells) to engulf and kill the microorganism. *Streptococcus pneumoniae*, for example, produces a capsule that is well known to aid in this bacterium's pathogenicity. As explained in section 2.2, capsules are difficult to stain for microscopy; negative staining techniques are typically used.

An S-layer is another type of cell envelope structure; it is composed of a mixture of structural proteins and glycoproteins. In bacteria, S-layers are found outside the cell wall, but in some archaea, the S-layer serves *as* the cell wall. The exact function of S-layers is not entirely understood, and they are difficult to study. Available evidence suggests that they may play a variety of functions in different prokaryotic cells, such as helping the cell withstand osmotic pressure and, for certain pathogens, interacting with the host immune system.

FILAMENTOUS APPENDAGES

Many bacterial cells have protein appendages embedded within their cell envelopes that extend outward, allowing interaction with the environment. These appendages can attach to other surfaces, transfer DNA, or provide movement. Filamentous appendages include fimbriae, pili, and flagella.

Fimbriae and Pili

Fimbriae and pili are structurally similar and, because differentiation between the two is problematic, these terms are often used interchangeably.[45] The term fimbriae commonly refers to short bristle-like proteins projecting from the cell surface by the hundreds. Fimbriae enable a cell to attach to surfaces and to other cells. For pathogenic bacteria, adherence to host cells is important for colonization, infectivity, and virulence. Adherence to surfaces is also important in biofilm formation.

The term pili (singular: pilus) commonly refers to longer, less numerous protein appendages that aid in attachment to surfaces (figure 2.12). A specific type of pilus, called the F pilus or sex pilus, is important in the transfer of DNA between bacterial cells, which occurs between members of the same generation when two cells physically transfer or exchange parts of their respective genomes.

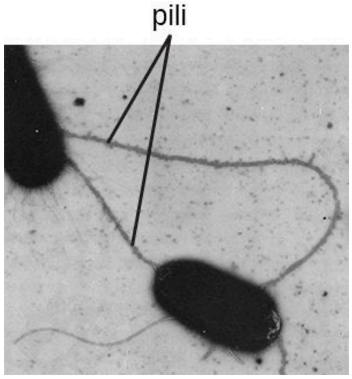


Figure 2.12: Bacteria may produce two different types of protein appendages that aid in surface attachment. Fimbriae typically are more numerous and shorter, whereas pili (shown here) are longer and less numerous per cell. *Figure description available at the end of the chapter.*

Flagella

Bacterial flagella act like propellers. They are stiff spiral filaments composed of flagellin protein subunits that extend outward from the cell and spin in solution. The basal body is the motor for the flagellum and is embedded in the plasma membrane (figure 2.13). A hook region connects the basal body to the filament. Gram-positive and gram-negative bacteria have different basal body configurations due to differences in cell wall structure.

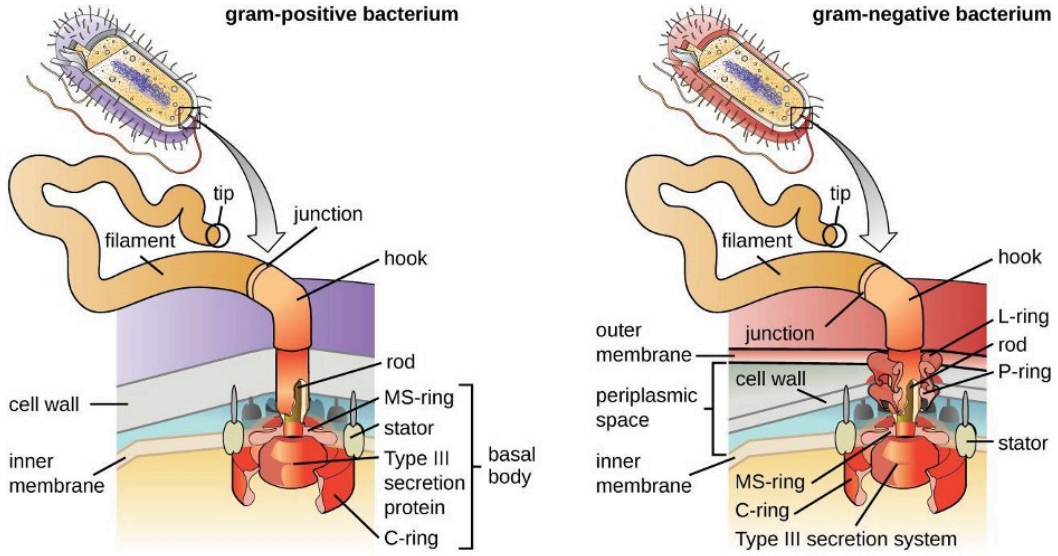


Figure 2.13: The basic structure of a bacterial flagellum consists of a basal body, hook, and filament. The basal body composition and arrangement differ between gram-positive and gram-negative bacteria. Figure description available at the end of the chapter.

Different types of motile bacteria exhibit different arrangements of flagella (figure 2.14). A bacterium with a singular flagellum, typically located at one end of the cell (polar), is said to have a monotrichous flagellum. An example of a monotrichously flagellated bacterial pathogen is *Vibrio cholerae*, the gram-negative bacterium that causes cholera. Cells with amphitrichous flagella have a flagellum or tufts of flagella at each end. An example is *Spirillum minor*, the cause of spirillary (Asian) rat-bite fever or sodoku. Cells with lophotrichous flagella have a tuft at one end of the cell. The gram-negative bacillus *Pseudomonas aeruginosa*, an opportunistic pathogen known for causing many infections including swimmer's ear and burn wound infections, has lophotrichous flagella. Flagella that cover the entire surface of a bacterial cell are called peritrichous flagella. The gram-negative bacterium *E. coli* shows a peritrichous arrangement of flagella.

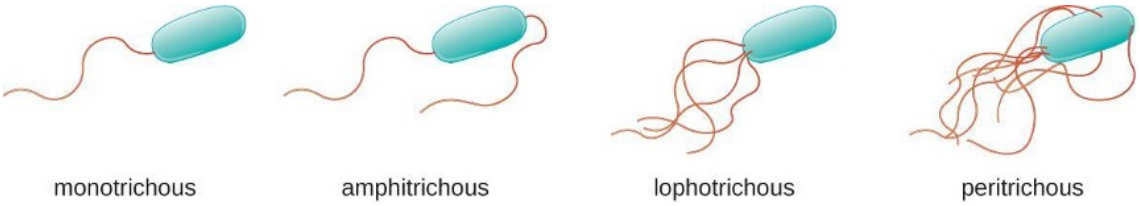


Figure 2.14: Flagellated bacteria may exhibit multiple arrangements of their flagella. Common arrangements include monotrichous, amphitrichous, lophotrichous, or peritrichous. Figure description available at the end of the chapter.

Directional movement depends on the configuration of the flagella. Bacteria can move in response to a variety of environmental signals, including light (phototaxis), magnetic fields (magnetotaxis) using magnetosomes, and, most commonly, chemical gradients (chemotaxis). In a peritrichous bacterium, the flagella are all bundled together in a notably streamlined way (figure 2.15), allowing for efficient movement.

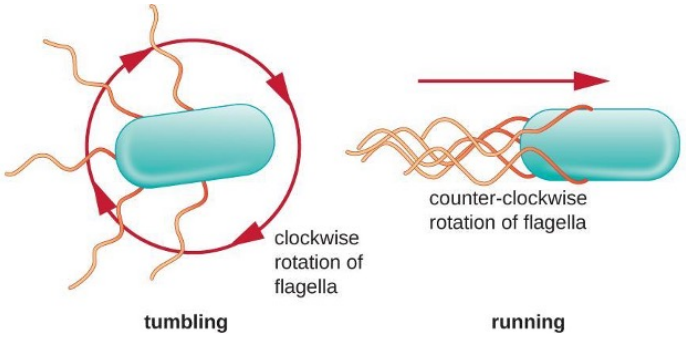


Figure 2.15: Bacteria achieve directional movement by changing the rotation of their flagella. In a cell with peritrichous flagella, the flagella bundle when they rotate in a counterclockwise direction, resulting in a run. However, when the flagella rotate in a clockwise direction, the flagella are no longer bundled, resulting in tumbles. *Figure description available at the end of the chapter.*

2.2 STAINING MICROSCOPIC SPECIMENS

GRAM STAINING

The Gram stain procedure is a differential staining procedure that involves multiple steps. It was developed by Danish microbiologist Hans Christian Gram in 1884 as an effective method to distinguish between bacteria with different types of cell walls. Even today it remains one of the most frequently used staining techniques. The steps of the Gram stain procedure are listed below and illustrated in table 2.5 and 2.6.

1. First, crystal violet, a primary stain, is applied to a heat-fixed smear, giving all of the cells a purple color.
2. Next, Gram's iodine, a mordant, is added. A mordant is a substance used to set or stabilize stains or dyes; in this case, Gram's iodine acts like a trapping agent that complexes with the crystal violet, making the crystal violet–iodine complex clump and stay contained in thick layers of peptidoglycan in the cell walls.
3. Next, a decolorizing agent is added, usually ethanol or an acetone/ethanol solution. Cells that have thick peptidoglycan layers in their cell walls are much less affected by the decolorizing agent; they generally retain the crystal violet dye and remain purple. However, the decolorizing agent more easily washes the dye out of cells with thinner peptidoglycan layers, making them again colorless.
4. Finally, a secondary counterstain, usually safranin, is added. This stains the decolorized cells pink and is less noticeable in the cells that still contain the crystal violet dye.

The purple, crystal-violet stained cells are referred to as gram-positive cells, while the red, safranin-dyed cells are gram-negative (figure 2.16). Besides their differing interactions with dyes and decolorizing agents, the chemical differences between gram-positive and gram-negative cells have other implications with clinical relevance. For example, Gram staining can help clinicians classify bacterial pathogens in a sample into categories associated with specific properties. Gram-negative bacteria tend to be more resistant to certain antibiotics than gram-positive bacteria. We will discuss this and other applications of Gram staining in more detail later.

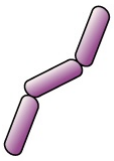
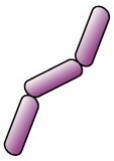
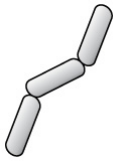
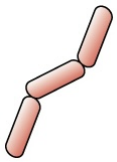
Gram staining steps	Cells effects	Gram-positive	Gram-negative
Step 1 **Crystal violet** *primary stain added to specimen smear*	Stains cells purple or blue.		
Step 2 **Iodine** *mordant makes dye less soluble so it adheres to cell walls.*	Stains cells purple or blue.		
Step 3 **Alcohol** *decolorizer washes away stain from gram-negative cell walls.*	Gram-positive cells remain purple or blue. Gram-negative cells are colorless.		
Step 4 **Safranin** *counterstain allows dye adherence to gram-negative cells.*	Gram-positive cells remain purple or blue. Gram-negative cells appear pink or red.		

Table 2.5: Gram staining process. Gram-staining is a differential staining technique that uses a primary stain and a secondary counterstain to distinguish between gram-positive and gram-negative bacteria.

ACID-FAST STAINS

Acid-fast staining (tables 2.5 and 2.6) is another commonly used, differential staining technique that can be an important diagnostic tool. An acid-fast stain is able to differentiate two types of gram-positive cells: those that have waxy mycolic acids in their cell walls and those that do not. Two different methods for acid-fast staining are the Ziehl-Neelsen technique and the Kinyoun technique. Both use carbolfuchsin as the primary stain. The waxy, acid-fast cells retain the carbolfuchsin even after a decolorizing agent (an acid-alcohol solution) is applied. A secondary counterstain, methylene blue, is then applied, which renders non–acid-fast cells blue.

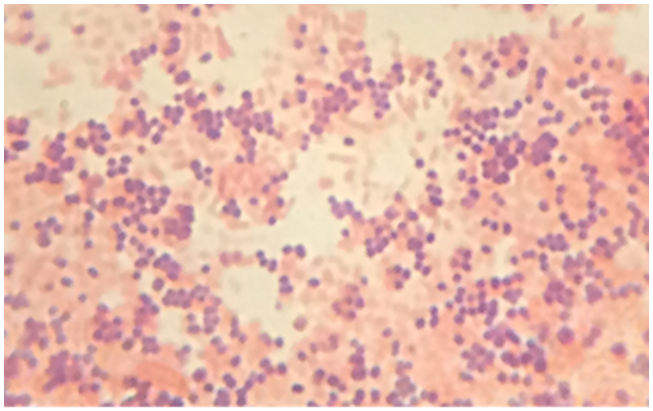


Figure 2.16: In this specimen, the gram-positive bacterium Staphylococcus aureus retains crystal violet dye even after the decolorizing agent is added. Gram-negative Escherichia coli, the most common Gram stain quality-control bacterium, is decolorized, and is only visible after the addition of the pink counterstain safranin. *Figure description available at the end of the chapter.*

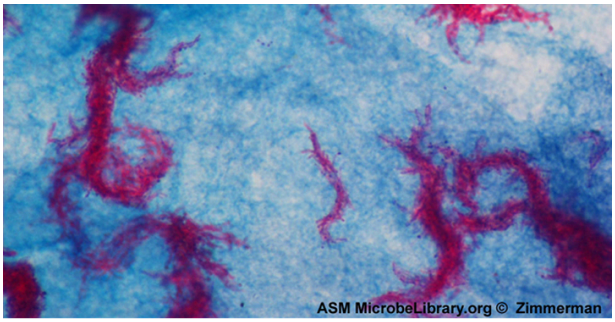


Figure 2.17: Ziehl-Neelsen staining has rendered these Mycobacterium tuberculosis cells red and the surrounding growth indicator methylene blue. Figure description available at the end of the chapter.

Mycobacterium tuberculosis, the bacterium that causes tuberculosis, can be detected in specimens based on the presence of acid-fast bacilli. Often, a smear is prepared from a sample of the patient's sputum and then stained using the Ziehl-Neelsen technique (figure 2.17). If acid-fast bacteria are confirmed, they are generally cultured to make a positive identification. Variations of this approach can be used as a first step in determining whether *M. tuberculosis* or other acid-fast bacteria are present, though samples from elsewhere in the body (such as urine) may contain other *Mycobacterium* species (figure 2.42).

An alternative approach for determining the presence of *M. tuberculosis* is immunofluorescence. In this technique, fluorochrome-labeled antibodies bind to *M. tuberculosis*, if present. Antibody-specific fluorescent dyes can be used to view the mycobacteria with a fluorescence microscope.

CAPSULE STAINING

Certain bacteria and yeasts have a protective outer structure called a capsule. Since the presence of a capsule is directly related to a microbe's virulence (its ability to cause disease), the ability to determine whether cells in a sample have capsules is an important diagnostic tool. Capsules do not absorb most basic dyes; therefore, a negative staining technique (staining around the cells) is typically used for capsule staining. The dye stains the background but does not penetrate the capsules, which appear like halos around the borders of the cell. The specimen does not need to be heat-fixed prior to negative staining.

One common negative staining technique for identifying encapsulated yeast and bacteria is to add a few drops of India ink or nigrosin to a specimen. Other capsular stains can also be used to negatively stain encapsulated cells (figure 2.18). Alternatively, positive and negative staining techniques can be combined to visualize capsules: The positive stain colors the body of the cell, and the negative stain colors the background but not the capsule, leaving a halo around each cell.

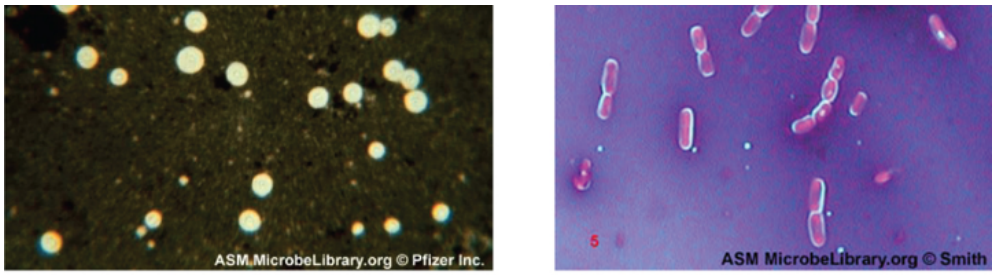


Figure 2.18: (a) India-ink was used to stain the background around these cells of the yeast Cryptococcus neoformans. The halos surrounding the cells are the polysaccharide capsules. (b) Crystal violet and copper sulfate dyes cannot penetrate the encapsulated Bacillus cells in this negatively stained sample. Encapsulated cells appear to have a light-blue halo. Figure description available at the end of the chapter.

ENDOSPORE STAINING

Endospores are structures produced within certain bacterial cells that allow them to survive harsh conditions. Gram staining alone cannot be used to visualize endospores, which appear clear when Gram-stained cells are viewed. Endospore staining uses two stains to differentiate endospores from the rest of the cell. The Schaeffer-Fulton method (the most commonly used endospore-staining technique) uses heat to permeabilize the cortex and protein spore coat, allowing the primary stain to penetrate into the endospore. Washing with water decolorizes the cell, but the endospore retains the green stain. The cell is then counterstained pink with safranin. The resulting image reveals the shape and location of endospores, if they are present. The green endospores will appear either within the pink vegetative cells or as separate from the pink cells altogether. If no endospores are present, then only the pink vegetative cells will be visible (figure 2.19).

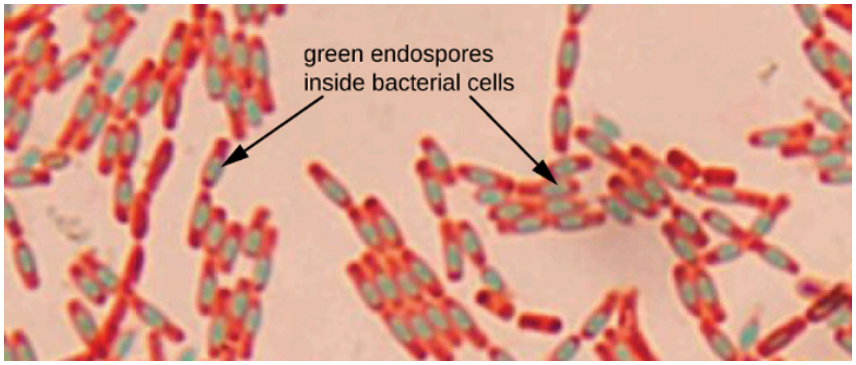


Figure 2.19: A stained preparation of Bacillus subtilis showing endospores as green and the vegetative cells as pink. *Figure description available at the end of the chapter.*

Endospore-staining techniques are important for identifying *Bacillus* and *Clostridium*, two genera of endospore-producing bacteria that contain clinically significant species. Among others, *B. anthracis* (which causes anthrax) has been of particular interest because of concern that its spores could be used as a bioterrorism agent. *C. difficile* is a particularly important species responsible for the typically hospital-acquired infection known as "C. diff."

FLAGELLA STAINING

Flagella (singular: flagellum) are tail-like cellular structures used for locomotion by some bacteria, archaea, and eukaryotes. Because they are so thin, flagella typically cannot be seen under a light microscope without a specialized flagella staining technique. Flagella staining thickens the flagella by first applying mordant (generally tannic acid, but sometimes potassium alum), which coats the flagella; then the specimen is stained with pararosaniline (most commonly) or basic fuchsin (figure 2.20).

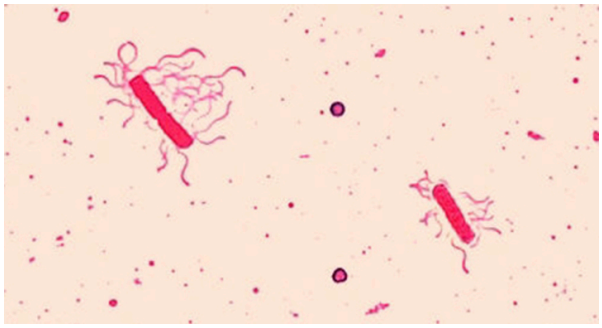


Figure 2.20: A flagella stain of Bacillus cereus, a common cause of foodborne illness, reveals that the cells have numerous flagella used for locomotion. *Figure description available at the end of the chapter.*

Though flagella staining is uncommon in clinical settings, the technique is commonly used by microbiologists, since the location and number of flagella can be useful in classifying and identifying bacteria in a sample.

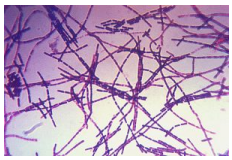
Stain Type	Specific Dyes	Purpose	Outcome	Sample Images
Basic stains	Methylene blue, crystal violet, malachite green, basic fuchsin, carbolfuchsin, safranin	Stain negatively charged molecules and structures such as nucleic acids and proteins	Positive stain	
Acidic stains	Eosin, acid fuchsin, rose bengal, Congo red	Stain positively charged molecules and structures such as proteins	Can be either a positive or negative stain, depending on the cells chemistry	
Negative stains	India ink, nigrosin	Stains background, not specimen	Dark background with light specimen	

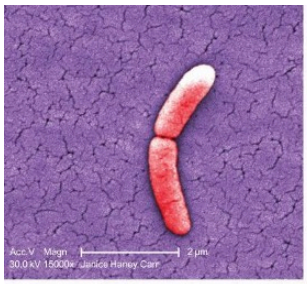
Table 2.6: Simple stains.

2.3 HOW MICROBES GROW

The bacterial cell cycle involves the formation of new cells through the replication of DNA and partitioning of cellular components into two daughter cells. In prokaryotes, reproduction is always asexual, although extensive genetic recombination in the form of horizontal gene transfer takes place. Most bacteria have a single circular chromosome; however, some exceptions exist. For example, *Borrelia burgdorferi*, the causative agent of Lyme disease, has a linear chromosome.

BINARY FISSION

The most common mechanism of cell replication in bacteria is a process called binary fission, which is depicted in figure 2.21. Before dividing, the cell grows and increases its number of cellular components. Next, the replication of DNA starts at a location on the circular chromosome called the origin of replication, where the chromosome is attached to the inner cell membrane. Replication continues in opposite directions along the chromosome until the terminus is reached.



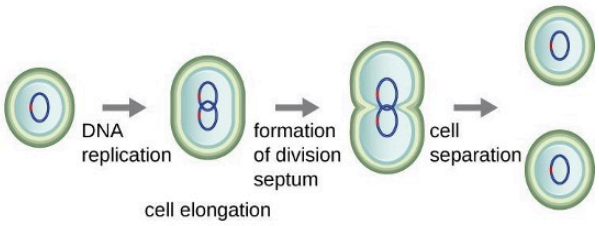


Figure 2.21: (a) The electron micrograph depicts two cells of Salmonella typhimurium after a binary fission event. (b) Binary fission in bacteria starts with the replication of DNA as the cell elongates. A division septum forms in the center of the cell. Two daughter cells of similar size form and separate, each receiving a copy of the original chromosome. *Figure description available at the end of the chapter.*

THE GROWTH CURVE

Microorganisms grown in a closed culture (also known as a batch culture), in which no nutrients are added and most waste is not removed, follow a reproducible growth pattern referred to as the growth curve. The culture density is defined as the number of cells per unit volume. In a closed environment, the culture density is also a measure of the number of cells in the population. Infections of the body do not always follow the growth curve, but correlations can exist depending upon the site and type of infection. When the number of live cells is plotted against time, distinct phases can be observed in the curve (figure 2.22).

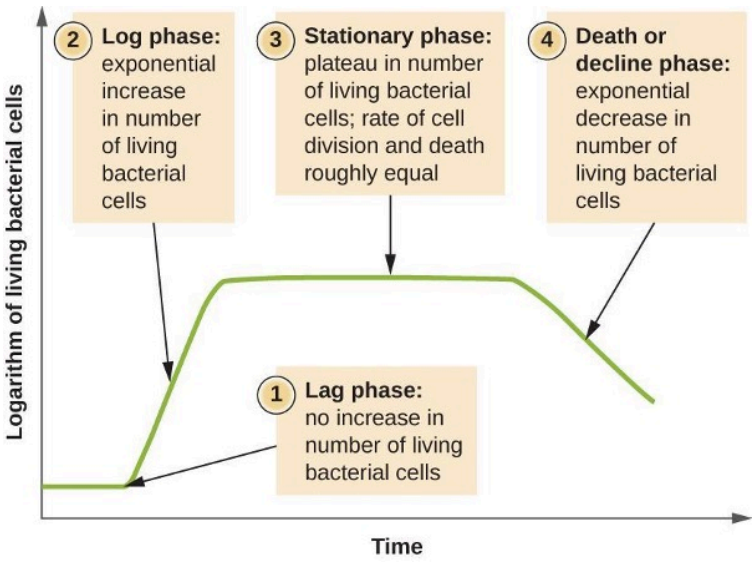


Figure 2.22: The growth curve of a bacterial culture is represented by the logarithm of the number of live cells plotted as a function of time. The graph can be divided into four phases according to the slope, each of which matches events in the cell. The four phases are lag, log, stationary, and death. *Figure description available at the end of the chapter.*

The Lag Phase

The beginning of the growth curve represents a small number of cells, referred to as an inoculum, that are added to a fresh culture medium, a nutritional broth that supports growth. The initial phase of the growth curve is called the lag phase, during which cells are gearing up for the next phase of growth. The number of cells does not change during the lag phase; however, cells grow larger and are metabolically active, synthesizing proteins needed to grow within the medium. If any cells were damaged or shocked during the transfer to the new medium, repair takes place during the lag phase. The duration of the lag phase is determined by many factors, including the species and genetic make-up of the cells, the composition of the medium, the temperature of the medium, and the size of the original inoculum.

The Log Phase

In the logarithmic (log) growth phase, sometimes called the exponential growth phase, the cells are actively dividing by binary fission and their number increases exponentially. For any given bacterial species, the generation time under specific growth conditions (nutrients, temperature, pH, and so forth) is genetically determined, and this generation time is called the intrinsic growth rate. During the log phase, the relationship between time and number of cells is not linear but exponential; however, the growth curve is often plotted on a semilogarithmic graph, as shown in figure 2.23, which gives the appearance of a linear relationship.

Cells in the log phase show constant growth rate and uniform metabolic activity. For this reason, cells in the log phase are preferentially used for industrial applications and research work. The log phase is also the stage where bacteria are the most susceptible to the action of disinfectants and common antibiotics that affect protein, DNA, and cell-wall synthesis.

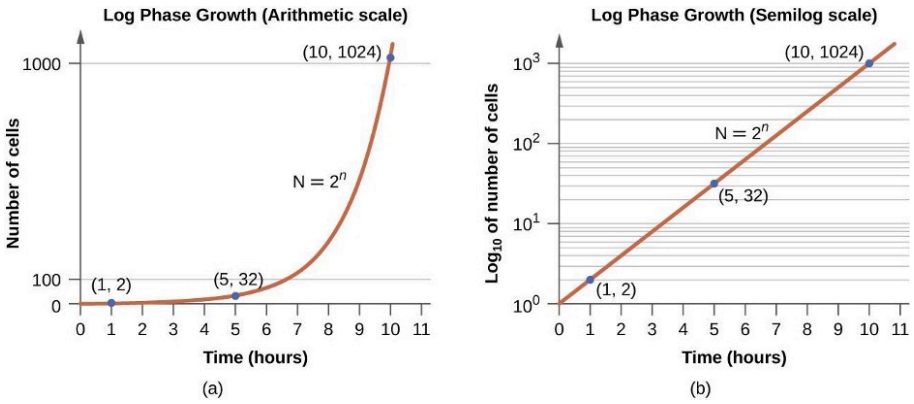


Figure 2.23: Both graphs illustrate population growth during the log phase for a bacterial sample with an initial population of one cell and a doubling time of 1 hour. (a) When plotted on an arithmetic scale, the growth rate resembles a curve. (b) When plotted on a semilogarithmic scale (meaning the values on the y-axis are logarithmic), the growth rate appears linear. *Figure description available at the end of the chapter.*

Stationary Phase

As the number of cells increases through the log phase, several factors contribute to a slowing of the growth rate. Waste products accumulate and nutrients are gradually used up. In addition, gradual depletion of oxygen begins to limit aerobic cell growth. This combination of unfavorable conditions slows and finally stalls population growth. The total number of live cells reaches a plateau referred to as the stationary phase (figure 2.22). In this phase, the number of new cells created by cell division is now equivalent to the number of cells dying; thus, the total population of living cells is relatively stagnant. The culture density in a stationary culture is constant. The culture's carrying capacity, or maximum culture density, depends on the types of microorganisms in the culture and the specific conditions of the culture; however, carrying capacity is constant for a given organism grown under the same conditions.

During the stationary phase, cells switch to a survival mode of metabolism. As growth slows, so too does the synthesis of peptidoglycans, proteins, and nucleic-acids; thus, stationary cultures are less susceptible to antibiotics that disrupt these processes. In bacteria capable of producing endospores, many cells undergo sporulation during the stationary phase. Secondary metabolites, including antibiotics, are synthesized in the stationary phase. In certain pathogenic bacteria, the stationary phase is also associated with the expression of virulence factors, products that contribute to a microbe's ability to survive, reproduce, and cause disease in a host organism. For example, quorum sensing in *Staphylococcus aureus* initiates the production of enzymes that can break down human tissue and cellular debris, clearing the way for bacteria to spread to new tissue where nutrients are more plentiful.

The Death Phase

As a culture medium accumulates toxic by-products and nutrients are exhausted, cells die in greater and greater numbers. Soon, the number of dying cells exceeds the number of dividing cells, leading to an exponential decrease in the number of cells (figure 2.22). This is the aptly named death phase, sometimes called the decline phase. Many cells lyse and release nutrients into the medium, allowing surviving cells to maintain viability and form endospores. A few cells, the so-called persisters, are characterized by a slow metabolic rate. Persister cells are medically important because they are associated with certain chronic infections, such as tuberculosis, that do not respond to antibiotic treatment.

2.4 PROTEOBACTERIA

In 1987, the American microbiologist Carl Woese (1928–2012) suggested that a large and diverse group of bacteria that he called "purple bacteria and their relatives" should be defined as a separate phylum within the domain *Bacteria* based on the similarity of the nucleotide sequences in their genome.[6] This phylum of gram-negative bacteria subsequently received the name Proteobacteria. It includes many bacteria that are part of the normal human microbiota as well as many pathogens. The Proteobacteria are further divided into five classes: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, and Epsilonproteobacteria.

ALPHAPROTEOBACTERIA

The first class of Proteobacteria is the Alphaproteobacteria, many of which are obligate or facultative intracellular bacteria. Some species are characterized as oligotrophs, organisms capable of living in low-nutrient environments such as deep oceanic sediments, glacial ice, or deep undersurface soil.

Among the Alphaproteobacteria are *rickettsias*, obligate intracellular pathogens, that require part of their life cycle to occur inside other cells called host cells. When not growing inside a host cell, *Rickettsia* are metabolically inactive outside the host cell. They cannot synthesize their own adenosine triphosphate (ATP), and, therefore, rely on cells for their energy needs.

Rickettsia spp. include a number of serious human pathogens. For example, *R. rickettsii* causes Rocky Mountain spotted fever, a life-threatening form of meningoencephalitis (inflammation of the membranes that wrap the brain). *R. rickettsii* infects ticks and can be transmitted to humans via a bite from an infected tick (figure 2.24).

Another species of *Rickettsia*, *R. prowazekii*, is spread by lice. It causes epidemic typhus, a severe infectious disease common during warfare and mass migrations of people. *R. prowazekii* infects human endothelial cells, causing inflammation of the inner lining of blood vessels, high fever, abdominal pain, and sometimes delirium. A relative, *R. typhi*, causes a less severe disease known as murine or endemic typhus, which is still observed in the southwestern United States during warm seasons.

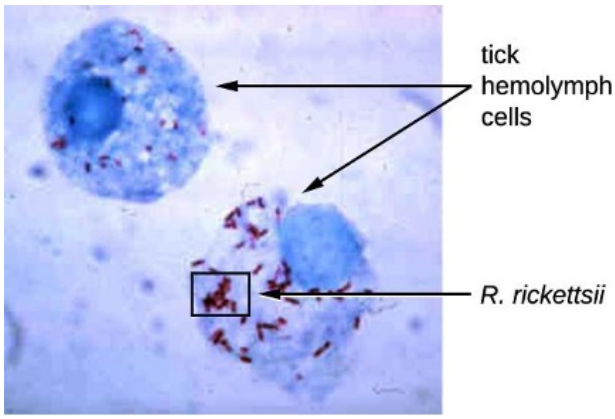


Figure 2.24: Rickettsias require special staining methods to see them under a microscope. Here, R. rickettsii, which causes Rocky Mountain spotted fever, is shown infecting the cells of a tick. *Figure description available at the end of the chapter.*

Table 2.7 summarizes the characteristics of important genera of Alphaproteobacteria.

Genus	Microscopic Morphology	Unique Characteristics
Agrobacterium	Gram-negative bacillus	Plant pathogen; one species, *A. tumefaciens*, causes tumors in plants
Bartonella	Gram-negative, pleomorphic, flagellated coccobacillus	Facultative intracellular bacteria, transmitted by lice and fleas, cause trench fever and cat scratch disease in humans
Brucella	Gram-negative, small, flagellated coccobacillus	Facultative intracellular bacteria, transmitted by contaminated milk from infected cows, cause brucellosis in cattle and humans
Caulobacter	Gram-negative bacillus	Used in studies on cellular adaptation and differentiation because of its peculiar life cycle (during cell division, forms swarm cells and stalked cells)
Coxiella	Small, gram-negative bacillus	Obligatory intracellular bacteria; cause Q fever; potential for use as biological weapon
Ehrlichia	Very small, gram-negative, coccoid or ovoid bacteria	Obligatory intracellular bacteria; can be transported from cell to cell; transmitted by ticks; cause ehrlichiosis (destruction of white blood cells and inflammation) in humans and dogs
Hyphomicrobium	Gram-negative bacilli; grows from a stalk	Similar to *Caulobacter* (above)
Methylocystis	Gram-negative, coccoid or short bacilli	Nitrogen-fixing aerobic bacteria
Rhizobium	Gram-negative, rectangular bacilli with rounded ends forming clusters	Nitrogen-fixing bacteria that live in soil and form symbiotic relationship with roots of legumes (e.g., clover, alfalfa, and beans)
Rickettsia	Gram-negative, highly pleomorphic bacteria (may be cocci, rods, or threads)	Obligate intracellular bacteria; transmitted by ticks; may cause Rocky Mountain spotted fever and typhus

Table 2.7: Class Alphaproteobacteria

BETAPROTEOBACTERIA

Betaproteobacteria are a diverse group of bacteria. The different bacterial species within this group utilize a wide range of metabolic strategies and can survive in a range of environments. Some genera include species that are human pathogens, able to cause severe, sometimes life-threatening disease. The genus *Neisseria*, for example, includes the bacteria *N. gonorrhoeae,* the causative agent of the STI gonorrhea, and *N. meningitides*, the causative agent of bacterial meningitis.

Neisseria are cocci that live on mucosal surfaces of the human body. They are fastidious, being difficult to culture as well as requiring high levels of moisture, nutrient supplements, and carbon dioxide. Also, *Neisseria* are microaerophilic, meaning that they require low levels of oxygen. For optimal growth and for the purposes of identification, *Neisseria* spp. are grown on chocolate agar (i.e., agar supplemented by partially hemolyzed red blood cells). Their characteristic pattern of growth in culture is diplococcal: pairs of cells resembling coffee beans (figure 2.25).

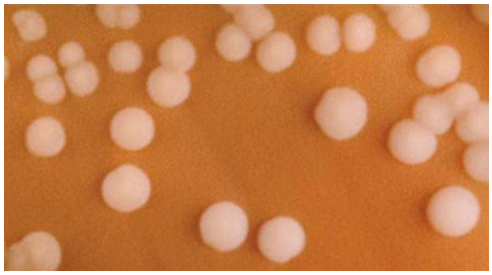


Figure 2.25: Neisseria meningitidis growing in colonies on a chocolate agar plate. Figure description available at the end of the chapter.

The pathogen responsible for pertussis (whooping cough) is also a member of Betaproteobacteria. The bacterium *Bordetella pertussis*, from the order Burkholderiales, produces several toxins that paralyze the movement of cilia in the human respiratory tract and directly damage cells of the respiratory tract, causing a severe cough.

Table 2.8 summarizes the characteristics of important genera of Betaproteobacteria.

Example Genus	Microscopic Morphology	Unique Characteristics
Bordetella	A small, gram-negative coccobacillus	Aerobic, very fastidious; *B. pertussis* causes pertussis (whooping cough)
Burkholderia	Gram-negative bacillus	Aerobic, aquatic, cause diseases in horses and humans (especially patients with cystic fibrosis); agents of nosocomial infections
Leptothrix	Gram-negative, sheathed, filamentous bacillus	Aquatic; oxidize iron and manganese; can live in wastewater treatment plants and clog pipes
Neisseria	Gram-negative, coffee bean-shaped coccus forming pairs	Require moisture and high concentration of carbon dioxide; oxidase positive, grow on chocolate agar; pathogenic species cause gonorrhea and meningitis
Thiobacillus	Gram-negative bacillus	Thermophilic, acidophilic, strictly aerobic bacteria; oxidize iron and sulfur

Table 2.8: Class Betaproteobacteria

GAMMAPROTEOBACTERIA

The most diverse class of gram-negative bacteria is Gammaproteobacteria, and it includes a number of human pathogens. For example, a large and diverse family, *Pseudomonaceae*, includes the genus *Pseudomonas*. Within this genus is the species *P. aeruginosa*, a pathogen responsible for diverse infections in various regions of the body. *P. aeruginosa* is a strictly aerobic, nonfermenting, highly motile bacterium. It often infects wounds and burns, can be the cause of chronic urinary tract infections, and can be an important cause of respiratory infections in patients with cystic fibrosis or patients on mechanical ventilators. Infections by *P. aeruginosa* are often difficult to treat because the bacterium is resistant to many antibiotics and has a remarkable ability to form biofilms. Other representatives of *Pseudomonas* include the fluorescent (glowing) bacterium *P. fluorescens* and the soil bacteria *P. putida*, which is known for its ability to degrade xenobiotics (substances not naturally produced or found in living organisms).

The *Pasteurellaceae* also includes several clinically relevant genera and species. This family includes several bacteria that are human and/or animal pathogens. For example, *Pasteurella haemolytica* causes severe pneumonia in sheep and goats. *P. multocida* is a species that can be transmitted from animals to humans through bites, causing infections of the skin and deeper tissues. The genus *Haemophilus* contains two human pathogens, *H. influenzae* and *H. ducreyi*. Despite its name, *H. influenzae* does not cause influenza (which is a viral disease). *H. influenzae* can cause both upper and lower respiratory tract infections, including sinusitis, bronchitis, ear infections, and pneumonia. Before the development of effective vaccination, strains of *H. influenzae* were a leading cause of more invasive diseases, like meningitis in children. *H. ducreyi* causes the STI known as chancroid.

The order *Vibrionales* includes the human pathogen *Vibrio cholerae*. This comma-shaped aquatic bacterium thrives in highly alkaline environments like shallow lagoons and sea ports. A toxin produced by *V. cholerae* causes hypersecretion of electrolytes and water in the large intestine, leading to profuse watery diarrhea and dehydration. *V. parahaemolyticus* is also a cause of gastrointestinal disease in humans, whereas *V. vulnificus* causes serious and potentially life-threatening cellulitis (infection of the skin and deeper tissues) and blood-borne infections. Another representative of *Vibrionales*, *Aliivibrio fischeri*, engages in a symbiotic relationship with squid. The squid provides nutrients for the bacteria to grow and the bacteria produce bioluminescence that protects the squid from predators (figure 2.26).

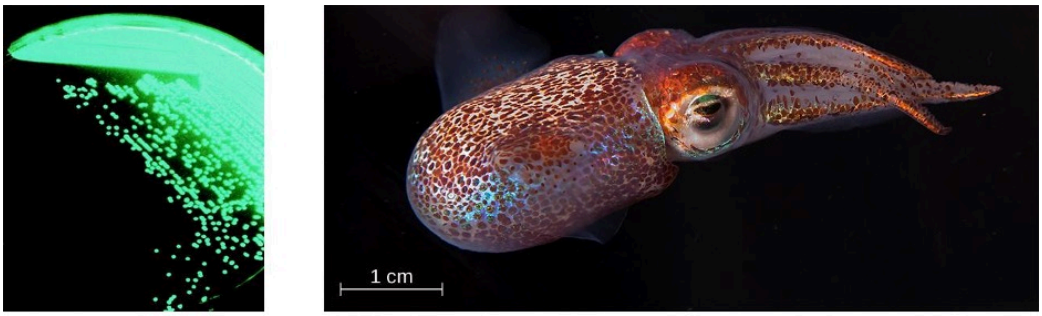


Figure 2.26: (a) Aliivibrio fischeri is a bioluminescent bacterium. (b) A. fischeri colonizes and lives in a mutualistic relationship with the Hawaiian bobtail squid (Euprymna scolopes). *Figure description available at the end of the chapter.*

The genus *Legionella* also belongs to the Gammaproteobacteria. *L. pneumophila,* the pathogen responsible for Legionnaires disease, is an aquatic bacterium that tends to inhabit pools of warm water, such as those found in the tanks of air conditioning units in large buildings (figure 2.27). Because the bacteria can spread in aerosols, outbreaks of Legionnaires disease often affect residents of a building in which the water has become contaminated with *Legionella.* In fact, these bacteria derive their name from the first known outbreak of Legionnaires disease, which occurred in a hotel hosting an American Legion veterans' association convention in Philadelphia in 1976.

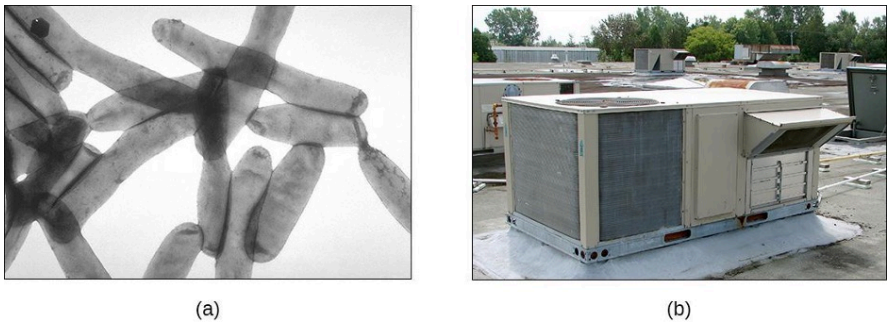


Figure 2.27: (a) Legionella pneumophila, the causative agent of Legionnaires disease, thrives in warm water. (b) Outbreaks of Legionnaires disease often originate in the air conditioning units of large buildings when water in or near the system becomes contaminated with L. pneumophila. *Figure description available at the end of the chapter.*

Enterobacteriaceae is a large family of enteric (intestinal) bacteria belonging to the Gammaproteobacteria. They are facultative anaerobes and are able to ferment carbohydrates. Within this family, microbiologists recognize two distinct categories. The first category is called the coliforms, after its prototypical bacterium species, *Escherichia coli*. Coliforms are able to ferment lactose completely (i.e., with the production of acid and gas). The second category, noncoliforms, either cannot ferment lactose or can only ferment it incompletely (producing either acid or gas, but not both). The noncoliforms include some notable human pathogens, such as *Salmonella* spp., *Shigella* spp., and *Yersinia pestis*.

E. coli has been perhaps the most studied bacterium since it was first described in 1886 by Theodor Escherich (1857–1911). Many strains of *E. coli* are in mutualistic relationships with humans. However, some strains produce a potentially deadly toxin called Shiga toxin. Shiga toxin is one of the most potent bacterial toxins identified. Upon entering target cells, Shiga toxin interacts with ribosomes, stopping protein synthesis. Lack of protein synthesis leads to cellular death and hemorrhagic colitis, characterized by inflammation of intestinal tract and bloody diarrhea. In the most severe cases, patients can develop a deadly hemolytic uremic syndrome. Other *E. coli* strains may cause traveler's diarrhea, a less severe but very widespread disease.

The genus *Salmonella*, which belongs to the noncoliform group of *Enterobacteriaceae*, is interesting in that there is still no consensus about how many species it includes. Scientists have reclassified many of the groups they once thought to be species as serotypes (also called serovars), which are strains or variations of the same species of bacteria. Their classification is based on patterns of reactivity by animal antisera against molecules on the surface of the bacterial cells. A number of serotypes of *Salmonella* can cause salmonellosis, characterized by inflammation of the small and the large intestine, accompanied by fever, vomiting, and diarrhea. The species *S. enterobacterica* (serovar *typhi*) causes typhoid fever, with symptoms including fever, abdominal pain, and skin rashes (figure 2.28).

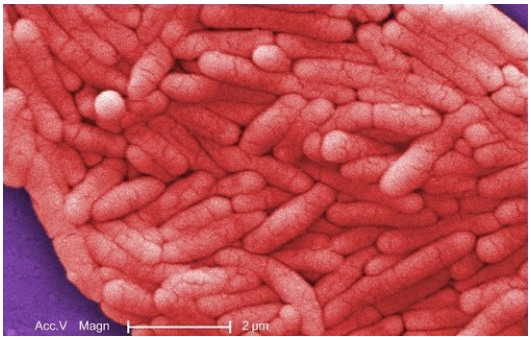


Figure 2.28: Salmonella typhi is the causative agent of typhoid fever. *Figure description available at the end of the chapter.*

Table 2.9 summarizes the characteristics of important genera of *Gammaproteobacteria*.

Example Genus	Microscopic Morphology	Unique Characteristics
Beggiatoa	Gram-negative bacteria; disc-shaped or cylindrical	Aquatic, live in water with high content of hydrogen disulfide; can cause problems for sewage treatment
Enterobacter	Gram-negative bacillus	Facultative anaerobe; cause urinary and respiratory tract infections in hospitalized patients; implicated in the pathogenesis of obesity
Erwinia	Gram-negative bacillus	Plant pathogen causing leaf spots and discoloration; may digest cellulose; prefer relatively low temperatures (25–30 °C)
Escherichia	Gram-negative bacillus	Facultative anaerobe; inhabit the gastrointestinal tract of warm-blooded animals; some strains are mutualists, producing vitamin K; others, like serotype *E. coli* O157:H7, are pathogens; *E. coli* has been a model organism for many studies in genetics and molecular biology

Example Genus	Microscopic Morphology	Unique Characteristics
Hemophilus	Gram-negative bacillus	Pleomorphic, may appear as coccobacillus, aerobe, or facultative anaerobe; grow on blood agar; pathogenic species can cause respiratory infections, chancroid, and other diseases
Klebsiella	Gram-negative bacillus; appears rounder and thicker than other members of Enterobacteriaceae	Facultative anaerobe, encapsulated, nonmotile; pathogenic species may cause pneumonia, especially in people with alcoholism
Legionella	Gram-negative bacillus	Fastidious, grow on charcoal-buffered yeast extract; *L. pneumophila* causes Legionnaires disease
Methylomonas	Gram-negative bacillus	Use methane as source of carbon and energy
Proteus	Gram-negative bacillus (pleomorphic)	Common inhabitants of the human gastrointestinal tract; motile; produce urease; opportunistic pathogens; may cause urinary tract infections and sepsis
Pseudomonas	Gram-negative bacillus	Aerobic; versatile; produce yellow and blue pigments, making them appear green in culture; opportunistic, antibiotic-resistant pathogens may cause wound infections, hospital-acquired infections, and secondary infections in patients with cystic fibrosis
Serratia	Gram-negative bacillus	Motile; may produce red pigment; opportunistic pathogens responsible for a large number of hospital-acquired infections
Shigella	Gram-negative bacillus	Nonmotile; dangerously pathogenic; produce Shiga toxin, which can destroy cells of the gastrointestinal tract; can cause dysentery
Vibrio	Gram-negative, comma- or curved rod-shaped bacteria	Inhabit seawater; flagellated, motile; may produce toxin that causes hypersecretion of water and electrolytes in the gastrointestinal tract; some species may cause serious wound infections
Yersinia	Gram-negative bacillus	Carried by rodents; human pathogens; *Y. pestis* causes bubonic plague and pneumonic plague; *Y. enterocolitica* can be a pathogen causing diarrhea in humans

Table 2.9: Class Gammaproteobacteria

DELTAPROTEOBACTERIA

The Deltaproteobacteria is a small class of gram-negative Proteobacteria that includes sulfate-reducing bacteria (SRBs), so named because they use sulfate as the final electron acceptor in the electron transport chain. Few SRBs are pathogenic. However, the SRB *Desulfovibrio orale* is associated with periodontal disease (disease of the gums).

Deltaproteobacteria also includes the genus *Bdellovibrio*, species of which are parasites of other gram-negative bacteria. *Bdellovibrio* invades the cells of the host bacterium, positioning itself in the periplasm, the space between the plasma membrane and the cell wall, feeding on the host's proteins and polysaccharides. The infection is lethal for the host cells.

Another type of Deltaproteobacteria, myxobacteria, lives in the soil, scavenging inorganic compounds. Motile and highly social, they interact with other bacteria within and outside their own group. They can form multicellular, macroscopic **fruiting bodies** (figure 2.29), structures that are still being studied by biologists and bacterial ecologists.[7] These bacteria can also form metabolically inactive myxospores.

Table 2.10 summarizes the characteristics of several important genera of Deltaproteobacteria.

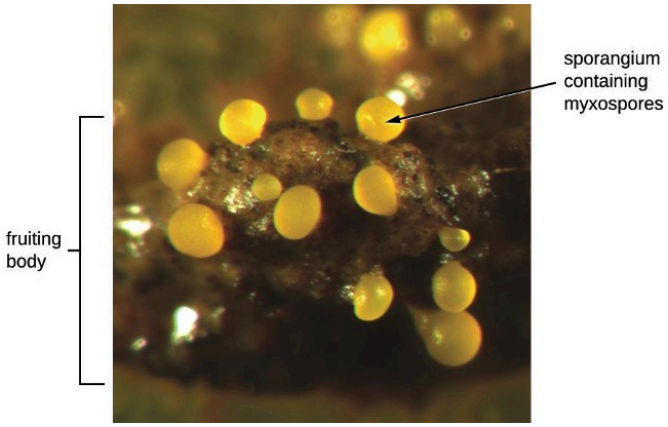


Figure 2.29: Myxobacteria form fruiting bodies. Figure description available at the end of the chapter.

Genus	Microscopic Morphology	Unique Characteristics
Bdellovibrio	Gram-negative, comma-shaped rod	Obligate aerobes; motile; parasitic (infecting other bacteria)
Desulfovibrio (formerly *Desufuromonas*)	Gram-negative, comma-shaped rod	Reduce sulfur; can be used for removal of toxic and radioactive waste
Myxobacterium	Gram-negative, coccoid bacteria forming colonies (swarms)	Live in soil; can move by gliding; used as a model organism for studies of intercellular communication (signaling)

Table 2.10: Class Deltaproteobacteria

EPSILONPROTEOBACTERIA

The smallest class of Proteobacteria is Epsilonproteobacteria, which are gram-negative microaerophilic bacteria (meaning they only require small amounts of oxygen in their environment). Two clinically relevant genera of Epsilonproteobacteria are *Campylobacter* and *Helicobacter*, both of which include human pathogens. *Campylobacter* can cause food poisoning that manifests as severe enteritis (inflammation in the small intestine). This condition, caused by the species *C. jejuni*, is rather common in developed countries, usually because of eating contaminated poultry products. Chickens often harbor *C. jejuni* in their gastrointestinal tract and feces, and their meat can become contaminated during processing.

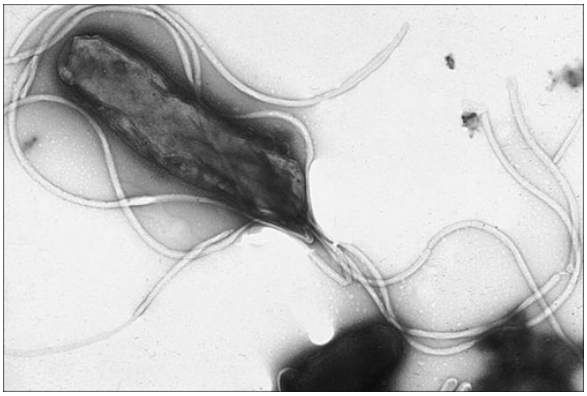


Figure 2.30: Helicobacter pylori can cause chronic gastritis, which can lead to ulcers and stomach cancer. Figure description available at the end of the chapter.

Within the genus *Helicobacter,* the helical, flagellated bacterium *H. pylori* has been identified as a beneficial member of the stomach microbiota, but it is also the most common cause of chronic gastritis and ulcers of the stomach and duodenum (figure 2.30). Studies have also shown that *H. pylori* is linked to stomach cancer.[8] *H. pylori* is somewhat unusual in its ability to survive in the highly acidic environment of the stomach. It produces urease and other enzymes that modify its environment to make it less acidic.

Table 2.11 summarizes the characteristics of the most clinically relevant genera of Epsilonproteobacteria.

Example Genus	Microscopic Morphology	Unique Characteristics
Campylobacter	Gram-negative, spiral-shaped rod	Aerobic (microaerophilic); often infects chickens; may infect humans via undercooked meat, causing severe enteritis
Helicobacter	Gram-negative, spiral-shaped rod	Aerobic (microaerophilic) bacterium; can damage the inner lining of the stomach, causing chronic gastritis, peptic ulcers, and stomach cancer

Table 2.11: Class Epsilonproteobacteria

2.5 NONPROTEOBACTERIA GRAM-NEGATIVE BACTERIA AND PHOTOTROPHIC BACTERIA

The majority of the gram-negative bacteria belong to the phylum Proteobacteria. Those that do not are called the nonproteobacteria. In this section, we will describe four classes of gram-negative nonproteobacteria: *Chlamydia*, the spirochetes, the CFB group, and the Planctomycetes. A diverse group of phototrophic bacteria that includes Proteobacteria and nonproteobacteria will be discussed at the end of this section.

CHLAMYDIA

C. trachomatis is a human pathogen that causes trachoma, a disease of the eyes, often leading to blindness. *C. trachomatis* also causes the sexually transmitted disease lymphogranuloma venereum (LGV). This disease is often mildly symptomatic, manifesting as regional lymph node swelling, or it may be asymptomatic, but it is extremely contagious and is common on college campuses.

Members of the genus *Chlamydia* are gram-negative, obligate intracellular pathogens that are extremely resistant to the cellular defenses, giving them the ability to spread from host to host rapidly via elementary bodies. The metabolically and reproductively inactive elementary bodies are an endospore-like form of intracellular bacteria that enter an epithelial cell, where they become active. Figure 2.31 illustrates the life cycle of *Chlamydia*.

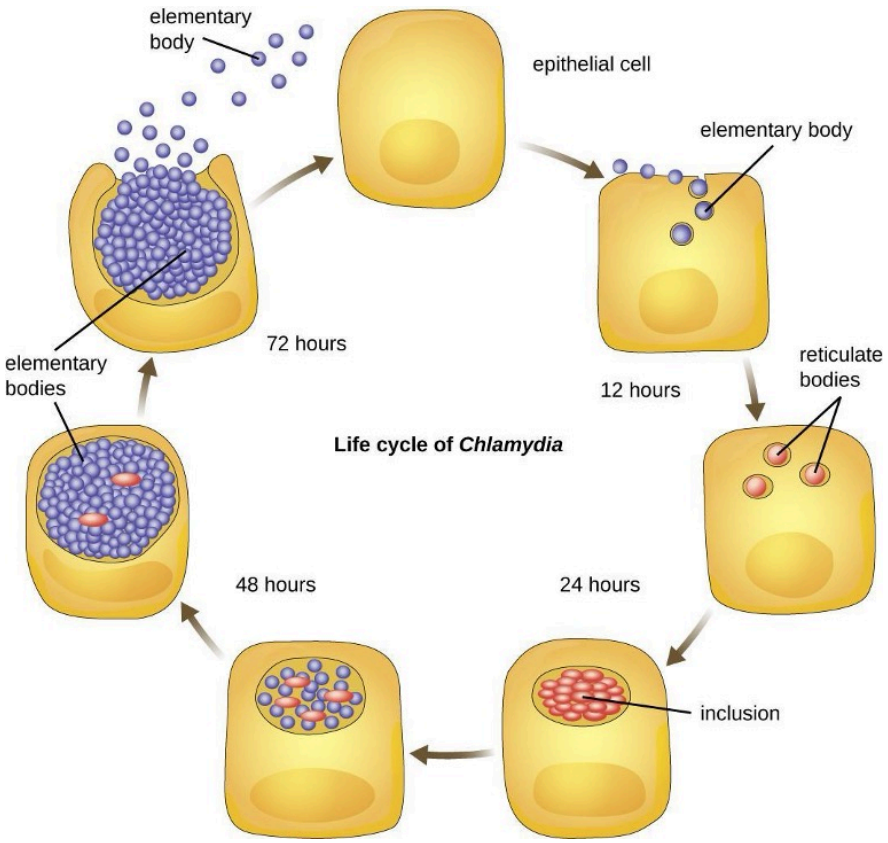


Figure 2.31: Chlamydia begins infection of a host when the metabolically inactive elementary bodies enter an epithelial cell. Once inside the host cell, the elementary bodies turn into active reticulate bodies. The reticulate bodies multiply and release more elementary bodies when the cell dies after the Chlamydia uses all of the host cell's ATP. *Figure description available at the end of the chapter.*

SPIROCHETES

Spirochetes are characterized by their long (up to 250 μm), spiral-shaped bodies. Most spirochetes are also very thin, which makes it difficult to examine gram-stained preparations under a conventional brightfield microscope. Darkfield fluorescent microscopy is typically used instead. Spirochetes are also difficult or even impossible to culture. They are highly motile, using their axial filament to propel themselves. The axial filament is similar to a flagellum, but it wraps around the cell and runs inside the cell body of a spirochete in the periplasmic space between the outer membrane and the plasma membrane (figure 2.32).

Several genera of spirochetes include human pathogens. For example, the genus *Treponema* includes a species *T. pallidum*, which is further classified into four subspecies: *T. pallidum pallidum*, *T. pallidum pertenue, T. pallidum carateum,* and *T. pallidum endemicum*. The subspecies *T. pallidum pallidum* causes the sexually transmitted infection known as syphilis, the third most prevalent sexually transmitted bacterial infection in the United States, after chlamydia and gonorrhea. The other subspecies of *T. pallidum* cause tropical infectious diseases of the skin, bones, and joints.

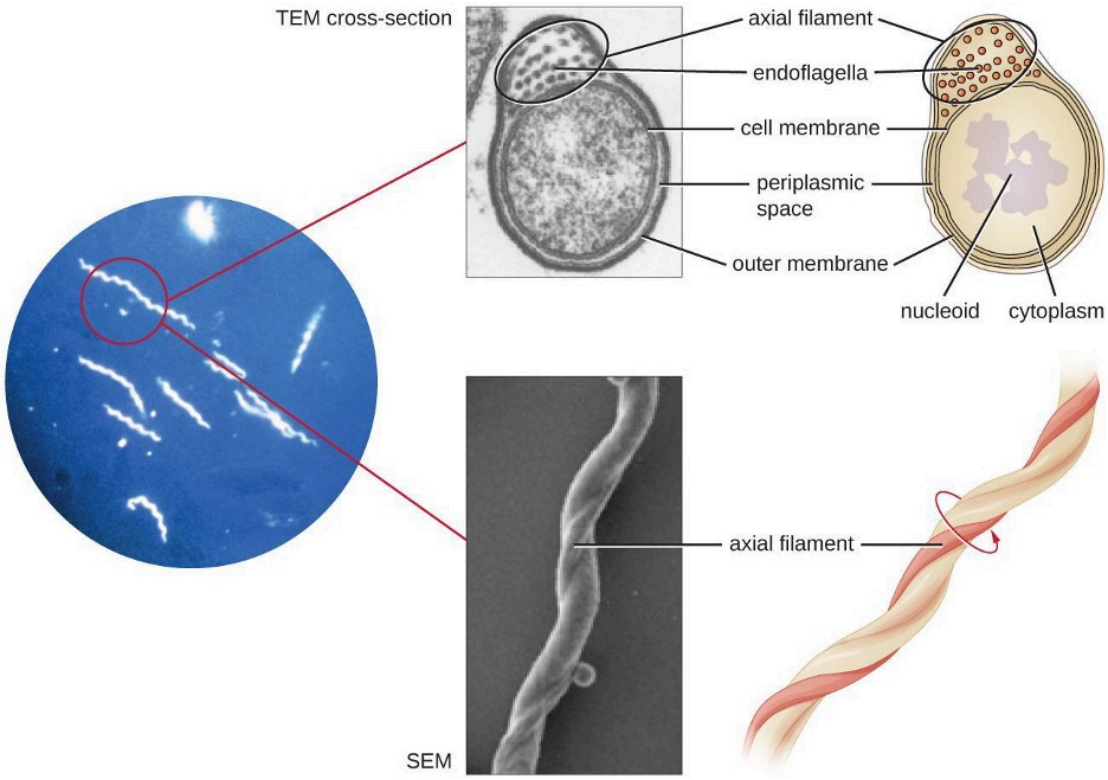


Figure 2.32: Spirochetes are typically observed using darkfield microscopy (left). However, electron microscopy (top center, bottom center) provides a more detailed view of their cellular morphology. The flagella found between the inner and outer membranes of spirochetes wrap around the bacterium, causing a twisting motion used for locomotion. *Figure description available at the end of the chapter.*

Another genus of spirochete, *Borrelia*, contains a number of pathogenic species. *B. burgdorferi* causes Lyme disease, which is transmitted by several genera of ticks (notably *Ixodes* and *Amblyomma*) and often produces a bull's eye rash, fever, fatigue, and, sometimes, debilitating arthritis. *B. recurrens* causes a condition known as relapsing fever.

CYTOPHAGA, *FUSOBACTERIUM*, AND *BACTEROIDES*

The gram-negative nonproteobacteria of the genera *Cytophaga*, *Fusobacterium*, and *Bacteroides* are classified together as a phylum and called the CFB group. Although they are phylogenetically diverse, bacteria of the CFB group share some similarities in the sequence of nucleotides in their DNA. They are rod-shaped bacteria adapted to anaerobic environments, such as the tissue of the gums, gut, and rumen of ruminating animals. CFB bacteria are avid fermenters, able to process cellulose in rumen, thus enabling ruminant animals to obtain carbon and energy from grazing.

Cytophaga are motile aquatic bacteria that glide. *Fusobacteria* inhabit the human mouth and may cause severe infectious diseases. The largest genus of the CFB group is *Bacteroides*, which includes dozens of species that are prevalent inhabitants of the human large intestine, making up about 30% of the entire gut microbiome (figure 2.33). One gram of human feces contains up to 100 billion *Bacteroides* cells. Most *Bacteroides* are mutualistic. They benefit from nutrients they find in the gut, and humans benefit from their ability to prevent pathogens from colonizing the large intestine. Indeed, when populations of *Bacteroides* are reduced in the gut—as often occurs when a patient takes antibiotics—the gut becomes a more favorable environment for pathogenic bacteria and fungi, which can cause secondary infections.

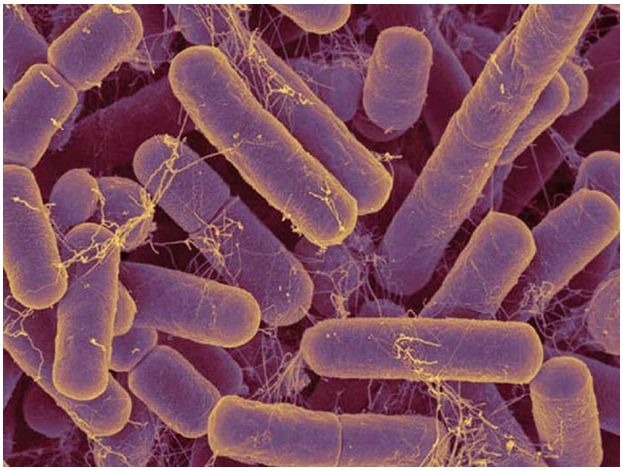


Figure 2.33: Bacteroides comprise up to 30% of the normal microbiota in the human gut. *Figure description available at the end of the chapter.*

Only a few species of *Bacteroides* are pathogenic. *B. melaninogenicus*, for example, can cause wound infections in patients with weakened immune systems.

PLANCTOMYCETES

The Planctomycetes are found in aquatic environments, inhabiting freshwater, saltwater, and brackish water. Planctomycetes are unusual in that they reproduce by budding, meaning that instead of one maternal cell splitting into two equal daughter cells in the process of binary fission, the mother cell forms a bud that detaches and lives as an independent cell. These so-called swarmer cells are motile and not attached to a surface. However, they will soon differentiate into sessile (immobile) cells with an appendage called a holdfast that allows them to attach to surfaces in the water (figure 2.34). Only the sessile cells are able to reproduce.

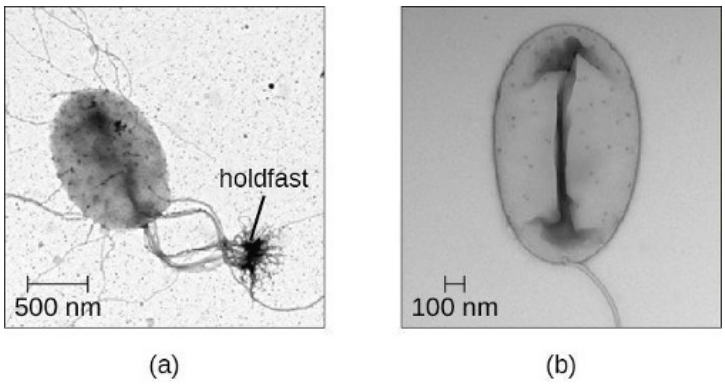


Figure 2.34: (a) Sessile Planctomycetes have a holdfast that allows them to adhere to surfaces in aquatic environments. (b) Swarmers are motile and lack a holdfast. *Figure description available at the end of the chapter.*

Table 2.12 summarizes the characteristics of some of the most clinically relevant genera of nonproteobacteria.

Example Genus	Microscopic Morphology	Unique Characteristics
Chlamydia	Gram-negative, coccoid or ovoid bacterium	Obligatory intracellular bacteria; some cause chlamydia, trachoma, and pneumonia
Bacteroides	Gram-negative bacillus	Obligate anaerobic bacteria; abundant in the human gastrointestinal tract; usually mutualistic, although some species are opportunistic pathogens
Cytophaga	Gram-negative bacillus	Motile by gliding; live in soil or water; decompose cellulose; may cause disease in fish
Fusobacterium	Gram-negative bacillus with pointed ends	Anaerobic; form biofilms; some species cause disease in humans (periodontitis, ulcers)
Leptospira	Spiral-shaped bacterium (spirochetes); gram negative-like (better viewed by darkfield microscopy); very thin	Aerobic, abundant in shallow water reservoirs; infect rodents and domestic animals; can be transmitted to humans by infected animals' urine; may cause severe disease
Borrelia	Gram-negative-like spirochete; very thin; better viewed by darkfield microscopy	*B. burgdorferi* causes Lyme disease and *B. recurrens* causes relapsing fever
Treponema	Gram-negative-like spirochete; very thin; better viewed by darkfield microscopy	Motile; do not grow in culture; *T. pallidum* (subspecies *T. pallidum pallidum*) causes syphilis

Table 2.12: Class nonproteobacteria

PHOTOTROPHIC BACTERIA

The phototrophic bacteria are a large and diverse category of bacteria that do not represent a taxon but, rather, a group of bacteria that use sunlight as their primary source of energy. This group contains both Proteobacteria and nonproteobacteria. They use solar energy to synthesize ATP through photosynthesis. When they produce oxygen, they perform oxygenic photosynthesis. When they do not produce oxygen, they perform anoxygenic photosynthesis. With the exception of some cyanobacteria, the majority of phototrophic bacteria perform anoxygenic photosynthesis.

One large group of phototrophic bacteria includes the purple or green bacteria that perform photosynthesis with the help of bacteriochlorophylls, which are green, purple, or blue pigments similar to chlorophyll in plants. Some of these bacteria have a varying amount of red or orange pigments called carotenoids. Their color varies from orange to red to purple to green (figure 2.35), and they are able to absorb light of various wavelengths. Traditionally, these bacteria are classified into sulfur and nonsulfur bacteria; they are further differentiated by color.

The sulfur bacteria perform anoxygenic photosynthesis, using sulfites as electron donors as well as releasing free elemental sulfur. Nonsulfur bacteria use organic substrates, such as succinate and malate, as donors of electrons.

The purple sulfur bacteria oxidize hydrogen sulfide into elemental sulfur and sulfuric acid. They get their purple color from the pigments bacteriochlorophylls and carotenoids. Bacteria of the genus *Chromatium* are purple sulfur Gammaproteobacteria. These microorganisms are strict anaerobes and live in water. They use carbon dioxide as their only source of carbon, but their survival and growth are possible only in the presence of sulfites, which they use as electron donors. *Chromatium* has been used as a model for studies of bacterial photosynthesis since the 1950s.[9]

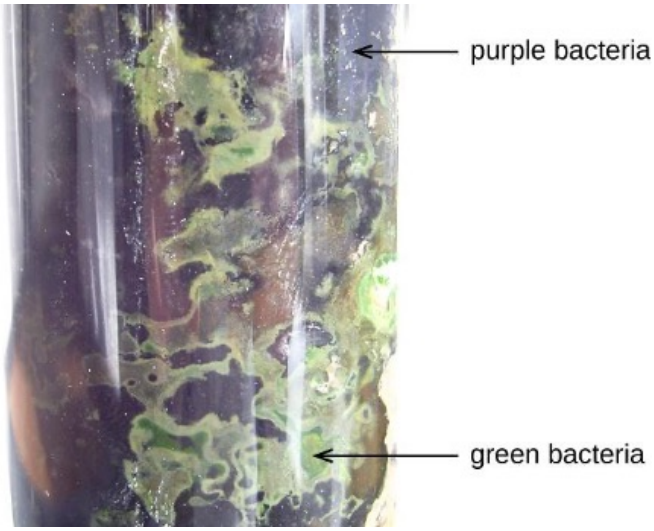


Figure 2.35: Purple and green sulfur bacteria use bacteriochlorophylls to perform photosynthesis. Figure description available at the end of the chapter.

The green sulfur bacteria use sulfide for oxidation and produce large amounts of green bacteriochlorophyll. The genus *Chlorobium* is a green sulfur bacterium that is implicated in climate change because it produces methane, a greenhouse gas. These bacteria use at least four types of chlorophyll for photosynthesis. The most prevalent of these, bacteriochlorophyll, is stored in special vesicle-like organelles called chlorosomes.

Purple nonsulfur bacteria are similar to purple sulfur bacteria, except that they use hydrogen rather than hydrogen sulfide for oxidation. Among the purple nonsulfur bacteria is the genus *Rhodospirillum*. These microorganisms are facultative anaerobes, which are actually pink rather than purple, and can metabolize ("fix") nitrogen. They may be valuable in the field of biotechnology because of their potential ability to produce biological plastic and hydrogen fuel.[10]

The green nonsulfur bacteria are similar to green sulfur bacteria except for their use of substrates other than sulfides for oxidation. *Chloroflexus* is an example of a green nonsulfur bacterium. It often has an orange color when it grows in the dark, but it becomes green when it grows in sunlight. It stores bacteriochlorophyll in chlorosomes, similar to *Chlorobium*, and performs anoxygenic photosynthesis, using organic sulfites (low concentrations) or molecular hydrogen as electron donors, so it can survive in the dark if oxygen is available. *Chloroflexus* does not have flagella but can glide, like *Cytophaga*. It grows at a wide range of temperatures, from 35 °C to 70 °C, thus can be thermophilic.

Another large, diverse group of phototrophic bacteria compose the phylum Cyanobacteria; they get their blue-green color from the chlorophyll contained in their cells (figure 2.36). Species of this group perform oxygenic photosynthesis, producing megatons of gaseous oxygen. Scientists hypothesize that cyanobacteria played a critical role in the change of our planet's anoxic atmosphere 1–2 billion years ago to the oxygen-rich environment we have today.[11]

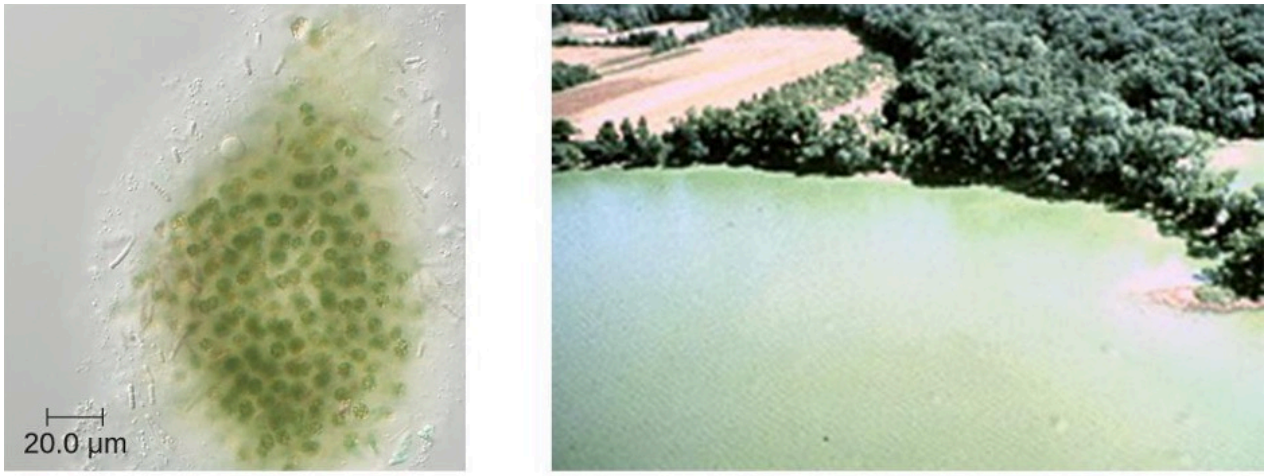


Figure 2.36: (a) Microcystis aeruginosa is a type of cyanobacteria commonly found in freshwater environments. (b) In warm temperatures, M. aeruginosa and other cyanobacteria can multiply rapidly and produce neurotoxins, resulting in blooms that are harmful to fish and other aquatic animals. *Figure description available at the end of the chapter.*

Cyanobacteria have other remarkable properties. Amazingly adaptable, they thrive in many habitats, including marine and freshwater environments, soil, and even rocks. They can live at a wide range of temperatures, even in the extreme temperatures of the Antarctic. They can live as unicellular organisms or in colonies, and they can be filamentous, forming sheaths or biofilms. Many of them fix nitrogen, converting molecular nitrogen into nitrites and nitrates that other bacteria, plants, and animals can use. The reactions of nitrogen fixation occur in specialized cells called heterocysts.

Photosynthesis in Cyanobacteria is oxygenic, using the same type of chlorophyll found in plants and algae as the primary photosynthetic pigment. Cyanobacteria also use phycocyanin and cyanophycin, two secondary photosynthetic pigments that give them their characteristic blue color. They are located in special organelles called phycobilisomes and in folds of the cellular membrane called thylakoids, which are remarkably similar to the photosynthetic apparatus of plants. Scientists hypothesize that plants originated from endosymbiosis of ancestral eukaryotic cells and ancestral photosynthetic bacteria.[12] Cyanobacteria are also an interesting object of research in biochemistry,[13] with studies investigating their potential as biosorbents[14] and products of human nutrition.[15]

Unfortunately, cyanobacteria can sometimes have a negative impact on human health. Genera such as *Microcystis* can form harmful cyanobacterial blooms, forming dense mats on bodies of water and producing large quantities of toxins that can harm wildlife and humans. These toxins have been implicated in tumors of the liver and diseases of the nervous system in animals and humans.[16]

Table 2.13 summarizes the characteristics of important phototrophic bacteria.

Phylum	Class	Example Genus or Species	Common Name	Oxygenic or Anoxygenic	Sulfur Deposition
Cyanobacteria	Cyanophyceae	*Microcystis aeruginosa*	Blue-green bacteria	Oxygenic	None
Chlorobi	Chlorobia	*Chlorobium*	Green sulfur bacteria	Anoxygenic	Outside the cell
Chloroflexi (Division)	Chloroflexi	*Chloroflexus*	Green nonsulfur bacteria	Anoxygenic	None
Proteobacteria	Alphaproteobacteria	*Rhodospirillum*	Purple nonsulfur bacteria	Anoxygenic	None
	Betaproteobacteria	*Rhodocyclus*	Purple nonsulfur bacteria	Anoxygenic	None
	Gammaproteobacteria	*Chromatium*	Purple sulfur bacteria	Anoxygenic	Inside the cell

Table 2.13: Phototrophic bacteria

2.6 GRAM-POSITIVE BACTERIA

Prokaryotes are identified as gram-positive if they have a multiple layer matrix of peptidoglycan forming the cell wall. Advances in nucleic acid biochemistry have revealed additional characteristics that can be used to classify gram-positive prokaryotes, namely the guanine to cytosine ratios (G+C) in DNA and the composition of 16S rRNA subunits. Microbiologists currently recognize two distinct groups of gram-positive, or weakly staining gram-positive, prokaryotes. The class Actinobacteria comprises the high G+C gram-positive bacteria, which have more than 50% guanine and cytosine nucleotides in their DNA. The class Bacilli comprises low G+C gram-positive bacteria, which have less than 50% of guanine and cytosine nucleotides in their DNA.

ACTINOBACTERIA: HIGH G+C GRAM-POSITIVE BACTERIA

The name Actinobacteria comes from the Greek words for *rays* and *small rod*, but Actinobacteria are very diverse. Their microscopic appearance can range from thin filamentous branching rods to coccobacilli. Most Actinobacteria live in the soil, but some are aquatic. The vast majority are aerobic. One distinctive feature of this group is the presence of several different peptidoglycans in the cell wall.

Actinomyces spp. play an important role in soil ecology, and some species are human pathogens. A number of *Actinomyces* spp. inhabit the human mouth and are opportunistic pathogens, causing infectious diseases like periodontitis (inflammation of the gums) and oral abscesses. The species *A. israelii* is an anaerobe notorious for causing endocarditis (inflammation of the inner lining of the heart) (figure 2.37).

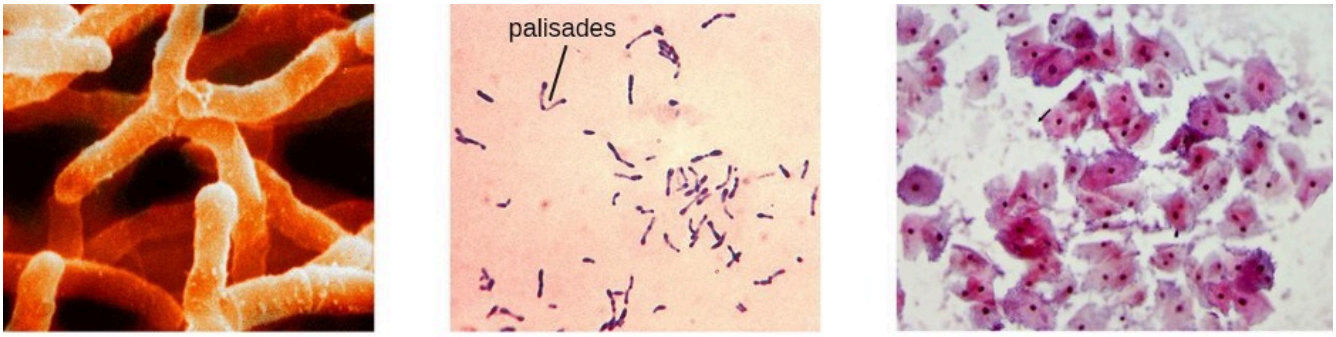


Figure 2.37: (a) Actinomyces israelii (false-color scanning electron micrograph [SEM]) has a branched structure. (b) Corynebacterium diphtheria causes the deadly disease diphtheria. Note the distinctive palisades. (c) The gram-variable bacterium Gardnerella vaginalis causes bacterial vaginosis in women. This micrograph shows a Pap smear from a woman with vaginosis. *Figure description available at the end of the chapter.*

The genus *Mycobacterium* is represented by bacilli covered with a mycolic acid coat. This waxy coat protects the bacteria from some antibiotics, prevents them from drying out, and blocks penetration by Gram stain reagents (see section 2.2). Because of this, a special acid-fast staining procedure is used to visualize these bacteria. The genus *Mycobacterium* is an important cause of a diverse group of infectious diseases. *M. tuberculosis* is the causative agent of tuberculosis, a disease that primarily impacts the lungs but can infect other parts of the body as well. It has been estimated that one-third of the world's population has been infected with *M. tuberculosis* and millions of new infections occur each year. Treatment of *M. tuberculosis* is challenging and requires patients to take a combination of drugs for an extended time. Complicating treatment even further is the development and spread of multidrug-resistant strains of this pathogen.

Another pathogenic species, *M. leprae*, is the cause of Hansen's disease (leprosy), a chronic disease that impacts peripheral nerves and the integrity of the skin and mucosal surface of the respiratory tract. Loss of pain sensation and the presence of skin lesions increase susceptibility to secondary injuries and infections with other pathogens.

Bacteria in the genus *Corynebacterium* contain diaminopimelic acid in their cell walls, and microscopically often form *palisades*, or pairs of rod-shaped cells resembling the letter *V*. Cells may contain metachromatic granules, intracellular storage of inorganic phosphates that are useful for identification of *Corynebacterium*. The vast majority of *Corynebacterium* spp. are nonpathogenic; however, *C. diphtheria* is the causative agent of diphtheria, a disease that can be fatal, especially in children (figure 2.37). *C. diphtheria* produces a toxin that forms a pseudomembrane in the patient's throat, causing swelling, difficulty breathing, and other symptoms that can become serious if untreated.

The genus *Bifidobacterium* consists of filamentous anaerobes, many of which are commonly found in the gastrointestinal tract, vagina, and mouth. In fact, *Bifidobacterium* spp. constitute a substantial part of the human gut microbiota and are frequently used as probiotics and in yogurt production.

The genus *Gardnerella*, contains only one species, *G. vaginalis*. This species is defined as gram-variable because its small coccobacilli do not show consistent results when Gram stained (figure 2.37). Based on its genome, it is placed into the high G+C gram-positive group. *G. vaginalis* can cause bacterial vaginosis in women; symptoms are typically mild or even undetectable, but can lead to complications during pregnancy.

Table 2.14 summarizes the characteristics of some important genera of Actinobacteria.

Example Genus	Microscopic Morphology	Unique Characteristics
Actinomyces	Gram-positive bacillus; in colonies, shows fungus-like threads (hyphae)	Facultative anaerobes; in soil, decompose organic matter; in the human mouth, may cause gum disease
Arthrobacter	Gram-positive bacillus (at the exponential stage of growth) or coccus (in stationary phase)	Obligate aerobes; divide by "snapping," forming *V*-like pairs of daughter cells; degrade phenol, can be used in bioremediation
Bifidobacterium	Gram-positive, filamentous actinobacterium	Anaerobes commonly found in human gut microbiota
Corynebacterium	Gram-positive bacillus	Aerobes or facultative anaerobes; form palisades; grow slowly; require enriched media in culture; *C. diphtheriae* causes diphtheria
Frankia	Gram-positive, fungus-like (filamentous) bacillus	Nitrogen-fixing bacteria; live in symbiosis with legumes
Gardnerella	Gram-variable coccobacillus	Colonize the human vagina, may alter the microbial ecology, thus leading to vaginosis
Micrococcus	Gram-positive coccus, form microscopic clusters	Ubiquitous in the environment and on the human skin; oxidase-positive (as opposed to morphologically similar *S. aureus*); some are opportunistic pathogens
Mycobacterium	Gram-positive, acid-fast bacillus	Slow growing, aerobic, resistant to drying and phagocytosis; covered with a waxy coat made of mycolic acid; *M. tuberculosis* causes tuberculosis; *M. leprae* causes leprosy
Nocardia	Weakly gram-positive bacillus; forms acid-fast branches	May colonize the human gingiva; may cause severe pneumonia and inflammation of the skin
Propionibacterium	Gram-positive bacillus	Aerotolerant anaerobe; slow-growing; *P. acnes* reproduces in the human sebaceous glands and may cause or contribute to acne
Rhodococcus	Gram-positive bacillus	Strict aerobe; used in industry for biodegradation of pollutants; *R. fascians* is a plant pathogen, and *R. equi* causes pneumonia in foals
Streptomyces	Gram-positive, fungus-like (filamentous) bacillus	Very diverse genus (>500 species); aerobic, spore-forming bacteria; scavengers, decomposers found in soil (give the soil its earthy odor); used in pharmaceutical industry as antibiotic producers (more than two-thirds of clinically useful antibiotics)

Table 2.14: Actinobacteria: High G+C gram-positive

LOW G+C GRAM-POSITIVE BACTERIA

The low G+C gram-positive bacteria have less than 50% guanine and cytosine in their DNA, and this group of bacteria includes a number of genera of bacteria that are pathogenic.

Clostridia

One large and diverse class of low G+C gram-positive bacteria is Clostridia. The best studied genus of this class is *Clostridium*. These rod-shaped bacteria are generally obligate anaerobes that produce endospores and can be found in anaerobic habitats like soil and aquatic sediments rich in organic nutrients. The endospores may survive for many years.

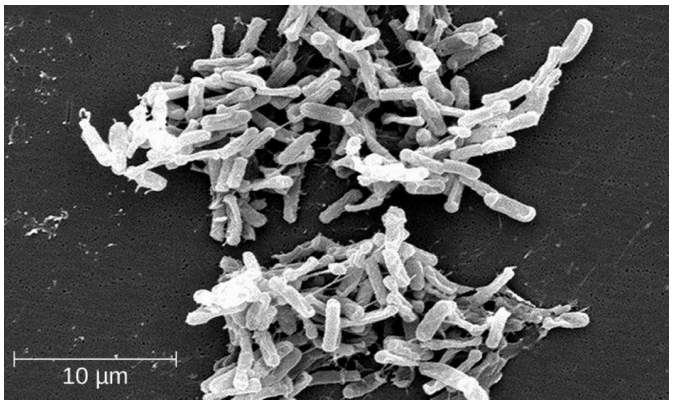


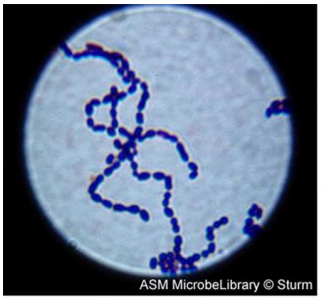
Figure 2.38: Clostridium difficile, a gram-positive, rod-shaped bacterium, causes severe colitis and diarrhea, often after the normal gut microbiota is eradicated by antibiotics. *Figure description available at the end of the chapter.*

Clostridium spp. produce more kinds of protein toxins than any other bacterial genus, and several species are human pathogens. *C. perfringens* is the third most common cause of food poisoning in the United States and is the causative agent of an even more serious disease called gas gangrene. Gas gangrene occurs when *C. perfringens* endospores enter a wound and germinate, becoming viable bacterial cells and producing a toxin that can cause the necrosis (death) of tissue. *C. tetani*, which causes tetanus, produces a neurotoxin that is able to enter neurons, travel to regions of the central nervous system where it blocks the inhibition of nerve impulses involved in muscle contractions, and cause a life-threatening spastic paralysis. *C. botulinum* produces botulinum neurotoxin, the most lethal biological toxin known. Botulinum toxin is responsible for rare but frequently fatal cases of botulism. The toxin blocks the release of acetylcholine in neuromuscular junctions, causing flaccid paralysis. In very small concentrations, botulinum toxin has been used to treat muscle pathologies in humans and in a cosmetic procedure to eliminate wrinkles. *C. difficile* is a common source of hospital-acquired infections (figure 2.38) that can result in serious and even fatal cases of colitis (inflammation of the large intestine). Infections often occur in patients who are immunosuppressed or undergoing antibiotic therapy that alters the normal microbiota of the gastrointestinal tract.

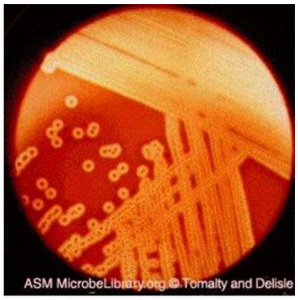
Lactobacillales

The order Lactobacillales comprises low G+C gram-positive bacteria that include both bacilli and cocci in the genera *Lactobacillus*, *Leuconostoc*, *Enterococcus*, and *Streptococcus*. Bacteria of the latter three genera typically are spherical or ovoid and often form chains.

Streptococcus, the name of which comes from the Greek word for *twisted chain*, is responsible for many types of infectious diseases in humans. Species from this genus, often referred to as streptococci, are usually classified by serotypes called Lancefield groups, and by their ability to lyse red blood cells when grown on blood agar.



(a)



(b)

Figure 2.39: (a) A gram-stained specimen of Streptococcus pyogenes shows the chains of cocci characteristic of this organism's morphology. (b) S. pyogenes on blood agar shows characteristic lysis of red blood cells, indicated by the halo of clearing around colonies. *Figure description available at the end of the chapter.*

S. pyogenes belongs to the Lancefield group A, β-hemolytic *Streptococcus*. This species is considered a pyogenic pathogen because of the associated pus production observed with infections it causes (figure 2.39). *S. pyogenes* is the most common cause of bacterial pharyngitis (strep throat); it is also an important cause of various skin infections that can be relatively mild (e.g., impetigo) or life threatening (e.g., necrotizing fasciitis, also known as flesh eating disease).

The nonpyogenic (i.e., not associated with pus production) streptococci are a group of streptococcal species that are not a taxon but are grouped together because they inhabit the human mouth. The nonpyogenic streptococci do not belong to any of the Lancefield groups. Most are commensals, but a few, such as *S. mutans*, are implicated in the development of dental caries.

S. pneumoniae (commonly referred to as pneumococcus), is a *Streptococcus* species that also does not belong to any Lancefield group. *S. pneumoniae* cells appear microscopically as diplococci, pairs of cells, rather than the long chains typical of most streptococci. Scientists have known since the 19th century that *S. pneumoniae* causes pneumonia and other respiratory infections. However, this bacterium can also cause a wide range of other diseases, including meningitis, septicemia, osteomyelitis, and endocarditis, especially in newborns, the elderly, and patients with immunodeficiency.

Bacilli

The name of the class Bacilli suggests that it is made up of bacteria that are bacillus in shape, but it is a morphologically diverse class that includes bacillus-shaped and cocccus-shaped genera. Among the many genera in this class are two that are very important clinically: *Bacillus* and *Staphylococcus*.

Bacteria in the genus *Bacillus* are bacillus in shape and can produce endospores. They include aerobes or facultative anaerobes. A number of *Bacillus* spp. are used in various industries, including the production of antibiotics (e.g., barnase), enzymes (e.g., alpha-amylase, BamH1 restriction endonuclease), and detergents (e.g., subtilisin).

Two notable pathogens belong to the *Bacillus* genus. *B. anthracis* is the pathogen that causes anthrax, a severe disease that affects wild and domesticated animals and can spread from infected animals to humans. Anthrax manifests in humans as charcoal-black ulcers on the skin, severe enterocolitis, pneumonia, and brain damage due to swelling. If untreated, anthrax is lethal. *B. cereus*, a closely related species, is a pathogen that may cause food poisoning. It is a rod-shaped species that forms chains. Colonies appear milky white with irregular shapes when cultured on blood agar (figure 2.40). One other important species is *B. thuringiensis*. This bacterium produces a number of substances used as insecticides because they are toxic for insects.

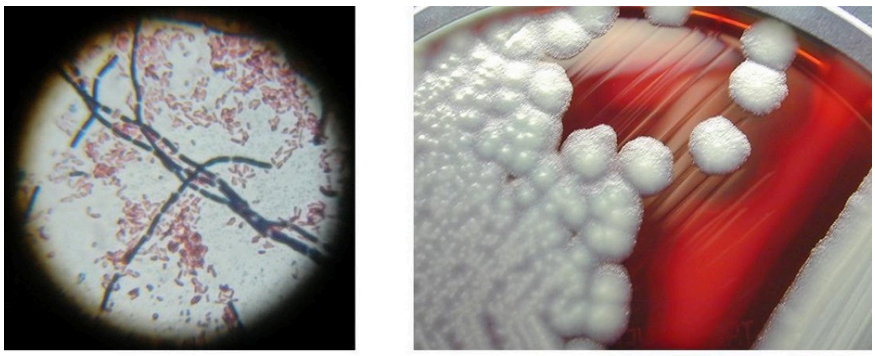


Figure 2.40: (a) In this gram-stained specimen, the violet rod-shaped cells forming chains are the gram-positive bacteria Bacillus cereus. The small, pink cells are the gram-negative bacteria Escherichia coli. (b) In this culture, white colonies of B. cereus have been grown on sheep blood agar. *Figure description available at the end of the chapter.*

The genus *Staphylococcus* also belongs to the class Bacilli, even though its shape is coccus rather than a bacillus. The name *Staphylococcus* comes from a Greek word for *bunches of grapes*, which describes their microscopic appearance in cultures (figure 2.41). *Staphylococcus* spp. are facultative anaerobic, halophilic, and nonmotile. The two best-studied species of this genus are *S. epidermidis* and *S. aureus*.

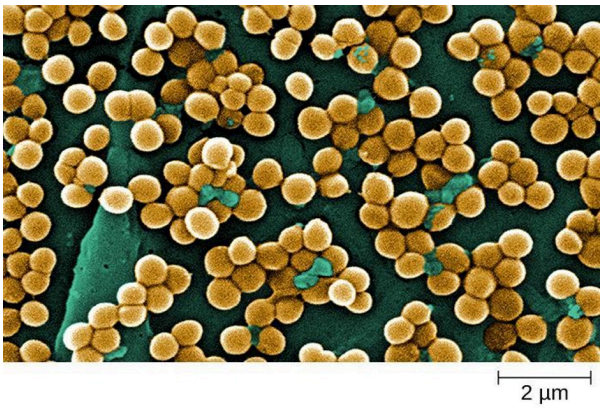


Figure 2.41: This SEM of Staphylococcus aureus illustrates the typical grape-like clustering of cells. *Figure description available at the end of the chapter.*

S. epidermidis, whose main habitat is the human skin, is thought to be nonpathogenic for humans with healthy immune systems, but in patients with immunodeficiency, it may cause infections in skin wounds and prostheses (e.g., artificial joints, heart valves). *S. epidermidis* is also an important cause of infections associated with intravenous catheters. This makes it a dangerous pathogen in hospital settings, where many patients may be immunocompromised.

Strains of *S. aureus* cause a wide variety of infections in humans, including skin infections that produce boils, carbuncles, cellulitis, or impetigo. Certain strains of *S. aureus* produce a substance called enterotoxin, which can cause severe enteritis, often called staph food poisoning. Some strains of *S. aureus* produce the toxin responsible for toxic shock syndrome, which can result in cardiovascular collapse and death.

Many strains of *S. aureus* have developed resistance to antibiotics. Some antibiotic-resistant strains are designated as methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA). These strains are some of the most difficult to treat because they exhibit resistance to nearly all available antibiotics, not just methicillin and vancomycin. Because they are difficult to treat with antibiotics, infections can be lethal. MRSA and VRSA are also contagious, posing a serious threat in hospitals, nursing homes, dialysis facilities, and other places where there are large populations of elderly, bedridden, and/or immunocompromised patients.

Mycoplasmas

Although *Mycoplasma* spp. do not possess a cell wall and, therefore, are not stained by Gram-stain reagents, this genus is still included with the low G+C gram-positive bacteria. The genus *Mycoplasma* includes more than 100 species, which share several unique characteristics. They are very small cells, some with a diameter of about 0.2 μm, which is smaller than some large viruses. They have no cell walls and, therefore, are pleomorphic, meaning that they may take on a variety of shapes and can even resemble very small animal cells. Because they lack a characteristic shape, they can be difficult to identify. One species, *M. pneumoniae*, causes the mild form of pneumonia known as "walking pneumonia" or "atypical pneumonia." This form of pneumonia is typically less severe than forms caused by other bacteria or viruses.

Table 2.15 summarizes the characteristics of notable genera low G+C Gram-positive bacteria.

Example Genus	Microscopic Morphology	Unique Characteristics
Bacillus	Large, gram-positive bacillus	Aerobes or facultative anaerobes; form endospores; *B. anthracis* causes anthrax in cattle and humans, *B. cereus* may cause food poisoning
Clostridium	Gram-positive bacillus	Strict anaerobes; form endospores; all known species are pathogenic, causing tetanus, gas gangrene, botulism, and colitis
Enterococcus	Gram-positive coccus; forms microscopic pairs in culture (resembling *Streptococcus pneumoniae*)	Anaerobic aerotolerant bacteria, abundant in the human gut, may cause urinary tract and other infections in the nosocomial environment
Lactobacillus	Gram-positive bacillus	Facultative anaerobes; ferment sugars into lactic acid; part of the vaginal microbiota; used as probiotics
Leuconostoc	Gram-positive coccus; may form microscopic chains in culture	Fermenter, used in food industry to produce sauerkraut and kefir
Mycoplasma	The smallest bacteria; appear pleomorphic under electron microscope	Have no cell wall; classified as low G+C Gram-positive bacteria because of their genome; *M. pneumoniae* causes "walking" pneumonia
Staphylococcus	Gram-positive coccus; forms microscopic clusters in culture that resemble bunches of grapes	Tolerate high salt concentration; facultative anaerobes; produce catalase; *S. aureus* can also produce coagulase and toxins responsible for local (skin) and generalized infections
Streptococcus	Gram-positive coccus; forms chains or pairs in culture	Diverse genus; classified into groups based on sharing certain antigens; some species cause hemolysis and may produce toxins responsible for human local (throat) and generalized disease
Ureaplasma	Similar to *Mycoplasma*	Part of the human vaginal and lower urinary tract microbiota; may cause inflammation, sometimes leading to internal scarring and infertility

Table 2.15: Bacilli: Low G+C gram-positive bacteria

2.7 UNICELLULAR EUKARYOTIC PARASITES

Eukaryotic microbes are an extraordinarily diverse group, including species with a wide range of life cycles, morphological specializations, and nutritional needs (table 2.16). Although more diseases are caused by viruses and bacteria than by microscopic eukaryotes, these eukaryotes are responsible for some diseases of great public health importance. The protist parasite *Giardia* causes a diarrheal illness (giardiasis) that is easily transmitted through contaminated water supplies. In the United States, *Giardia* is the most common human intestinal parasite (figure 2.44). Even in developed countries, these worms are notable parasites affecting humans and domestic animals. There are fewer fungal pathogens, but these are also relevant causes of illness, as well. At the same time,, fungi have been important in producing antimicrobial substances such as penicillin.

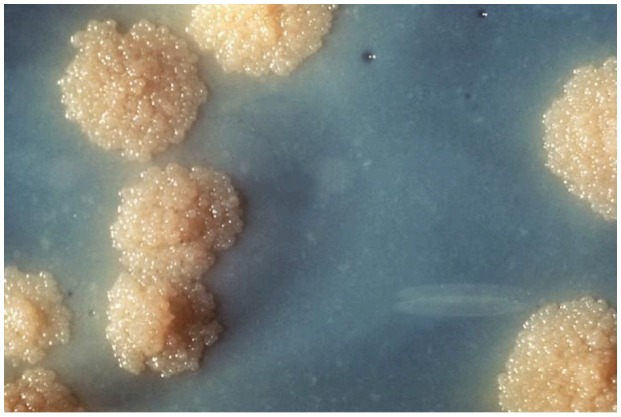


Figure 2.42: M. tuberculosis grows on Löwenstein-Jensen (LJ) agar in distinct colonies. *Figure description available at the end of the chapter.*

In this section, we will examine characteristics of protists, worms, and fungi while considering their roles in causing disease.

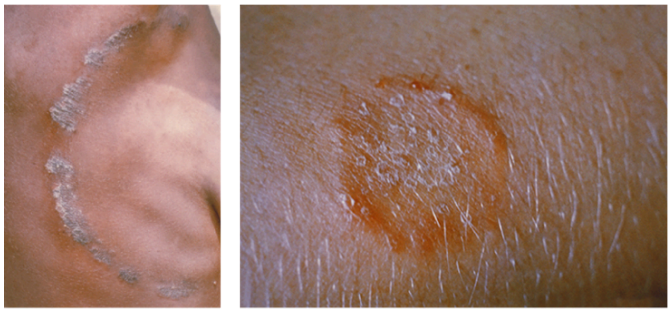


Figure 2.43: Ringworm presents as a raised ring, which is gray or brown on brown or black skin (a), and red on lighter skin (b). *Figure description available at the end of the chapter.*

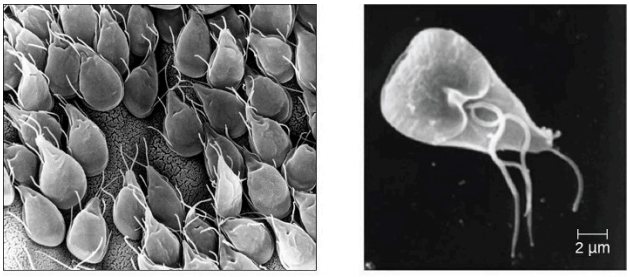


Figure 2.44: (a) A scanning electron micrograph shows many Giardia parasites in the trophozoite, or feeding stage, in a gerbil intestine. (b) An individual trophozoite of G. lamblia, visualized here in a scanning electron micrograph. This waterborne protist causes severe diarrhea when ingested. *Figure description available at the end of the chapter.*

CHARACTERISTICS OF PROTISTS

Protozoans inhabit a wide variety of habitats, both aquatic and terrestrial. Many are free-living, while others are parasitic, carrying out a life cycle within a host or hosts and potentially causing illness. There are also beneficial symbionts that provide metabolic services to their hosts. During the feeding and growth part of their life cycle, they are called trophozoites; these feed on small particulate food sources such as bacteria. While some types of protozoa exist exclusively in the trophozoite form, others can develop from trophozoite to an encapsulated cyst stage when environmental conditions are too harsh for the trophozoite. A cyst is a cell with a protective wall, and the process by which a trophozoite becomes a cyst is called encystment. When conditions become more favorable, these cysts are triggered by environmental cues to become active again through excystment.

One protozoan genus capable of encystment is *Eimeria*, which includes some human and animal pathogens. Figure 2.45 illustrates the life cycle of *Eimeria*.

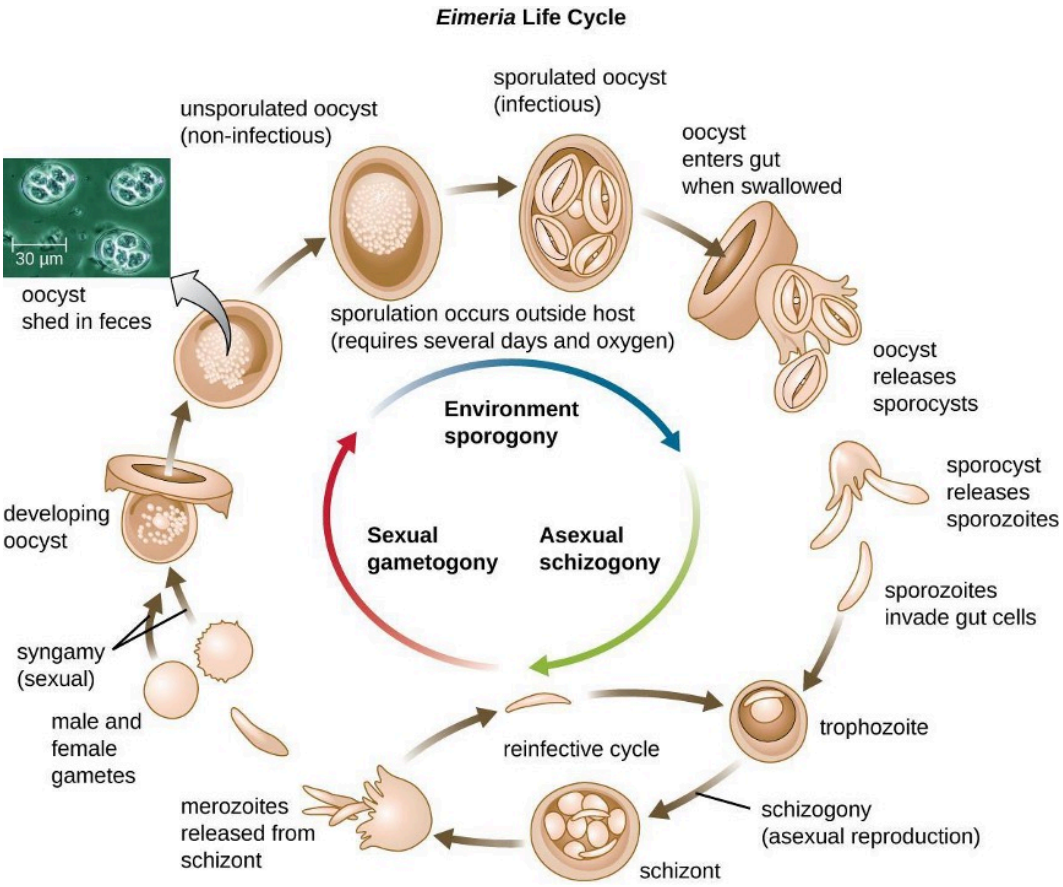


Figure 2.45: In the sexual/asexual life cycle of Eimeria, oocysts (inset) are shed in feces and may cause disease when ingested by a new host. *Figure description available at the end of the chapter.*

Protozoans have a variety of reproductive mechanisms. Some protozoans reproduce asexually and others reproduce sexually; still others are capable of both sexual and asexual reproduction. In protozoans, asexual reproduction occurs by binary fission, budding, or schizogony. In schizogony, the nucleus of a cell divides multiple times before the cell divides into many smaller cells. The products of schizogony are called merozoites and they are stored in structures known as schizonts. Protozoans may also reproduce sexually, which increases genetic diversity and can lead to complex life cycles. Protozoans can produce haploid gametes that fuse through syngamy. However, they can also exchange genetic material by joining to exchange DNA in a process called con-

jugation. This is a different process than the conjugation that occurs in bacteria. The term protist conjugation refers to a true form of eukaryotic sexual reproduction between two cells of different mating types. It is found in ciliates, a group of protozoans.

All protozoans have a plasma membrane, or plasmalemma, and some have bands of protein just inside the membrane that add rigidity, forming a structure called the pellicle. Some protists, including protozoans, have distinct layers of cytoplasm under the membrane. In these protists, the outer gel layer (with microfilaments of actin) is called the ectoplasm. Inside this layer is a sol (fluid) region of cytoplasm called the endoplasm. These structures contribute to complex cell shapes in some protozoans, whereas others (such as amoebas) have more flexible shapes (figure 2.46).

Different groups of protozoans have specialized feeding structures. They may have a specialized structure for taking in food through phagocytosis, called a cytostome, as well as a specialized structure for the exocytosis of wastes called a cytoproct. Oral grooves leading to cytostomes are lined with hair-like cilia to sweep in food particles. Protozoans are heterotrophic. Protozoans that are holozoic ingest whole food particles through phagocytosis. Forms that are saprozoic ingest small, soluble food molecules.

Many protists have whip-like flagella or hair-like cilia made of microtubules that can be used for locomotion (figure 2.46). Other protists use cytoplasmic extensions known as pseudopodia ("false feet") to attach the cell to a surface; they then allow cytoplasm to flow into the extension, thus moving themselves forward.

Protozoans have a variety of unique organelles and sometimes lack organelles found in other cells. Some have contractile vacuoles, organelles that can be used to move water out of the cell for osmotic regulation (salt and water balance) (figure 2.46). Mitochondria may be absent in parasites or can be altered to kinetoplastids (modified mitochondria) or hydrogenosomes.

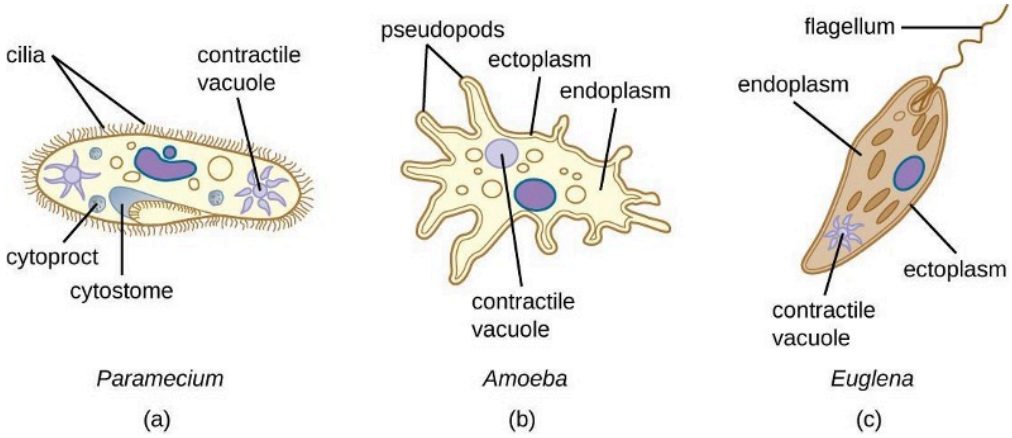


Figure 2.46: (a) Paramecium spp. have hair-like appendages called cilia for locomotion. (b) Amoeba spp. use lobe-like pseudopodia to anchor the cell to a solid surface and pull forward. (c) Euglena spp. use a whip-like structure called a flagellum to propel the cell. *Figure description available at the end of the chapter.*

Table 2.16 lists the eukaryote supergroups and some examples.

Supergroup	Subgroups	Distinguishing Features	Examples	Clinical notes
Excavata	Fornicata	Form cysts Pair of equal nuclei No mitochondria Often parasitic Four free flagella	*Giardia lamblia*	Giardiasis
	Parabasalids	No mitochondria Four free flagella One attached flagella No cysts Parasitic or symbiotic Basal bodies Kinetoplastids	*Trichomonas*	Trichomoniasis
	Euglenozoans	Photosynthetic or heterotrophic Flagella	*Euglena*	N/a
			Trypanosoma	African sleeping sickness, Chagas disease
			Leishmania	Leishmaniasis
Chromalveolata	Dinoflagellates	Cellulose theca Two dissimilar flagella	*Gonyaulax*	Red tides
			Alexandrium	Paralytic shellfish poisoning
			Pfiesteria	Harmful algal blooms
	Apicomplexans	Intracellular parasite Apical organelles	*Plasmodium*	Malaria
			Cryptosporidium	Cryptosporidiosis
			Theileria (Babesia)	Babesiosis
			Toxoplasma	Toxoplasmosis
	Ciliates	Cilia	*Balantidium*	Balantidiasis
			Paramecium	N/a
			Stentor	N/a
	Oomycetes/ peronosporomycetes	"Water molds" Generally diploid Cellulose cell walls	*Phytophthora*	Disease in crops
Rhizaria	Formaninifera	Amoeboid Threadlike pseudopodia Calcium carbonate shells	*Astrolonche*	N/a
	Radiolaria	Amoeboid Threadlike pseudopodia Silica shells	*Actinomma*	N/a
	Cercozoa	Amoeboid Threadlike pseudopodia Complex shells Parasitic forms	*Spongospora subterranea*	Powdery scab (potato disease
			Plasmodiophora brassicae	Cabbage clubroot

Supergroup	Subgroups	Distinguishing Features	Examples	Clinical notes
Archaeplastida	Red algae	Chlorophyll *a* Phycoerythrin Phycocyanin Floridean starch Agar in cell walls	*Gelidium*	Source of agar
			Gracilaria	Source of agar
	Chlorophytes	Chlorophyll *a* Chlorophyll *b* Cellulose cell walls Starch storage	*Acetabularia*	N/a
			Ulva	N/a
Amoebozoa	Slime molds	Plasmodial and cellular forms	*Dictyostelium*	N/a
	Entamoebas	Trophozoites Form cysts	*Entamoeba*	Amoebiasis
			Naegleria	Primary amoebic meningoencephalitis
			Acanthamoeba	Keratitis, granulomatous amoebic encephalitis
Opisthokonta	Fungi	Chitin cell walls Unicellular or multicellular Often hyphae	Zygomycetes	Zygomycosis
			Ascomycetes	Candidiasis
			Basidiomycetes	Cryptococcosis
			Microsporidia	Microsporidiosis
	Animals	Multicellular heterotrophs No cell walls	Nematoda	Trichinosis; hookworm and pinworm infections
			Trematoda	Schistosomiasis
			Cestoda	Tapeworm infections

Table 2.16: The eukaryote supergroups and some examples

Amoebozoa

The supergroup Amoebozoa includes protozoans that use amoeboid movement. Actin microfilaments produce pseudopodia, into which the remainder of the protoplasm flows, thereby moving the organism. The genus *Entamoeba* includes commensal or parasitic species, including the medically important *E. histolytica*, which is transmitted by cysts in feces and is the primary cause of amoebic dysentery. Another member of this group that is pathogenic to humans is *Acanthamoeba*, which can cause keratitis (corneal inflammation) and blindness. The notorious "brain eating amoeba," *Naegleria fowleri*, is a considered a distant relative of the Amoebozoa and is classified in the phylum Percolozoa.

The Eumycetozoa are an unusual group of organisms called slime molds, which have previously been classified as animals, fungi, and plants (figure 2.47). Slime molds can be divided into two types: cellular slime molds and plasmodial slime molds. The cellular slime molds exist as individual amoeboid cells that periodically aggregate into a mobile slug. The aggregate then forms a fruiting body that produces haploid spores. Plasmodial slime molds exist as large, multinucleate amoeboid cells that form reproductive stalks to produce spores that divide into gametes. One cellular slime mold, *Dictyostelium discoideum*, has been an important study organism

for understanding cell differentiation, because it has both single-celled and multicelled life stages, with the cells showing some degree of differentiation in the multicelled form. Figure 2.48 and figure 2.49 illustrate the life cycles of cellular and plasmodial slime molds, respectively.

Figure 2.47: (a) The cellular slime mold Dictyostelium discoideum can be grown on agar in a Petri dish. In this image, individual amoeboid cells (visible as small spheres) are streaming together to form an aggregation that is beginning to rise in the upper right corner of the image. The primitively multicellular aggregation consists of individual cells that each have their own nucleus. (b) Fuligo septica is a plasmodial slime mold. This brightly colored organism consists of a large cell with many nuclei. *Figure description available at the end of the chapter.*

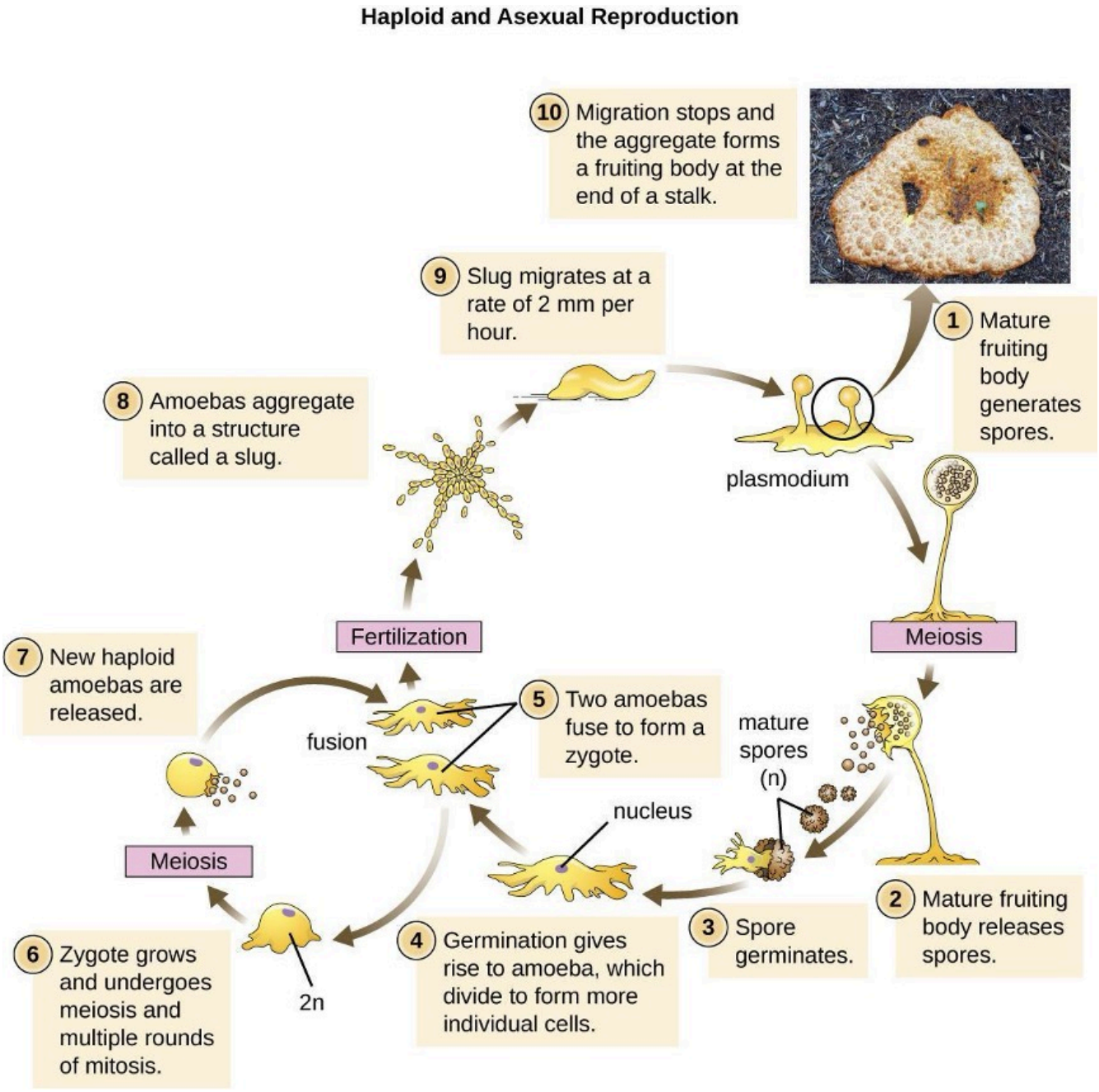


Figure 2.48: The life cycle of the cellular slime mold Dictyostelium discoideum primarily involves individual amoebas but includes the formation of a multinucleate plasmodium formed from a uninucleate zygote (the result of the fusion of two individual amoeboid cells). The plasmodium is able to move and forms a fruiting body that generates haploid spores. *Figure description available at the end of the chapter.*

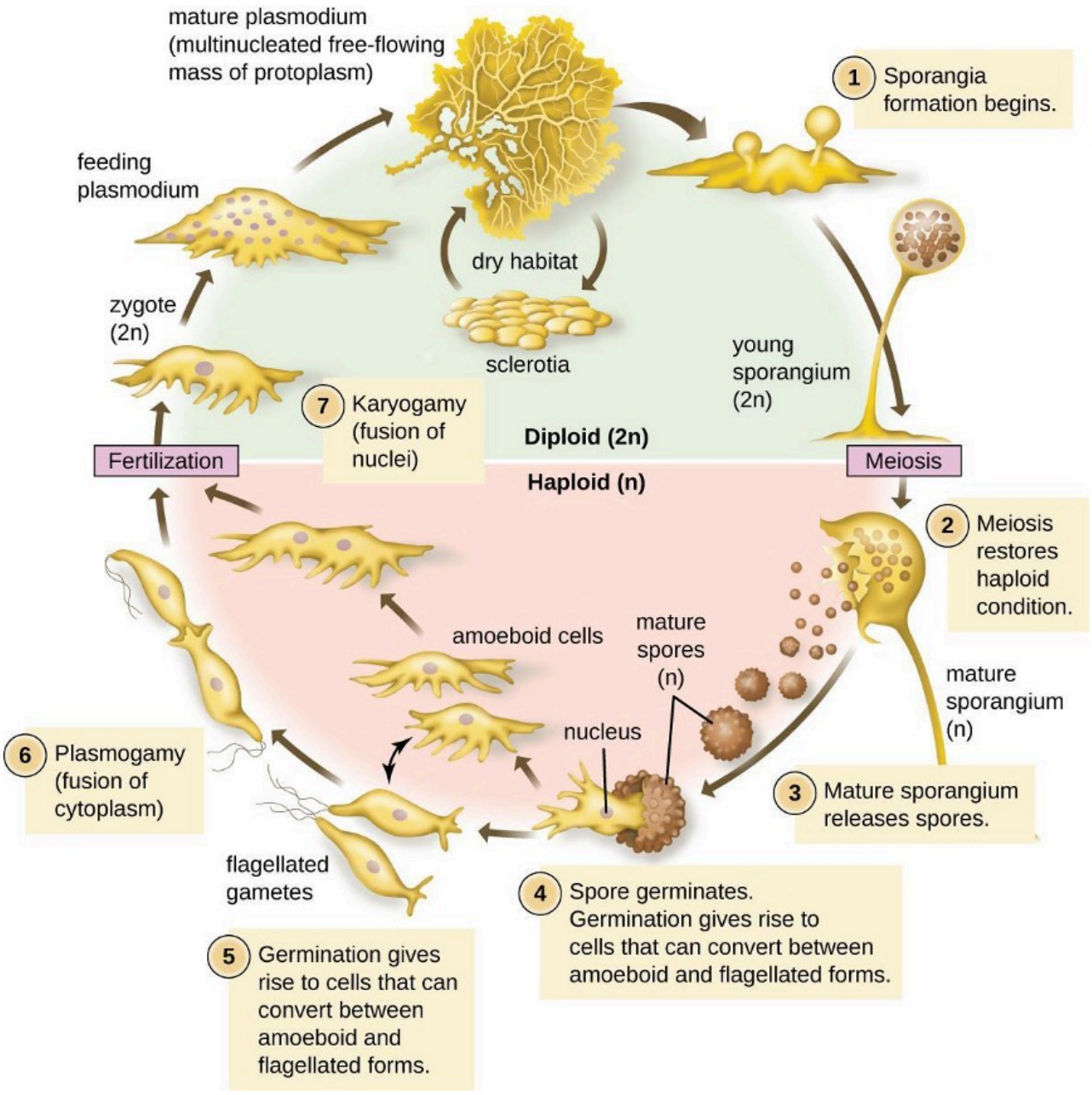


Figure 2.49: Plasmodial slime molds exist as large multinucleate amoeboid cells that form reproductive stalks to produce spores that divide into gametes. *Figure description available at the end of the chapter.*

Chromalveolata

The supergroup Chromalveolata is united by similar origins of its members' plastids and includes the apicomplexans, ciliates, diatoms, and dinoflagellates, among other groups. The apicomplexans are intra- or extracellular parasites that have an apical complex at one end of the cell. The apical complex is a concentration of organelles, vacuoles, and microtubules that allows the parasite to enter host cells (figure 2.50). Apicomplexans have complex life cycles that include an infective sporozoite that undergoes schizogony to make many merozoites (see the example in figure 2.45). Many are capable of infecting a variety of animal cells, from insects to livestock to humans, and their life cycles often depend on transmission between multiple hosts. The genus *Plasmodium* is an example of this group.

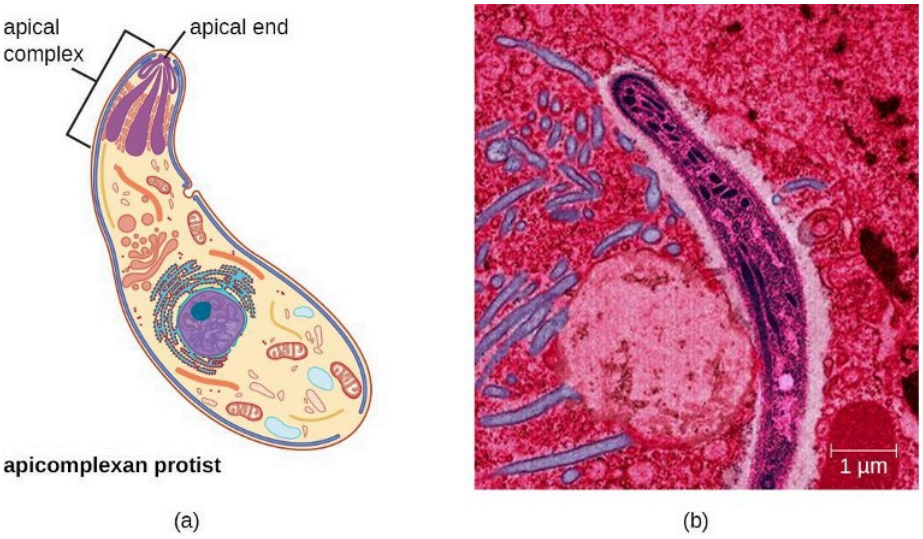


Figure 2.50: (a) Apicomplexans are parasitic protists. They have a characteristic apical complex that enables them to infect host cells. (b) A colorized electron microscope image of a Plasmodium sporozoite. *Figure description available at the end of the chapter.*

Other apicomplexans are also medically important. *Cryptosporidium parvum* causes intestinal symptoms and can cause epidemic diarrhea when the cysts contaminate drinking water. *Theileria (Babesia) microti*, transmitted by the tick *Ixodes scapularis*, causes recurring fever that can be fatal and is becoming a common transfusion-transmitted pathogen in the United States (*Theileria* and *Babesia* are closely related genera and there is some debate about the best classification). Finally, *Toxoplasma gondii* causes toxoplasmosis and can be transmitted from cat feces, unwashed fruit and vegetables, or from undercooked meat. Because toxoplasmosis can be associated with serious birth defects, pregnant women need to be aware of this risk and use caution if they are exposed to the feces of potentially infected cats. A national survey found the frequency of individuals with antibodies for toxoplasmosis (and thus who presumably have a current latent infection) in the United States to be 11%. Rates are much higher in other countries, including some developed countries.[17] There is also evidence and a good deal of theorizing that the parasite may be responsible for altering infected humans' behavior and personality traits.[18]

The ciliates (Ciliaphora), also within the Chromalveolata, are a large, very diverse group characterized by the presence of cilia on their cell surface. Although the cilia may be used for locomotion, they are often used for feeding as well, and some forms are nonmotile. *Balantidium coli* (figure 2.51) is the only parasitic ciliate that affects humans by causing intestinal illness, although it rarely causes serious medical issues except in the immunocompromised (those having a weakened immune system). Perhaps the most familiar ciliate is *Paramecium*, a motile organism with a clearly visible cytostome and cytoproct that is often studied in biology laboratories (figure 2.52). Another ciliate, *Stentor*, is sessile and uses its cilia for feeding (figure 2.53). Generally, these organisms have a micronucleus that is diploid, somatic, and used for sexual reproduction by conjugation. They also have a macronucleus that is derived from the micronucleus; the macronucleus becomes polyploid (multiple sets of duplicate chromosomes), and has a reduced set of metabolic genes.

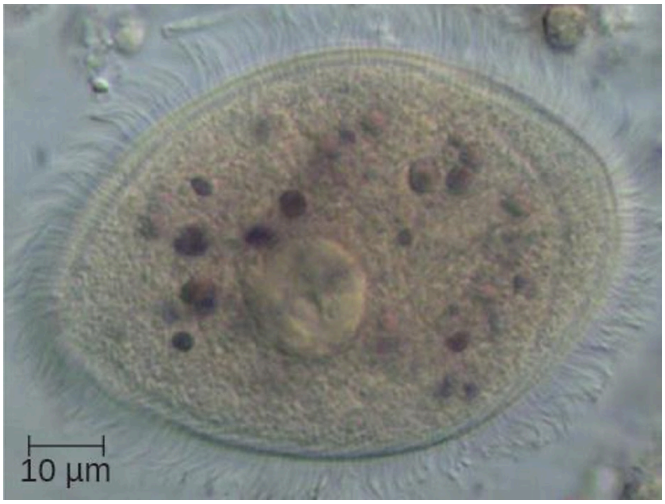


Figure 2.51: This specimen of the ciliate Balantidium coli is a trophozoite form isolated from the gut of a primate. B. coli is the only ciliate capable of parasitizing humans. *Figure description available at the end of the chapter.*

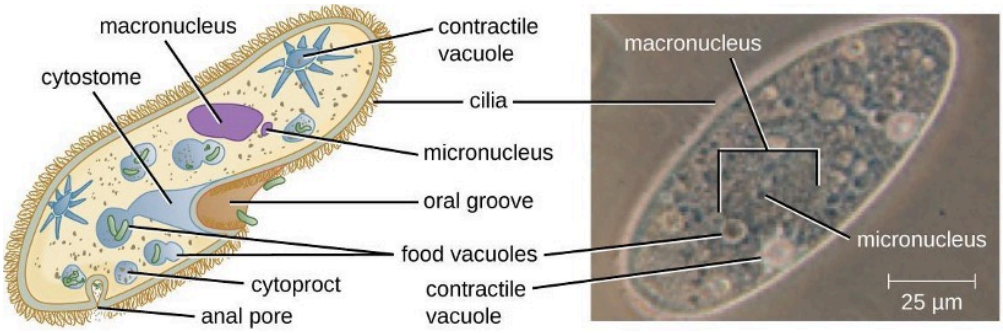


Figure 2.52: Paramecium has a primitive mouth (called an oral groove) to ingest food, and an anal pore to excrete it. Contractile vacuoles allow the organism to excrete excess water. Cilia enable the organism to move. *Figure description available at the end of the chapter.*

Ciliates are able to reproduce through conjugation, in which two cells attach to each other. In each cell, the diploid micronuclei undergo meiosis, producing eight haploid nuclei each. Then, all but one of the haploid micronuclei and the macronucleus disintegrate; the remaining (haploid) micronucleus undergoes mitosis. The two cells then exchange one micronucleus each, which fuses with the remaining micronucleus to form a new, genetically different, diploid micronucleus. The diploid micronucleus undergoes two mitotic divisions, so each cell has four micronuclei. Two of these four micronuclei then combine to form a new macronucleus. The chromosomes in the macronucleus then replicate repeatedly, the macronucleus reaches its polyploid state, and the two cells separate. The two cells are now genetically different from each other and from their previous versions.

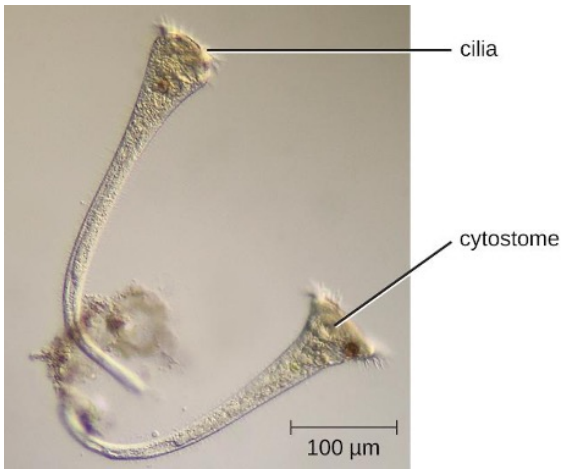


Figure 2.53: This differential interference contrast micrograph (magnification: ×65) of Stentor roeselie shows cilia present on the margins of the structure surrounding the cytostome; the cilia move food particles. *Figure description available at the end of the chapter.*

Figure 2.54: A saprobic oomycete, or water mold, engulfs a dead insect. *Figure description available at the end of the chapter.*

Öomycetes, also called water molds, have similarities to fungi and were once classified with them. However, they differ from fungi in several important ways. Unlike the chitinous cell walls of fungi, Öomycetes have walls composed of cellulose. Additionally, they are generally diploid, whereas the dominant life forms of fungi are typically haploid. *Phytophthora*, the plant pathogen found in the soil that caused the Irish potato famine, is classified within this group (figure 2.54).

Excavata

The third and final supergroup to be considered in this section is the Excavata, which includes primitive eukaryotes and many parasites with limited metabolic abilities. These organisms have complex cell shapes and structures, often including a depression on the surface of the cell called an excavate. The group Excavata includes the subgroups Fornicata, Parabasalia, and Euglenozoa. The Fornicata lack mitochondria but have flagella. This group includes *Giardia lamblia* (also known as *G. intestinalis* or *G. duodenalis)*, a widespread pathogen that causes diarrheal illness and can be spread through cysts from feces that contaminate water supplies (figure 2.44). Parabasalia are frequent animal endosymbionts; they live in the guts of animals like termites and cockroaches. They have basal bodies and modified mitochondria (kinetoplastids). They also have a large, complex cell structure with an undulating membrane and often have many flagella. The trichomonads (a subgroup of the Parabasalia) include pathogens such as *Trichomonas vaginalis*, which causes the human sexually transmitted disease trichomoniasis. Trichomoniasis often does not cause symptoms in men, but men are able to transmit the infection. In women, it causes vaginal discomfort and discharge and may cause complications in pregnancy if left untreated.

The Euglenozoa are common in the environment and include photosynthetic and nonphotosynthetic species. Members of the genus *Euglena* are typically not pathogenic. Their cells have two flagella, a pellicle, a stigma (eyespot) to sense light, and chloroplasts for photosynthesis (figure 2.55). The pellicle of *Euglena* is made of a series of protein bands surrounding the cell; it supports the cell membrane and gives the cell shape.

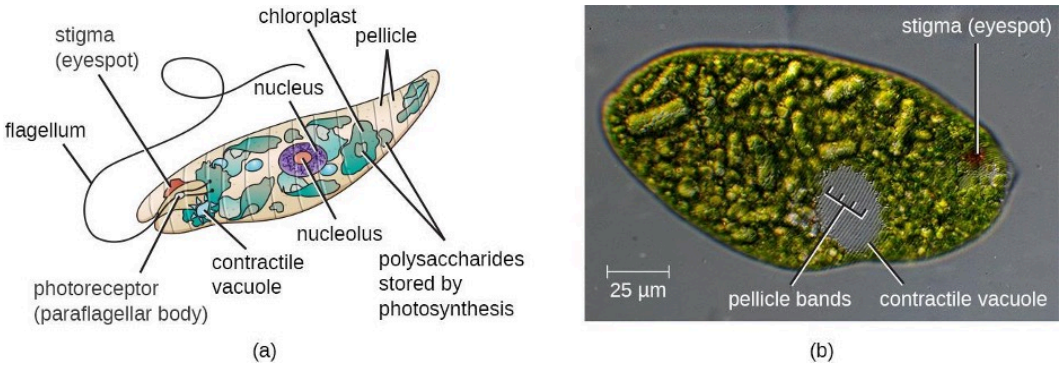


Figure 2.55: (a) This illustration of a Euglena shows the characteristic structures, such as the stigma and flagellum. (b) The pellicle, under the cell membrane, gives the cell its distinctive shape and is visible in this image as delicate parallel striations over the surface of the entire cell (especially visible over the grey contractile vacuole). *Figure description available at the end of the chapter.*

The Euglenozoa also include the trypanosomes, which are parasitic pathogens. The genus *Trypanosoma* includes *T. brucei*, which causes African trypanosomiasis (African sleeping sickness and *T. cruzi*, which causes American trypanosomiasis (Chagas disease). These tropical diseases are spread by insect bites. In African sleeping sickness, *T. brucei* colonizes the blood and the brain after being transmitted via the bite of a tsetse fly (*Glossina* spp.) (figure 2.56). The early symptoms include confusion, difficulty sleeping, and lack of coordination. Left untreated, it is fatal.

Chagas' disease originated and is most common in Latin America. The disease is transmitted by *Triatoma* spp., insects often called "kissing bugs," and affects either the heart tissue or tissues of the digestive system. Untreated cases can eventually lead to heart failure or significant digestive or neurological disorders.

The genus *Leishmania* includes trypanosomes that cause disfiguring skin disease and sometimes systemic illness as well.

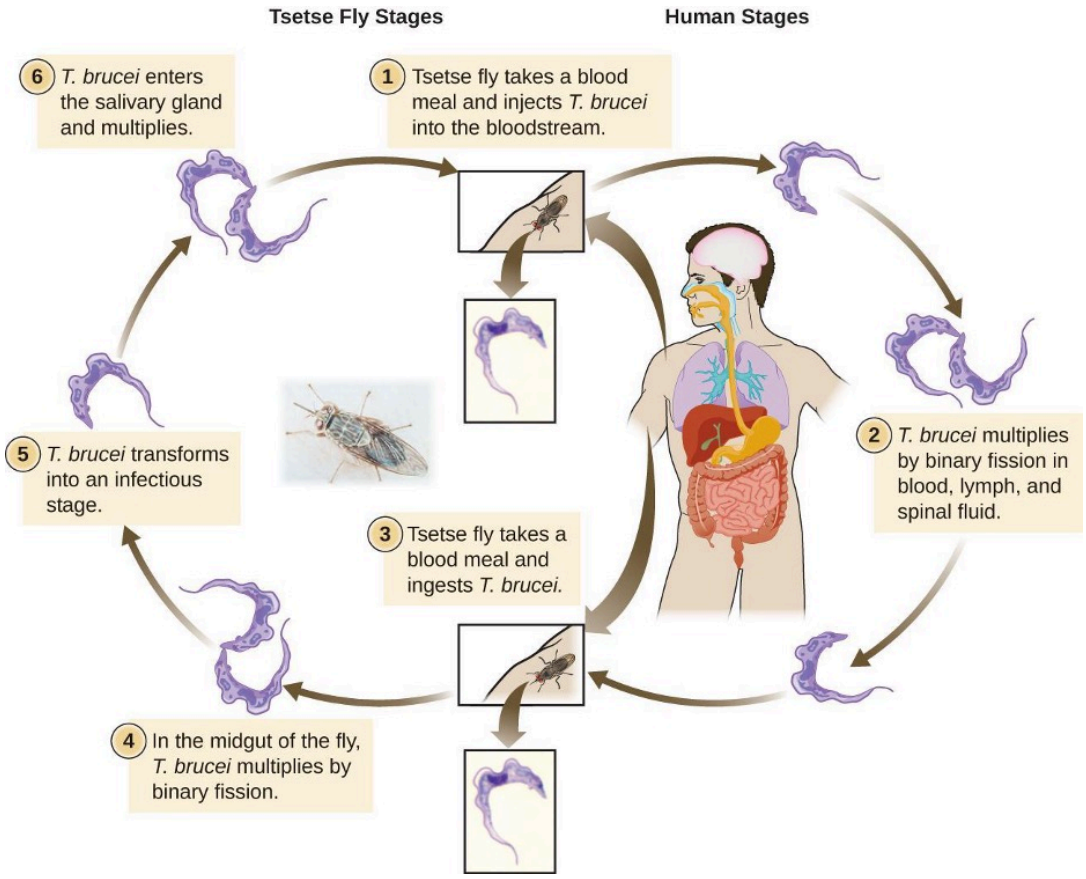


Figure 2.56: Trypanosoma brucei, the causative agent of African trypanosomiasis, spends part of its life cycle in the tsetse fly and part in humans. *Figure description available at the end of the chapter.*

2.8 PARASITIC HELMINTHS

There are two major groups of parasitic helminths: the roundworms (Nematoda) and flatworms (Platyhelminthes). Of the many species that exist in these groups, about half are parasitic and some are important human pathogens. As animals, they are multicellular and have organ systems. However, the parasitic species often have limited digestive tracts, nervous systems, and locomotor abilities. Parasitic forms may have complex reproductive cycles with several different life stages and more than one type of host. Some are monoecious, having both male and female reproductive organs in a single individual, while others are dioecious, each having either male or female reproductive organs.

NEMATODA (ROUNDWORMS)

Phylum Nematoda (the roundworms) is a diverse group containing more than 15,000 species, of which several are notable human parasites (figure 2.57). These unsegmented worms have a full digestive system even when parasitic. Some are common intestinal parasites, and their eggs can sometimes be identified in feces or around the anus of infected individuals. *Ascaris lumbricoides* is the largest nematode intestinal parasite found in humans; females may reach lengths greater than 1 meter. *A. lumbricoides* is also very widespread, even in developed nations, although it is now a relatively uncommon problem in the United States. It may cause symptoms ranging from relatively mild (such as a cough and mild abdominal pain) to severe (such as intestinal blockage and impaired growth).

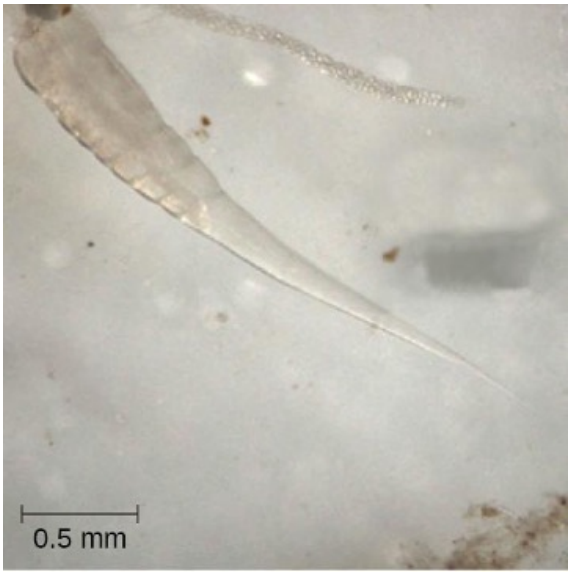


Figure 2.57: A micrograph of the nematode Enterobius vermicularis, also known as the pinworm. Figure description available at the end of the chapter.

Of all nematode infections in the United States, pinworm (caused by *Enterobius vermicularis*) is the most common. Pinworm causes sleeplessness and itching around the anus, where the female worms lay their eggs during the night. *Toxocara canis* and *T. cati* are nematodes found in dogs and cats, respectively, that can be transmitted to humans, causing toxocariasis. Antibodies to these parasites have been found in approximately 13.9% of the U.S. population, suggesting that exposure is common.[19] Infection can cause larval migrans, which can result in vision loss and eye inflammation, or fever, fatigue, coughing, and abdominal pain, depending on whether the organism infects the eye or the viscera. Another common nematode infection is hookworm, which is caused by *Necator americanus* (the New World or North American hookworm) and *Ancylostoma duodenale* (the Old World hookworm). Symptoms of hookworm infection can include abdominal pain, diarrhea, loss of appetite, weight loss, fatigue, and anemia.

Trichinellosis, also called trichinosis, caused by *Trichinella spiralis*, is contracted by consuming undercooked meat, which releases the larvae and allows them to encyst in muscles. Infection can cause fever, muscle pains, and digestive system problems; severe infections can lead to lack of coordination, breathing and heart problems, and even death. Finally, heartworm in dogs and other animals is caused by the nematode *Dirofilaria immitis*, which is transmitted by mosquitoes. Symptoms include fatigue and cough; when left untreated, death may result.

PLATYHELMINTHES (FLATWORMS)

Phylum Platyhelminthes (the platyhelminthes) are flatworms. This group includes the flukes, tapeworms, and the turbellarians, which include planarians. The flukes and tapeworms are medically relevant parasites (figure 2.58).

The flukes (trematodes) are nonsegmented flatworms that have an oral sucker (figure 2.59) (and sometimes a second ventral sucker) and attach to the inner walls of intestines, lungs, large blood vessels, or the liver. Trematodes have complex life cycles, often with multiple hosts. Several important examples are the liver flukes (*Clonorchis* and *Opisthorchis*), the intestinal fluke (*Fasciolopsis buski*), and the oriental lung fluke (*Paragonimus westermani*). Schistosomiasis is a serious parasitic disease, considered second in the scale of its impact on human populations only to malaria. The parasites *Schistosoma mansoni, S. haematobium*, and *S. japonicum* (found in freshwater snails) are responsible for schistosomiasis. Immature forms burrow through the skin into the blood. They migrate to the lungs, then to the liver and, later, other organs. Symptoms include anemia, malnutrition, fever, abdominal pain, fluid buildup, and sometimes death. Figure 2.60 presents the life cycle of *Schistosoma* spp.

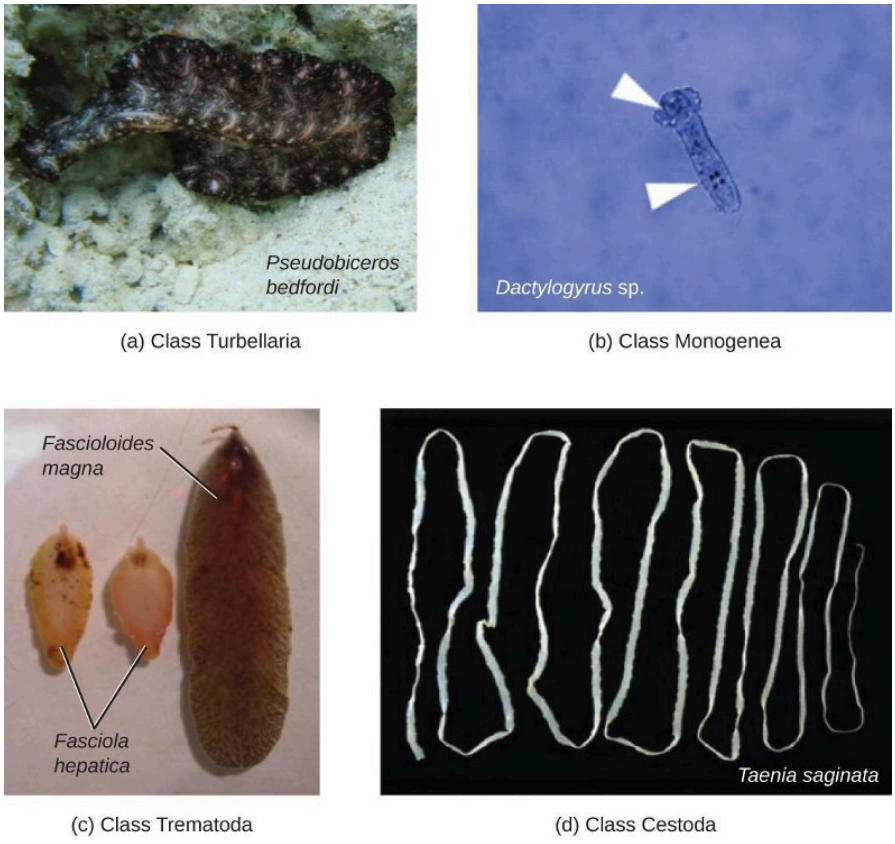


Figure 2.58: Phylum Platyhelminthes is divided into four classes. (a) Class Turbellaria includes the Bedford's flatworm (Pseudobiceros bedfordi), which is about 8–10 cm long. (b) The parasitic class Monogenea includes Dactylogyrus spp. Worms in this genus are commonly called gill flukes. The specimen pictured here is about 0.2 mm long and has two anchors, indicated by arrows, that it uses to latch onto the gills of host fish. (c) The Trematoda class includes the common liver fluke Fasciola hepatica and the giant liver fluke Fascioloides magna (right). The F. magna specimen shown here is about 7 cm long. (d) Class Cestoda includes tapeworms such as Taenia saginata, which infects both cattle and humans and can reach lengths of 4–10 meters; the specimen shown here is about 4 meters long. *Figure description available at the end of the chapter.*

The other medically important group of platyhelminths are commonly known as tapeworms (cestodes) and are segmented flatworms that may have suckers or hooks at the scolex (head region) (figure 2.59). Tapeworms use these suckers or hooks to attach to the wall of the small intestine. The body of the worm is made up of segments called proglottids that contain reproductive structures; these detach when the gametes are fertilized, releasing gravid proglottids with eggs. Tapeworms often have an intermediate host that consumes the eggs, which then hatch into a larval form called an oncosphere. The oncosphere migrates to a particular tissue or organ in the intermediate host, where it forms cysticerci. After being eaten by the definitive host, the cysticerci develop into adult tapeworms in the host's digestive system (figure 2.61). *Taenia saginata* (the beef tapeworm) and *T. solium* (the pork tapeworm) enter humans through ingestion of undercooked, contaminated meat. The adult worms develop and reside in the intestine, but the larval stage may migrate and be found in other body locations such as skeletal and smooth muscle. The beef tapeworm is relatively benign, although it can cause digestive problems and, occasionally, allergic reactions. The pork tapeworm can cause more serious problems when the larvae leave the intestine and colonize other tissues, including those of the central nervous system. *Diphylobothrium latum* is the largest human tapeworm and can be ingested in undercooked fish. It can grow to a length of 15 meters. *Echinococcus granulosus,* the dog tapeworm, can parasitize humans and uses dogs as a host.

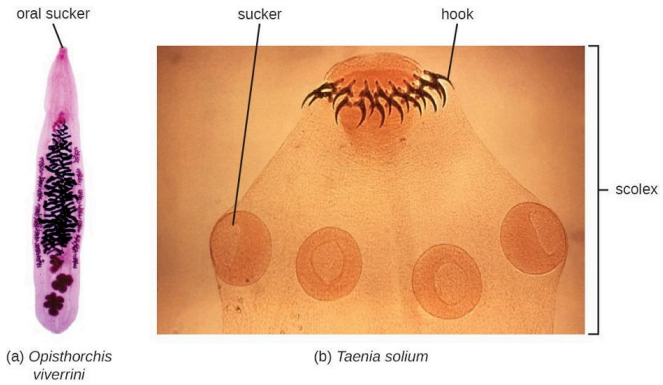


Figure 2.59: (a) The oral sucker is visible on the anterior end of this liver fluke, Opisthorchis viverrini. (b) This micrograph shows the scolex of the cestode Taenia solium, also known as the pork tapeworm. The visible suckers and hooks allow the worm to attach itself to the inner wall of the intestine. *Figure description available at the end of the chapter.*

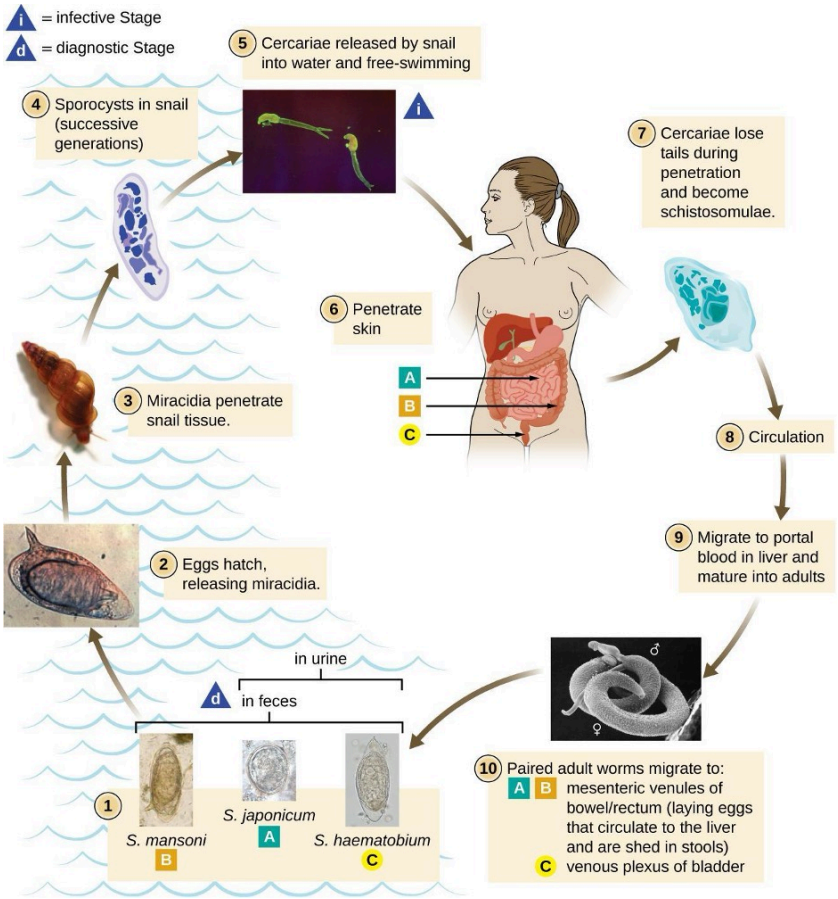


Figure 2.60: The life cycle of Schistosoma spp. includes several species of water snails, which serve as secondary hosts. The parasite is transmitted to humans through contact with contaminated water and takes up residence in the veins of the digestive system. Eggs escape the host in the urine or feces and infect a snail to complete the life cycle. *Figure description available at the end of the chapter.*

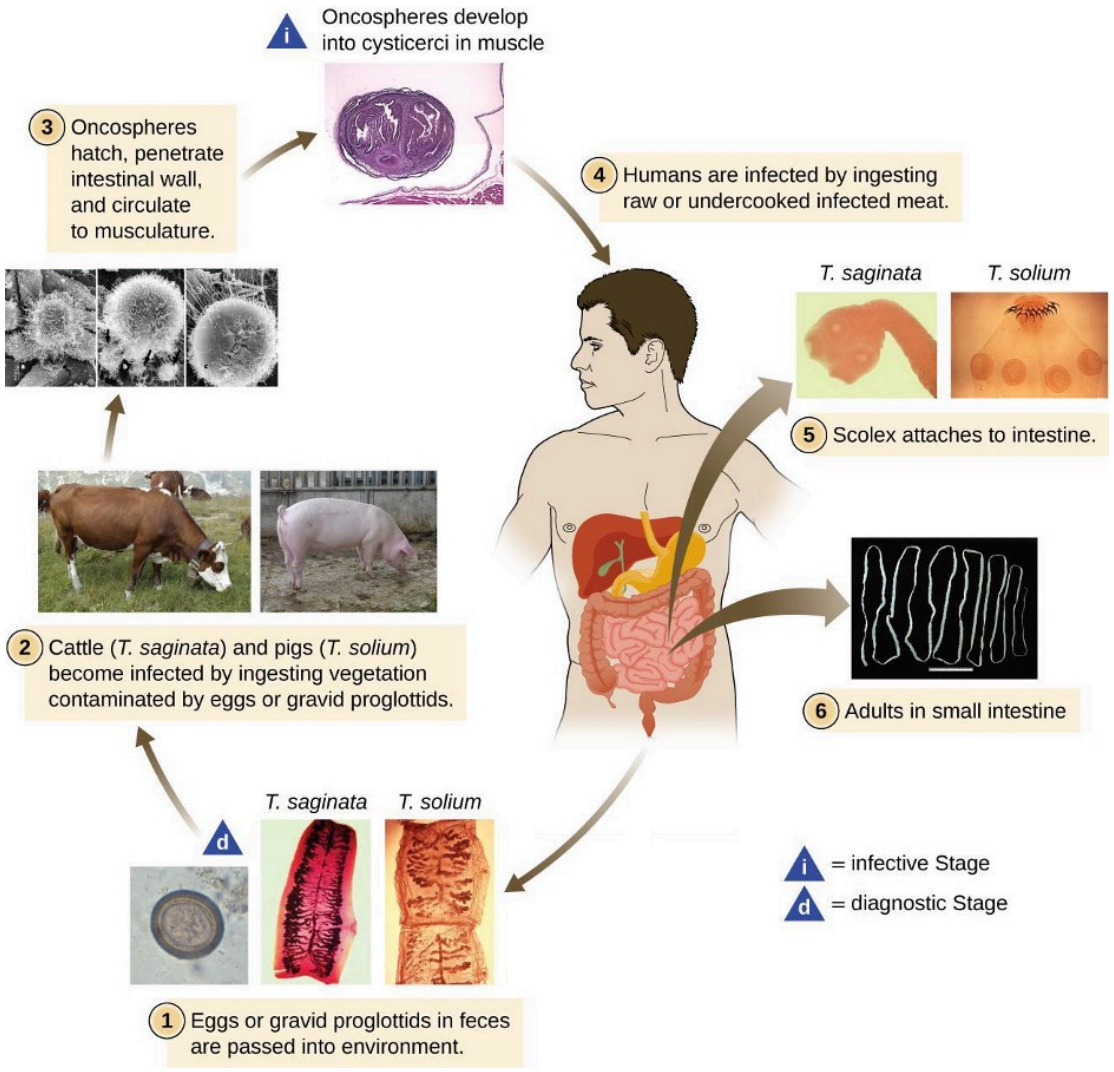


Figure 2.61: Life cycle of a tapeworm. *Figure description available at the end of the chapter.*

2.9 FUNGI

Fungi are important to humans in a variety of ways. Both microscopic and macroscopic fungi have medical relevance, with some pathogenic species that can cause mycoses (illnesses caused by fungi). Some pathogenic fungi are opportunistic, meaning that they mainly cause infections when the host's immune defenses are compromised and do not normally cause illness in healthy individuals. Fungi are important in other ways as well. They act as decomposers in the environment, and they are critical for the production of certain foods such as cheeses. Fungi are also major sources of antibiotics, such as penicillin from the fungus *Penicillium*.

CHARACTERISTICS OF FUNGI

Fungi have well-defined characteristics that set them apart from other organisms. Most multicellular fungal bodies, commonly called molds, are made up of filaments called hyphae. Hyphae can form a tangled network called a mycelium and form the thallus (body) of fleshy fungi. Hyphae that have walls between the cells are called septate hyphae; hyphae that lack walls and cell membranes between the cells are called nonseptate or coenocytic hyphae) (figure 2.62).

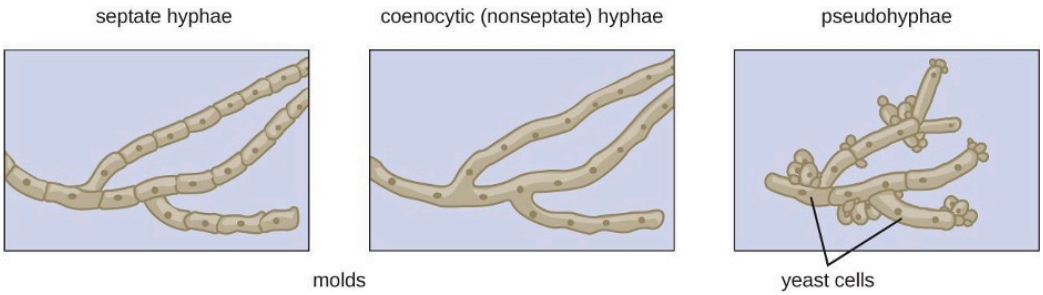


Figure 2.62: Multicellular fungi (molds) form hyphae, which may be septate or nonseptate. Unicellular fungi (yeasts) cells form pseudohyphae from individual yeast cells. *Figure description available at the end of the chapter.*

In contrast to molds, yeasts are unicellular fungi. The budding yeasts reproduce asexually by budding off a smaller daughter cell; the resulting cells may sometimes stick together as a short chain or pseudohyphae (figure 2.62).

Some fungi are dimorphic, meaning they have more than one appearance during their life cycle. These dimorphic fungi may be able to appear as yeasts or molds, which can be important for infectivity. They are capable of changing their appearance in response to environmental changes such as nutrient availability or fluctuations in temperature, growing as a mold, for example, at 25 °C (77 °F), and as yeast cells at 37 °C (98.6 °F). This ability helps dimorphic fungi to survive in diverse environments. Two examples of dimorphic yeasts are the human pathogens *Histoplasma capsulatum* and *Candida albicans*. *H. capsulatum* causes the lung disease histoplasmosis, and *C. albicans* is associated with vaginal yeast infections, oral thrush, and candidiasis of the skin (figure 2.63).

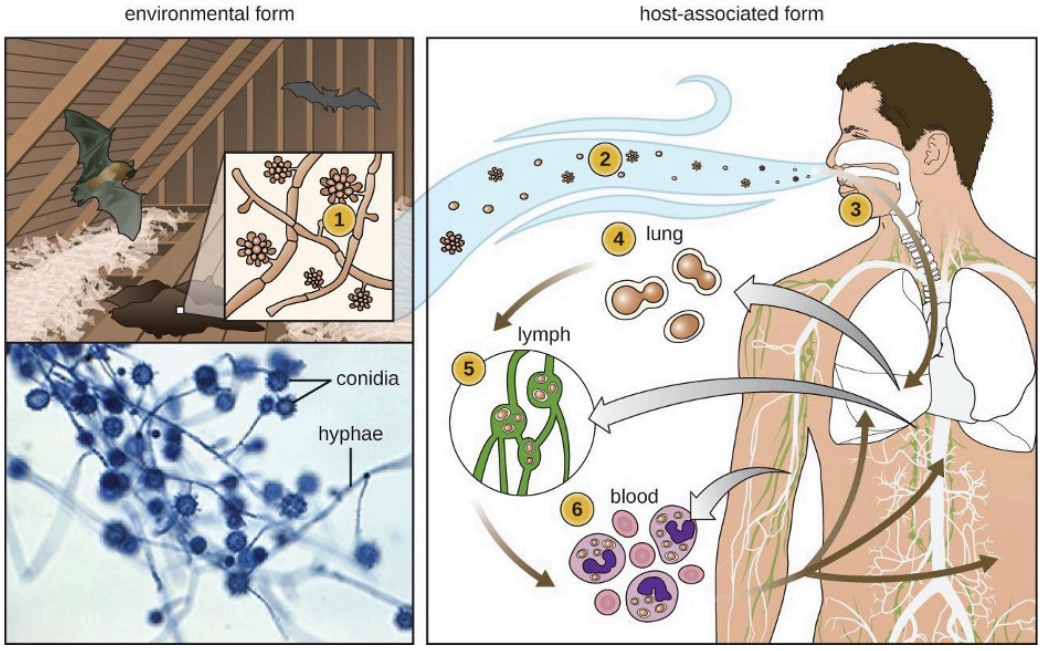


Figure 2.63: Histoplasma capsulatum is a dimorphic fungus that grows in soil exposed to bird feces or bat feces (guano) (top left). It can change forms to survive at different temperatures. In the outdoors, it typically grows as a mycelium (as shown in the micrograph, bottom left), but when the spores are inhaled (right), it responds to the high internal temperature of the body (37 °C [98.6 °F]) by turning into a yeast that can multiply in the lungs, causing the chronic lung disease histoplasmosis. *Figure description available at the end of the chapter.*

There are notable unique features in fungal cell walls and membranes. Fungal cell walls contain chitin, as opposed to the cellulose found in the cell walls of plants and many protists. Additionally, whereas animals have cholesterol in their cell membranes, fungal cell membranes have different sterols called ergosterols. Ergosterols are often exploited as targets for antifungal drugs.

Fungal life cycles are unique and complex. Fungi reproduce sexually either through cross- or self-fertilization. Haploid fungi form hyphae that have gametes at the tips. Two different mating types (represented as + type and – type) are involved. The cytoplasms of the + and – type gametes fuse (in an event called plasmogamy), producing a cell with two distinct nuclei (a dikaryotic cell). Later, the nuclei fuse (in an event called karyogamy) to create a diploid zygote. The zygote undergoes meiosis to form spores that germinate to start the haploid stage, which eventually creates more haploid mycelia (figure 2.64). Depending on the taxonomic group, these sexually produced spores are known as zygospores (in Zygomycota), ascospores (in Ascomycota), or basidiospores (in Basidiomycota) (figure 2.65).

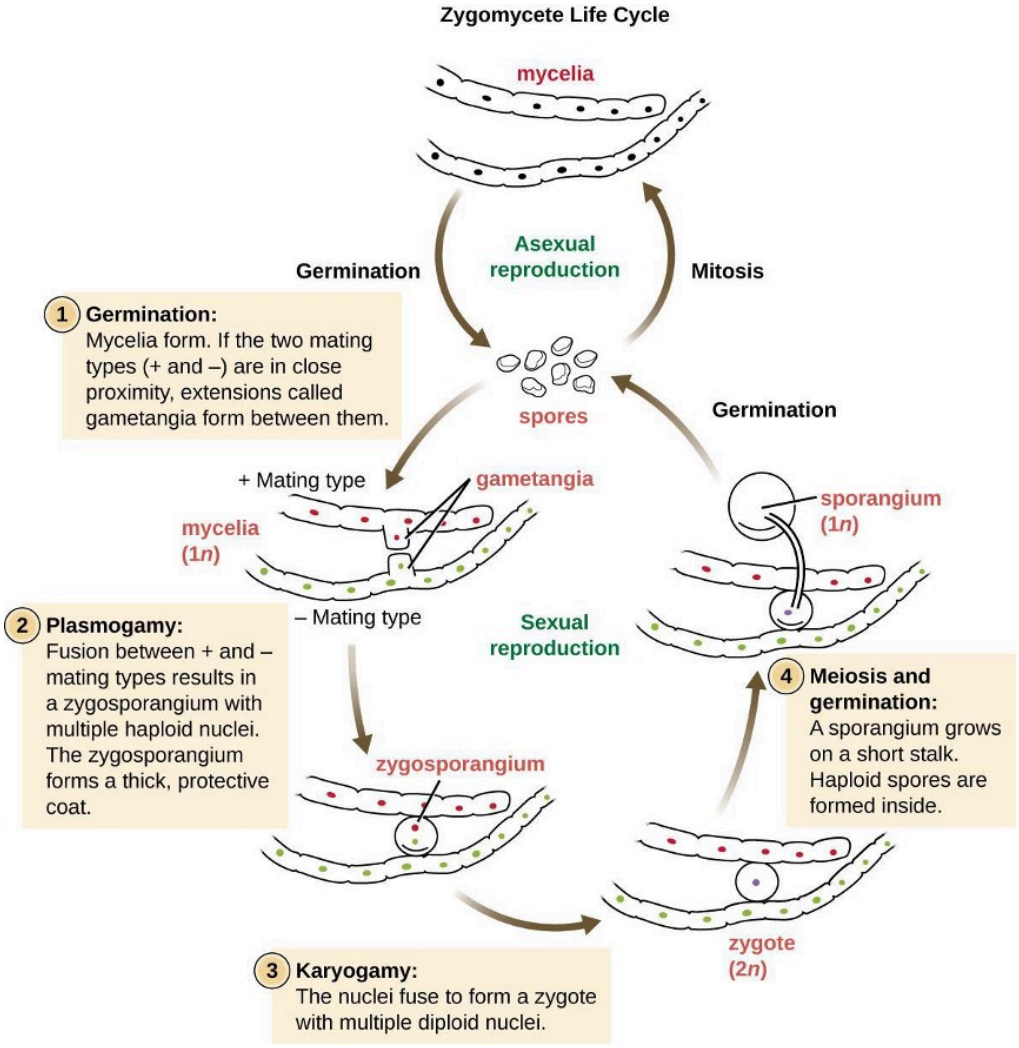


Figure 2.64: Zygomycetes have sexual and asexual life cycles. In the sexual life cycle, + and – mating types conjugate to form a zygosporangium. *Figure description available at the end of the chapter.*

Fungi may also exhibit asexual reproduction by mitosis, mitosis with budding, fragmentation of hyphae, and formation of asexual spores by mitosis. These spores are specialized cells that, depending on the organism, may have unique characteristics for survival, reproduction, and dispersal. Fungi exhibit several types of asexual spores and these can be important in classification.

Figure 2.65: These images show asexually produced spores. (a) This brightfield micrograph shows the release of spores from a sporangium at the end of a hypha called a sporangiophore. The organism is a Mucor sp. fungus, a mold often found indoors. (b) Sporangia grow at the ends of stalks, which appear as the white fuzz seen on this bread mold, Rhizopus stolonifer. The tips of bread mold are the dark, spore-containing sporangia. *Figure description available at the end of the chapter.*

FUNGAL DIVERSITY

The fungi are very diverse, comprising seven major groups. Not all of the seven groups contain pathogens. Some of these groups are generally associated with plants and include plant pathogens. For example, Urediniomycetes and Ustilagomycetes include the plant rusts and smuts, respectively. These form reddish or dark masses, respectively, on plants as rusts (red) or smuts (dark). Some species have substantial economic impact because of their ability to reduce crop yields. Glomeromycota includes the mycorrhizal fungi, important symbionts with plant roots that can promote plant growth by acting like an extended root system. The Glomeromycota are obligate symbionts, meaning that they can only survive when associated with plant roots; the fungi receive carbohydrates from the plant and the plant benefits from the increased ability to take up nutrients and minerals from the soil. The Chytridiomycetes (chytrids) are small fungi, but are extremely ecologically important. Chytrids are generally aquatic and have flagellated, motile gametes; specific types are implicated in amphibian declines around the world. Because of their medical importance, we will focus on Zygomycota, Ascomycota, Basidiomycota, and Microsporidia. Table 2.17 summarizes the characteristics of these medically important groups of fungi.

The Zygomycota (zygomycetes) are mainly saprophytes with coenocytic hyphae and haploid nuclei. They use sporangiospores for asexual reproduction. The group name comes from the zygospores that they use for sexual reproduction (figure 2.64), which have hard walls formed from the fusion of reproductive cells from two individuals. Zygomycetes are important for food science and as crop pathogens. One example is *Rhizopus stolonifer* (figure 2.65), an important bread mold that also causes rice seedling blight. *Mucor* is a genus of fungi that can potentially cause necrotizing infections in humans, although most species are intolerant of temperatures found in mammalian bodies (figure 2.65).

The Ascomycota include fungi that are used as food (edible mushrooms, morels, and truffles), others that are common causes of food spoilage (bread molds and plant pathogens), and still others that are human pathogens. Ascomycota may have septate hyphae and cup-shaped fruiting bodies called ascocarps. Some genera of Ascomycota use sexually produced ascospores as well as asexual spores called conidia, but sexual phases have not been discovered or described for others. Some produce an ascus containing ascospores within an ascocarp (figure 2.66).

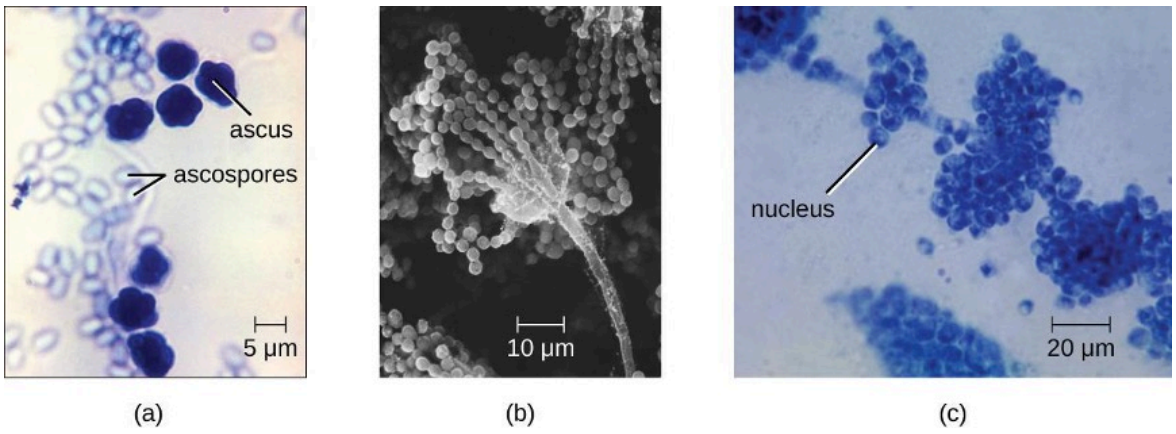


Figure 2.66: (a) This brightfield micrograph shows ascospores being released from asci in the fungus Talaromyces flavus var. flavus. (b) This electron micrograph shows the conidia (spores) borne on the conidiophore of Aspergillus, a type of toxic fungus found mostly in soil and plants. (c) This brightfield micrograph shows the yeast Candida albicans, the causative agent of candidiasis and thrush. *Figure description available at the end of the chapter.*

Examples of the Ascomycota include several bread molds and minor pathogens, as well as species capable of causing more serious mycoses. Species in the genus *Aspergillus* are important causes of allergy and infection, and are useful in research and in the production of certain fermented alcoholic beverages such as Japanese *sake*. The fungus *Aspergillus flavus,* a contaminant of nuts and stored grains, produces an aflatoxin that is both a toxin and the most potent known natural carcinogen. *Neurospora crassa* is of particular use in genetics research because the spores produced by meiosis are kept inside the ascus in a row that reflects the cell divisions that produced them, giving a direct view of segregation and assortment of genes (figure 2.67). *Penicillium* produces the antibiotic penicillin (figure 2.66).

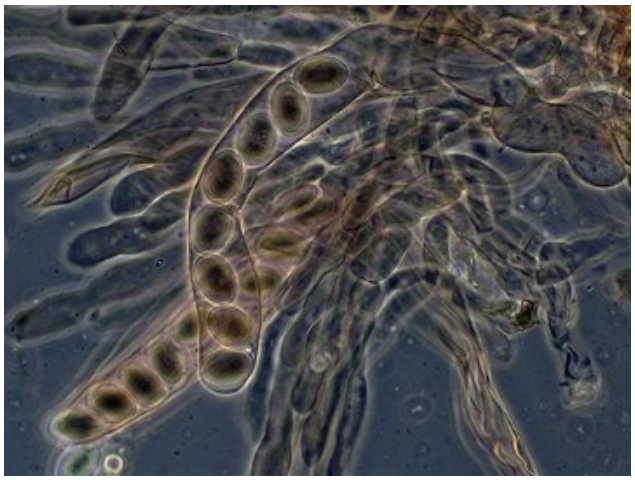


Figure 2.67: These ascospores, lined up within an ascus, are produced sexually. *Figure description available at the end of the chapter.*

Many species of ascomycetes are medically important. A large number of species in the genera *Trichophyton*, *Microsporum*, and *Epidermophyton* are dermatophytes, pathogenic fungi capable of causing skin infections such as athlete's foot, jock itch, and ringworm (figure 2.42). *Blastomyces dermatitidis* is a dimorphic fungus that can cause blastomycosis, a respiratory infection that, if left untreated, can become disseminated to other body sites, sometimes leading to death. Another important respiratory pathogen is the dimorphic fungus *Histoplasma capsulatum* (figure 2.63), which is associated with birds and bats in the Ohio and Mississippi river valleys. *Coccidioides immitis* causes the serious lung disease Valley fever. *Candida albicans*, the most common cause of vaginal and other yeast infections, is also an ascomycete fungus; it is a part of the normal microbiota of the skin, intestine, genital tract, and ear (figure 2.66). Ascomycetes also cause plant diseases, including ergot infections, Dutch elm disease, and powdery mildews.

Saccharomyces yeasts, including the baker's yeast *S. cerevisiae*, are unicellular ascomycetes with haploid and diploid stages (figure 2.68). This and other *Saccharomyces* species are used for brewing beer.

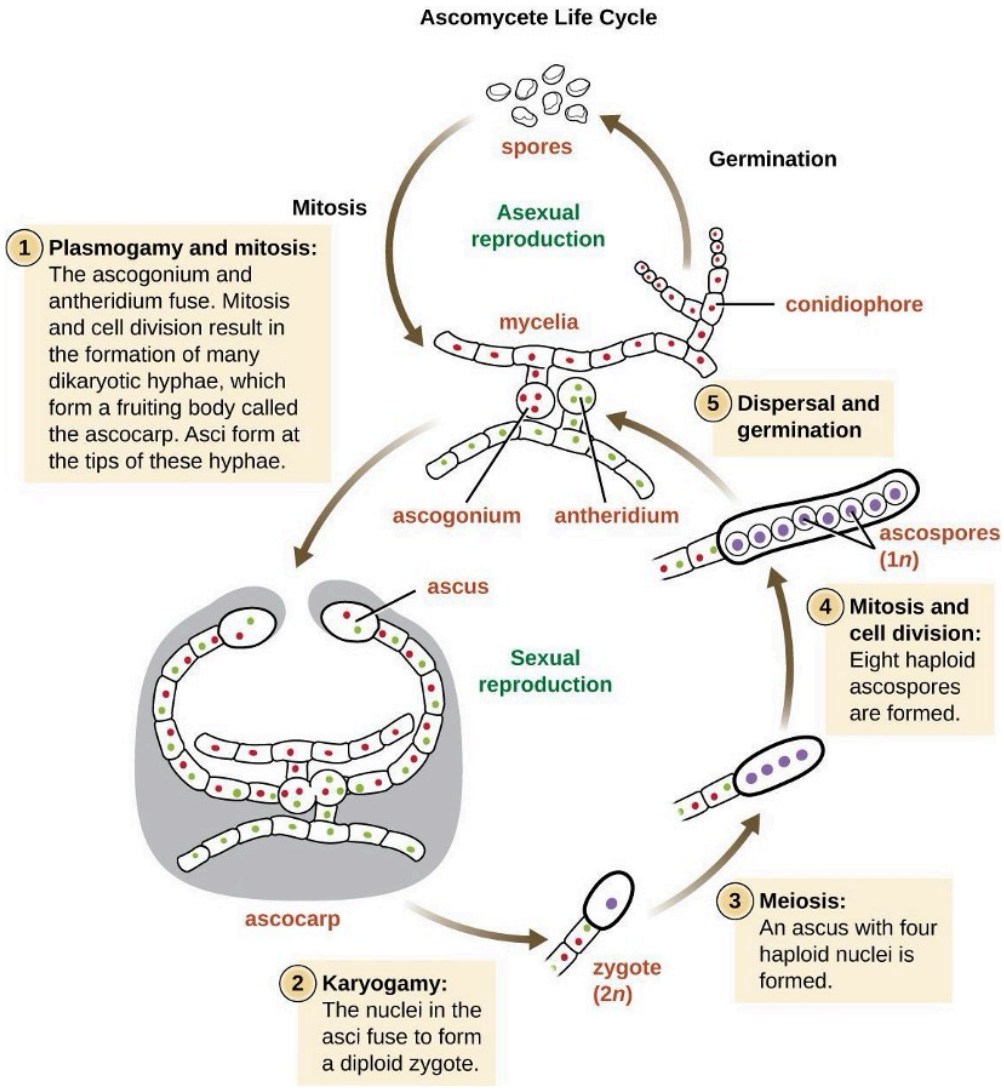


Figure 2.68: The life cycle of an ascomycete is characterized by the production of asci during the sexual phase. The haploid phase is the predominant phase of the life cycle. Whether spores are produced through sexual or asexual processes, they can germinate into haploid hyphae. *Figure description available at the end of the chapter.*

The Basidiomycota (basidiomycetes) are fungi that have basidia (club-shaped structures) that produce basidiospores (spores produced through budding) within fruiting bodies called basidiocarps (figure 2.69). They are important as decomposers and as food. This group includes rusts, stinkhorns, puffballs, and mushrooms. Several species are of particular importance. *Cryptococcus neoformans*, a fungus commonly found as a yeast in the environment, can cause serious lung infections when inhaled by individuals with weakened immune systems. The edible meadow mushroom, *Agricus campestris*, is a basidiomycete, as is the poisonous mushroom *Amanita phalloides*, known as the death cap. The deadly toxins produced by *A. phalloides* have been used to study transcription.

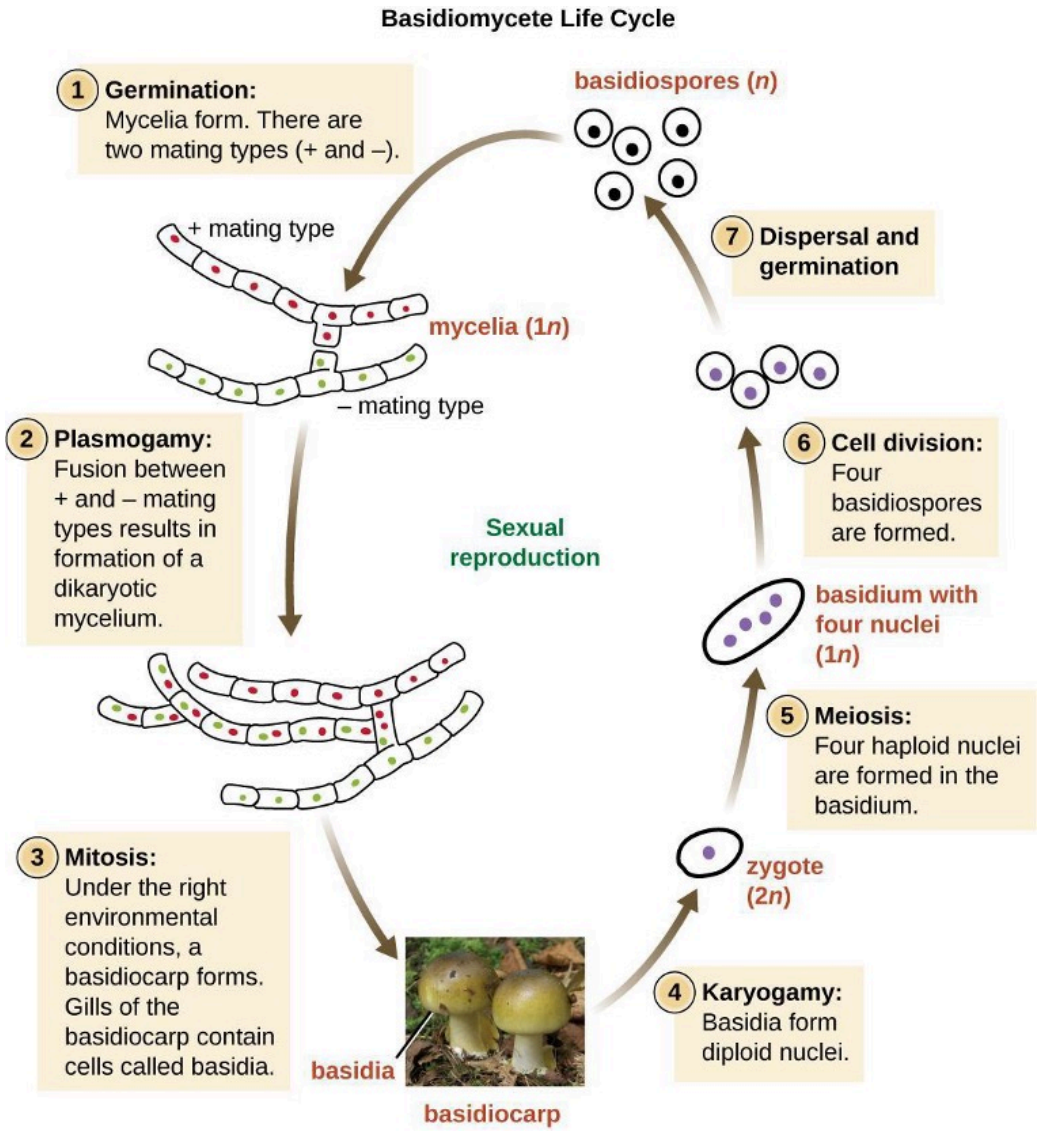


Figure 2.69: The life cycle of a basidiomycete alternates a haploid generation with a prolonged stage in which two nuclei (dikaryon) are present in the hyphae. *Figure description available at the end of the chapter.*

Finally, the Microsporidia are unicellular fungi that are obligate intracellular parasites. They lack mitochondria, peroxisomes, and centrioles, but their spores release a unique polar tubule that pierces the host cell membrane to allow the fungus to gain entry into the cell. A number of microsporidia are human pathogens, and infections with microsporidia are called microsporidiosis. One pathogenic species is *Enterocystozoan bieneusi*, which can cause symptoms such as diarrhea, cholecystitis (inflammation of the gall bladder), and in rare cases, respiratory illness.

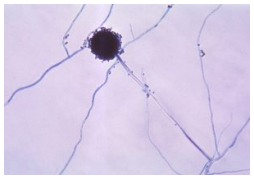
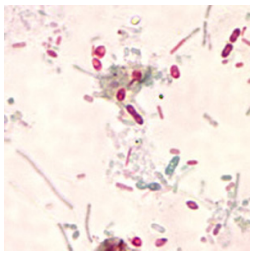
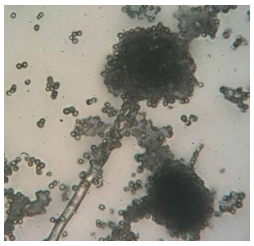
Group	Characteristics	Examples	Medically Important Species	Image
Ascomycota	Septate hyphae Ascus with ascospores in ascocarp Condidiospores	Cup fungi Edible mushrooms Morels Truffles *Neurospora* *Penicillium*	*Aspergillus* spp. *Trichophyton* spp. *Microsporum* spp. *Epidermonphyton* spp. *Blastomyces dermititus* *Histoplasma capsulatum*	 *Aspergillus niger*
Basidiomycota	Basidia produce basidiospores in a basidiocarp	Club fungi Rusts Stinkhorns Puffballs Mushrooms *Cryptococcus neoformans* *Amanita phalloides*	*Crytococcus neoformans*	 *Amanita phalloides*
Microsporidia	Lack mitochondria, peroxisomes, centrioles Spores produce a polar tube	*Enterocystozoan bieneusi*	*Enterocystozoan bieneusi*	 Microsporidia (unidentified)
Zygomycota	Mainly saprophytes Coenocytic hyphae Haploid nuclei Zygospores	*Rhizopus stolonifera*	*Mucor* spp.	 *Rhizopus sp.*

Table 2.17: Select groups of fungi

2.10 VIRUSES

Viruses are distinct biological entities; however, their evolutionary origin is still a matter of speculation. In terms of taxonomy, they are not included in the tree of life because they are acellular (not consisting of cells). In order to survive and reproduce, viruses must infect a cellular host, making them obligate intracellular parasites. The genome of a virus enters a host cell and directs the production of the viral components, proteins and nucleic acids, needed to form new virus particles called virions. New virions are made in the host cell by assembly of viral components. The new virions transport the viral genome to another host cell to carry out another round of infection. Table 2.18 summarizes the properties of viruses.

Characteristics of Viruses
Infectious, acellular pathogens
Obligate intracellular parasites with host and cell-type specificity
DNA or RNA genome (never both)
Genome is surrounded by a protein capsid and, in some cases, a phospholipid membrane studded with viral glycoproteins
Lack genes for many products needed for successful reproduction, requiring exploitation of host-cell genomes to reproduce

Table 2.18: Characteristics of viruses

HOSTS AND VIRAL TRANSMISSION

Viruses can be transmitted through direct contact, indirect contact with fomites, or through a vector: an animal that transmits a pathogen from one host to another. Arthropods such as mosquitoes, ticks, and flies, are typical vectors for viral diseases, and they may act as mechanical vectors or biological vectors. Mechanical transmission occurs when the arthropod carries a viral pathogen on the outside of its body and transmits it to a new host by physical contact. Biological transmission occurs when the arthropod carries the viral pathogen inside its body and transmits it to the new host through biting.

Viruses that can be transmitted from an animal host to a human host can cause zoonoses. For example, the avian influenza virus originates in birds but can cause disease in humans. Reverse zoonoses are caused by infection of an animal by a virus that originated in a human.

VIRAL STRUCTURES

In general, virions (viral particles) are small and cannot be observed using a regular light microscope. They are much smaller than prokaryotic and eukaryotic cells; this is an adaptation allowing viruses to infect these larger cells (see figure 2.70). The size of a virion can range from 20 nm for small viruses up to 900 nm for typical, large viruses (see figure 2.72). Recent discoveries, however, have identified new giant viral species, such as *Pandoravirus salinus* and *Pithovirus sibericum*, with sizes approaching that of a bacterial cell.[20]

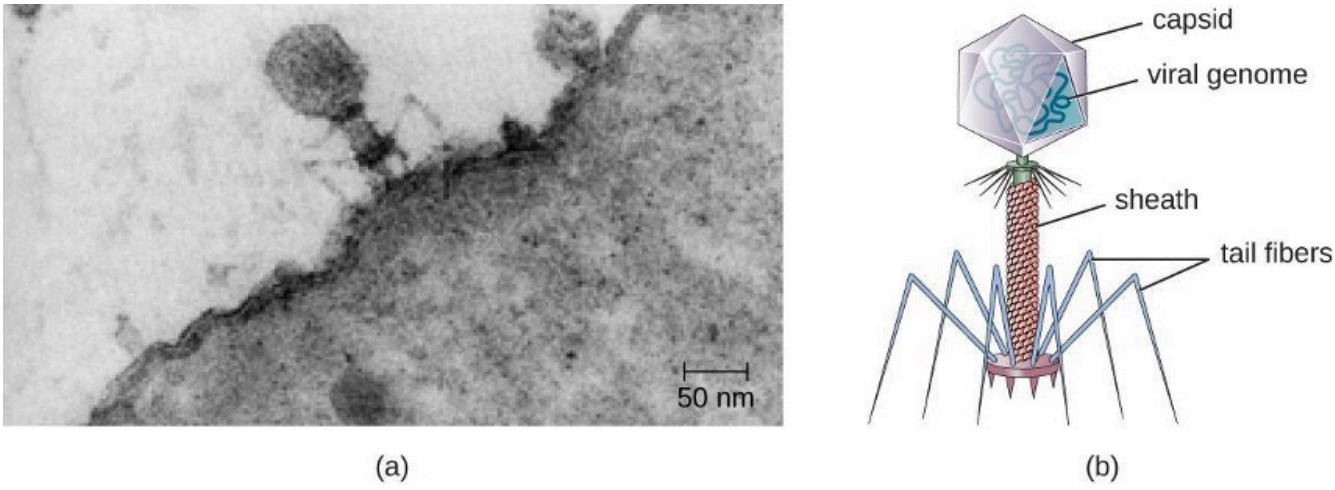


Figure 2.70: (a) In this transmission electron micrograph, a bacteriophage (a virus that infects bacteria) is dwarfed by the bacterial cell it infects. (b) An illustration of the bacteriophage in the micrograph. *Figure description available at the end of the chapter.*

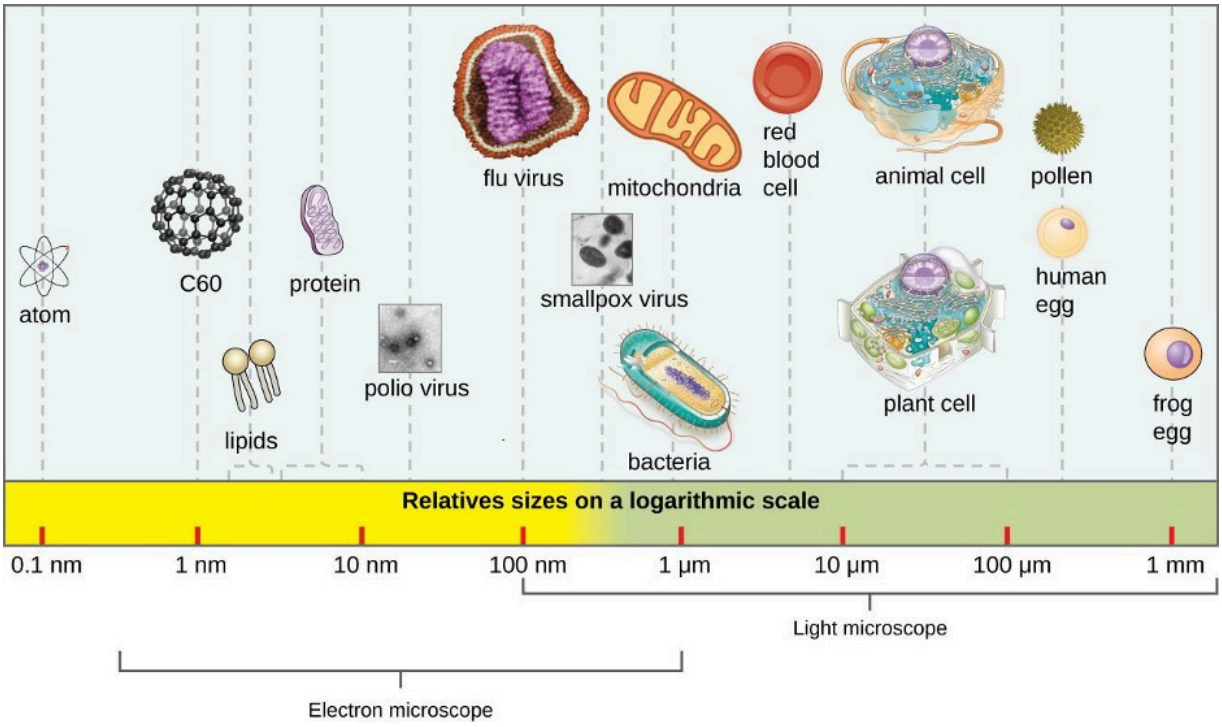


Figure 2.71: The size of a virus is small relative to the size of most bacterial and eukaryotic cells and their organelles. Figure description available at the end of the chapter.

There are two categories of viruses based on general composition. Viruses formed from only a nucleic acid and capsid are called naked viruses or nonenveloped viruses. Viruses formed with a nucleic-acid packed capsid surrounded by a lipid layer are called enveloped viruses (see figure 2.72). The viral envelope is a small portion of phospholipid membrane obtained as the virion buds from a host cell. The viral envelope may either be intracellular or cytoplasmic in origin.

Extending outward and away from the capsid on some naked viruses and enveloped viruses are protein structures called spikes. At the tips of these spikes are structures that allow the virus to attach and enter a cell, like the influenza virus hemagglutinin spikes (H) or enzymes like the neuraminidase (N) influenza virus spikes that allow the virus to detach from the cell surface during release of new virions. Influenza viruses are often identified by their H and N spikes. For example, H1N1 influenza viruses were responsible for the pandemics in 1918 and 2009,[21] H2N2 for the pandemic in 1957, and H3N2 for the pandemic in 1968.

Viruses vary in the shape of their capsids, which can be either helical, polyhedral, or complex. A helical capsid forms the shape of tobacco mosaic virus (TMV), a naked helical virus, and Ebola virus, an enveloped helical virus. The capsid is cylindrical or rod shaped, with the genome fitting just inside the length of the capsid. Polyhedral capsids form the shapes of poliovirus and rhinovirus, and they consist of a nucleic acid surrounded by a polyhedral (many-sided) capsid in the form of an icosahedron. An icosahedral capsid is a three-dimensional, 20-sided structure with 12 vertices. These capsids somewhat resemble a soccer ball. Both helical and polyhedral viruses can have envelopes. Viral shapes seen in certain types of bacteriophages, such as T4 phage, and poxviruses like vaccinia virus, may have features of both polyhedral and helical viruses so they are described as a complex viral shape (see figure 2.73). In the bacteriophage complex form, the genome is located within the polyhedral head and the sheath connects the head to the tail fibers and tail pins that help the virus attach to receptors on the host cell's surface. Poxviruses that have complex shapes are often brick shaped, with intricate surface characteristics not seen in the other categories of capsid.

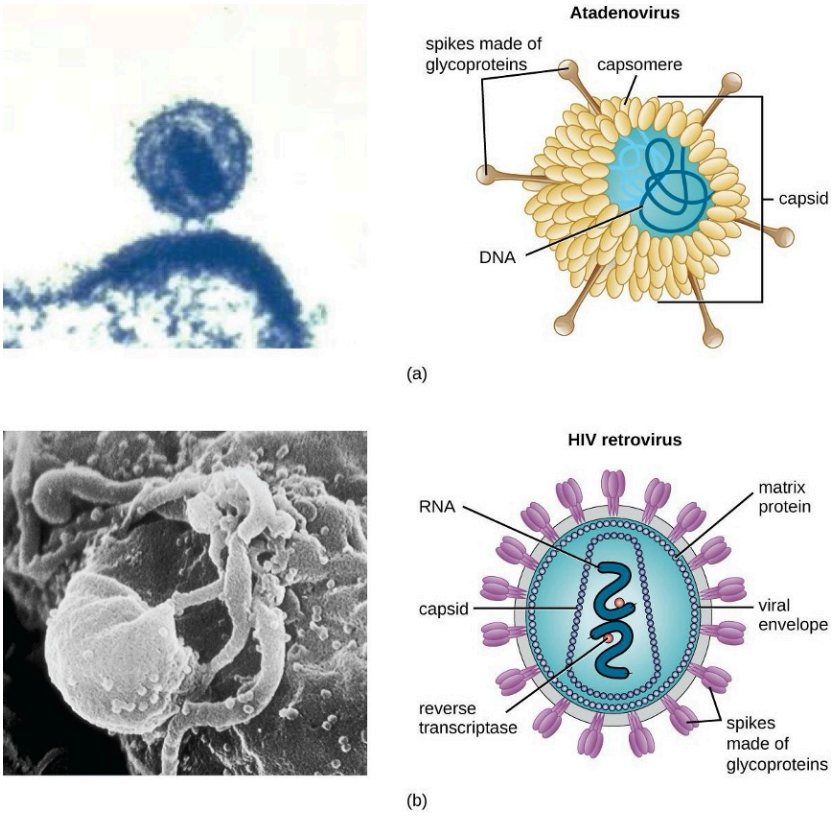


Figure 2.72: (a) The naked atadenovirus uses spikes made of glycoproteins from its capsid to bind to host cells. (b) The enveloped human immunodeficiency virus uses spikes made of glycoproteins embedded in its envelope to bind to host cells. Figure description available at the end of the chapter.

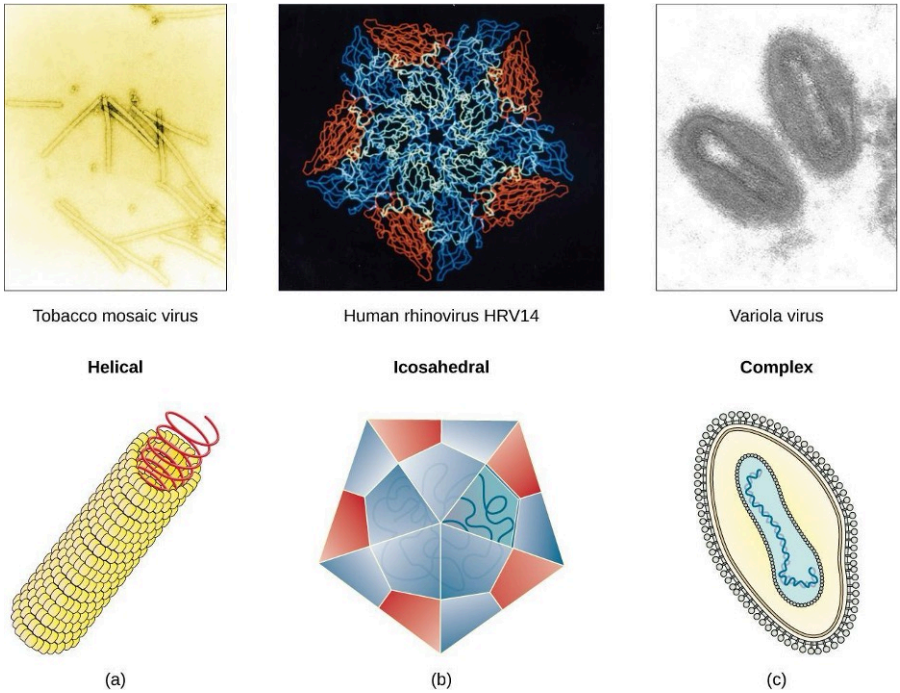


Figure 2.73: Viral capsids can be (a) helical, (b) polyhedral, or (c) have a complex shape. Used under fair use. Figure description available at the end of the chapter.

CLASSIFICATION AND TAXONOMY OF VIRUSES

To date, the ICTV has classified known viruses in seven orders, 96 families, and 350 genera. Viral family names end in *–viridae* (e.g, *Parvoviridae*) and genus names end in *–virus* (e.g., *Parvovirus*). The names of viral orders, families, and genera are all italicized. When referring to a viral species, we often use a genus and species epithet such as *Pandoravirus dulcis* or *Pandoravirus salinus.*

The Baltimore classification system is an alternative to ICTV nomenclature. The Baltimore system classifies viruses according to their genomes (DNA or RNA, single versus double stranded, and mode of replication). This system thus creates seven groups of viruses that have common genetics and biology.

Aside from formal systems of nomenclature, viruses are often informally grouped into categories based on chemistry, morphology, or other characteristics they share in common. Categories may include naked or enveloped structure, single-stranded (ss) or double-stranded (ds) DNA or ss or ds RNA genomes, segmented or non-segmented genomes, and positive-strand (+) or negative-strand (–) RNA. For example, herpes viruses can be classified as a dsDNA enveloped virus; human immunodeficiency virus (HIV) is a +ssRNA enveloped virus, and tobacco mosaic virus is a +ssRNA virus. Other characteristics such as host specificity, tissue specificity, capsid shape, and special genes or enzymes may also be used to describe groups of similar viruses. Table 2.19 lists some of the most common viruses that are human pathogens by genome type.

Genome	Family	Example Virus	Clinical Features
dsDNA, enveloped	*Poxviridae*	*Orthopoxvirus*	Skin papules, pustules, lesions
	Poxviridae	*Parapoxvirus*	Skin lesions
	Herpesviridae	*Simplexvirus*	Cold sores, genital herpes, sexually transmitted disease
dsDNA, naked	*Adenoviridae*	*Atadenovirus*	Respiratory infection (common cold)
	Papillomaviridae	*Papillomavirus*	Genital warts, common, and plantar warts cervical, vaginal or oropharyngeal cancer
	Reoviridae	*Reovirus*	Gastroenteritis severe diarrhea (stomach flu)
ssDNA, naked	*Parvoviridae*	*Adeno-associated dependoparvovirus A*	Respiratory tract infection
	Parvoviridae	*Adeno-associated dependoparvovirus B*	Respiratory tract infection
dsRNA, naked	*Reoviridae*	*Rotavirus*	Gastroenteritis
+ssRNA, naked	*Picornaviridae*	*Enterovirus C*	Poliomyelitis
	Picornaviridae	*Rhinovirus*	Upper respiratory tract infection (common cold)
	Picornaviridae	*Hepatovirus*	Hepatitis
+ssRNA, enveloped	*Togaviridae*	*Alphavirus*	Encephalitis, hemorrhagic fever
	Togaviridae	*Rubivirus*	Rubella

Genome	Family	Example Virus	Clinical Features
	Retroviridae	*Lentivirus*	Acquired immune deficiency syndrome (AIDS)
−ssRNA, enveloped	*Filoviridae*	*Zaire Ebolavirus*	Hemorrhagic fever
	Orthomyxoviridae	*Influenzavirus A, B, C*	Flu
	Rhabdoviridae	*Lyssavirus*	Rabies

Table 2.19: Common pathogenic viruses

2.11 THE VIRAL LIFE CYCLE

All viruses depend on cells for reproduction and metabolic processes. By themselves, viruses do not encode for all of the enzymes necessary for viral replication. But within a host cell, a virus can commandeer cellular machinery to produce more viral particles. Bacteriophages replicate only in the cytoplasm, since prokaryotic cells do not have a nucleus or organelles. In eukaryotic cells, most DNA viruses can replicate inside the nucleus, with an exception observed in the large DNA viruses, such as the poxviruses, that can replicate in the cytoplasm. With a few exceptions, RNA viruses that infect animal cells replicate in the cytoplasm. An important exception that will be highlighted later is Influenza virus.

THE LIFE CYCLE OF VIRUSES WITH PROKARYOTE HOSTS

The life cycle of bacteriophages has been a good model for understanding how viruses affect the cells they infect, since similar processes have been observed for eukaryotic viruses, which can cause immediate death of the cell or establish a latent or chronic infection. Virulent phages typically lead to the death of the cell through cell lysis. Temperate phages, on the other hand, can become part of a host chromosome and are replicated with the cell genome until such time as they are induced to make newly assembled viruses, or progeny viruses.

The Lytic Cycle

During the lytic cycle of virulent phage, the bacteriophage takes over the cell, reproduces new phages, and destroys the cell. T-even phage is a good example of a well-characterized class of virulent phages. There are five stages in the bacteriophage lytic cycle (see figure 2.74). Attachment is the first stage in the infection process in which the phage interacts with specific bacterial surface receptors (e.g., lipopolysaccharides and OmpC protein on host surfaces). Most phages have a narrow host range and may infect one species of bacteria or one strain within a species. This unique recognition can be exploited for targeted treatment of bacterial infection by phage therapy or for phage typing to identify unique bacterial subspecies or strains. The second stage of infection is entry or penetration. This occurs through contraction of the tail sheath, which acts like a hypodermic needle to inject the viral genome through the cell wall and membrane. The phage head and remaining components remain outside the bacteria.

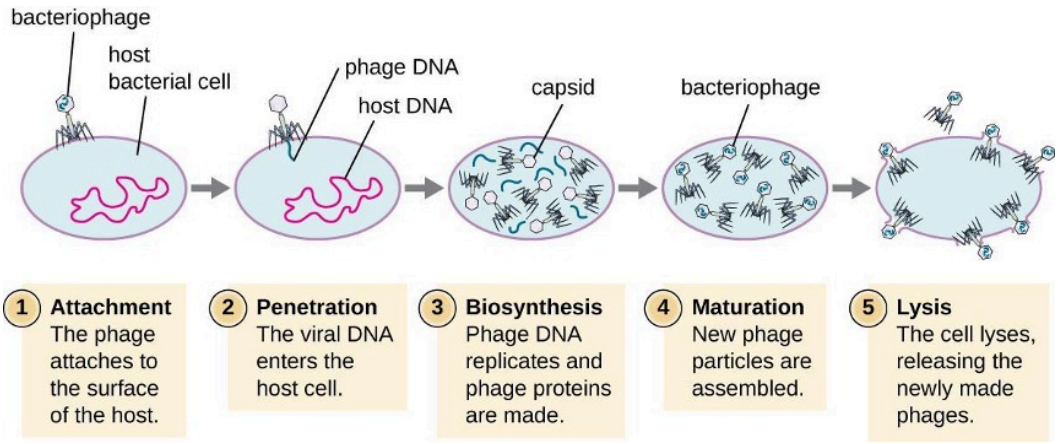


Figure 2.74: A virulent phage shows only the lytic cycle pictured here. In the lytic cycle, the phage replicates and lyses the host cell. *Figure description available at the end of the chapter.*

The third stage of infection is biosynthesis of new viral components. After entering the host cell, the virus synthesizes virus-encoded endonucleases to degrade the bacterial chromosome. It then hijacks the host cell to replicate, transcribe, and translate the necessary viral components (capsomeres, sheath, base plates, tail fibers, and viral enzymes) for the assembly of new viruses. Polymerase genes are usually expressed early in the cycle, while capsid and tail proteins are expressed later. During the maturation phase, new virions are created. To liberate free phages, the bacterial cell wall is disrupted by phage proteins such as holin or lysozyme. The final stage is the release of the newly made phages. Mature viruses burst out of the host cell in a process called lysis and the progeny viruses are liberated into the environment to infect new cells.

The Lysogenic Cycle

In a lysogenic cycle, the phage genome also enters the cell through attachment and penetration. A prime example of a phage with this type of life cycle is the lambda phage. During the lysogenic cycle, instead of killing the host, the phage genome integrates into the bacterial chromosome and becomes part of the host. The integrated phage genome is called a prophage. A bacterial host with a prophage is called a lysogen. The process in which a bacterium is infected by a temperate phage is called lysogeny. It is typical of temperate phages to be latent or inactive within the cell. As the bacterium replicates its chromosome, it also replicates the phage's DNA and passes it on to new daughter cells during reproduction. The presence of the phage may alter the phenotype of the bacterium, since it can bring in extra genes (e.g., toxin genes that can increase bacterial virulence). This change in the host phenotype is called lysogenic conversion or phage conversion. Some bacteria, such as *Vibrio cholerae* and *Clostridium botulinum*, are less virulent in the absence of the prophage. The phages infecting these bacteria carry the toxin genes in their genome and enhance the virulence of the host when the toxin genes are expressed. In the case of *V. cholera*, phage-encoded toxins can cause severe diarrhea; in *C. botulinum*, the toxin can cause paralysis. During lysogeny, the prophage will persist in the host chromosome until induction, which results in the excision of the viral genome from the host chromosome. After induction has occurred the temperate phage can proceed through a lytic cycle and then undergo lysogeny in a newly infected cell (see figure 2.75).

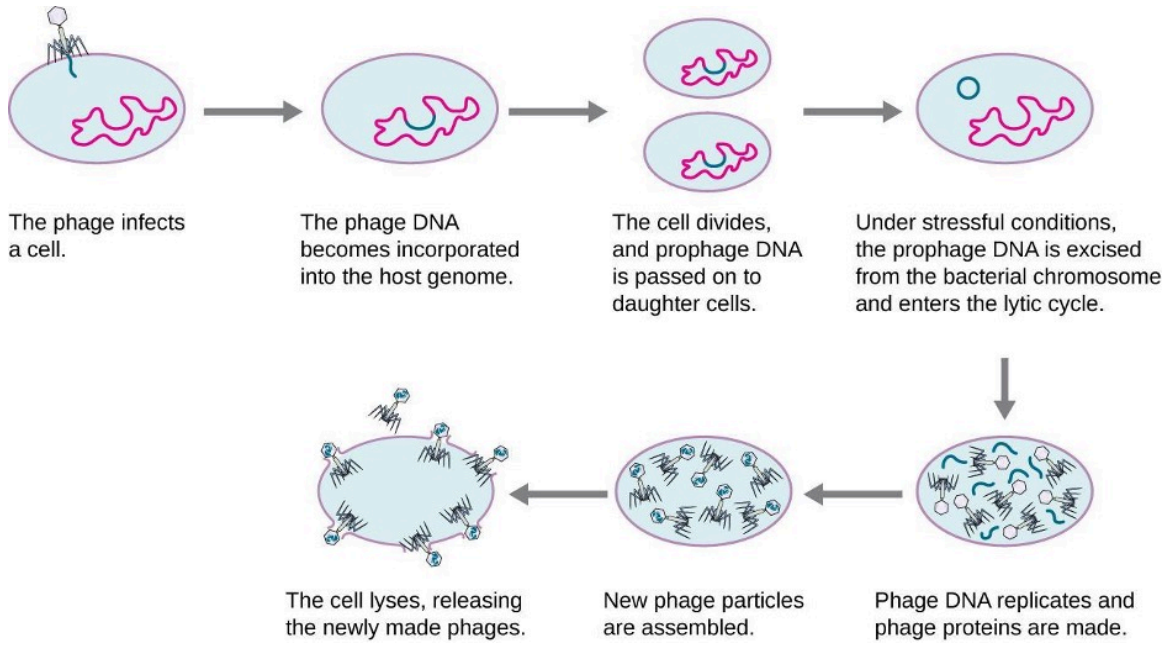


Figure 2.75: A temperate bacteriophage has both lytic and lysogenic cycles. In the lysogenic cycle, phage DNA is incorporated into the host genome, forming a prophage, which is passed on to subsequent generations of cells. Environmental stressors such as starvation or exposure to toxic chemicals may cause the prophage to be excised and enter the lytic cycle. *Figure description available at the end of the chapter.*

TRANSDUCTION

Transduction occurs when a bacteriophage transfers bacterial DNA from one bacterium to another during sequential infections. There are two types of transduction: generalized and specialized transduction. During the lytic cycle of viral replication, the virus hijacks the host cell, degrades the host chromosome, and makes more viral genomes. As it assembles and packages DNA into the phage head, packaging occasionally makes a mistake. Instead of packaging viral DNA, it takes a random piece of host DNA and inserts it into the capsid. Once released, this virion will then inject the former host's DNA into a newly infected host. The asexual transfer of genetic information can allow for DNA recombination to occur, thus providing the new host with new genes (e.g., an antibiotic-resistance gene, or a sugar-metabolizing gene). Generalized transduction occurs when a random piece of bacterial chromosomal DNA is transferred by the phage during the lytic cycle. Specialized transduction occurs at the end of the lysogenic cycle, when the prophage is excised and the bacteriophage enters the lytic cycle. Since the phage is integrated into the host genome, the prophage can replicate as part of the host. However, some conditions (e.g., ultraviolet light exposure or chemical exposure) stimulate the prophage to undergo induction, causing the phage to excise from the genome, enter the lytic cycle, and produce new phages to leave host cells. During the process of excision from the host chromosome, a phage may occasionally remove some bacterial DNA near the site of viral integration. The phage and host DNA from one or both ends of the integration site are packaged within the capsid. From here they are transferred to the new, infected host. Since the DNA transferred by the phage is not randomly packaged but is instead a specific piece of DNA near the site of integration, this mechanism of gene transfer is referred to as specialized transduction (see figure 2.76). The DNA can then recombine with the host chromosome, giving the latter new characteristics. Transduction seems to play an important role in the evolutionary process of bacteria, giving them a mechanism for asexual exchange of genetic information.

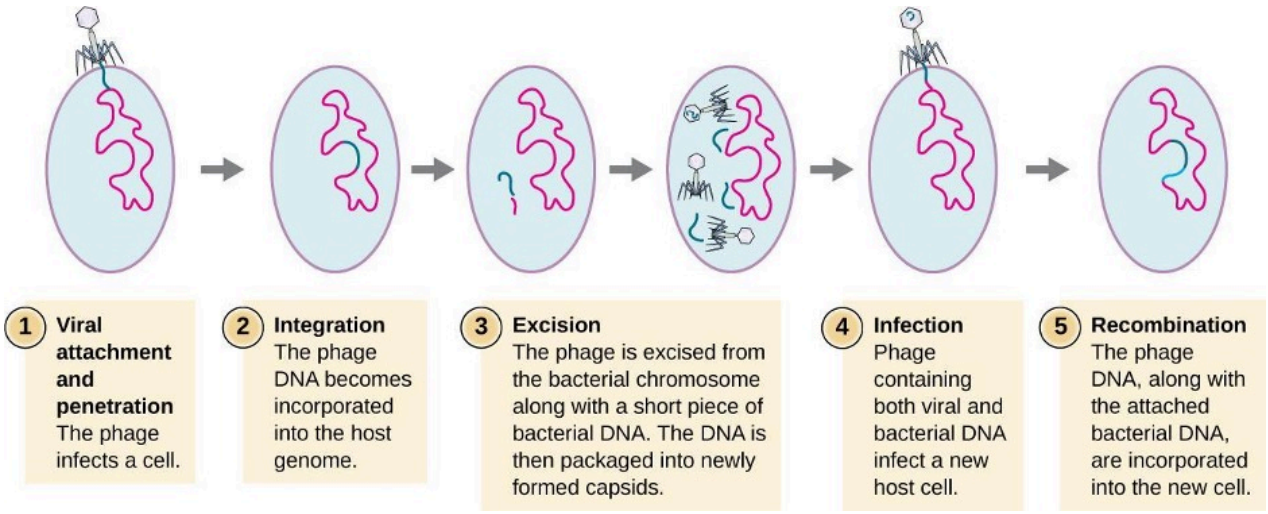


Figure 2.76: This flowchart illustrates the mechanism of specialized transduction. An integrated phage excises, bringing with it a piece of the DNA adjacent to its insertion point. On reinfection of a new bacterium, the phage DNA integrates along with the genetic material acquired from the previous host. *Figure description available at the end of the chapter.*

LIFE CYCLE OF VIRUSES WITH ANIMAL HOSTS

Lytic animal viruses follow similar infection stages to bacteriophages: attachment, penetration, biosynthesis, maturation, and release (see figure 2.77). However, the mechanisms of penetration, nucleic-acid biosynthesis, and release differ between bacterial and animal viruses. After binding to host receptors, animal viruses enter through endocytosis (engulfment by the host cell) or through membrane fusion (viral envelope with the host cell membrane). Many viruses are host specific, meaning they only infect a certain type of host; and most viruses only infect certain types of cells within tissues. This specificity is called a tissue tropism. Examples of this are demonstrated by the poliovirus, which exhibits tropism for the tissues of the brain and spinal cord, or the influenza virus, which has a primary tropism for the respiratory tract.

Animal viruses do not always express their genes using the normal flow of genetic information—from DNA to RNA to protein. Some viruses have a dsDNA genome like cellular organisms and can follow the normal flow. However, others may have ssDNA, dsRNA, or ssRNA genomes. The nature of the genome determines how the genome is replicated and expressed as viral proteins. If a genome is ssDNA, host enzymes will be used to synthesize a second strand that is complementary to the genome strand, thus producing dsDNA. The dsDNA can now be replicated, transcribed, and translated similar to host DNA.

If the viral genome is RNA, a different mechanism must be used. There are three types of RNA genome: dsRNA, positive (+) single-strand (+ssRNA) or negative (–) single-strand RNA (–ssRNA). If a virus has a +ssRNA genome, it can be translated directly to make viral proteins. Viral genomic +ssRNA acts like cellular mRNA. However, if a virus contains a –ssRNA genome, the host ribosomes cannot translate it until the –ssRNA is replicated into +ssRNA by viral RNA-dependent RNA polymerase (RdRP) (see figure 2.78). The RdRP is brought in by the virus and can be used to make +ssRNA from the original –ssRNA genome. The RdRP is also an important enzyme for the replication of dsRNA viruses, because it uses the negative strand of the double-stranded genome as a template to create +ssRNA. The newly synthesized +ssRNA copies can then be translated by cellular ribosomes.

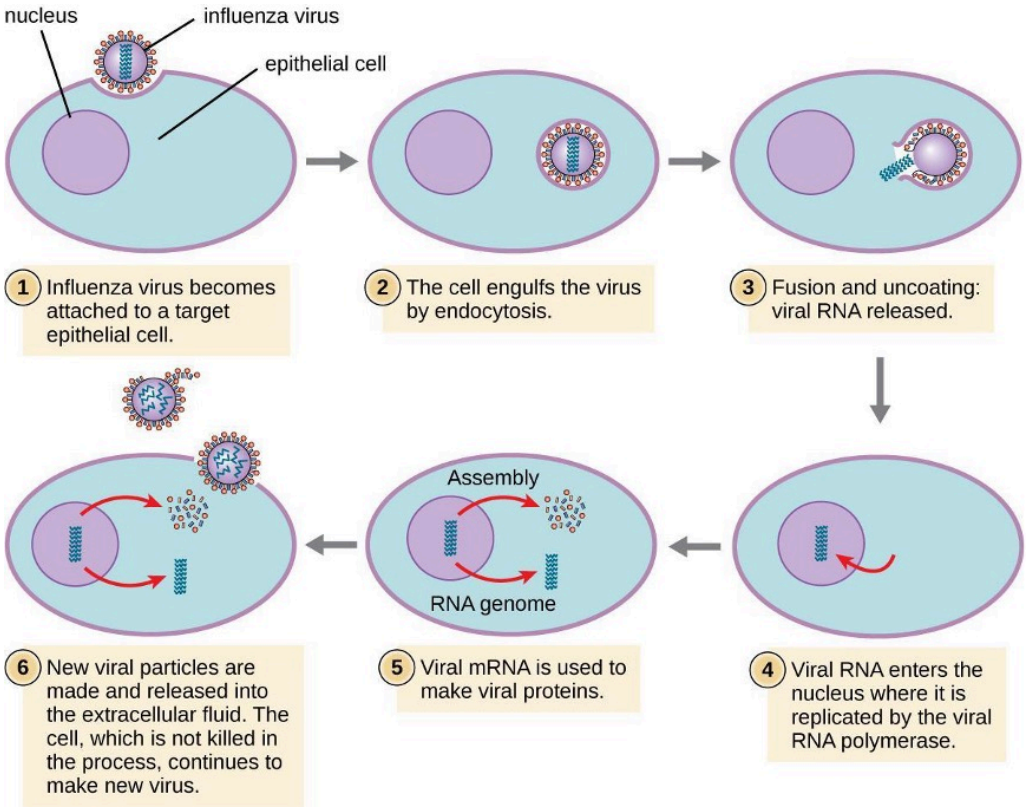


Figure 2.77: Influenza virus is one of the few RNA viruses that replicates in the nucleus of cells. In influenza virus infections, viral glycoproteins attach the virus to a host epithelial cell. As a result, the virus is engulfed. Viral RNA and viral proteins are made and assembled into new virions that are released by budding. *Figure description available at the end of the chapter.*

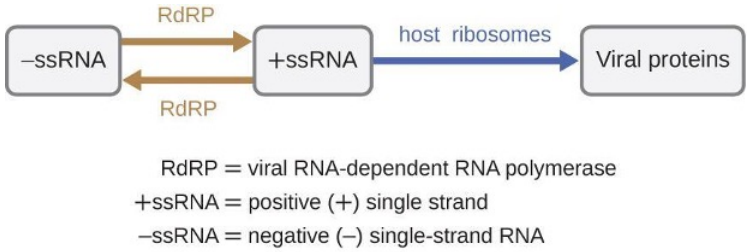


Figure 2.78: RNA viruses can contain +ssRNA that can be directly read by the ribosomes to synthesize viral proteins. Viruses containing −ssRNA must first use the −ssRNA as a template for the synthesis of +ssRNA before viral proteins can be synthesized. *Figure description available at the end of the chapter.*

An alternative mechanism for viral nucleic acid synthesis is observed in the retroviruses, which are +ssRNA viruses (see figure 2.79). Single-stranded RNA viruses such as HIV carry a special enzyme called reverse transcriptase within the capsid that synthesizes a complementary ssDNA (cDNA) copy using the +ssRNA genome as a template. The ssDNA is then made into dsDNA, which can integrate into the host chromosome and become a permanent part of the host. The integrated viral genome is called a provirus. The virus can now remain in the host for a long time to establish a chronic infection. The provirus stage is similar to the prophage stage in a bacterial infection during the lysogenic cycle. However, unlike prophage, the provirus does not undergo excision after splicing into the genome.

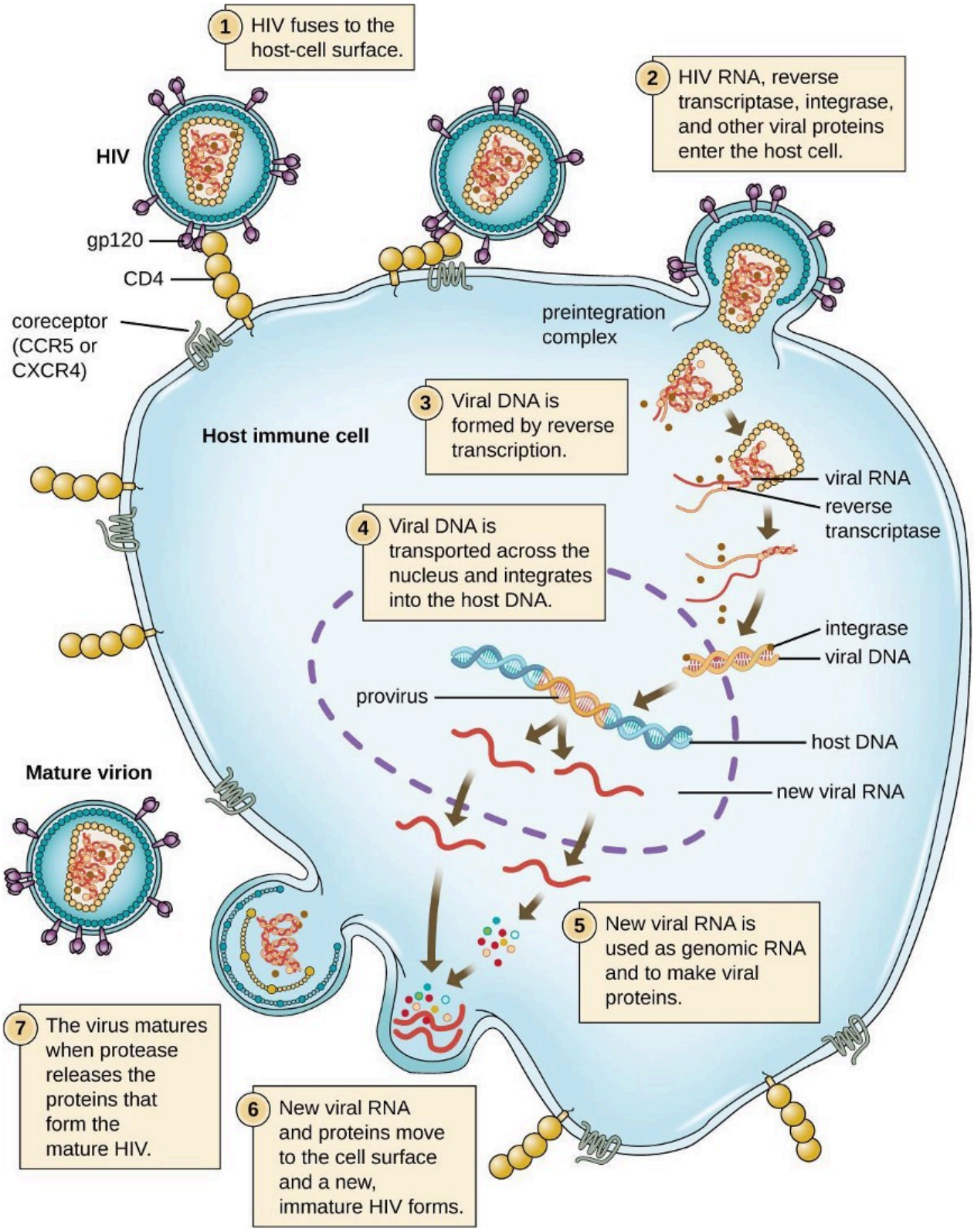


Figure 2.79: HIV, an enveloped, icosahedral retrovirus, attaches to a cell surface receptor of an immune cell and fuses with the cell membrane. Viral contents are released into the cell, where viral enzymes convert the single-stranded RNA genome into DNA and incorporate it into the host genome. *Figure description available at the end of the chapter.*

PERSISTENT INFECTIONS

Persistent infection occurs when a virus is not completely cleared from the system of the host but stays in certain tissues or organs of the infected person. The virus may remain silent or undergo productive infection without seriously harming or killing the host. Mechanisms of persistent infection may involve the regulation of the viral or host gene expressions or the alteration of the host immune response. The two primary categories of persistent infections are latent infection and chronic infection. Examples of viruses that cause latent infections include herpes simplex virus (oral and genital herpes), varicella-zoster virus (chickenpox and shingles), and Epstein-Barr virus (mononucleosis). Hepatitis C virus and HIV are two examples of viruses that cause long-term chronic infections.

Latent Infection

Not all animal viruses undergo replication by the lytic cycle. There are viruses that are capable of remaining hidden or dormant inside the cell in a process called latency. These types of viruses are known as latent viruses and may cause latent infections. In some cases, viruses capable of latency may initially cause an acute infection before becoming dormant.

For example, the varicella-zoster virus infects many cells throughout the body and causes chickenpox, characterized by a rash of blisters covering the skin. About 10 to 12 days post infection, the disease resolves and the virus goes dormant, living within nerve-cell ganglia for years. During this time, the virus does not kill the nerve cells or continue replicating. It is not clear why the virus stops replicating within the nerve cells and expresses few viral proteins but, in some cases, typically after many years of dormancy, the virus is reactivated and causes a new disease called shingles (figure 2.80). Whereas chickenpox affects many areas throughout the body, shingles is a nerve cell-specific disease emerging from the ganglia in which the virus was dormant.

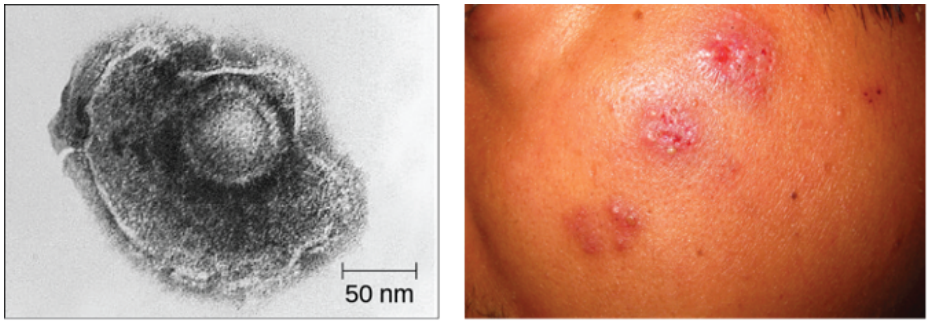


Figure 2.80: (a) Varicella-zoster, the virus that causes chickenpox, has an enveloped icosahedral capsid visible in this transmission electron micrograph. Its double-stranded DNA genome becomes incorporated in the host DNA. (b) After a period of latency, the virus can reactivate in the form of shingles, usually manifesting as a painful, localized rash on one side of the body. Used under fair use. *Figure description available at the end of the chapter.*

Latent viruses may remain dormant by existing as circular viral genome molecules outside of the host chromosome. Others become proviruses by integrating into the host genome. During dormancy, viruses do not cause any symptoms of disease and may be difficult to detect. A patient may be unaware that he or she is carrying the virus unless a viral diagnostic test has been performed.

Chronic Infection

A chronic infection is a disease with symptoms that are recurrent or persistent over a long time. Some viral infections can be chronic if the body is unable to eliminate the virus. HIV is an example of a virus that produces a chronic infection, often after a long period of latency. Once a person becomes infected with HIV, the virus can be detected in tissues continuously thereafter, but untreated patients often experience no symptoms for years. However, the virus maintains chronic persistence through several mechanisms that interfere with immune function, including preventing expression of viral antigens on the surface of infected cells, altering immune cells themselves, restricting expression of viral genes, and rapidly changing viral antigens through mutation. Eventually, the damage to the immune system results in progression of the disease leading to acquired immunodeficiency syndrome (AIDS). The various mechanisms that HIV uses to avoid being cleared by the immune system are also used by other chronically infecting viruses, including the hepatitis C virus.

VIRAL GROWTH CURVE

Unlike the growth curve for a bacterial population, the growth curve for a virus population over its life cycle does not follow a sigmoidal curve. During the initial stage, an inoculum of virus causes infection. In the eclipse phase, viruses bind and penetrate the cells with no virions detected in the medium. The chief difference that next appears in the viral growth curve compared to a bacterial growth curve occurs when virions are released from the lysed host cell at the same time. Such an occurrence is called a burst, and the number of virions per bacterium released is described as the burst size. In a one-step multiplication curve for bacteriophage, the host cells lyse, releasing many viral particles to the medium, which leads to a very steep rise in viral titer (the number of virions per unit volume). If no viable host cells remain, the viral particles begin to degrade during the decline of the culture (see figure 2.81).

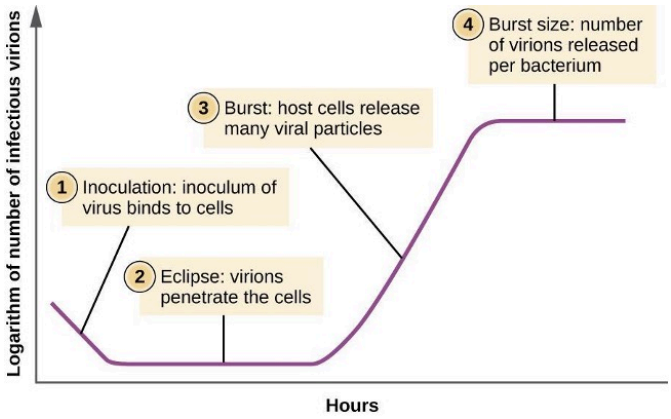


Figure 2.81: The one-step multiplication curve for a bacteriophage population follows three steps: 1) inoculation, during which the virions attach to host cells; 2) eclipse, during which entry of the viral genome occurs; and 3) burst, when sufficient numbers of new virions are produced and emerge from the host cell. The burst size is the maximum number of virions produced per bacterium. *Figure description available at the end of the chapter.*

DETECTION OF A VIRUS

Regardless of the method of cultivation, once a virus has been introduced into a whole host organism, embryo, or tissue-culture cell, a sample can be prepared from the infected host, embryo, or cell line for further analysis under a brightfield, electron, or fluorescent microscope. Cytopathic effects (CPEs) are distinct observable cell abnormalities due to viral infection. CPEs can include loss of adherence to the surface of the container, changes in cell shape from flat to round, shrinkage of the nucleus, vacuoles in the cytoplasm, fusion of cytoplasmic membranes and the formation of multinucleated syncytia, inclusion bodies in the nucleus or cytoplasm, and complete cell lysis (see table 2.20).

Further pathological changes include viral disruption of the host genome and altering normal cells into transformed cells, which are the types of cells associated with carcinomas and sarcomas. The type or severity of the CPE depends on the type of virus involved. Table 2.20 lists CPEs for specific viruses.

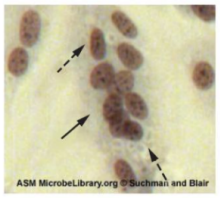
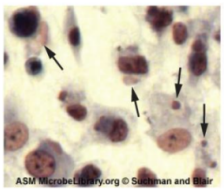
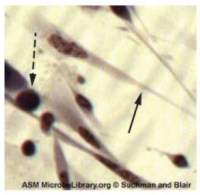
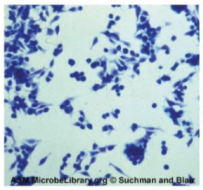
Virus	Cytopathic Effect	Example
Paramyxovirus	Syncytium and faint basophilic cytoplasmic inclusion bodies	
Poxvirus	Pink eosinophilic cytoplasmic inclusion bodies (arrows) and cell swelling	
Herpesvirus	Cytoplasmic stranding (arrow) and nuclear inclusion bodies (dashed arrow)	
Adenovirus	Cell enlargement, rounding, and distinctive "grape-like" clusters	

Table 2.20: Cytopathic effects of specific viruses

Hemagglutination Assay

A serological assay is used to detect the presence of certain types of viruses in patient serum. Serum can be used in a direct assay called a hemagglutination assay to detect specific types of viruses in the patient's sample. Hemagglutination is the agglutination (clumping) together of erythrocytes (red blood cells). Many viruses produce surface proteins or spikes called hemagglutinins that can bind to receptors on the membranes of erythrocytes and cause the cells to agglutinate. Hemagglutination is observable without using the microscope, but this method does not always differentiate between infectious and noninfectious viral particles, since both can agglutinate erythrocytes.

To identify a specific pathogenic virus using hemagglutination, we must use an indirect approach. Proteins called antibodies, generated by the patient's immune system to fight a specific virus, can be used to bind to components such as hemagglutinins that are uniquely associated with specific types of viruses. The binding of the antibodies with the hemagglutinins found on the virus subsequently prevent erythrocytes from directly interacting with the virus. So when erythrocytes are added to the antibody-coated viruses, there is no appearance of agglutination; agglutination has been inhibited. We call these types of indirect assays for virus-specific antibodies hemagglutination inhibition (HAI) assays. HAI can be used to detect the presence of antibodies specific to many types of viruses that may be causing or have caused an infection in a patient even months or years after infection (see figure 2.82).

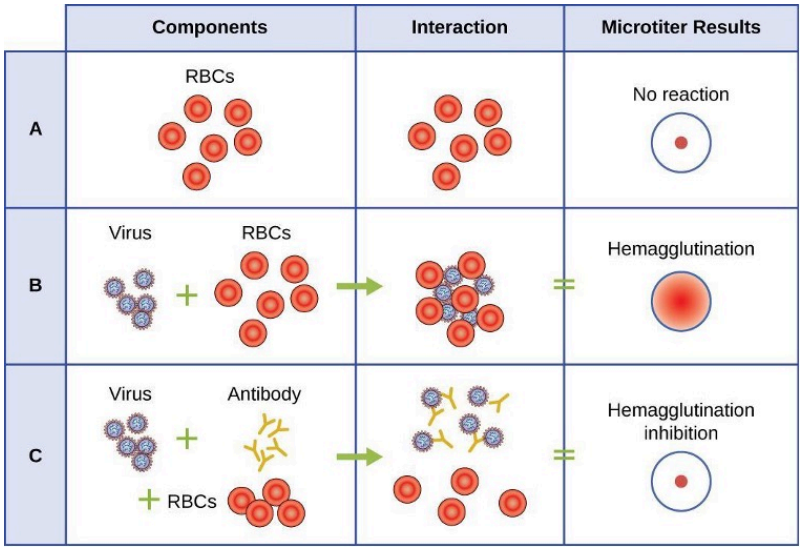


Figure 2.82: This figure shows the possible outcomes of a hemagglutination test. Row A: Erythrocytes do not bind together and will sink to the bottom of the well plate; this becomes visible as a red dot in the center of the well. Row B: Many viruses have hemagglutinins that cause agglutination of erythrocytes; the resulting hemagglutination forms a lattice structure that results in red color throughout the well. Row C: Virus-specific antibody, the viruses, and the erythrocytes are added to the well plate. The virus-specific antibodies inhibit agglutination, as can be seen as a red dot in the bottom of the well. *Figure description available at the end of the chapter.*

Nucleic Acid Amplification Test

Nucleic acid amplification tests (NAAT) are used in molecular biology to detect unique nucleic acid sequences of viruses in patient samples. Polymerase chain reaction (PCR) is an NAAT used to detect the presence of viral DNA in a patient's tissue or body fluid sample. PCR is a technique that amplifies (i.e., synthesizes many copies) of a viral DNA segment of interest. Using PCR, short nucleotide sequences called primers bind to specific sequences of viral DNA, enabling identification of the virus.

Reverse transcriptase-PCR (RT-PCR) is an NAAT used to detect the presence of RNA viruses. RT-PCR differs from PCR in that the enzyme reverse transcriptase (RT) is used to make cDNA from the small amount of viral RNA in the specimen. The cDNA can then be amplified by PCR. Both PCR and RT-PCR are used to detect and confirm the presence of the viral nucleic acid in patient specimens.

Enzyme immunoassays

Enzyme immunoassays (EIAs) rely on the ability of antibodies to detect and attach to specific biomolecules called antigens. The detecting antibody attaches to the target antigen with a high degree of specificity in what might be a complex mixture of biomolecules. Also included in this type of assay is a colorless enzyme attached to the detecting antibody. The enzyme acts as a tag on the detecting antibody and can interact with a colorless substrate, leading to the production of a colored end product. EIAs often rely on layers of antibodies to capture and react with antigens, all of which are attached to a membrane filter (see figure 2.83). EIAs for viral antigens are often used as preliminary screening tests. If the results are positive, further confirmation will require tests with even greater sensitivity, such as a western blot or an NAAT.

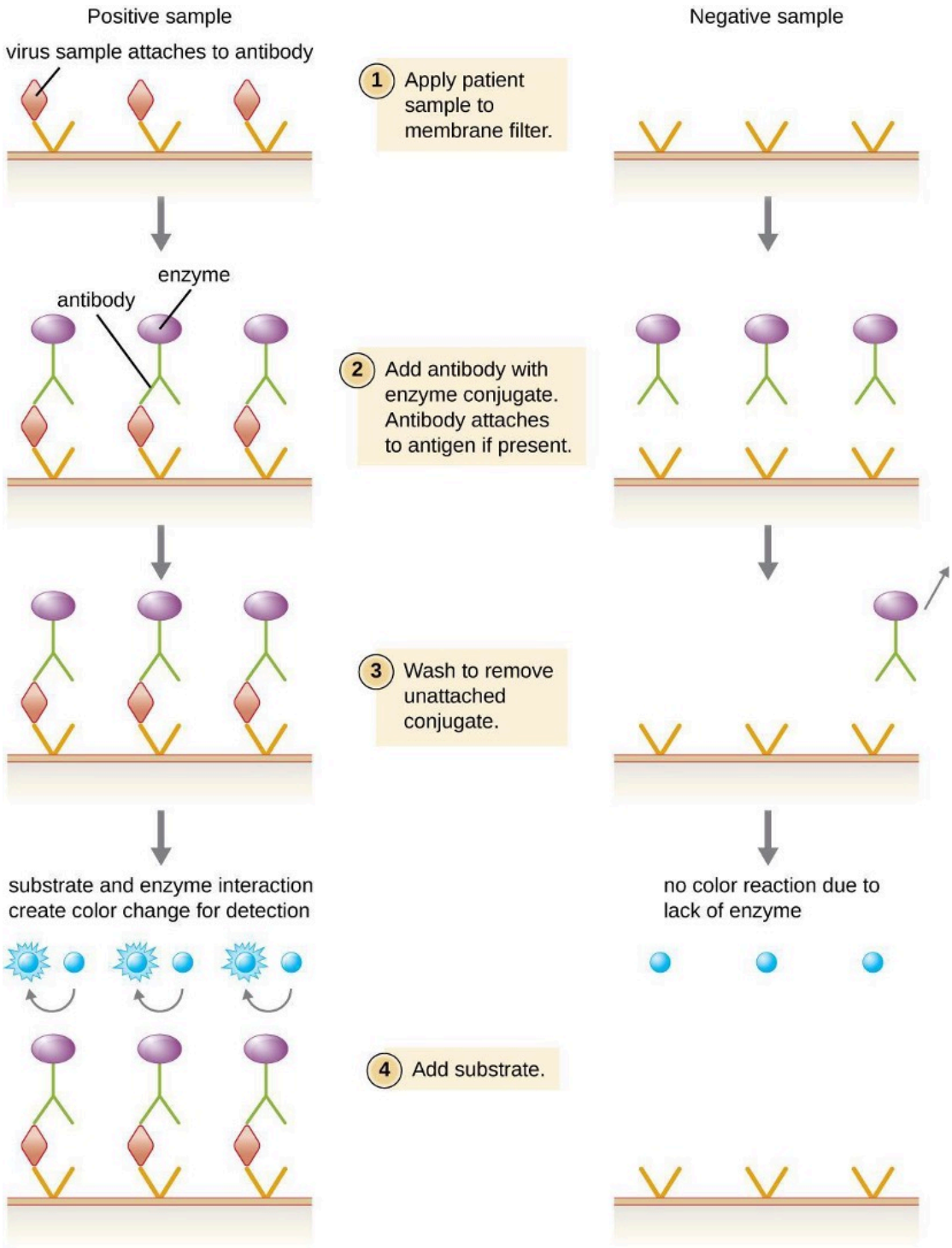


Figure 2.83: Similar to rapid, over-the-counter pregnancy tests, EIAs for viral antigens require a few drops of diluted patient serum or plasma applied to a membrane filter (1). The membrane filter has been previously modified and embedded with antibody to viral antigen and internal controls. Antibody conjugate is added to the filter (2), with the targeted antibody attached to the antigen (in the case of a positive test). Excess conjugate is washed off the filter (3). Substrate is added (4) to activate the enzyme-mediated reaction to reveal the color change of a positive test. *Figure description available at the end of the chapter.*

2.12 VIROIDS, VIRUSOIDS, AND PRIONS

Research attempts to discover the causative agents of previously uninvestigated diseases have led to the discovery of nonliving disease agents quite different from viruses. These include particles consisting only of RNA or only of protein that, nonetheless, are able to self-propagate at the expense of a host—a key similarity to viruses that allows them to cause disease conditions. To date, these discoveries include viroids, virusoids, and the proteinaceous prions.

PRIONS

At one time, scientists believed that any infectious particle must contain DNA or RNA. Then, in 1982, Stanley Prusiner, a medical doctor studying scrapie (a fatal, degenerative disease in sheep) discovered that the disease was caused by proteinaceous infectious particles, or prions. Because proteins are acellular and do not contain DNA or RNA, Prusiner's findings were originally met with resistance and skepticism; however, his research was eventually validated, and he received the Nobel Prize in Physiology or Medicine in 1997.

A prion is a misfolded rogue form of a normal protein (PrPc) found in the cell. This rogue prion protein (PrPsc), which may be caused by a genetic mutation or occur spontaneously, can be infectious, stimulating other endogenous normal proteins to become misfolded, forming plaques (see figure 2.84). Today, prions are known to cause various forms of transmissible spongiform encephalopathy (TSE) in humans and animals. TSE is a rare degenerative disorder that affects the brain and nervous system. The accumulation of rogue proteins causes the brain tissue to become sponge-like, killing brain cells and forming holes in the tissue, leading to brain damage, loss of motor coordination, and dementia (see figure 2.85). Infected individuals are mentally impaired and become unable to move or speak. There is no cure, and the disease progresses rapidly, eventually leading to death within a few months or years.

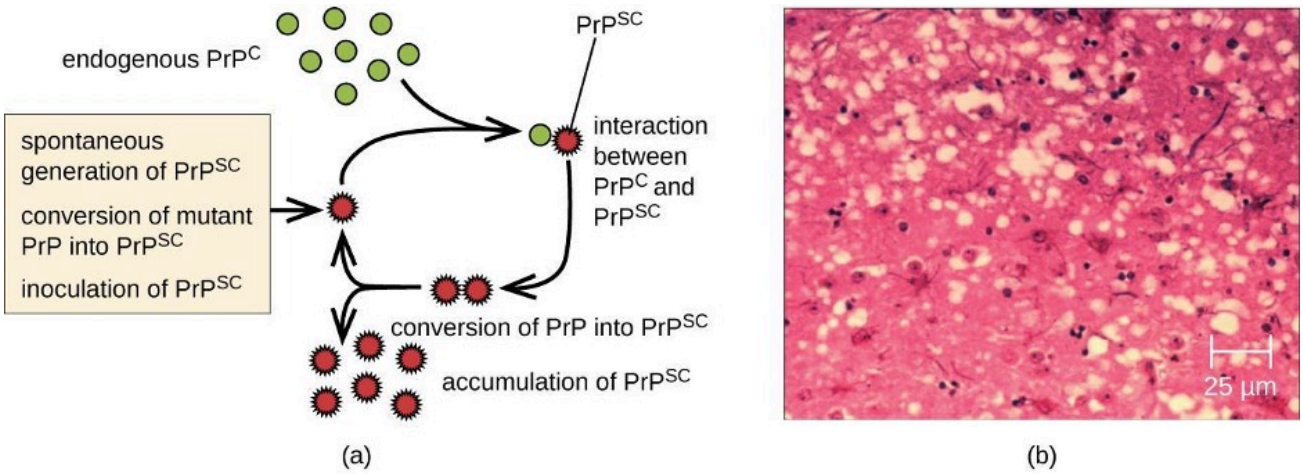


Figure 2.84: Endogenous normal prion protein (PrPc) is converted into the disease-causing form (PrPsc) when it encounters this variant form of the protein. PrPsc may arise spontaneously in brain tissue, especially if a mutant form of the protein is present, or it may originate from misfolded prions consumed in food that eventually find their way into brain tissue. *Figure description available at the end of the chapter.*

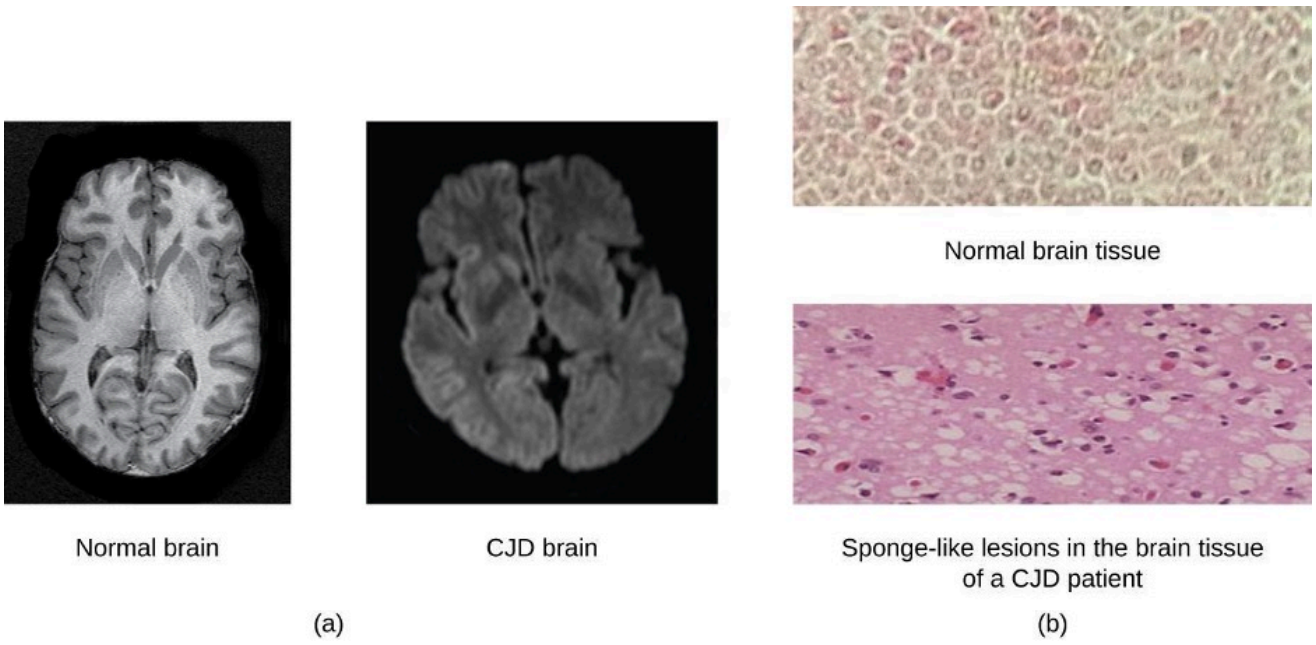


Figure 2.85: Creutzfeldt-Jakob disease (CJD) is a fatal disease that causes degeneration of neural tissue. (a) These brain scans compare a normal brain to one with CJD. (b) Compared to a normal brain, the brain tissue of a CJD patient is full of sponge-like lesions, which result from abnormal formations of prion protein. *Figure description available at the end of the chapter.*

TSEs in humans include kuru, fatal familial insomnia, Gerstmann-Straussler-Scheinker disease, and Creutzfeldt-Jakob disease (see figure 2.85). TSEs in animals include mad cow disease, scrapie (in sheep and goats), and chronic wasting disease (in elk and deer). TSEs can be transmitted between animals and from animals to humans by eating contaminated meat or animal feed. Transmission between humans can occur through heredity (as is often the case with GSS and CJD) or by contact with contaminated tissue, as might occur during a blood transfusion or organ transplant. There is no evidence for transmission via casual contact with an infected person. Table 2.21 lists TSEs that affect humans and their modes of transmission.

Disease	Mechanism(s) of Transmission*
Sporadic CJD (sCJD)	Not known; possibly by alteration of normal prior protein (PrP) to rogue form due to somatic mutation
Variant CJD (vCJD)	Eating contaminated cattle products and by secondary bloodborne transmission
Familial CJD (fCJD)	Mutation in germline PrP gene
Iatrogenic CJD (iCJD)	Contaminated neurosurgical instruments, corneal graft, gonadotrophic hormone, and, secondarily, by blood transfusion
Kuru	Eating infected meat through ritualistic cannibalism
Gerstmann-Straussler-Scheinker disease (GSS)	Mutation in germline PrP gene
Fatal familial insomnia (FFI)	Mutation in germline PrP gene

*Table 2.21: Transmissible spongiform encephalopathies (TSEs) in humans. *National Institute of Neurological Disorders and Stroke. "Creutzfeldt-Jakob Disease Fact Sheet." http://www.ninds.nih.gov/disorders/cjd/detail_cjd.htm (accessed December 31, 2015).*

Prions are extremely difficult to destroy because they are resistant to heat, chemicals, and radiation. Even standard sterilization procedures do not ensure the destruction of these particles. Currently, there is no treatment or cure for TSE disease, and contaminated meats or infected animals must be handled according to federal guidelines to prevent transmission.

2.13 HOW PATHOGENS CAUSE DISEASE

For most infectious diseases, the ability to accurately identify the causative pathogen is a critical step in finding or prescribing effective treatments. Today's physicians, patients, and researchers owe a sizable debt to the physician Robert Koch (1843–1910), who devised a systematic approach for confirming causative relationships between diseases and specific pathogens.

KOCH'S POSTULATES

In 1884, Koch published four postulates (table 2.22) that summarized his method for determining whether a particular microorganism was the cause of a particular disease. Each of Koch's postulates represents a criterion that must be met before a disease can be positively linked with a pathogen. In order to determine whether the criteria are met, tests are performed on laboratory animals and cultures from healthy and diseased animals are compared (figure 2.86).

Koch's Postulates
(1) The suspected pathogen must be found in every case of disease and not be found in healthy individuals.
(2) The suspected pathogen can be isolated and grown in pure culture.
(3) A healthy test subject infected with the suspected pathogen must develop the same signs and symptoms of disease as seen in postulate 1.
(4) The pathogen must be re-isolated from the new host and must be identical to the pathogen from postulate 2.

Table 2.22: Koch's postulates

In many ways, Koch's postulates are still central to our current understanding of the causes of disease. However, advances in microbiology have revealed some important limitations in Koch's criteria. Koch made several assumptions that we now know are untrue in many cases. The first relates to postulate 1, which assumes that pathogens are only found in diseased, not healthy, individuals. This is not true for many pathogens.

Koch's second faulty assumption was that all healthy test subjects are equally susceptible to disease. We now know that individuals are not equally susceptible to disease. Individuals are unique in terms of their microbiota and the state of their immune system at any given time. The makeup of the resident microbiota can influence an individual's susceptibility to an infection. Members of the normal microbiota play an important role in immunity by inhibiting the growth of transient pathogens. In some cases, the microbiota may prevent a pathogen from establishing an infection; in others, it may not prevent an infection altogether but may influence the severity or type of signs and symptoms.

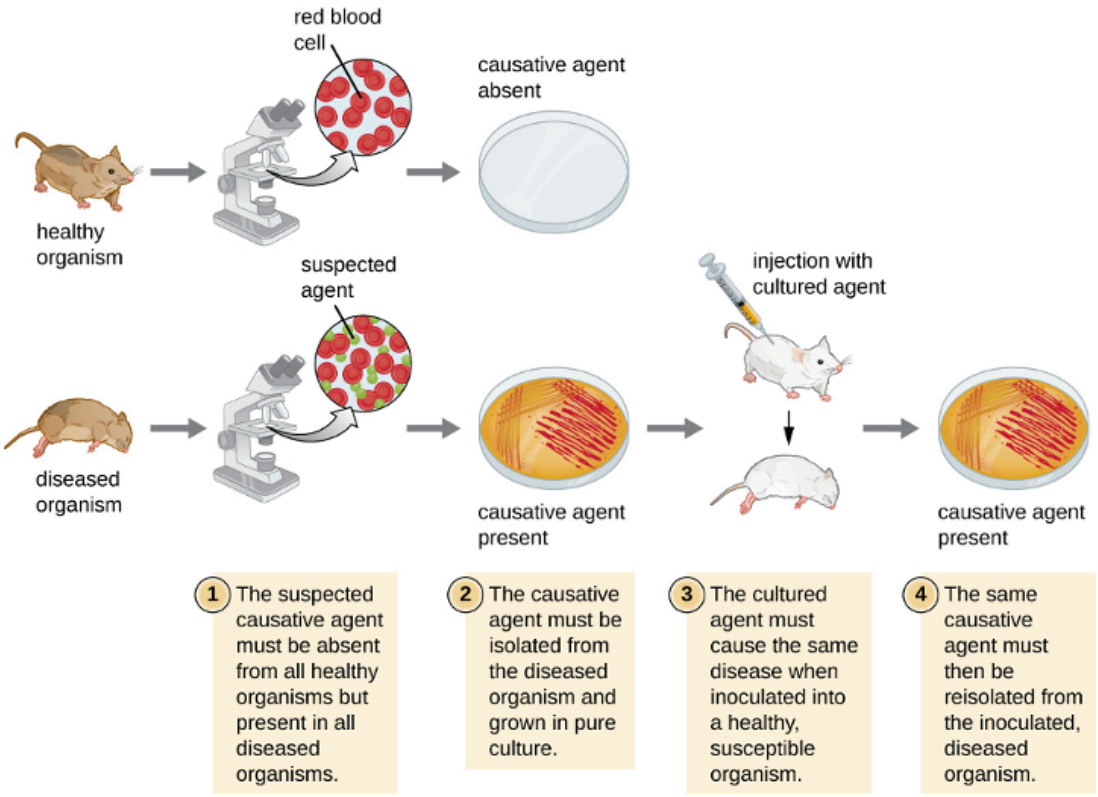


Figure 2.86: The steps for confirming that a pathogen is the cause of a particular disease using Koch's postulates. *Figure description available at the end of the chapter.*

Koch also assumed that all pathogens are microorganisms that can be grown in pure culture (postulate 2) and that animals could serve as reliable models for human disease. However, we now know that not all pathogens can be grown in pure culture, and many human diseases cannot be reliably replicated in animal hosts. Viruses and certain bacteria, including *Rickettsia* and *Chlamydia*, are obligate intracellular pathogens that can grow only when inside a host cell. If a microbe cannot be cultured, a researcher cannot move past postulate 2. Likewise, without a suitable non-human host, a researcher cannot evaluate postulate 3 without deliberately infecting humans, which presents obvious ethical concerns. AIDS is an example of such a disease because the human immunodeficiency virus (HIV) only causes disease in humans.

MOLECULAR KOCH'S POSTULATES

In 1988, Stanley Falkow (1934–) proposed a revised form of Koch's postulates known as molecular Koch's postulates. These are listed in the left column of table 2.23. The premise for molecular Koch's postulates is not in the ability to isolate a particular pathogen but rather to identify a gene that may cause the organism to be pathogenic.

Falkow's modifications to Koch's original postulates explain not only infections caused by intracellular pathogens but also the existence of pathogenic strains of organisms that are usually nonpathogenic. For example, the predominant form of the bacterium *Escherichia coli* is a member of the normal microbiota of the human intestine and is generally considered harmless. However, there are pathogenic strains of *E. coli* such as enterotoxigenic *E. coli* (ETEC) and enterohemorrhagic *E. coli* (O157:H7) (EHEC). We now know ETEC and EHEC exist because of the acquisition of new genes by the once-harmless *E. coli*, which, in the form of these pathogenic strains, is now capable of producing toxins and causing illness.

Molecular Koch's Postulates	Application to EHEC
(1) The phenotype (sign or symptom of disease) should be associated only with pathogenic strains of a species.	EHEC causes intestinal inflammation and diarrhea, whereas nonpathogenic strains of *E. coli* do not.
(2) Inactivation of the suspected gene(s) associated with pathogenicity should result in a measurable loss of pathogenicity.	One of the genes in EHEC encodes for Shiga toxin, a bacterial toxin (poison) that inhibits protein synthesis. Inactivating this gene reduces the bacteria's ability to cause disease.
(3) Reversion of the inactive gene should restore the disease phenotype.	By adding the gene that encodes the toxin back into the genome (e.g., with a phage or plasmid), EHEC's ability to cause disease is restored.

Table 2.23: Molecular Koch's postulates applied to EHEC

PATHOGENICITY AND VIRULENCE

The ability of a microbial agent to cause disease is called **pathogenicity**, and the degree to which an organism is pathogenic is called **virulence**. Virulence is a continuum. On one end of the spectrum are organisms that are avirulent (not harmful) and on the other are organisms that are highly virulent. Highly virulent pathogens will almost always lead to a disease state when introduced to the body, and some may even cause multi-organ and body system failure in healthy individuals. Less virulent pathogens may cause an initial infection, but may not always cause severe illness. Pathogens with low virulence would more likely result in mild signs and symptoms of disease, such as low-grade fever, headache, or muscle aches. Some individuals might even be asymptomatic.

Virulence of a pathogen can be quantified using controlled experiments with laboratory animals. Two important indicators of virulence are the median infectious dose (ID50) and the median lethal dose (LD50), both of which are typically determined experimentally using animal models. The ID50 is the number of pathogen cells or virions required to cause active infection in 50% of inoculated animals. The LD50 is the number of pathogenic cells, virions, or amount of toxin required to kill 50% of infected animals. To calculate these values, each group of animals is inoculated with one of a range of known numbers of pathogen cells or virions. In graphs like the one shown in figure 2.87, the percentage of animals that have been infected (for ID50) or killed (for LD50) is plotted against the concentration of pathogen inoculated. Figure 2.87 represents data graphed from a hypothetical experiment measuring the LD50 of a pathogen. Interpretation of the data from this graph indicates that the LD50 of the pathogen for the test animals is 104 pathogen cells or virions (depending upon the pathogen studied).

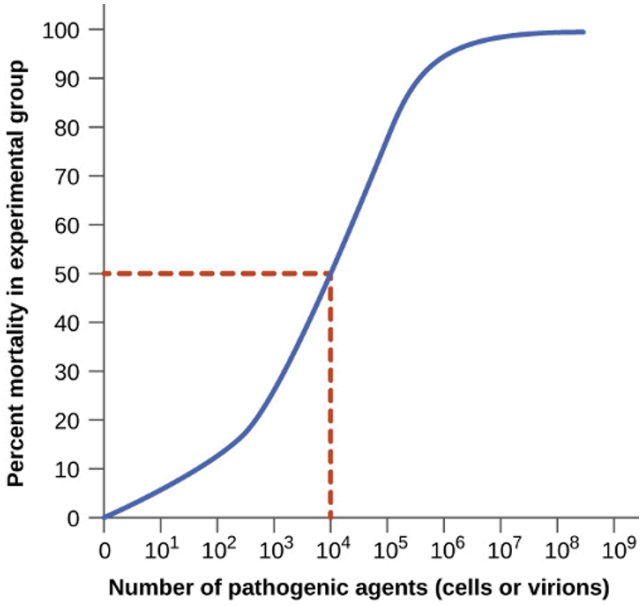


Figure 2.87: A graph like this is used to determine LD50 by plotting pathogen concentration against the percent of infected test animals that have died. In this example, the LD50 = 104 pathogenic particles. *Figure description available at the end of the chapter.*

Table 2.24 lists selected foodborne pathogens and their ID50 values in humans (as determined from epidemiologic data and studies on human volunteers). Keep in mind that these are *median* values. The actual infective dose for an individual can vary widely, depending on factors such as route of entry; the age, health, and immune status of the host; and environmental and pathogen-specific

factors such as susceptibility to the acidic pH of the stomach. It is also important to note that a pathogen's infective dose does not necessarily correlate with disease severity. For example, just a single cell of *Salmonella enterica* serotype Typhimurium can result in an active infection. The resultant disease, *Salmonella* gastroenteritis or salmonellosis, can cause nausea, vomiting, and diarrhea, but has a mortality rate of less than 1% in healthy adults. In contrast, *S. enterica* serotype Typhi has a much higher ID50, typically requiring as many as 1,000 cells to produce infection. However, this serotype causes typhoid fever, a much more systemic and severe disease that has a mortality rate as high as 10% in untreated individuals.

Pathogen	ID50
Viruses	
Hepatitis A virus	10–100
Norovirus	1–10
Rotavirus	10–100
Bacteria	
Escherichia coli, enterohemorrhagic (EHEC, serotype O157)	10–100
E. coli, enteroinvasive (EIEC)	200–5,000
E. coli, enteropathogenic (EPEC)	10,000,000–10,000,000,000
E. coli, enterotoxigenic (ETEC)	10,000,000–10,000,000,000
Salmonella enterica serovar Typhi	<1,000
S. enterica serovar Typhimurium	≥1
Shigella dysenteriae	10–200
Vibrio cholerae (serotypes O139, O1)	1,000,000
V. parahemolyticus	100,000,000
Protozoa	
Giardia lamblia	1
Cryptosporidium parvum	10–100

Table 2.24: ID50 for selected foodborne diseases (credit: Food and Drug Administration. "Bad Bug Book, Foodborne Pathogenic Microorganisms and Natural Toxins." 2nd ed. Silver Spring, MD: US Food and Drug Administration; 2012.)

PRIMARY PATHOGENS VERSUS OPPORTUNISTIC PATHOGENS

Pathogens can be classified as either primary pathogens or opportunistic pathogens. A primary pathogen can cause disease in a host regardless of the host's resident microbiota or immune system. An opportunistic pathogen, by contrast, can only cause disease in situations that compromise the host's defenses, such as the body's protective barriers, immune system, or normal microbiota. Individuals susceptible to opportunistic infections include the very young, the elderly, women who are pregnant, patients undergoing chemotherapy, people with immunodeficiencies (such as acquired immunodeficiency syndrome [AIDS]), patients who are recovering from surgery, and those who have had a breach of protective barriers (such as a severe wound or burn).

An example of a primary pathogen is enterohemorrhagic *E. coli* (EHEC), which produces a virulence factor known as Shiga toxin. This toxin inhibits protein synthesis, leading to severe and bloody diarrhea, inflammation, and renal failure, even in patients with healthy immune systems. *Staphylococcus epidermidis*, on the other hand, is an opportunistic pathogen that is among the most frequent causes of nosocomial disease.[22] *S. epidermidis* is a member of the normal microbiota of the skin, where it is generally avirulent. However, in hospitals, it can also grow in biofilms that form on catheters, implants, or other devices that are inserted into the body during surgical procedures. Once inside the body, *S. epidermidis* can cause serious infections such as endocarditis, and it produces virulence factors that promote the persistence of such infections.

Other members of the normal microbiota can also cause opportunistic infections under certain conditions. This often occurs when microbes that reside harmlessly in one body location end up in a different body system, where they cause disease. For example, *E. coli* normally found in the large intestine can cause a urinary tract infection if it enters the bladder. This is the leading cause of urinary tract infections among women.

Members of the normal microbiota may also cause disease when a shift in the environment of the body leads to overgrowth of a particular microorganism. For example, the yeast *Candida* is part of the normal microbiota of the skin, mouth, intestine, and vagina, but its population is kept in check by other organisms of the microbiota. If an individual is taking antibacterial medications, however, bacteria that would normally inhibit the growth of *Candida* can be killed off, leading to a sudden growth in the population of *Candida*, which is not affected by antibacterial medications because it is a fungus. An overgrowth of *Candida* can manifest as oral thrush (growth on mouth, throat, and tongue), a vaginal yeast infection, or cutaneous candidiasis. Other scenarios can also provide opportunities for *Candida* infections. Untreated diabetes can result in a high concentration of glucose in the saliva, which provides an optimal environment for the growth of *Candida,* resulting in thrush. Immunodeficiencies such as those seen in patients with HIV, AIDS, and cancer also lead to higher incidence of thrush. Vaginal yeast infections can result from decreases in estrogen levels during the menstruation or menopause. The amount of glycogen available to lactobacilli in the vagina is controlled by levels of estrogen; when estrogen levels are low, lactobacilli produce less lactic acid. The resultant increase in vaginal pH allows overgrowth of *Candida* in the vagina.

STAGES OF PATHOGENESIS

To cause disease, a pathogen must successfully achieve four steps or stages of pathogenesis: exposure (contact), adhesion (colonization), invasion, and infection. The pathogen must be able to gain entry to the host, travel to the location where it can establish an infection, evade or overcome the host's immune response, and cause damage (i.e., disease) to the host. In many cases, the cycle is completed when the pathogen exits the host and is transmitted to a new host.

Exposure

An encounter with a potential pathogen is known as exposure or contact. The food we eat and the objects we handle are all ways that we can come into contact with potential pathogens. Yet, not all contacts result in infection and disease. For a pathogen to cause disease, it needs to be able to gain access into host tissue. An anatomic site through which pathogens can pass into host tissue is called a portal of entry. These are locations where the host cells are in direct contact with the external environment. Major portals of entry are identified in figure 2.88 and include the skin, mucous membranes, and parenteral routes.

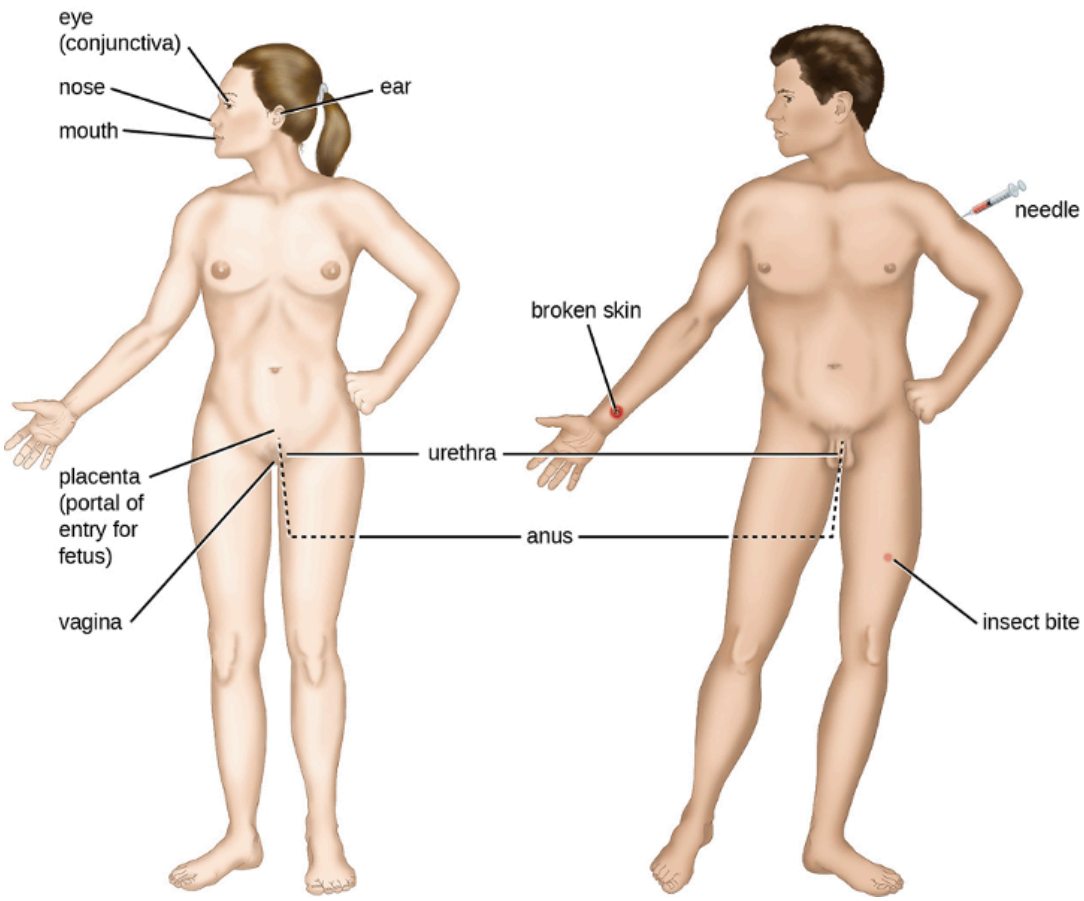


Figure 2.88: Shown are different portals of entry where pathogens can gain access into the body. With the exception of the placenta, which is only present during pregnancy, many of these locations are directly exposed to external environments. *Figure description available at the end of the chapter.*

Mucosal surfaces are the most important portals of entry for microbes; these include the mucous membranes of the respiratory tract, the gastrointestinal tract, and the genitourinary tract. Although most mucosal surfaces are in the interior of the body, some are contiguous with the external skin at various body openings, including the eyes, nose, mouth, urethra, and anus.

Most pathogens are suited to a particular portal of entry. A pathogen's portal specificity is determined by the organism's environmental adaptations and by the enzymes and toxins they secrete. The respiratory and gastrointestinal tracts are particularly vulnerable portals of entry because particles that include microorganisms are constantly inhaled or ingested, respectively.

Pathogens can also enter through a breach in the protective barriers of the skin and mucous membranes. Pathogens that enter the body in this way are said to enter by the parenteral route. For example, the skin is a good natural barrier to pathogens, but breaks in the skin (e.g., wounds, insect bites, animal bites, needle pricks) can provide a parenteral portal of entry for microorganisms.

In pregnant women, the placenta normally prevents microorganisms from passing from the mother to the fetus. However, a few pathogens are capable of crossing the blood-placental barrier. The gram-positive bacterium *Listeria monocytogenes*, which causes the foodborne disease listeriosis, is one example that poses a serious risk to the fetus and can sometimes lead to spontaneous abortion. Other pathogens that can pass the placental barrier to infect the fetus are known collectively by the acronym TORCH (table 2.25).

Transmission of infectious diseases from mother to baby is also a concern at the time of birth when the baby passes through the birth canal. Babies whose mothers have active chlamydia or gonorrhea infections may be exposed to the causative pathogens in the vagina, which can result in eye infections that lead to blindness. To prevent this, it is standard practice to administer antibiotic drops to infants' eyes shortly after birth.

	Disease	Pathogen
T	Toxoplasmosis	*Toxoplasma gondii* (protozoan)
O*	Syphilis Chickenpox Hepatitis B HIV Fifth disease (erythema infectiosum)	*Treponema pallidum* (bacterium) Varicella-zoster virus (human herpesvirus 3) Hepatitis B virus (hepadnavirus) Retrovirus Parvovirus B19
R	Rubella (German measles)	Togavirus
C	Cytomegalovirus	Human herpesvirus 5
H	Herpes	Herpes simplex viruses (HSV) 1 and 2
*The O in TORCH stands for "other."		

Table 2.25: Pathogens capable of crossing the placental barrier (TORCH infections)

Adhesion

Following the initial exposure, the pathogen adheres at the portal of entry. The term adhesion refers to the capability of pathogenic microbes to attach to the cells of the body using adhesion factors, and different pathogens use various mechanisms to adhere to the cells of host tissues.

Molecules (either proteins or carbohydrates) called adhesins are found on the surface of certain pathogens and bind to specific receptors (glycoproteins) on host cells. Adhesins are present on the fimbriae and flagella of bacteria, the cilia of protozoa, and the capsids or membranes of viruses. Protozoans can also use hooks and barbs for adhesion; spike proteins on viruses also enhance viral adhesion. The production of glycocalyces (slime layers and capsules) (figure 2.89), with their high sugar and protein content, can also allow certain bacterial pathogens to attach to cells.

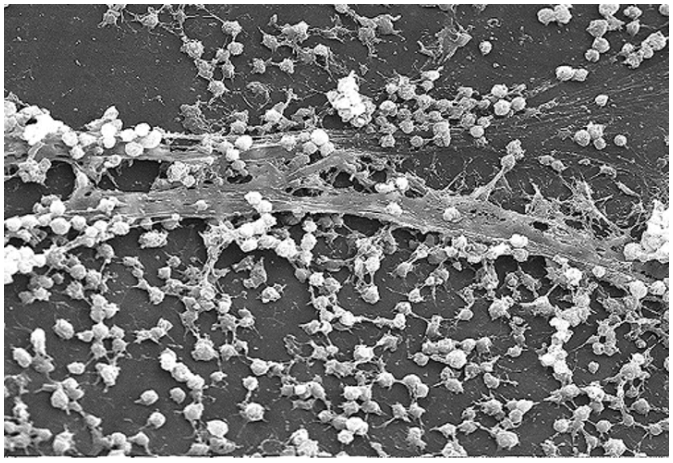


Figure 2.89: Glycocalyx produced by bacteria in a biofilm allows the cells to adhere to host tissues and to medical devices such as the catheter surface shown here. *Figure description available at the end of the chapter.*

Biofilm growth can also act as an adhesion factor. A biofilm is a community of bacteria that produce a glycocalyx, known as extra polymeric substance (EPS), that allows the biofilm to attach to a surface. Persistent *Pseudomonas aeruginosa* infections are common in patients suffering from cystic fibrosis, burn wounds, and middle-ear infections (otitis media) because *P. aeruginosa* produces a biofilm. The EPS allows the bacteria to adhere to the host cells and makes it harder for the host to physically remove the pathogen. The EPS not only allows for attachment but provides protection against the immune system and antibiotic treatments, preventing antibiotics from reaching the bacterial cells within the

biofilm. In addition, not all bacteria in a biofilm are rapidly growing; some are in a stationary phase. Since antibiotics are most effective against rapidly growing bacteria, portions of bacteria in a biofilm are protected against antibiotics.[23]

Invasion

Once adhesion is successful, invasion can proceed. Invasion involves the dissemination of a pathogen throughout local tissues or the body. Pathogens may produce exoenzymes or toxins, which serve as virulence factors that allow them to colonize and damage host tissues as they spread deeper into the body. Pathogens may also produce virulence factors that protect them against immune system defenses. A pathogen's specific virulence factors determine the degree of tissue damage that occurs. Figure 2.90 shows the invasion of *H. pylori* into the tissues of the stomach, causing damage as it progresses.

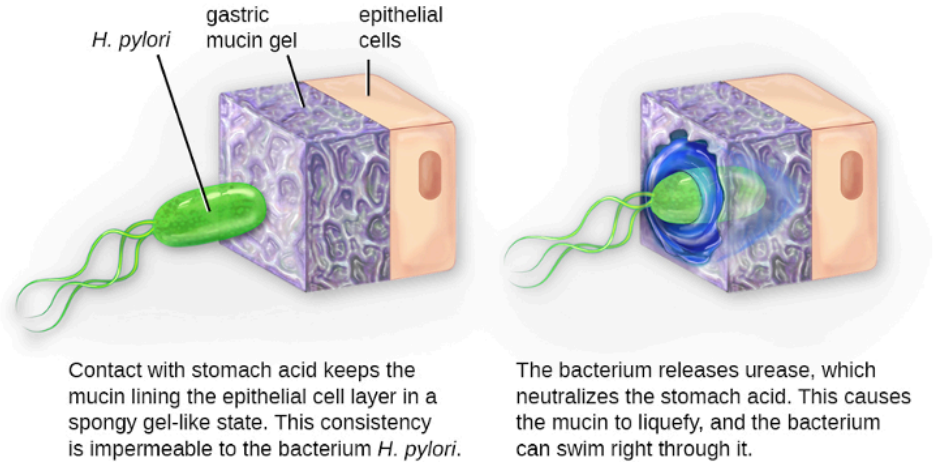


Figure 2.90: H. pylori is able to invade the lining of the stomach by producing virulence factors that enable it to pass through the mucin layer covering epithelial cells. *Figure description available at the end of the chapter.*

Intracellular pathogens achieve invasion by entering the host's cells and reproducing. Some are obligate intracellular pathogens (meaning they can only reproduce inside of host cells) and others are facultative intracellular pathogens (meaning they can reproduce either inside or outside of host cells). By entering the host cells, intracellular pathogens are able to evade some mechanisms of the immune system while also exploiting the nutrients in the host cell.

Entry to a cell can occur by endocytosis. For most kinds of host cells, pathogens use one of two different mechanisms for endocytosis and entry. One mechanism relies on effector proteins secreted by the pathogen; these effector proteins trigger entry into the host cell. This is the method that *Salmonella* and *Shigella* use when invading intestinal epithelial cells. When these pathogens come in contact with epithelial cells in the intestine, they secrete effector molecules that cause protrusions of membrane ruffles that bring the bacterial cell in. This process is called membrane ruffling. The second mechanism relies on surface proteins expressed on the pathogen that bind to receptors on the host cell, resulting in entry. For example, *Yersinia pseudotuberculosis* produces a surface protein known as invasin that binds to beta-1 integrins expressed on the surface of host cells.

Some host cells, such as white blood cells and other phagocytes of the immune system, actively endocytose pathogens in a process called phagocytosis. Although phagocytosis allows the pathogen to gain entry to the host cell, in most cases, the host cell kills and degrades the pathogen by using digestive enzymes. Normally, when a pathogen is ingested by a phagocyte, it is enclosed within a phagosome in the cytoplasm; the phagosome fuses

with a lysosome to form a phagolysosome, where digestive enzymes kill the pathogen (see section 1.6). However, some intracellular pathogens have the ability to survive and multiply within phagocytes. Examples include *Listeria monocytogenes* and *Shigella*; these bacteria produce proteins that lyse the phagosome before it fuses with the lysosome, allowing the bacteria to escape into the phagocyte's cytoplasm where they can multiply. Bacteria such as *Mycobacterium tuberculosis*, *Legionella pneumophila*, and *Salmonella* species use a slightly different mechanism to evade being digested by the phagocyte. These bacteria prevent the fusion of the phagosome with the lysosome, thus remaining alive and dividing within the phagosome.

Infection

Following invasion, successful multiplication of the pathogen leads to infection. Infections can be described as local, focal, or systemic depending on the extent of the infection. A local infection is confined to a small area of the body, typically near the portal of entry. For example, a hair follicle infected by *Staphylococcus aureus* infection may result in a boil around the site of infection, but the bacterium is largely contained to this small location. Other examples of local infections that involve more extensive tissue involvement include urinary tract infections confined to the bladder or pneumonia confined to the lungs.

In a focal infection, a localized pathogen, or the toxins it produces, can spread to a secondary location. For example, a dental hygienist nicking the gum with a sharp tool can lead to a local infection in the gum by *Streptococcus* bacteria of the normal oral microbiota. These *Streptococcus* spp. may then gain access to the bloodstream and make their way to other locations in the body, resulting in a secondary infection.

When an infection becomes disseminated throughout the body, we call it a systemic infection. For example, infection by the varicella-zoster virus typically gains entry through a mucous membrane of the upper respiratory system. It then spreads throughout the body, resulting in the classic red skin lesions associated with chickenpox. Since these lesions are not sites of initial infection, they are signs of a systemic infection.

Sometimes a primary infection, the initial infection caused by one pathogen, can lead to a secondary infection by another pathogen. For example, the immune system of a patient with the primary infection HIV becomes compromised, making the patient more susceptible to secondary diseases like oral thrush and others caused by opportunistic pathogens. Similarly, a primary infection by the influenza virus damages and decreases the defense mechanisms of the lungs, making patients more susceptible to an infection from secondary pneumonia caused by a bacterial pathogen like *Haemophilus influenzae* or *Streptococcus pneumoniae*. Some secondary infections can even develop as a result of treatment for a primary infection. Antibiotic therapy targeting the primary pathogen can cause collateral damage to the normal microbiota, creating an opening for opportunistic pathogens.

TRANSMISSION OF DISEASE

For a pathogen to persist, it must put itself in a position to be transmitted to a new host, leaving the infected host through a portal of exit (figure 2.91). As with portals of entry, many pathogens are adapted to use a particular portal of exit. Similar to portals of entry, the most common portals of exit include the skin and the respiratory, urogenital, and gastrointestinal tracts. Coughing and sneezing can expel pathogens from the respiratory tract. A single sneeze can send thousands of virus particles into the air. Secretions and excretions can transport pathogens out of other portals of exit. Feces, urine, semen, vaginal secretions, tears, sweat, and shed skin cells can all serve as vehicles for a pathogen to leave the body. Pathogens that rely on insect vectors for transmission exit the body in the blood extracted by a biting insect. Similarly, some pathogens exit the body in blood extracted by needles.

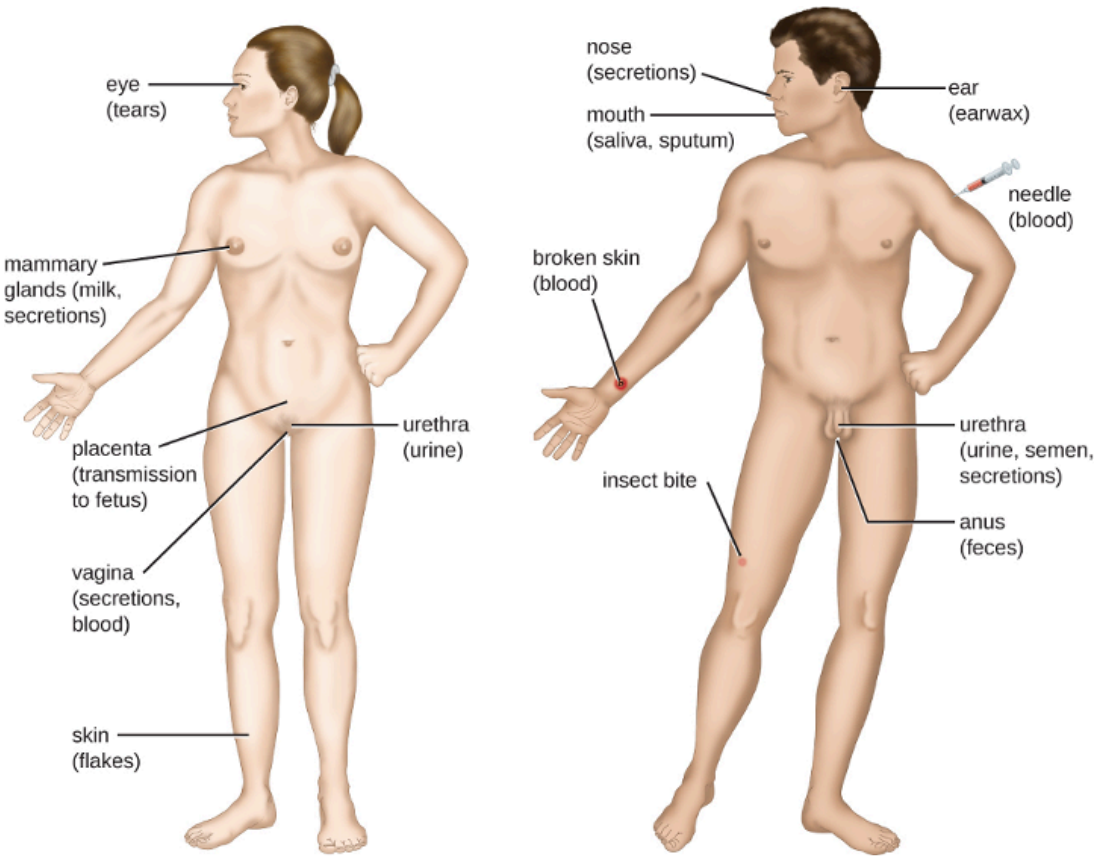


Figure 2.91: Pathogens leave the body of an infected host through various portals of exit to infect new hosts.
Figure description available at the end of the chapter.

2.14 VIRULENCE FACTORS OF BACTERIAL AND VIRAL PATHOGENS

Some pathogens are more virulent than others. This is due to the unique virulence factors produced by individual pathogens, which determine the extent and severity of disease they may cause. A pathogen's virulence factors are encoded by genes that can be identified using molecular Koch's postulates. When genes encoding virulence factors are inactivated, virulence in the pathogen is diminished. In this section, we examine various types and specific examples of virulence factors and how they contribute to each step of pathogenesis.

VIRULENCE FACTORS FOR ADHESION

The first two steps in pathogenesis are exposure and adhesion. Recall that an adhesin is a protein or glycoprotein found on the surface of a pathogen that attaches to receptors on the host cell. Adhesins are found on bacterial, viral, fungal, and protozoan pathogens. One example of a bacterial adhesin is type 1 fimbrial adhesin, a molecule found on the tips of fimbriae of enterotoxigenic *E. coli* (ETEC). Recall that fimbriae are hairlike protein bristles on the cell surface. Type 1 fimbrial adhesin allows the fimbriae of ETEC cells to attach to the mannose glycans expressed on intestinal epithelial cells. Table 2.26 lists common adhesins found in some of the pathogens we have discussed or will be seeing later in this chapter.

Pathogen	Disease	Adhesin	Attachment Site
Streptococcus pyogenes	Strep throat	Protein F	Respiratory epithelial cells
Streptococcus mutans	Dental caries	Adhesin P1	Teeth
Neisseria gonorrhoeae	Gonorrhea	Type IV pili	Urethral epithelial cells
Enterotoxigenic E. coli (ETEC)	Traveler's diarrhea	Type 1 fimbriae	Intestinal epithelial cells
Vibrio cholerae	Cholera	N-methylphenylalanine pili	Intestinal epithelial cells

Table 2.26: Some bacterial adhesins and their host attachment sites

BACTERIAL EXOENZYMES AND TOXINS AS VIRULENCE FACTORS

After exposure and adhesion, the next step in pathogenesis is invasion, which can involve enzymes and toxins. Many pathogens achieve invasion by entering the bloodstream, an effective means of dissemination because blood vessels pass close to every cell in the body. The downside of this mechanism of dispersal is that the blood also includes numerous elements of the immune system. Various terms ending in –emia are used to describe the presence of pathogens in the bloodstream. The presence of bacteria in blood is called bacteremia. Bacteremia involving pyogenes (pus-forming bacteria) is called pyemia. When viruses are found in the blood, it is called viremia. The term toxemia describes the condition when toxins are found in the blood. If bacteria are both present and multiplying in the blood, this condition is called septicemia.

Patients with septicemia are described as septic, which can lead to shock, a life-threatening decrease in blood pressure (systolic pressure <90 mm Hg) that prevents cells and organs from receiving enough oxygen and nutrients. Some bacteria can cause shock through the release of toxins (virulence factors that can cause tissue damage) and lead to low blood pressure. Gram-negative bacteria are engulfed by immune system phagocytes, which then release tumor necrosis factor, a molecule involved in inflammation and fever. Tumor necrosis factor binds to blood capillaries to increase their permeability, allowing fluids to pass out of blood vessels and into tissues, causing swelling, or edema (figure 2.92). With high concentrations of tumor necrosis factor, the inflammatory reaction is severe and enough fluid is lost from the circulatory system that blood pressure decreases to dangerously low levels. This can have dire consequences because the heart, lungs, and kidneys rely on normal blood pressure for proper function; thus, multi-organ failure, shock, and death can occur.

Figure 2.92: This patient has edema in the tissue of the right hand. Such swelling can occur when bacteria cause the release of pro-inflammatory molecules from immune cells and these molecules cause an increased permeability of blood vessels, allowing fluid to escape the bloodstream and enter tissue. *Figure description available at the end of the chapter.*

Exoenzymes

Some pathogens produce extracellular enzymes, or exoenzymes, that enable them to invade host cells and deeper tissues. Exoenzymes have a wide variety of targets. Some general classes of exoenzymes and associated pathogens are listed in table 2.27. Each of these exoenzymes functions in the context of a particular tissue structure to facilitate invasion or support its own growth and defend against the immune system. For example, hyaluronidase S, an enzyme produced by pathogens like *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Clostridium perfringens*, degrades the glycoside hyaluronan (hyaluronic acid), which acts as an intercellular cement between adjacent cells in connective tissue (figure 2.93). This allows the pathogen to pass through the tissue layers at the portal of entry and disseminate elsewhere in the body (figure 2.93).

Class	Example	Function
Glycohydrolases	Hyaluronidase S in *Staphylococcus aureus*	Degrades hyaluronic acid that cements cells together to promote spreading through tissues
Nucleases	DNAse produced by *S. aureus*	Degrades DNA released by dying cells (bacteria and host cells) that can trap the bacteria, thus promoting spread
Phospholipases	Phospholipase C of *Bacillus anthracis*	Degrades phospholipid bilayer of host cells, causing cellular lysis, and degrade membrane of phagosomes to enable escape into the cytoplasm
Proteases	Collagenase in *Clostridium perfringens*	Degrades collagen in connective tissue to promote spread

Table 2.27: Some classes of exoenzymes and their targets

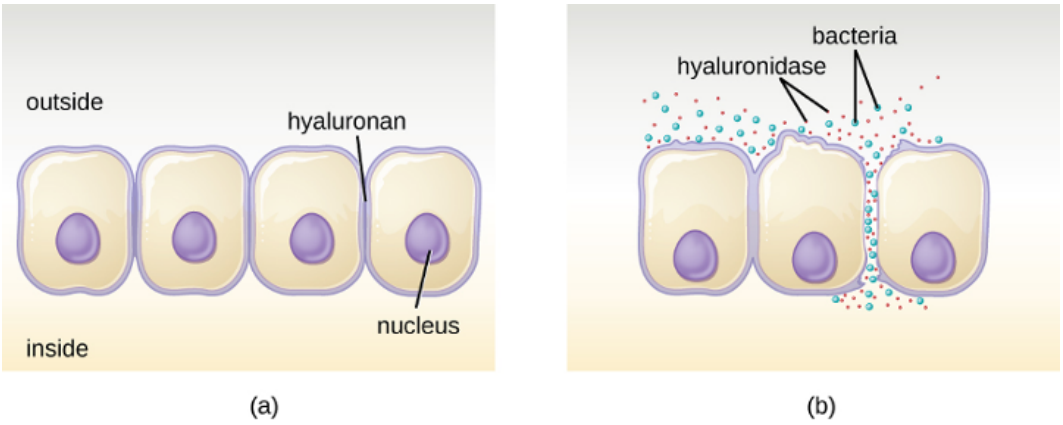


Figure 2.93: (a) Hyaluronan is a polymer found in the layers of epidermis that connect adjacent cells. (b) Hyaluronidase produced by bacteria degrades this adhesive polymer in the extracellular matrix, allowing passage between cells that would otherwise be blocked. *Figure description available at the end of the chapter.*

Pathogen-produced nucleases, such as DNAse produced by *S. aureus,* degrade extracellular DNA as a means of escape and spreading through tissue. As bacterial and host cells die at the site of infection, they lyse and release their intracellular contents. The DNA chromosome is the largest of the intracellular molecules, and masses of extracellular DNA can trap bacteria and prevent their spread. *S. aureus* produces a DNAse to degrade the mesh of extracellular DNA so it can escape and spread to adjacent tissues. This strategy is also used by *S. aureus* and other pathogens to degrade and escape webs of extracellular DNA produced by immune system phagocytes to trap the bacteria.

Enzymes that degrade the phospholipids of cell membranes are called phospholipases. Their actions are specific in regard to the type of phospholipids they act upon and where they enzymatically cleave the molecules. The pathogen responsible for anthrax, *B. anthracis,* produces phospholipase C. When *B. anthracis* is ingested by phagocytic cells of the immune system, phospholipase C degrades the membrane of the phagosome before it can fuse with the lysosome, allowing the pathogen to escape into the cytoplasm and multiply. Phospholipases can also target the membrane that encloses the phagosome within phagocytic cells. As described earlier in this chapter, this is the mechanism used by intracellular pathogens such as *L. monocytogenes* and *Rickettsia* to escape the phagosome and multiply within the cytoplasm of phagocytic cells. The role of phospholipases in bacterial virulence is not restricted to phagosomal escape. Many pathogens produce phospholipases that act to degrade cell membranes and cause lysis of target cells. These phospholipases are involved in lysis of red blood cells, white blood cells, and tissue cells.

Bacterial pathogens also produce various protein-digesting enzymes, or proteases. Proteases can be classified according to their substrate target (e.g., serine proteases target proteins with the amino acid serine) or if they contain metals in their active site (e.g., zinc metalloproteases contain a zinc ion, which is necessary for enzymatic activity).

Toxins

In addition to exoenzymes, certain pathogens are able to produce toxins, biological poisons that assist in their ability to invade and cause damage to tissues. The ability of a pathogen to produce toxins to cause damage to host cells is called toxigenicity.

Toxins can be categorized as endotoxins or exotoxins. The lipopolysaccharide (LPS) found on the outer membrane of gram-negative bacteria is called endotoxin (figure 2.94). During infection and disease, gram-negative bacterial pathogens release endotoxin either when the cell dies, resulting in the disintegration of the membrane, or when the bacterium undergoes binary fission. The lipid component of endotoxin, lipid A, is responsible for the toxic properties of the LPS molecule. Lipid A is relatively conserved across different genera of gram-negative bacteria; therefore, the toxic properties of lipid A are similar regardless of the gram-negative pathogen. In a manner similar to that of tumor necrosis factor, lipid A triggers the immune system's inflammatory response (see section 1.7). If the concentration of endotoxin in the body is low, the inflammatory response may provide the host an effective defense against infection; on the other hand, high concentrations of endotoxin in the blood can cause an excessive inflammatory response, leading to a severe drop in blood pressure, multi-organ failure, and death.

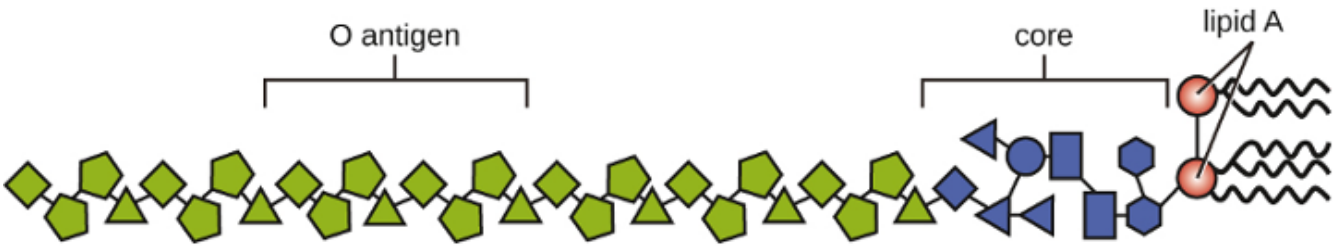


Figure 2.94: Lipopolysaccharide is composed of lipid A, a core glycolipid, and an O-specific polysaccharide side chain. Lipid A is the toxic component that promotes inflammation and fever. *Figure description available at the end of the chapter.*

A classic method of detecting endotoxin is by using the *Limulus* amebocyte lysate (LAL) test. In this procedure, the blood cells (amebocytes) of the horseshoe crab (*Limulus polyphemus*) are mixed with a patient's serum. The amebocytes will react to the presence of any endotoxin. This reaction can be observed either chromogenically (color) or by looking for coagulation (clotting reaction) to occur within the serum. An alternative method that has been used is an enzyme-linked immunosorbent assay (ELISA) that uses antibodies to detect the presence of endotoxin.

Unlike the toxic lipid A of endotoxin, exotoxins are protein molecules that are produced by a wide variety of living pathogenic bacteria. Although some gram-negative pathogens produce exotoxins, the majority are produced by gram-positive pathogens. Exotoxins differ from endotoxin in several other key characteristics, summarized in table 2.28. In contrast to endotoxin, which stimulates a general systemic inflammatory response when released, exotoxins are much more specific in their action and the cells they interact with. Each exotoxin targets specific receptors on specific cells and damages those cells through unique molecular mechanisms. Endotoxin remains stable at high temperatures, and requires heating at 121 °C (250 °F) for 45 minutes to inactivate. By contrast, most exotoxins are heat labile because of their protein structure, and many are denatured (inactivated) at temperatures above 41 °C (106 °F). As discussed earlier, endotoxin can stimulate a lethal inflammatory response at very high concentrations and has a measured LD50 of 0.24 mg/kg. By contrast, very small concentrations of exotoxins can be lethal. For example, botulinum toxin, which causes botulism, has an LD50 of 0.000001 mg/kg (240,000 times more lethal than endotoxin).

Characteristic	Endotoxin	Exotoxin
Source	Gram-negative bacteria	Gram-positive (primarily) and gram-negative bacteria
Composition	Lipid A component of lipopolysaccharide	Protein
Effect on host	General systemic symptoms of inflammation and fever	Specific damage to cells dependent upon receptor-mediated targeting of cells and specific mechanisms of action
Heat stability	Heat stable	Most are heat labile, but some are heat stable
LD_{50}	High	Low

Table 2.28: Comparison of endotoxin and exotoxins produced by bacteria

The exotoxins can be grouped into three categories based on their target: intracellular targeting, membrane disrupting, and superantigens. Table 2.29 provides examples of well-characterized toxins within each of these three categories.

The intracellular targeting toxins comprise two components: A for activity and B for binding. Thus, these types of toxins are known as A-B exotoxins (figure 2.95). The B component is responsible for the cellular specificity of the toxin and mediates the initial attachment of the toxin to specific cell surface receptors. Once the A-B toxin binds to the host cell, it is brought into the cell by endocytosis and entrapped in a vacuole. The A and B subunits separate as the vacuole acidifies. The A subunit then enters the cell cytoplasm and interferes with the specific internal cellular function that it targets.

Category	Example	Pathogen	Mechanism and Disease
Intracellular-targeting toxins	Cholera toxin	*Vibrio cholerae*	Activation of adenylate cyclase in intestinal cells, causing increased levels of cyclic adenosine monophosphate (cAMP) and secretion of fluids and electrolytes out of cell, causing diarrhea
	Tetanus toxin	*Clostridium tetani*	Inhibits the release of inhibitory neurotransmitters in the central nervous system, causing spastic paralysis
	Botulinum toxin	*Clostridium botulinum*	Inhibits release of the neurotransmitter acetylcholine from neurons, resulting in flaccid paralysis
	Diphtheria toxin	*Corynebacterium diphtheriae*	Inhibition of protein synthesis, causing cellular death
Membrane-disrupting toxins	Streptolysin	*Streptococcus pyogenes*	Proteins that assemble into pores in cell membranes, disrupting their function and killing the cell
	Pneumolysin	*Streptococcus pneumoniae*	
	Alpha-toxin	*Staphylococcus aureus*	
	Alpha-toxin	*Clostridium perfringens*	Phospholipases that degrade cell membrane phospholipids, disrupting membrane function and killing the cell
	Phospholipase C	*Pseudomonas aeruginosa*	
	Beta-toxin	*Staphylococcus aureus*	
Superantigens	Toxic shock syndrome toxin	*Staphylococcus aureus*	Stimulates excessive activation of immune system cells and release of cytokines (chemical mediators) from immune system cells. Life-threatening fever, inflammation, and shock are the result.
	Streptococcal mitogenic exotoxin	*Streptococcus pyogenes*	
	Streptococcal pyrogenic toxins	*Streptococcus pyogenes*	

Table 2.29: Some common exotoxins and associated bacterial pathogens

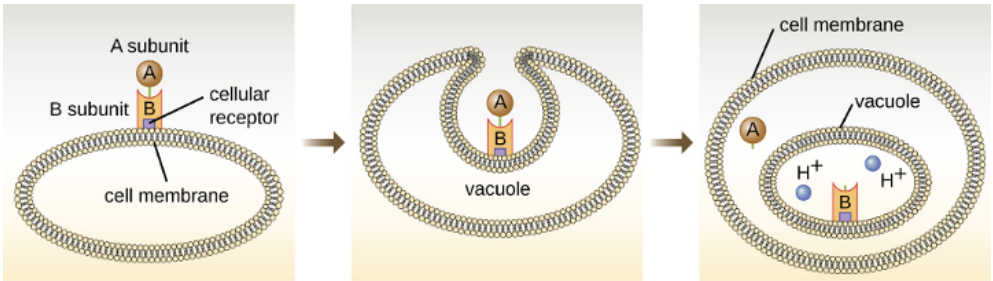


Figure 2.95: (a) In A-B toxins, the B component binds to the host cell through its interaction with specific cell surface receptors. (b) The toxin is brought in through endocytosis. (c) Once inside the vacuole, the A component (active component) separates from the B component and the A component gains access to the cytoplasm. *Figure description available at the end of the chapter.*

Four unique examples of A-B toxins are the diphtheria, cholera, botulinum, and tetanus toxins. The diphtheria toxin is produced by the gram-positive bacterium *Corynebacterium diphtheriae*, the causative agent of nasopharyngeal and cutaneous diphtheria. After the A subunit of the diphtheria toxin separates and gains access to the cytoplasm, it facilitates the transfer of adenosine diphosphate (ADP)-ribose onto an elongation-factor protein (EF-2) that is needed for protein synthesis. Hence, diphtheria toxin inhibits protein synthesis in the host cell, ultimately killing the cell (figure 2.96).

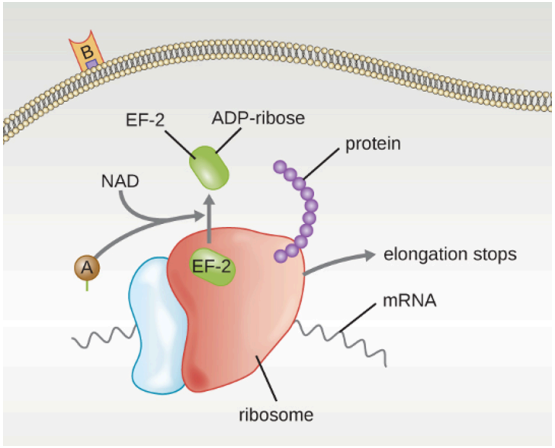


Figure 2.96: The mechanism of the diphtheria toxin inhibiting protein synthesis. The A subunit inactivates elongation factor 2 by transferring an ADP-ribose. This stops protein elongation, inhibiting protein synthesis and killing the cell. *Figure description available at the end of the chapter.*

Cholera toxin is an enterotoxin produced by the gram-negative bacterium *Vibrio cholerae* and is composed of one A subunit and five B subunits. The mechanism of action of the cholera toxin is complex. The B subunits bind to receptors on the intestinal epithelial cell of the small intestine. After gaining entry into the cytoplasm of the epithelial cell, the A subunit activates an intracellular G protein. The activated G protein, in turn, leads to the activation of the enzyme adenylyl cyclase, which begins to produce an increase in the concentration of cyclic AMP (a secondary messenger molecule). The increased cAMP disrupts the normal physiology of the intestinal epithelial cells and causes them to secrete excessive amounts of fluid and electrolytes into the lumen of the intestinal tract, resulting in severe "rice-water stool" diarrhea characteristic of cholera.

Botulinum toxin (also known as botox) is a neurotoxin produced by the gram-positive bacterium *Clostridium botulinum*. It is the most acutely toxic substance known to date. The toxin is composed of a light A subunit and heavy protein chain B subunit. The B subunit binds to neurons to allow botulinum toxin to enter the neurons at the neuromuscular junction. The A subunit acts as a protease, cleaving proteins involved in the neuron's release of acetylcholine, a neurotransmitter molecule. Normally, neurons release acetylcholine to induce muscle fiber contractions. The toxin's ability to block acetylcholine release results in the inhibition of muscle contractions, leading to muscle relaxation. This has the potential to stop breathing and cause death. Because of its action, low concentrations of botox are used for cosmetic and medical procedures, including the removal of wrinkles and treatment of overactive bladder.

Another neurotoxin is tetanus toxin, which is produced by the gram-positive bacterium *Clostridium tetani*. This toxin also has a light A subunit and heavy protein chain B subunit. Unlike botulinum toxin, tetanus toxin binds to inhibitory interneurons, which are responsible for release of the inhibitory neurotransmitters glycine and gamma-aminobutyric acid (GABA). Normally, these neurotransmitters bind to neurons at the neuromuscular junction, resulting in the inhibition of acetylcholine release. Tetanus toxin inhibits the release of glycine and GABA from the interneuron, resulting in permanent muscle contraction. The first symptom is typically stiffness of the jaw (lockjaw). Violent muscle spasms in other parts of the body follow, typically culminating with respiratory failure and death. Figure 2.97 shows the actions of both botulinum and tetanus toxins.

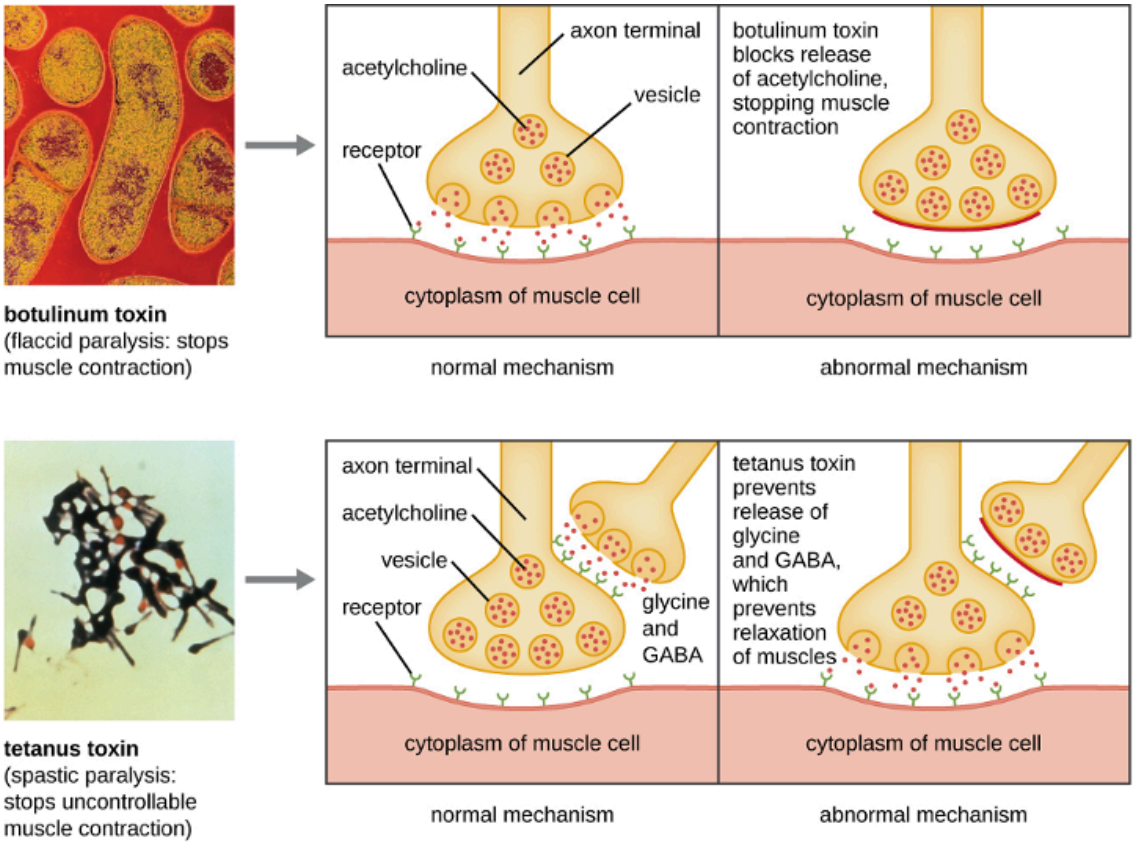


Figure 2.97: Mechanisms of botulinum and tetanus toxins. *Figure description available at the end of the chapter.*

Membrane-disrupting toxins affect cell membrane function either by forming pores or by disrupting the phospholipid bilayer in host cell membranes. Two types of membrane-disrupting exotoxins are hemolysins and leukocidins, which form pores in cell membranes, causing leakage of the cytoplasmic contents and cell lysis. These toxins were originally thought to target red blood cells (erythrocytes) and white blood cells (leukocytes), respectively, but we now know they can affect other cells as well. The gram-positive bacterium *Streptococcus pyogenes* produces streptolysins, water-soluble hemolysins that bind to the cholesterol moieties in the host cell membrane to form a pore. The two types of streptolysins, O and S, are categorized by their ability to cause hemolysis in erythrocytes in the absence or presence of oxygen. Streptolysin O is not active in the presence of oxygen, whereas streptolysin S is active in the presence of oxygen. Other important pore-forming membrane-disrupting toxins include alpha toxin of *Staphylococcus aureus* and pneumolysin of *Streptococcus pneumoniae*.

Bacterial phospholipases are membrane-disrupting toxins that degrade the phospholipid bilayer of cell membranes rather than forming pores. We have already discussed the phospholipases associated with *B. anthracis, L. pneumophila,* and *Rickettsia* species that enable these bacteria to affect the lysis of phagosomes. These same phospholipases are also hemolysins. Other phospholipases that function as hemolysins include the alpha toxin of *Clostridium perfringens*, phospholipase C of *P. aeruginosa*, and beta toxin of *Staphylococcus aureus*.

Some strains of *S. aureus* also produce a leukocidin called Panton-Valentine leukocidin (PVL). PVL consists of two subunits, S and F. The S component acts like the B subunit of an A-B exotoxin in that it binds to glycolipids on the outer plasma membrane of animal cells. The F-component acts like the A subunit of an A-B exotoxin and carries the enzymatic activity. The toxin inserts and assembles into a pore in the membrane. Genes that encode PVL are more frequently present in *S. aureus* strains that cause skin infections and pneumonia.[24] PVL promotes skin infections by causing edema, erythema (reddening of the skin due to blood vessel dilation), and skin necro-

sis. PVL has also been shown to cause necrotizing pneumonia. PVL promotes pro-inflammatory and cytotoxic effects on alveolar leukocytes. This results in the release of enzymes from the leukocytes, which, in turn, cause damage to lung tissue.

The third class of exotoxins is the superantigens. These are exotoxins that trigger an excessive, nonspecific stimulation of immune cells to secrete cytokines (chemical messengers). The excessive production of cytokines, often called a cytokine storm, elicits a strong immune and inflammatory response that can cause life-threatening high fevers, low blood pressure, multi-organ failure, shock, and death. The prototype superantigen is the toxic shock syndrome toxin of *S. aureus*. Most toxic shock syndrome cases are associated with vaginal colonization by toxin-producing *S. aureus* in menstruating women; however, colonization of other body sites can also occur. Some strains of *Streptococcus pyogenes* also produce superantigens; they are referred to as the streptococcal mitogenic exotoxins and the streptococcal pyrogenic toxins.

VIRULENCE FACTORS FOR SURVIVAL IN THE HOST AND IMMUNE EVASION

Evading the immune system is also important to invasiveness. Bacteria use a variety of virulence factors to evade phagocytosis by cells of the immune system. For example, many bacteria produce capsules, which are used in adhesion but also aid in immune evasion by preventing ingestion by phagocytes. The composition of the capsule prevents immune cells from being able to adhere and then phagocytose the cell. In addition, the capsule makes the bacterial cell much larger, making it harder for immune cells to engulf the pathogen (figure 2.100). A notable capsule-producing bacterium is the gram-positive pathogen *Streptococcus pneumoniae*, which causes pneumococcal pneumonia, meningitis, septicemia, and other respiratory tract infections. Encapsulated strains of *S. pneumoniae* are more virulent than nonencapsulated strains and are more likely to invade the bloodstream and cause septicemia and meningitis.

Some pathogens can also produce proteases to protect themselves against phagocytosis. The human immune system produces antibodies that bind to surface molecules found on specific bacteria (e.g., capsules, fimbriae, flagella, LPS). This binding initiates phagocytosis and other mechanisms of antibacterial killing and clearance. Proteases combat antibody-mediated killing and clearance by attacking and digesting the antibody molecules (figure 2.98).

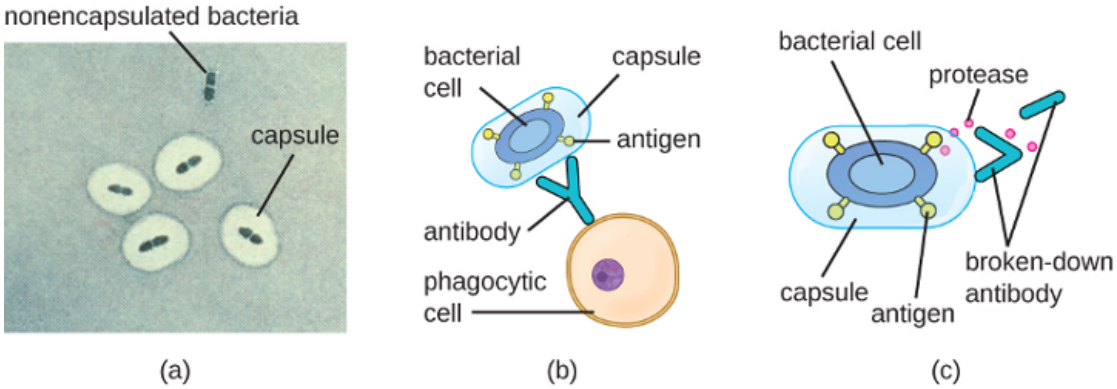


Figure 2.98: (a) A micrograph of capsules around bacterial cells. (b) Antibodies normally function by binding to antigens, molecules on the surface of pathogenic bacteria. Phagocytes then bind to the antibody, initiating phagocytosis. (c) Some bacteria also produce proteases, virulence factors that break down host antibodies to evade phagocytosis. *Figure description available at the end of the chapter.*

In addition to capsules and proteases, some bacterial pathogens produce other virulence factors that allow them to evade the immune system. The fimbriae of certain species of *Streptococcus* contain M protein, which alters the surface of *Streptococcus* and inhibits phagocytosis by blocking the binding of the complement molecules that assist phagocytes in ingesting bacterial pathogens. The acid-fast bacterium *Mycobacterium tuberculosis* (the causative agent of tuberculosis) produces a waxy substance known as mycolic acid in its cell envelope. When it is engulfed by phagocytes in the lung, the protective mycolic acid coat enables the bacterium to resist some of the killing mechanisms within the phagolysosome.

Some bacteria produce virulence factors that promote infection by exploiting molecules naturally produced by the host. For example, most strains of *Staphylococcus aureus* produce the exoenzyme coagulase, which exploits the natural mechanism of blood clotting to evade the immune system. Normally, blood clotting is triggered in response to blood vessel damage; platelets begin to plug the clot, and a cascade of reactions occurs in which fibrinogen, a soluble protein made by the liver, is cleaved into fibrin. Fibrin is an insoluble, thread-like protein that binds to blood platelets, cross-links, and contracts to form a mesh of clumped platelets and red blood cells. The resulting clot prevents further loss of blood from the damaged blood vessels. However, if bacteria release coagulase into the bloodstream, the fibrinogen-to-fibrin cascade is triggered in the absence of blood vessel damage. The resulting clot coats the bacteria in fibrin, protecting the bacteria from exposure to phagocytic immune cells circulating in the bloodstream.

Whereas coagulase causes blood to clot, kinases have the opposite effect by triggering the conversion of plasminogen to plasmin, which is involved in the digestion of fibrin clots. By digesting a clot, kinases allow pathogens trapped in the clot to escape and spread, similar to the way that collagenase, hyaluronidase, and DNAse facilitate the spread of infection. Examples of kinases include staphylokinases and streptokinases, produced by *Staphylococcus aureus* and *Streptococcus pyogenes*, respectively. It is intriguing that *S. aureus* can produce both coagulase to promote clotting and staphylokinase to stimulate the digestion of clots. The action of the coagulase provides an important protective barrier from the immune system, but when nutrient supplies are diminished or other conditions signal a need for the pathogen to escape and spread, the production of staphylokinase can initiate this process.

A final mechanism that pathogens can use to protect themselves against the immune system is called antigenic variation, which is the alteration of surface proteins so that a pathogen is no longer recognized by the host's immune system. For example, the bacterium *Borrelia burgdorferi*, the causative agent of Lyme disease, contains a surface lipoprotein known as VlsE. Because of genetic recombination during DNA replication and repair, this bacterial protein undergoes antigenic variation. Each time fever occurs, the VlsE protein in *B. burgdorferi* can differ so much that antibodies against previous VlsE sequences are not effective. It is believed that this variation in the VlsE contributes to the ability of *B. burgdorferi* to cause chronic disease. Another important human bacterial pathogen that uses antigenic variation to avoid the immune system is *Neisseria gonorrhoeae*, which causes the sexually transmitted disease gonorrhea. This bacterium is well known for its ability to undergo antigenic variation of its type IV pili to avoid immune defenses.

VIRAL VIRULENCE

Although viral pathogens are not similar to bacterial pathogens in terms of structure, some of the properties that contribute to their virulence are similar. Viruses use adhesins to facilitate adhesion to host cells, and certain enveloped viruses rely on antigenic variation to avoid the host immune defenses. These virulence factors are discussed in more detail in the following sections.

Viral Adhesins

One of the first steps in any viral infection is adhesion of the virus to specific receptors on the surface of cells. This process is mediated by adhesins that are part of the viral capsid or membrane envelope. The interaction of viral adhesins with specific cell receptors defines the tropism (preferential targeting) of viruses for specific cells, tissues, and organs in the body. The spike protein hemagglutinin found on the influenza virus is an example of a viral adhesin; it allows the virus to bind to the sialic acid on the membrane of host respiratory and intestinal cells. Another viral adhesin is the glycoprotein gp20, found on HIV. For HIV to infect cells of the immune system, it must interact with two receptors on the surface of cells. The first interaction involves binding between gp120 and the CD4 cellular marker that is found on some essential immune system cells. However, before viral entry into the cell can occur, a second interaction between gp120 and one of two chemokine receptors (CCR5 and CXCR4) must occur. Table 2.30 lists the adhesins for some common viral pathogens and the specific sites to which these adhesins allow viruses to attach.

Pathogen	Disease	Adhesin	Attachment Site
Influenza virus	Influenza	Hemagglutinin	Sialic acid of respiratory and intestinal cells
Herpes simplex virus I or II	Oral herpes, genital herpes	Glycoproteins gB, gC, gD	Heparan sulfate on mucosal surfaces of the mouth and genitals
Human immunodeficiency virus	HIV/AIDS	Glycoprotein gp120	CD4 and CCR5 or CXCR4 of immune system cells

Table 2.30: Some viral adhesins and their host attachment sites

Antigenic Variation in Viruses

Antigenic variation also occurs in certain types of enveloped viruses, including influenza viruses, which exhibit two forms of antigenic variation: antigenic drift and antigenic shift (figure 2.99). Antigenic drift is the result of point mutations causing slight changes in the spike proteins hemagglutinin (H) and neuraminidase (N). On the other hand, antigenic shift is a major change in spike proteins due to gene reassortment. This reassortment for antigenic shift occurs typically when two different influenza viruses infect the same host.

The rate of antigenic variation in influenza viruses is very high, making it difficult for the immune system to recognize the many different strains of influenza virus. Although the body may develop immunity to one strain through natural exposure or vaccination, antigenic variation results in the continual emergence of new strains that the immune system will not recognize. This is the main reason that vaccines against the influenza virus must be given annually. Each year's influenza vaccine provides protection against the most prevalent strains for that year, but new or different strains may be more prevalent the following year.

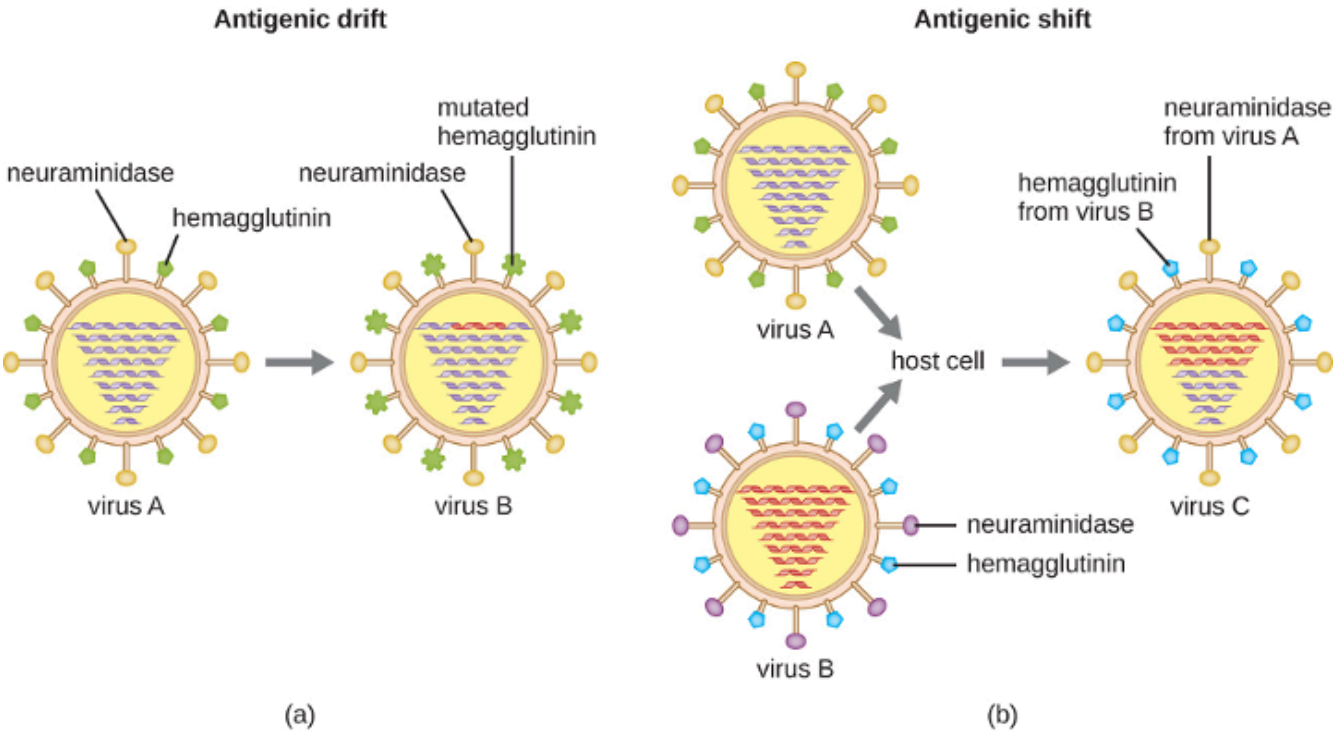


Figure 2.99: Antigenic drift and antigenic shift in influenza viruses. (a) In antigenic drift, mutations in the genes for the surface proteins neuraminidase and/or hemagglutinin result in small antigenic changes over time. (b) In antigenic shift, simultaneous infection of a cell with two different influenza viruses results in mixing of the genes. The resultant virus possesses a mixture of the proteins of the original viruses. Influenza pandemics can often be traced to antigenic shifts. *Figure description available at the end of the chapter.*

Figure Descriptions

Figure 2.1: A diagram of a rod-shaped prokaryotic cell. The thick outer layer is called the capsule, inside of that is a thinner cell wall and inside of that is an even thinner plasma membrane. Inside of the plasma membrane is a fluid called the cytoplasm, little dots called ribosomes, small spheres called inclusions, a small loop of DNA called a plasmid, and a large folded loop of DNA called the nucleoid. Long projections start at the plasma membrane and extend out of the capsule; these are called flagella (singular: flagellum). A shorter projection is labeled pilus. And many very short projections are labeled fimbriae.

Figure 2.2: A micrograph of an oval cell with a lighter region in the center of the cell. The lighter region takes up approximately one third of the volume of the cell and is labeled nucleoid.

Figure 2.3: There are three prokaryote-specific mechanisms leading to horizontal gene transfer in prokaryotes. a) In transformation, the cell takes up DNA directly from the environment. The DNA may remain separate as a plasmid or be incorporated into the host genome. b) In transduction, a bacteriophage injects DNA that is a hybrid of viral DNA and DNA from a previously infected bacterial cell. c) In conjugation, DNA is transferred between cells through a cytoplasmic bridge after a conjugation pilus draws the two cells close enough to form the bridge.

Figure 2.4: A drawing showing that the complete ribosome is made of a small subunit and a large subunit. The small subunit is about half the size of the large one. The small subunit has a size of 30S, the large subunit has a size of 50S and the complete ribosome (containing both the small and large subunit) has a size of 70S.

Figure 2.5: a) A micrograph showing gray spheres each containing 2-8 smaller white spheres. The gray spheres are approximately 600 nm in diameter B) A micrograph showing thin ribbons of approximately 100 μm length; each ribbon contains many dark spots in a line down the center of the ribbon. C) A micrograph showing a gray sphere of approximately 4 μm diameter with a cluster of smaller white spheres at the bottom of the larger sphere. D) A micrograph showing a larger sphere of approximately 10 μm diameter with many smaller spheres of approximately 1 μm diameter inside of the larger sphere. 3) a micrograph showing a long ribbon over 500 nm in length with small dots in the center. A closeup shows the dots to be a chain of spheres approximately 20 nm in diameter.

Figure 2.6: a) A diagram showing the process of sporulation. Step 1 – the DNA replicates. The image shows a rod shaped cell with 2 loops of DNA; one in the center and one towards the end of the cell. Step 2 – Membranes form around the DNA. The drawing shows lines encircling the loop of DNA at the end of the cell. Step 3 – Forespore forms additional membranes. The lines around the loop of DNA are thickened. Step 4 – Protective cortex forms around the spore. The lines around the loop of DNA are thickened even more. Step 5 – protein coat forma around the cortex. The lines around the loop of DNA are thickened even more and the outer cell lyses. Step 6 – the endospore is released. A small spherical structure with DNA inside of many thick layers is shown. B) A micrograph of an endospore shows a dark central core inside a lighter region; these are surrounded by thick layers on the outside. C) a micrograph showing red rods in chains; many of the rods have a green dot in their center.

Figure 2.7: The diagram of the gram-positive cell wall shows alternative NAG (N-acetylglucosamine) and NAM (N-acetylmuramic acid) in a chain; these are shown as alternative red and blue spheres. The chains or red and blue spheres are connected to other chains with smaller yellow spheres in a chain labeled pentapeptide and smaller green spheres labeled tetrapeptide. Each NAG in the chain is connected to the NAG in the chains next to it by both a tetrapeptide connected to a pentapeptide. The diagram of the gram-negative cell wall has the same NAG and NAM chains. But this time, they are linked with a direct line to the chains next to them.

Figure 2.8: The gram-positive bacterial cell wall diagram shows a plasma membrane on top of the cytoplasm. The cell wall is shown as a thick layer of peptidoglycans connected to the plasma membrane by teichoic acids. The gram-negative cell wall also has a plasma membrane on top of the cytoplasm. On top of the plasma membrane is a thin periplasmic space. On top of that is a thin peptidoglycan cell wall. On top of that is an outer membrane that contains murein lipoproteins that connect the outer membrane to the peptidoglycan cell wall. Lipid A, O antigens, and lipopolysaccharides sit on top of the outer membrane. Proins are tubes that connect the outside of the outer membrane with the region of the peptidoglycan cell wall.

Figure 2.9: A) A diagram of gram-positive acid-fast bacteria. The plasma membrane is shown on top of the cytoplasm and a thick layer of peptidoglycan makes up the cell wall outside the plasma membrane. Teichoic acids connect the peptidoglycans to the plasma membrane. On top of the peptidoglycans are mycolic acids, lipomannan, and arabinoglycans. B) A micrograph of red cells labeled acid-fast bacteria.

Figure 2.10: A diagram of the outer membrane of gram-negative bacteria. At the top of the diagram is a long chain of structures labeled O antigen. Below that is a shorter chain labeled core. Below that are two spheres labeled lipid A. Attached to the lipid A are squiggly tails labeled fatty acids.

Figure 2.11: a) A diagram showing the outer structures of bacterial cells. The thick outer layer is labeled capsule. Below that is a thinner cell wall and below that is an even thinner plasma membrane. B) A micrograph showing capsules as clearings outside of red stained cells; the background of the micrograph is a pale pink.

Figure 2.12: a) A diagram showing the outer structures of bacterial cells. The thick outer layer is labeled capsule. Below that is a thinner cell wall and below that is an even thinner plasma membrane. B) A micrograph showing capsules as clearings outside of red stained cells; the background of the micrograph is a pale pink.

Figure 2.13: A diagram showing the attachment point of flagella in gram-positive and gram-negative bacteria. The gram-positive diagram shows the filament on the outside of the cell wall; a bent elbow labeled hook connects the filament to the cell wall. A thin line between the filament and hook is labeled junction. The hook connects to a rod which connects to a basal body in the inner membrane. The basal body is a complex structure with a C-ring on the bottom. In the center of this ring is a sphere labeled type III secretion protein. Outside of this are oval structures labeled stator. On top of the secretion protein is a ring labeled MS-ring. The gram-negative flagellum is similar. There is a filament attached to a junction attached to hook. In the outer membrane is a ring labeled L-ring that connects to a rod in the periplasmic space. A P-ring sits in the cell wall. In the inner membrane is the C-ring, type III secretion system, MS ring and stator.

Figure 2.14: Diagrams of flagellar arrangements. Monotrichous bacteria have a single flagellum at one end. Amphitrichouls bacteria have one flagellum at each end. Lophotrichous bacteria have a tuft of flagella at one end. Peritrichous bacteria have flagella all the way around the outside of the cell.

Figure 2.15: A diagram showing the run and tumble of bacterial motion. The tumble is when a clockwise rotation of flagella cause the bacterial cell to tumble about. The run is when a counter-clockwise rotation of the flagella cause the bacterial cell to move in a linear direction.

Figure 2.16: A micrograph shows purple circles and pink rods.

Figure 2.17: A micrograph shows red chains of cells on a blue background.

Figure 2.18: Micrograph a shows clear circles on a black background. Micrograph b shows red rods with a clear halo on a dark background.

Figure 2.19: A micrograph shows chains of red rods. Each red rod contains a green oval. An arrow pointing to the green ovals states: green endospore inside bacterial cells.

Figure 2.20: Three red rectangles on a clear background are shown. Each rectangle has many thin, wiggly lines projecting from it.

Figure 2.21: A) A micrograph of two rod shaped cells attached at their ends. B) A diagram of binary fission. First a cell replicates its DNA and elongates. Then, as the cell continues to elongate, each loop of DNA travels to one end or the other. The cell then starts to constrict in the center. This results in two cells each containing a loop of DNA.

Figure 2.22: A graph with time on the X axis and logarithm of living bacterial cells on the Y axis. The line of the graph begins towards the bottom of the Y axis and is flat for a short time. This is labeled 1) lag phase: no increase in number of living bacterial cells. Next the line slopes upwards. This is labeled Log phase: exponential increase in number of living bacterial cells. Next the line flattens again. This is labeled 3) Stationary phase: plateau in number of living bacterial cells; rate of cell division and death roughly equal. Final the line slopes downwards. This is labeled 4) Death or decline phase: exponential decrease in number of living bacterial cells.

Figure 2.23: a) An arithmetic scale graph showing log phase growth. The X axis is time (hours), and the Y axis is number of cells. The line on this graph starts fairly flat with readings of (1,2) and (5,32) but quickly slopes up steeply with a final reading of (10, 1024). B) is a semilog scale graph of log phase growth. The X axis is time (hours) and the Y axis is log 10 number of cells. The Y axis more clearly shows the difference between 10 superscript 1 and 10 superscript 3. The line on this graph is a straight line diagonally across the graph with points of (1,2), (5,32) ,and (10,1024) – just like the first graph. The equation for both graphs is N=2 superscript n.

Figure 2.24: A micrograph of blue cells labeled tick hemolymph cells. Inside of these cells are small red cells labeled R. rickettsia.

Figure 2.25: A photograph showing round domes on a brown background.

Figure 2.26: a) A photograph of shining green dots on a black background. B) A photograph of a glowing squid.

Figure 2.27: A) A micrograph of rod shaped cells. B) A photograph of an air conditioner.

Figure 2.28: A micrograph of many rod shaped cells.

Figure 2.29: An image of a round structure labeled fruiting body. Smaller spheres on this structure are labeled sporangium containing myxospores.

Figure 2.30: A micrograph of a rod shaped cell with many long projections.

Figure 2.31: A diagram showing the life cycle of Chlamydia. An epithelial cell is infected by small spheres labeled elementary bodies. Within 12 hours, these form into reticulate bodies which divide to form inclusions within 24 hours. Within the inclusions more elementary bodies are formed and within 72 hours these are released when the cell ruptures.

Figure 2.32: A light micrograph of long spiral shaped cells. A TEM cross-section of these shows a circle outlined by a cell membrane. Inside the cell is the cytoplasm and a darker region labeled nucleoid. Outside of this is the periplasmic space and outside of that is an outer membrane. A bulge within the periplasmic space is labeled axial filament. Small dots within the axial filament are labeled endoflagella. An SEM from the original light micrograph shows what looks like a thin rope wound around a thicker rope. The thin rope is labeled axial filament.

Figure 2.33: A micrograph of many rod shaped cells.

Figure 2.34: a) A micrograph of an oval cell with long projections attached to a root-shaped structure labeled holdfast. The oval cell is approximately 500 nm in diameter. B) A micrograph of a similar looking cell with a long projection that is not attached to a holdfast.

Figure 2.35: A thick glass tube filled with purple regions labeled purple bacteria and green regions labeled green bacteria.

Figure 2.36: a) A micrograph of green spherical cells. B) A photo of a green lake.

Figure 2.37: a) A micrograph of branched cells. B) A micrograph of cells arranged in a V-shape – these are labeled palisades. C) A micrograph of corn-flake shaped cells with a nucleus. Smaller cells outside of these are identified with an arrow.

Figure 2.38: A micrograph of many rod shaped cells.

Figure 2.39: a) A micrograph of spherical cells in a chain. B) A photograph of colonies on agar. The agar is red, and there is a clearing around each colony.

Figure 2.40: a) A micrograph of rod shaped cells in a chain. B) A photograph of colonies on agar. The agar is red and the colonies are white and fluffy looking.

Figure 2.41: A micrograph of clusters of spherical cells.

Figure 2.42: A photograph of colonies on agar. The agar is blue and the colonies look like a pile of beads.

Figure 2.43: Two photos of a rash on skin, the first of a large raised grayish ring, and the second of a raised red ring.

Figure 2.44: a) A micrograph of kite-shaped parasites. B) A single triangular parasite with multiple flagella.

Figure 2.45: Eimera life cycle. Environment sporogony is the process of sporulation occurring outside the host; this requires several days and oxygen. A non-infectious unsporulated oocyst becomes an infectious sporulated oocyst. These enter the gut when swallowed and begin the process of asexual schizogony. Oocysts release sporocysts which release sporozoites. Sporozoites invade gut cells and form trophozoites. Trophozoites undergo schizogony (asexual reproduction) to form schizont which releases merozoites. Merozoites can reinfect and become trophozoites again or continue with sexual gametogony where the merozoites form male and female gametes. The gametes undergo syngamy (sexual reproduction) to form a developing oocyst, which matures into an unsporulated non-infectious oocyst. This brings us back to the beginning of the environment sporogony stage of the cycle.

Figure 2.46: a) Paramecium cell with short strands on the outside labeled cilia. An indent in the outer layer is labeled cytostome. A sphere inside the cell at the base of the cytostome is labeled cytoproct. A star-shaped structure inside the cell is labeled contractile vacuole. B) Amoeba cell with projections on the outside labeled pseudopods. The outer layer of the cell is labeled ectoplasm and the inner layer is labeled endoplasm. A sphere inside the cell is labeled contractile vacuole. C) Euglena with a single long flagellum on the outside. The outer layer of the cell is labeled ectoplasm; the inner layer is labeled endoplasm. A star-shaped structure is labeled contractile vacuole.

Figure 2.47: a) A micrograph showing a circular dome with long branches emanating outward. B) A photograph showing a yellow structure that looks like foam on a branch.

Figure 2.48: A mature fruiting body produces a tall stalk with a sphere that generates spores via meiosis. The mature fruiting body releases spores. The haploid spores germinate and give rise to amoeba which divide to form more individual cells. Two amoeba fuse to form a zygote. The zygote can grow and undergo meiosis and multiple rounds of mitosis. The new haploid amoeba is released. Fertilization produces amoebas that aggregate into a structure called a slug. The slug migrates at a rate of 2 mm per hour. The migration stops the aggregate forms a fruiting body at the end of a stalk. This brings us back to the fruiting body in the life cycle.

Figure 2.49: A mature plasmodium (multinucleated free-flowing mass of protoplasm) can produce sclerotium (small cells) in a dry habitat. The mature plasmodium also produces diploid sporangia which produces haploid spores via meiosis. The mature sporangium releases mature spores which germinate. Germination gives rise to cells that can convert between ameboid and flagellated forms. Plasmogamy is the fusion of cytoplasm of two cells. Karyogamy is the fusion of nuclei and leads to the production of a diploid zygote. The zygote divides to form a multi-nucleated feeding plasmodium. This takes us back to the beginning of plasmodium stage of the life cycle.

Figure 2.50: a) A diagram of an apicomplexan protist. The cell is a long oval with an apical complex at the apical end. B) A micrograph of the protist showing a long oval.

Figure 2.51: A micrograph of an oval cell with many short projections.

Figure 2.52: Paramecium cell with short strands on the outside labeled cilia. An indent in the outer layer is labeled cytostome. The outside edge of the cytostome is an indent in the cell labeled oral groove. A sphere inside the cell at the base of the cytostome is labeled food vacuole, another nearby sphere is labeled cytoproct. A smaller opening in the cell is labeled anal pore. A star-shaped structure inside the cell is labeled contractile vacuole. A large oval is labeled macronucleus, and a smaller oval is labeled micronucleus.

Figure 2.53: A micrograph of long trumpet shaped cells. The wide part of the cell has an oval structure labeled cytostome and small projections labeled cilia.

Figure 2.54: A photograph of an insect covered in white fuzz labeled water mold.

Figure 2.55: An oval cell with a long flagellum at one end near the photoreceptor (paraflagellar body). A large oval inside the cell is labeled nucleus and contains a smaller oval labeled nucleolus. Green structures are labeled chloroplasts. A red circle is labeled stigma (eyespot). Another sphere is labeled contractile vacuole and a large sphere is labeled pellicle bands. Gray structures are labeled polysaccharides stored by photosynthesis.

Figure 2.56: The life cycle of Trypanosoma brucei takes place in both tsetse fly and humans. When the tsetse fly takes a blood meal it injects T. brucei into the bloodstream of a human. There the T. brucei multiplies by binary fission in blood, lymph, and spinal fluid. When another tsetse fly takes a blood meal it ingests T. brucei which multiplies by binary fission in the midgut of the fly. The T. brucei then transforms into an infectious stage which enters the salivary glands and multiplies. This can then be spread to another human.

Figure 2.57: A micrograph of a small clear tapered worm.

Figure 2.58: a) Class Turbellaria – a photograph of a flat oval-shaped worm labeled Pseudobiceros bedfordi. B) Class Monogenea – a micrograph of a rectangular cell with a bulb at one end. Labeled Dactylogyrus sp. C) Class Trematoda – A photograph of a long oval organism labeled Fascioloides magna and two smaller oval organisms labeled Fasciola hepatica. D) Class Cestoda – A photograph of a very long tapeworm labeled Taenia saginata.

Figure 2.59: a) A micrograph of Opisthorchis viverrini; an oval cell with a projection at one end. B) A micrograph of one end of Taenia solium showing a whorl of small projections above an area labeled scolex.

Figure 2.60: Schistosoma mansoni, japonicum, and haematobium are found in feces; S. japonicum and S. haematobium are also found in urine. These can be diagnosed in the water and produce eggs which hatch releasing miracidia. The miracidia penetrate snail tissues and produce sporocysts in the snail (successive generations). The Cercariae released by snail into the water are free flowing and are the infective stage which can penetrate skin. S. mansoni travels to the large intestines, S. japonicum travels to the small intestines, and S. haematobium travels to the rectum. The cercariae lose their tails during penetration and become schistosomula. These enter circulation and migrate to portal blood in liver and mature into adults. The paired adult worms migrate to the mesenteric venules of the bowels/rectum (laying eggs that circulate to the liver and are shed in stools) – for S. mansoni and S. Japonicum. S. haematobium migrates to the venous plexus of the bladder.

Figure 2.61: Eggs or gravid proglottids from an infected individual are passed into the environment; this is the diagnostic stage. Cattle (T. saginata) and pigs (T. solium) become infected by ingesting vegetation contaminated by eggs or gravid proglottids. Oncospheres hatch, penetrating intestinal wall and circulate to musculature. The oncospheres develop into cysticerci in muscles and become infective. Humans are infected by ingesting raw or undercooked infected meat. The scolex attaches to intestine and adults are found in the small intestine.

Figure 2.62: Molds can have septate hyphae – long strands with cell walls separating the nuclei. Or they can have coenocytic (nonseptate) hyphae – long strands with no cell wall separating the nuclei. Or they can have pseudohyphae which look like chains of cells with small clusters at intervals.

Figure 2.63: Drawing of bats in an attic. Fungal body is shown in the guano. A micrograph of the fungus shows hyphae (long strands) with spheres labeled conidia. The life cycle shows a person inhaling spores which then travel to the lungs and divide into a yeast form. They then travel to the lymph and blood.

Figure 2.64: Zygomycete life cycle. The mycelia can undergo asexual reproduction by forming spores via mitosis. The spores then form mycelia by germination. The haploid spores can also undergo sexual reproduction. The first step is germination when mycelia form. If the two mating types (+ and -) are in close proximity, extensions called gametangia form between them. Next is plasmogamy. This is the fusion between the + and – mating types, resulting in a zygosporangium with multiple haploid nuclei. The zygosporangium forms a thick, protective coat. Next the nuclei fuse to form a zygote with multiple diploid nuclei in karyogamy. This forms a diploid zygote. Next is mitosis and germination where the sporangium grows on a short stalk and the haploid spores are formed inside. The spores are released in germination and we are back to the spore stage of the life cycle.

Figure 2.65: a) A micrograph of long strands labeled hyphae and a sphere (labeled sporangium) on the end of one of the long strands. B) A photograph of bread mold. The white fuzz has black dots labeled sporangia.

Figure 2.66: a) a micrograph of a large oval (10 μm) labeled ascus and smaller ovals (5 μm) labeled ascospores. B) a micrograph of a long stalk with strands of spheres emanating from a sphere on the tip. The spheres are about 2 μm in diameter. C) A long strand with clusters of spheres. A small dot in each sphere is labeled nucleus.

Figure 2.67: A micrograph showing a thick tube with 8 ovals lined up within the tube.

Figure 2.68: Ascomycete life cycle. Mycelia produce conidiophores which use mitosis to asexually produce spores. These spores then germinate into new mycelia. Sexual reproduction begins one hyphae produces an ascogonium and another produces an antheridium. In plasmogamy the ascogonium and antheridium fuse. Mitosis and cell division result in the formation of many dikaryotic hyphae, which form a fruiting body called the ascocarp. Asci form at the tips of these hyphae. In karyogamy the nuclein in the asci fuse to form a diploid zygote. Then meiosis produces four haploid nuclei in the ascus. Then mitosis and cell division results in eight haploid ascospores in the ascus. These ascospores then disperse and germinate into new mycelia.

Figure 2.69: Basidiomycete life cycle. Haploid basidiospores germinate to form mycelia. There are two mating types (+ and -_). In plasmogamy, fusion between + and – mating types results in formation of a dikaryotic mycelium. Under the right environmental conditions, a basidiocarp forms via mitosis. Gills of the basidiocarp contain cells called basidia. A photo of a mushroom labels the mushroom as basidiocarp and basidia within the gills. Basidia form diploid nuclei via karyogamy; this produces a diploid zygote. Four haploid nuclei are formed in the basidium via meiosis. Cell division produces four haploid basidiospores. These spores then disperse and germinate into new mycelia.

Figure 2.70: Figure a is an electron micrograph showing a virus on the surface of a bacterial cell. The virus has a large head region, a thick neck and thin spider-like legs attached to the bacterium. Figure b is a drawing that labels the outside of the head as the capsid with the viral genome inside. The neck as the sheath and the legs as tail fibers.

Figure 2.71: A scale is showing sizes of various entities. The largest is a frog egg and 1 mm. Human egg cells and pollen are approximately 400 μm. Typical plant and animal cells range from 10 to 100 μm. Red blood cells are under 10 μm. Mitochondria and bacteria are approximately 1 μm. Smallpox is approximately 500 nm. Flu virus is approximately 100 nm. Polio virus is approximately 50 nm. Proteins range from 5 – 10 nm. Lipids range from 1 – 5 nm. Atoms are approximately 0.1 nm.

Figure 2.72: Part A shows a micrograph of atadenovirus, which looks like a wispy sphere that has a larger, flatter structure attached to the bottom. To the right of that is an illustration of the atadenovirus that labels capsomeres, capsids, DNA, and spikes made of glycoproteins. Part B shows the enveloped human immunodeficiency virus in black and white. To the right is an illustration that labels the matrix protein, viral envelope, spikes made of glycoproteins, reverse transcriptase, capsids, and RNA.

Figure 2.73: Figure a is a helical virus which has a long linear structure. The outer proteins are small spheres arranged into a long, hollow tube. Inside the tube is the genetic material. Tobacco mosaic virus is an example of a helical virus. Figure b is an Icosahedral viruses have a polyhedron structure. The example shown is human rhinovirus which has a pentagon structure. Complex viruses have a more complex structure. The example is variola which has an ovoid structure.

Figure 2.74: This figure outlines the stages of the lytic cycle. Step 1 is attachment when the phage attaches to the surface of the host. The bacteriophage is shown sitting on the surface of the bacterial host cell. Step 2 is penetration when the viral DNA enters the host cell. The image shows DNA from within the virus being injected into the host DNA. Step 3 is biosynthesis when the phage DNA replicates and the phage proteins are made. The image shows various pieces of virus being built within the cell. Step 4 is maturation when the new phage particles are assembled. This shows the viral components being put together in the cell. The fifth step is lysis when the cell lyses and the newly made phages are released. This shows the cell bursting and built viruses being released.

Figure 2.75: The steps of the lytic and lysogenic cycles. First the phage infects a cell; this shows the virus sitting on the outside of a cell and injecting DNA into the cell. In the next step the phage DNA becomes incorporated into the host genome. In the next step, the cell divides and prophage DNA is passed to the daughter cells. The image shows the cell dividing and the viral DNA within the host genome also being passed to the daughter cell. The next step shows the viral DNA jumping out of the host genome. Under stressful conditions, the prophage DNA is excised from the bacterial chromosomes and enters the lytic cycle. Next, the phage DNA replicates and phage proteins are made. This shows viral pieces being made within the cell. The next step is when the new phage particles are assembled. This shows the virus being build. The final step is when the cell lyses and releases the newly made phages.

Figure 2.76: The steps of specialized transduction. Step 1 is viral attachment and penetration. This is when the phage infects a cell. This shows the virus sitting on the outside of a cell and injecting DNA into the cell. Step 2 is integration when the phage DNA becomes incorporated into the host genome. Step 3 is excision when the phage is excised from the bacterial chromosomes along with a short piece of bacterial DNA. The DNA is then packaged into newly formed capsids. When the virus particles are assembled the DNA contains both viral and host segments. Step 4 is infection when the phage contains both viral and bacterial DNA infects a new host cell. Step 5 is recombination when the phage DNA along with the attached bacterial DNA are incorporated into a new cell. The image shows a new bacterial cell with virus DNA as well as other bacterial DNA in its genome.

Figure 2.77: Steps of influenza infection. Step 1 is attachment when the influenza virus becomes attached to a target epithelial cell. This image shows a spherical virus binding to the surface of a host cell. Step 2 is penetration when the cell engulfs the virus by endocytosis; this shows the virus within a vacuole. Step 3 is uncoating when the viral contents are released; the image shows the virus being released from the vacuole. Step 4 is biosynthesis when the viral RNA enters the nucleus where it is replicated by RNA polymerase. Step 5 is assembly when the new phage particles are assembled. Step 6: release when new viral particles are made and released into the extracellular fluid. The cell, which is not killed in the process continues to make new viruses.

Figure 2.78: Viruses with –ssRNA (negative single-stranded RNA) use RdRP (viral RNA-dependent RNA polymerase) to make +ssRNA (positive single-stranded RNA). RdRP can also be used to convert +ssRNA to –ssRNA. +ssRNA uses host ribosomes to make viral proteins.

Figure 2.79: The HIV viral cycle. Step 1: the HIV fuses to the host-cell surface. Specifically, the gp120 proteins on the surface of the virus binds to the CD4. This then binds to a smaller coreceptor (CCR5 or CXCR4). Step 2: HIV RNA, reverse transcriptase, integrase, and other viral proteins enter the host cell. The virus is brought into the host cell and uncoated; both viral RNA and reverse transcriptase are loosed into the cell. Step 3: Viral DNA is formed by reverse transcription. Step 4: Viral DNA is transported across the nucleus and integrates into the host DNA. Integrase found on the viral DNA. The viral DNA in the host DNA is called provirus. Step 5: New viral RNA is used as genomic RNA and to make viral proteins. New viral RNA strands are made and leave the nucleus. Step 6: New viral RNA and proteins move to the cell surface and a new immature HIV forms. The viral is assembled in an out bulging of the host cell. Step 7: The virus matures when protease releases the proteins that form the mature HIV. Mature virion is released.

Figure 2.80: Figure A is an electron micrograph that shows a sphere within a larger blob-shaped structure. Figure B shows raised red dots on a person's face.

Figure 2.81: The Y axis of the graph is "logarithm of number of infectious virions"; the X axis of the graph is "hours". The beginning of the line has a downward slope and is labeled "1: Inoculation: inoculum of virus binds to cells". Next is a flat region of the line labeled "2: Eclipse: virions penetrate the cell". Next is an upward slope labeled "3: Burst: host cells release many viral particles". Next is another flat region labeled "4: Burst size: number of virions released per bacterium".

Figure 2.82: This chart has three columns labeled components, interactions, and microtiter results. In row A the components are the red blood cells which do not interact with anything and show no reaction in a microtiter result. The lack of reaction is seen as a small red dot in the center of the well. In row B the components are viruses and red blood cells. The viruses and red blood cells clump together and this is seen in a microtiter result as redness throughout the well. This is called hemagglutination. In row C, the components are viruses, red blood cells and antibodies. The viruses and antibodies clump together but the red blood cells do not clump with anything. This is again seen as no reaction; this is called hemagglutination inhibition.

Figure 2.83: The explanation of EIA is separated to show what occurs in a positive sample and what occurs in a negative sample. First patient sample is applied to a membrane filter. If the sample contains viruses they are trapped by the filter. Next, antibody with enzyme conjugate is added. Antibody will attach to antigen if present. Next is a wash step. If the virus is present the enzyme binds to the virus, otherwise the enzyme washes away. Finally substrate is added. If the antibody is present (because it is bound to the virus) the attached enzyme causes a color change. If no enzyme linked antibody is present, no color change occurs.

Figure 2.84: Figure A shows the process of how normal prion protein is converted to disease causing forms. Endogenous PrPc interact with PrpSC. This converts PRPC into PRPSC. The PRPSC accumulate. Figure B is a micrograph that shows holes in brain tissue.

Figure 2.85: The CJD brain has larger spaces as seen by more black regions in the image of the whole brain. The micrograph shows holes in the brain tissue.

Figure 2.86: A diagram Koch's postulates. 1 – The suspected causative agent must be absent from all healthy organisms but present in all diseased organisms. This is demonstrated by looking at slides under a microscope from a sick mouse and seeing the suspected agent. A slide from a healthy mouse only shows healthy red blood cells. 2 – The causative agent must be isolated from the diseased organism and grown in pure culture. This is demonstrated by showing growth on a petri plate from the sick mouse and no growth from the healthy mouse. 3 – The cultured agent must cause the same disease when inoculated into a healthy, susceptible organism. This is demonstrated by injecting a healthy mouse with the cultured agent and having that mouse get sick. 4 – The same causative agent must then be reisolated from the inoculated diseased organism. This is demonstrated by a Petri plate from this last mouse showing the growth of the causative agent.

Figure 2.87: A graph with "number of pathogenic agents (cells or virions)" on the X axis and Percent mortality in experimental group on the Y axis. The graph begins at 0,0 and increases until there is nearly 100% death at 10 to the 5. The line then plateaus at 100%. A 50% death rate occurs at 10 to the 4. This is the LD 50.

Figure 2.88: Portals of entry: eye (conjunctiva), nose, mouth, ear, needle, broken skin, insect bite, urethra, vagina, anus, placenta (portal of entry for fetus).

Figure 2.89: Micrograph of round cells attached to a surface by long strands.

Figure 2.90: Diagram of H. pylori invading the lining of the stomach. In the first image the H. pylori (an oval cell with 3 flagella is not able to penetrate the gastric mucin gel on top of the epithelial cells. Contact with stomach acid keeps the mucin lining the epithelial cell layer in a spongy gel-like state. This consistency is impermeable to the bacterium H. pylori. The second image shows the bacterium entering the lining. The bacterium releases urease, which neutralizes the stomach acid. This causes the mucin to liquefy and the bacterium can swim right through it.

Figure 2.91: Portals of exit: eye (tears), needle, mammary glands (milk, secretions), placenta (transmission to fetus), vagina (secretions, blood), urethra (urine), broken skin, broken skin (blood), skin (flakes), nose (secretions), mouth (saliva, sputum), ear (earwax), urethra (urine, semen, secretions), anus (feces).

Figure 2.92: A picture of a person with a swollen right hand.

Figure 2.93: a) A diagram of epithelial cells that are connected along their membranes. Hyaluronidases enter at these connection points. B) after the hyaluronidases break down the connections between the cells, bacteria can flow through the openings.

Figure 2.94: A long chain of O antigens is drawn as various geometric shapes in a long row. Next is a core; a shorter region of similar shapes. Next is 2 circles labeled lipid A. Each of these has 2 or 3 long wavy lines projecting from them.

Figure 2.95: Diagram of how the A-B toxin works. The B subunit binds to a cellular receptor on the cell membrane. The A subunit is bound to the B subunit at this point. The cell engulfs the toxins into a vacuole. Inside the vacuole, which is acidic, the a subunit dissociates and escapes into the cytoplasm.

Figure 2.96: A diagram of the mechanism of diphtheria toxin. On the outside is a membrane with the B subunit attached. Inside is the A subunit binding with NAD. This block EF-2 by binding ADP-ribose. The diagram also shows mRNA bound to a ribosome and protein being made. The A subunit causes elongation of the protein to stop.

Figure 2.97: Botulinum toxin causes flaccid paralysis and stops muscle contraction. The diagram shows that the normal mechanism has acetylcholine released at the axon terminal. The acetylcholine then binds to receptors on the membrane of a muscle cell. The abnormal mechanism – botulinum toxin blocks the release of acetylcholine stopping muscle contraction. Tetanus toxin causes spastic paralysis; uncontrollable muscle contraction. The normal mechanism shows glycine and GABA released from one axon terminal and binding to receptors on another axon terminal. This causes acetylcholine to remain in the vesicles and not bind to the receptors on the muscle cell. In the abnormal mechanism tetanus toxin prevents the release of glycine and GABA. Therefore acetylcholine is released and binds to receptors on the muscle. This prevents the relaxation of muscles.

Figure 2.98: A) a micrograph showing nonencapsulated cells as blue ovals on a light background. Encapsulated cells have a thick clear ring around the blue cells. B) Antibodies on phagocytic cells bind to antigens on the bacterial cell. Capsules on the bacterial cell cover the antigen and prevent the antibody from binding to the antigen. C) A bacterial cell is releasing small dots labeled proteases that are breaking down an antibody.

Figure 2.99: A) antigenic drift results from genetic mutations. Virus A is shown with different shaped pieces on the outside labeled neuraminidase and hemagglutinin. The mutated hemagglutinin has a different shape. B) Antigenic shift results from genetic reassortment. Virus A has green hemagglutinin and orange neuraminidase on the outside. Virus B has purple neuraminidase and blue hemagglutinin. These both enter the same host cell. Virus C is then produced which has the neuraminidase from virus A and the hemagglutinin from virus B.

Figure References

Figure 2.1: A typical prokaryotic cell contains a cell membrane, chromosomal DNA that is concentrated in a nucleoid, ribosomes, and a cell wall. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Table 2.1: Common prokaryotic cell shapes. Illustrations: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology. Coccus micrograph: modification of work by Janice Haney Carr, Centers for Disease Control and Prevention. Public Domain. Bacillus micrograph: modification of work by National Institute of Allery and Infectious Diseases. Public Domain. https://commons.wikimedia.org/wiki/File:E._coli_Bacteria_(7316101966).jpg. Vibrio micrograph: modification of work by Janice Haney Carr, Centers for Disease Control and Prevention. Public Domain. https://phil.cdc.gov/Details.aspx?pid=6937. Coccobacillus micrograph: modification of work by Janice Carr, Centers for Disease Control and Prevention. Public Domain. Spirillum micrograph: modification of work by Wolframm Adlassnig. Public Domain. https://commons.wikimedia.org/wiki/File:Spirillen.jpg Spirochete micrograph: modification of work by Centers for Disease Control and Prevention. Public Domain.

Table 2.2: Common prokaryotic cell arrangements. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.2: The nucleoid region (the area enclosed by the green dashed line) is a condensed area of DNA found within prokaryotic cells. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.3: There are three prokaryote-specific mechanisms leading to horizontal gene transfer in prokaryotes. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.4: Prokaryotic ribosomes (70S) are composed of two subunits: the 30S (small subunit) and the 50S (large subunit), each of which are composed of protein and rRNA components. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.5: Prokaryotic cells may have various types of inclusions. Image a. Figure 5A in Moore, R.A., Tuanyok, A. & Woods, D.E. Survival of Burkholderia pseudomallei in Water. BMC Res Notes 1, 11 (2008). https://doi.org/10.1186/1756-0500-1-11. CC BY 2.0, Images b, c, d: (c) American Society of Microbiology. Redistribution authorized with attribution. Image e: Modified from Figure 3 in Blondeau, M., Guyodo, Y., Guyot, F. et al. Magnetic-field induced rotation of magnetosome chains in silicified magnetotactic bacteria. Sci Rep 8, 7699 (2018). https://doi.org/10.1038/s41598-018-25972-x. CC BY 4.0. Images b, c, d: (c) American Society of Microbiology. Redistribution authorized with attribution.

Figure 2.6: Sporulation begins following asymmetric cell division. Image a: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology. Image b: (c) Jonathan Eisen. CC BY 1.0 Generic. https://commons.wikimedia.org/wiki/File:Electron_micrograph_of_endospore_of_the_bacterium_Carboxydothermus_hydrogenoformans.png. Image c: Centers for Disease Control and Prevention. Public Domain. https://phil.cdc.gov/Details.aspx?pid=1894

Figure 2.7: Peptidoglycan is composed of polymers of alternating NAM and NAG subunits, which are cross-linked by peptide bridges linking NAM subunits from various glycan chains. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.8: Bacteria contain two common cell wall structural types. (c) Rice University. CC BY 4.0. Modification of work (c) Franciscosp2 "Bacteria cell wall2". CC BY 3.0 Unported. https://commons.wikimedia.org/wiki/File:Bacteria_cell_wall2.svg

Figure 2.9: Some gram-positive bacteria, including members of the Mycobacteriaceae, produce waxy mycolic acids found exterior to their structurally-distinct peptidoglycan. Left: (c) Rice University CC BY 4.0. Modification of work by (c) Franciscosp2 "Bacteria cell wall2". CC BY 3.0 Unported. https://commons.wikimedia.org/wiki/File:Bacteria_cell_wall2.svg. Right: Modification of work by Centers for Disease Control and Prevention/Dr George P. Kobica. Public Domain. https://commons.wikimedia.org/wiki/File:Mycobacterium_tuberculosis_Ziehl-Neelsen_stain_02.jpg

Figure 2.10: The outer membrane of a gram-negative bacterial cell contains lipopolysaccharide (LPS), a toxin composed of Lipid A embedded in the outer membrane, a core polysaccharide, and the O-side chain. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.11: Capsules are a type of glycocalyx composed of an organized layer of polysaccharides. Left: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology. Right: (c) American Society of Microbiology. Redistribution authorized with attribution.

Figure 2.12: Bacteria may produce two different types of protein appendages that aid in surface attachment. (c) American Society of Microbiology. Redistribution authorized with attribution.

Figure 2.13: The basic structure of a bacterial flagellum consists of a basal body, hook, and filament. Modification of work by LadyofHats/Mariana Ruiz Villareal. Public Domain. https://commons.wikimedia.org/wiki/File:Flagellum_base_diagram-en.svg

Figure 2.14: Flagellated bacteria may exhibit multiple arrangements of their flagella. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.15: Bacteria achieve directional movement by changing the rotation of their flagella. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Table 2.5: Gram staining process. Gram-staining is a differential staining technique that uses a primary stain and a secondary counterstain to distinguish between gram-positive and gram-negative bacteria. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.16: In this specimen, the gram-positive bacterium Staphylococcus aureus retains crystal violet dye even after the decolorizing agent is added. Modification of work (c) Nina Parker. CC BY 4.0.

Figure 2.17: Ziehl-Neelsen staining has rendered these Mycobacterium tuberculosis cells red and the surrounding growth indicator methylene blue. (c) American Society of Microbiology. Redistribution authorized with attribution.

Figure 2.18: India-ink was used to stain the background around these cells of the yeast Cryptococcus neoformans. (c) American Society of Microbiology. Redistribution authorized with attribution.

Figure 2.19: A stained preparation of Bacillus subtilis showing endospores as green and the vegetative cells as pink. (c) American Society of Microbiology. Redistribution authorized with attribution.

Figure 2.20: A flagella stain of Bacillus cereus, a common cause of foodborne illness, reveals that the cells have numerous flagella used for locomotion. Modification of work by Centers for Disease Control and Prevention. Public Domain.

Table 2.6: Simple stains. Top: Modification of work by Centers for Disease Control and Prevention. Public Domain. Middle: Modification of Figure 1C in Danovaro, R., Dell'Anno, A., Pusceddu, A. et al. The first metazoa living in permanently anoxic conditions. BMC Biol 8, 30 (2010). https://doi.org/10.1186/1741-7007-8-30. CC BY 2.0. Bottom: (c) modification of work by Anh-Hue Tu. CC BY 4.0.

Figure 2.21: The electron micrograph depicts two cells of Salmonella typhimurium after a binary fission event. Left: modification of work by Centers for Disease Control and Prevention. Public Domain. Right: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.22: The growth curve of a bacterial culture is represented by the logarithm of the number of live cells plotted as a function of time. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.23: Both graphs illustrate population growth during the log phase for a bacterial sample with an initial population of one cell and a doubling time of 1 hour. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.24: Rickettsias require special staining methods to see them under a microscope. modification of work by Centers for Disease Control and Prevention. Public Domain.

Figure 2.25: Neisseria meningitidis growing in colonies on a chocolate agar plate. Centers for Disease Control and Prevention. Public Domain.

Figure 2.26: Aliivibrio fischeri is a bioluminescent bacterium. Left: (c) American Society of Microbiology. Redistribution authorized with attribution. Right: modification of work (c) Margaret McFall-Ngai. CC BY 4.0. https://commons.wikimedia.org/wiki/File:Hawaiian_Bobtail_squid.tiff

Figure 2.27: Legionella pneumophila, the causative agent of Legionnaires disease, thrives in warm water. Left: Modification of work by Centers for Disease Control and Prevention. Public Domain. Right: Public Domain.

Figure 2.28: Salmonella typhi is the causative agent of typhoid fever. Centers for Disease Control and Prevention. Public Domain.

Figure 2.29: Myxobacteria form fruiting bodies. Modification of work by Michiel Vos in (2005) Antisocial Behavior in Cooperative Bacteria (or, Why Can't Bacteria Just Get Along?). PLoS Biol 3(11): e398. https://doi.org/10.1371/journal.pbio.0030398. https://commons.wikimedia.org/wiki/File:Myxococcus_xanthus.png. CC BY 2.5.

Figure 2.30: Helicobacter pylori can cause chronic gastritis, which can lead to ulcers and stomach cancer. By Yutaka Tsutsumi. Permission granted. https://commons.wikimedia.org/wiki/File:EMpylori.jpg

Figure 2.31: Chlamydia begins infection of a host when the metabolically inactive elementary bodies enter an epithelial cell. modification of work by Centers for Disease Control and Prevention. Public Domain.

Figure 2.32: Spirochetes are typically observed using darkfield microscopy (left). Left: modification of work by Centers for Disease Control and Prevention. Public Domain. Middle: (c) modification of Figure 2a and e by Guyard C, Raffel SJ, Schrumpf ME, Dahlstrom E, Sturdevant D, Ricklefs SM, et al. (2013) Periplasmic Flagellar Export Apparatus Protein, FliH, Is Involved in Post-Transcriptional Regulation of FlaB, Motility and Virulence of the Relapsing Fever Spirochete Borrelia hermsii. PLoS ONE 8(8): e72550. https://doi.org/10.1371/journal.pone.0072550. CC0/Public Domain. Right: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology

Figure 2.33: Bacteroides comprise up to 30% of the normal microbiota in the human gut. NOAA. Public domain.

Figure 2.34: (a) Sessile Planctomycetes have a holdfast that allows them to adhere to surfaces in aquatic environments. (c) American Society of Microbiology. Redistribution authorized with attribution.

Figure 2.35: Purple and green sulfur bacteria use bacteriochlorophylls to perform photosynthesis. Modification of work by kOchstudiO. Public Domain. https://commons.wikimedia.org/wiki/File:Green_d_winogradsky.jpg

Figure 2.36: (a) Microcystis aeruginosa is a type of cyanobacteria commonly found in freshwater environments. Credit a: modification of work by Dr. Barry H. Rosen/U.S. Geological Survey; credit b: modification of work by NOAA. Public domain.

Figure 2.37: Actinomyces israelii. Credit a: Modification of work by Graham Beards. CC BY 3.0 https://commons.wikimedia.org/wiki/File:Actinomyces_israelii.jpg. Credit b: modification of work by Centers for Disease Control and Prevention. Public Domain https://commons.wikimedia.org/wiki/File:Corynebacterium_diphtheriae_Gram_stain.jpg. Credit c: modification of Figure 3 in Mwakigonja, A.R., Torres, L.M.M., Mwakyoma, H.A. et al. Cervical cytological changes in HIV-infected patients attending care and treatment clinic at Muhimbili National Hospital, Dar es Salaam, Tanzania. Infect Agents Cancer 7, 3 (2012). https://doi.org/10.1186/1750-9378-7-3. CC BY 2.0.

Figure 2.38: Clostridium difficile, a gram-positive, rod-shaped bacterium, causes severe colitis and diarrhea, often after the normal gut microbiota is eradicated by antibiotics. modification of work by Centers for Disease Control and Prevention. Public Domain.

Figure 2.39: A gram-stained specimen of Streptococcus pyogenes shows the chains of cocci characteristic of this organism's morphology. (c) American Society of Microbiology. Redistribution authorized with attribution.

Figure 2.40: In this gram-stained specimen, the violet rod-shaped cells forming chains are the gram-positive bacteria Bacillus cereus. Credit a: modification of work by (c) Bibliomaniac 15. CC BY 3.0 Unported. https://commons.wikimedia.org/wiki/File:Bacillus_cereus_and_Escherichia_coli.jpg; credit b: modification of work by Centers for Disease Control and Prevention. Public Domain. https://commons.wikimedia.org/wiki/File:Bacillus_cereus_01.png

Figure 2.41: This SEM of Staphylococcus aureus illustrates the typical grape-like clustering of cells. modification of work by Centers for Disease Control and Prevention. Public Domain.

Figure 2.42: M. tuberculosis grows on Löwenstein-Jensen (LJ) agar in distinct colonies. Centers for Disease Control and Prevention. Public Domain.

Figure 2.43: Ringworm presents as a raised ring, which is gray or brown on brown or black skin (a), and red on lighter skin (b). Centers for Disease Control and Prevention. Public Domain.

Figure 2.44 A scanning electron micrograph shows many Giardia parasites in the trophozoite, or feeding stage, in a gerbil intestine. modification of work by Centers for Disease Control and Prevention. Public Domain.

Figure 2.45: In the sexual/asexual life cycle of Eimeria, oocysts (inset) are shed in feces and may cause disease when ingested by a new host. "life cycle," "micrograph": modification of work by USDA. Public domain.

Figure 2.46: Paramecium spp. have hair-like appendages called cilia for locomotion. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.47: The cellular slime mold Dictyostelium discoideum. By Bruno in Columbus. Public Domain. https://commons.wikimedia.org/wiki/File:Dictyostelium_Aggregation.JPG. (b) Fuligo septica. By Siga. Public Domain. https://commons.wikimedia.org/wiki/File:Fuligo_septica_bl1.JPG

Figure 2.48: The life cycle of the cellular slime mold Dictyostelium discoideum. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology. Modification of photo (c) thatredhead4. CC BY 2.0. https://flic.kr/p/6JdBCD

Figure 2.49: Plasmodial slime molds exist as large multinucleate amoeboid cells that form reproductive stalks to produce spores that divide into gametes. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.50: Apicomplexans are parasitic protists. Left: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology. Right: Modification of work by (c) Ute Frevert. CC BY 42.5 Generic. https://commons.wikimedia.org/wiki/File:Malaria.jpg

Figure 2.51: This specimen of the ciliate Balantidium coli is a trophozoite form isolated from the gut of a primate. modification of Figure 2g in Kouassi RY, McGraw SW, Yao PK, Abou-Bacar A, Brunet J, Pesson B, Bonfoh B, N'goran EK, Candolfi E. Diversity and prevalence of gastrointestinal parasites in seven non-human primates of the Taï National Park, Côte d'Ivoire. Parasite. 2015;22:1. doi: 10.1051/parasite/2015001. CC BY 4.0. https://commons.wikimedia.org/wiki/File:Balantidium_coli_trophozoite.jpg

Figure 2.52: Paramecium has a primitive mouth (called an oral groove) to ingest food, and an anal pore to excrete it. Left: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology. Right: Luis Fernández García. CC BY SA 3.0 Unported. https://commons.wikimedia.org/wiki/File:Paramecium.jpg

Figure 2.53: This differential interference contrast micrograph (magnification: ×65) of Stentor roeselie shows cilia present on the margins of the structure surrounding the cytostome; the cilia move food particles. Modification of work (c) picturepest. https://flic.kr/p/oRgy8Q. CC BY 2.0.

Figure 2.54: A saprobic oomycete, or water mold, engulfs a dead insect. Modification of work (c) Thomas Bresson. https://commons.wikimedia.org/wiki/File:Dead_insect_(6).jpg. CC BY 2.0.

Figure 2.55: This illustration of a Euglena shows the characteristic structures, such as the stigma and flagellum. Left: Modification of work (c) Claudio Miklos. Public Domain. https://commons.wikimedia.org/wiki/File:Euglena_diagram.jpg. Right: Modification of work by David Shykind. Public Domain. https://commons.wikimedia.org/wiki/File:Euglena_pellicle_2.jpg

Figure 2.56: Trypanosoma brucei, the causative agent of African trypanosomiasis, spends part of its life cycle in the tsetse fly and part in humans. modification of work by Centers for Disease Control and Prevention; credit "photo": DPDx/Centers for Disease Control and Prevention. Public Domain.

Figure 2.57: A micrograph of the nematode Enterobius vermicularis, also known as the pinworm. modification of work by Centers for Disease Control and Prevention. Public Domain.

Figure 2.58: Phylum Platyhelminthes is divided into four classes. Images a, b, d. Public Domain. Image c: Modification of work (c) Flukeman CC BY 2.5 Generic. https://commons.wikimedia.org/wiki/File:F.magna_1_a_F.hepatica_2.jpg

Figure 2.59: The oral sucker is visible on the anterior end of this liver fluke, Opisthorchis viverrini. Left (c) modification of Figure 2 in Sripa B., Kaewkes S., Sithithaworn P., Mairiang E., Laha T., et al. (2007). "Liver Fluke Induces Cholangiocarcinoma". PLoS Medicine 4(7): e201. doi:10.1371/journal.pmed.0040201. CC BY 2.5 Generic. https://commons.wikimedia.org/wiki/File:Opisthorchis_viverrini_3.png. Right: modification of work by Centers for Disease Control and Prevention. Public Domain.

Figure 2.60: The life cycle of Schistosoma spp. includes several species of water snails, which serve as secondary hosts. Illustration (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology. Modification of work by Centers for Disease Control and Prevention. Public Domain. https://www.cdc.gov/dpdx/schistosomiasis/modules/Schistomes_LifeCycle_lg.jpg. Step 3 photo: modification of Figure 1 in (c) Lewis FA, Liang Y-s, Raghavan N, Knight M (2008) The NIH-NIAID Schistosomiasis Resource Center. PLoS Negl Trop Dis 2(7): e267. https://doi.org/10.1371/journal.pntd.0000267. CC BY.

Figure 2.61: Life cycle of a tapeworm. Illustration: Modification of work by Centers for Disease Control and Prevention. Step 1, 2, 4, 5, 6 Images: Public Domain. Step 3 Images: (c) American Society of Microbiology. Redistribution authorized with attribution.

Figure 2.62: Multicellular fungi (molds) form hyphae, which may be septate or nonseptate. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.63: Histoplasma capsulatum is a dimorphic fungus that grows in soil exposed to bird feces or bat feces (guano) (top left). modification of work by Centers for Disease Control and Prevention. Public Domain.

Figure 2.64: Zygomycetes have sexual and asexual life cycles. In the sexual life cycle, + and – mating types conjugate to form a zygosporangium. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.65: These images show asexually produced spores. Left: modification of work by Centers for Disease Control and Prevention. Public Domain. Right: modification of work by Ciar. Public Domain. https://commons.wikimedia.org/wiki/File:Moldy_old_bread.JPG

Figure 2.66: This brightfield micrograph shows ascospores being released from asci in the fungus Talaromyces flavus var. flavus. modification of work by Centers for Disease Control and Prevention. Public Domain.

Figure 2.67: These ascospores, lined up within an ascus, are produced sexually. (c) Peter G. Werner. CC BY 3.0 Unported. https://commons.wikimedia.org/wiki/File:Morelasci.jpg

Figure 2.68: The life cycle of an ascomycete is characterized by the production of asci during the sexual phase. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.69: The life cycle of a basidiomycete alternates a haploid generation with a prolonged stage in which two nuclei (dikaryon) are present in the hyphae. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Table 2.17: Select groups of fungi. Image a: Ascomycota micrograph. modification of work by Dr. Lucille Georg, Centers for Disease Control and Prevention. Public Domain. https://commons.wikimedia.org/wiki/File:Aspergillus_niger_01.jpg. Image b: Basidiomycota. modification of work by Sanislaw Skowron. Public Domain. https://commons.wikimedia.org/wiki/File:Amanita_phalloides_young.jpg. Image c: Microsporidia micrograph: modification of work by Centers for Disease Control and Prevention. Public Domain. https://www.cdc.gov/dpdx/microsporidiosis/images/2/Microsporidia_2012.jpg. Image d: Zygomycota micrographs. Modification of work by Agong. Public Domain. https://commons.wikimedia.org/wiki/File:Rhizopus_fungus.jpg

Figure 2.70: (a) In this transmission electron micrograph, a bacteriophage (a virus that infects bacteria) is dwarfed by the bacterial cell it infects. Left: modification of work by U.S. Department of Energy, Office of Science, LBL, PBD. Public domain. Right: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.71: The size of a virus is small relative to the size of most bacterial and eukaryotic cells and their organelles. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.72: The naked atadenovirus uses spikes made of glycoproteins from its capsid to bind to host cells. Top Left: modification of work by NIAID. Public Domain. Bottom Left: modification of work by Centers for Disease Control and Prevention. Public Domain. Right: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.73: Viral capsids can be (a) helical, (b) polyhedral, or (c) have a complex shape. Top Left: modification of work by USDA ARS. Public Domain. Top Middle: Modification of work by U.S. Department of Energy. Public Domain. Top Right: University of Wisconsin-La Crosse, Microbiology program. Fair Use. https://web.archive.org/web/20220531010814/https://commons.wikimedia.org/wiki/File:Monkeypox.gif. Bottom: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology

Figure 2.74: A virulent phage shows only the lytic cycle pictured here. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.75: A temperate bacteriophage has both lytic and lysogenic cycles. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.76: This flowchart illustrates the mechanism of specialized transduction. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.77: Influenza virus is one of the few RNA viruses that replicates in the nucleus of cells. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.78: RNA viruses can contain +ssRNA that can be directly read by the ribosomes to synthesize viral proteins. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.79: HIV, an enveloped, icosahedral retrovirus, attaches to a cell surface receptor of an immune cell and fuses with the cell membrane. Modification of work by NIAID, NIH. Public domain.

Figure 2.80: Varicella-zoster, the virus that causes chickenpox. Left: Modification of work by Erskine Palmer and B.G. Partin/CDC. https://commons.wikimedia.org/wiki/File:Varicella_(Chickenpox)_Virus_PHIL_1878_lores.jpg. Scale-bar data from Matt Russell. CC BY 4.0. Right: (c) Paulo O. Fair Use. https://flic.kr/p/8Dt2fc

Figure 2.81: The one-step multiplication curve for a bacteriophage population follows three steps. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.
Table 2.20: Cytopathic effects of specific viruses. (c) American Society of Microbiology. Redistribution authorized with attribution.

Table 2.20: Cytopathic effects of specific viruses. (c) American Society of Microbiology. Redistribution authorized with attribution.

Figure 2.82: This figure shows the possible outcomes of a hemagglutination test. modification of work by Centers for Disease Control and Prevention. Public Domain.

Figure 2.83: Similar to rapid, over-the-counter pregnancy tests, EIAs for viral antigens require a few drops of diluted patient serum or plasma applied to a membrane filter (1). Modification of work by "Cavitri. CC BY 3.0 Unported. https://commons.wikimedia.org/wiki/File:ELISA_diagram.png

Figure 2.84: Endogenous normal prion protein (PrPc) is converted into the disease-causing form (PrPsc) when it encounters this variant form of the protein. Left: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology. Right: Modification of work by USDA/Dr. Al Jenny. Public Domain. https://commons.wikimedia.org/wiki/File:Histology_bse.jpg

Figure 2.85: Creutzfeldt-Jakob disease (CJD) is a fatal disease that causes degeneration of neural tissue. Left: modification of work (c) Dr. Laughlin Dawes CC BY 4.0. Top Right: Modification of work (c) Suzanne Wakim. CC BY 4.0. Bottom right: modification of work by Centers for Disease Control and Prevention. Public Domain.

Figure 2.86: The steps for confirming that a pathogen is the cause of a particular disease using Koch's postulates. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.87: A graph like this is used to determine LD50 by plotting pathogen concentration against the percent of infected test animals that have died. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.88: Shown are different portals of entry where pathogens can gain access into the body. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.89: Glycocalyx produced by bacteria in a biofilm allows the cells to adhere to host tissues and to medical devices such as the catheter surface shown here. Modification of work by Centers for Disease Control and Prevention. Public Domain.

Figure 2.90: H. pylori is able to invade the lining of the stomach by producing virulence factors that enable it to pass through the mucin layer covering epithelial cells. Modification of work by Zina Deretsky, National Science Foundation. Public domain.

Figure 2.91: Pathogens leave the body of an infected host through various portals of exit to infect new hosts. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.92: This patient has edema in the tissue of the right hand. Attributed to OpenStaxCNX. CC BY 3.0 Unported. https://commons.wikimedia.org/wiki/File:2707_Edema_of_Right_Hand_Due_to_Allergic_Reaction.jpg

Figure 2.93: Hyaluronan is a polymer found in the layers of epidermis that connect adjacent cells. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.94: Lipopolysaccharide is composed of lipid A, a core glycolipid, and an O-specific polysaccharide side chain. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.95: In A-B toxins, the B component binds to the host cell through its interaction with specific cell surface receptors. Modification of work by (c) "Biology Discussion Forum"/YouTube. CC BY 4.0.

Figure 2.96: The mechanism of the diphtheria toxin inhibiting protein synthesis. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.97: Mechanisms of botulinum and tetanus toxins. Left: modification of work by Centers for Disease Control and Prevention. Right: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.98: (a) A micrograph of capsules around bacterial cells. Left: modification of work by Centers for Disease Control and Prevention. Public domain. Middle and right: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 2.99: Antigenic drift and antigenic shift in influenza viruses. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

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CHAPTER 3.

SYSTEMIC INFECTIONS OF THE SKIN

3.1 INTRODUCTION TO THE ANATOMY AND NORMAL MICROBIOTA OF THE SKIN AND EYES

Human skin is an important part of the innate immune system. In addition to serving a wide range of other functions, the skin serves as an important barrier to microbial invasion. Not only is it a physical barrier to penetration of deeper tissues by potential pathogens, but it also provides an inhospitable environment for the growth of many pathogens. In this section, we will provide a brief overview of the anatomy and normal microbiota of the skin and eyes, along with general symptoms associated with skin and eye infections.

LAYERS OF THE SKIN

Human skin is made up of several layers and sublayers. The two main layers are the epidermis and the dermis. These layers cover a third layer of tissue called the hypodermis, which consists of fibrous and adipose connective tissue (figure 3.1).

The epidermis is the outermost layer of the skin and its thickness varies based on location. Areas such as the soles of the feet and palms of the hands display a thick epidermis to protect against mechanical forces, while the epidermis of areas such as the face are much thinner. The exterior surface of the epidermis, called the stratum corneum, primarily consists of dead skin cells. This layer of dead cells limits direct contact between the outside world and live cells. The stratum corneum is rich in keratin, a tough, fibrous scleroprotein that is a major structural intermediate filament of epithelial cells and is also found in hair and nails. Keratin helps make the outer surface of the skin relatively tough and waterproof. It also helps to keep the surface of the skin dry, which reduces microbial growth. However, some microbes are still able to live on the surface of the skin, and some of these can be shed with dead skin cells in the process of desquamation, which is the shedding and peeling of skin that occurs as a normal process but that may be accelerated when infection is present.

Beneath the epidermis lies a thicker skin layer called the dermis. The dermis contains connective tissue and embedded structures such as blood vessels, nerves, and muscles. Structures called hair follicles (from which hair grows) are located within the dermis, even though much of their structure consists of epidermal tissue. Indeed, the other epithelial layer of the hair follicle is actually an invagination of the overlying squamous epithelium. The dermis also contains the two major types of glands found in human skin: sweat (apocrine)glands (tubular glands that produce sweat) and sebaceous glands (which are associated with hair follicles and produce sebum, a lipid-rich substance containing proteins and minerals).

Perspiration (sweat) provides some moisture to the epidermis, which can increase the potential for microbial growth. For this reason, more microbes are found on the regions of the skin that produce the most sweat, such as the skin of the underarms and groin. However, in addition to water, sweat also contains substances that inhibit microbial growth, such as salts, lysozyme, and antimicrobial peptides. Sebum also serves to protect the skin and reduce water loss. Although some of the lipids and fatty acids in sebum inhibit microbial growth, sebum contains compounds that provide nutrition for certain microbes.

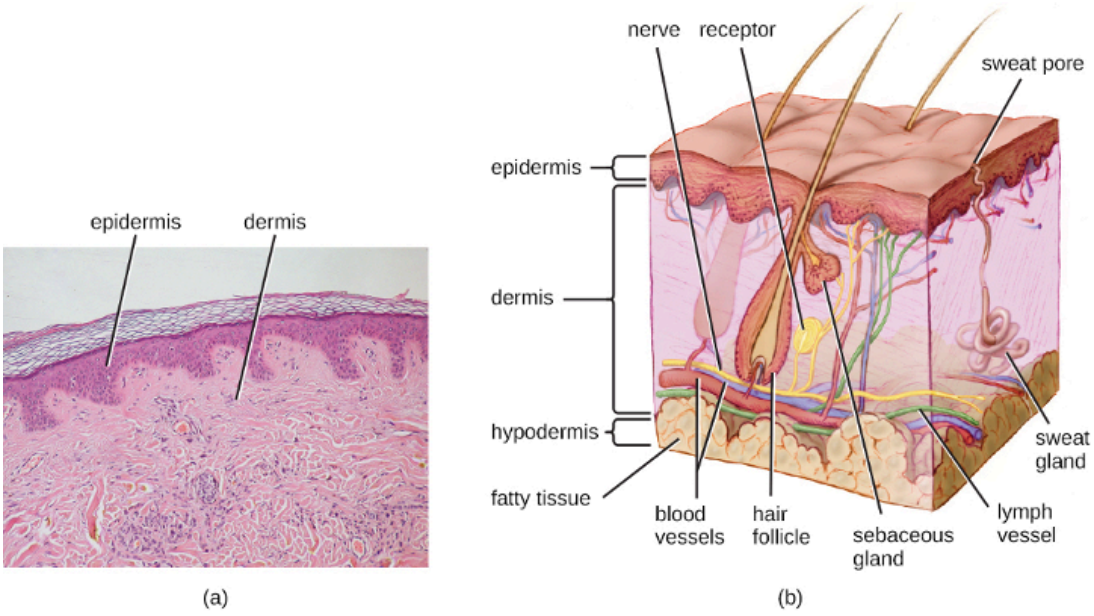


Figure 3.1: (a) A micrograph of a section through human skin shows the epidermis and dermis. (b) The major layers of human skin are the epidermis, dermis, and hypodermis. *Figure description available at the end of the chapter.*

NORMAL MICROBIOTA OF THE SKIN

The skin is home to a wide variety of normal microbiota, consisting of commensal organisms that derive nutrition from skin cells and secretions such as sweat and sebum. The normal microbiota of skin tends to inhibit transient-microbe colonization by producing antimicrobial substances and outcompeting other microbes that land on the surface of the skin. This helps to protect the skin from pathogenic infection.

The skin's properties differ from one region of the body to another, as does the composition of the skin's microbiota. The availability of nutrients and moisture partly dictates which microorganisms will thrive in a particular region of the skin. Relatively moist skin, such as that of the nares (nostrils) and underarms, has a much different microbiota than the dryer skin on the arms, legs, hands, and top of the feet. Some areas of the skin have higher densities of sebaceous glands. These sebum-rich areas, which include the back, the folds at the side of the nose, and the back of the neck, harbor distinct microbial communities that are less diverse than those found on other parts of the body.

Different types of bacteria dominate the dry, moist, and sebum-rich regions of the skin. The most abundant microbes typically found in the dry and sebaceous regions are Betaproteobacteria and Propionibacteria, respectively. In the moist regions, *Corynebacterium* and *Staphylococcus* are most commonly found (figure 3.2). Viruses and fungi are also found on the skin; *Malassezia* is the most common type of fungus found within the normal microbiota. The role and populations of viruses in the microbiota, known as viromes, are still not well understood, and there are limitations to the techniques used to identify them. However, Circoviridae, Papillomaviridae, and Polyomaviridae appear to be the most common residents in the healthy skin virome.[123]

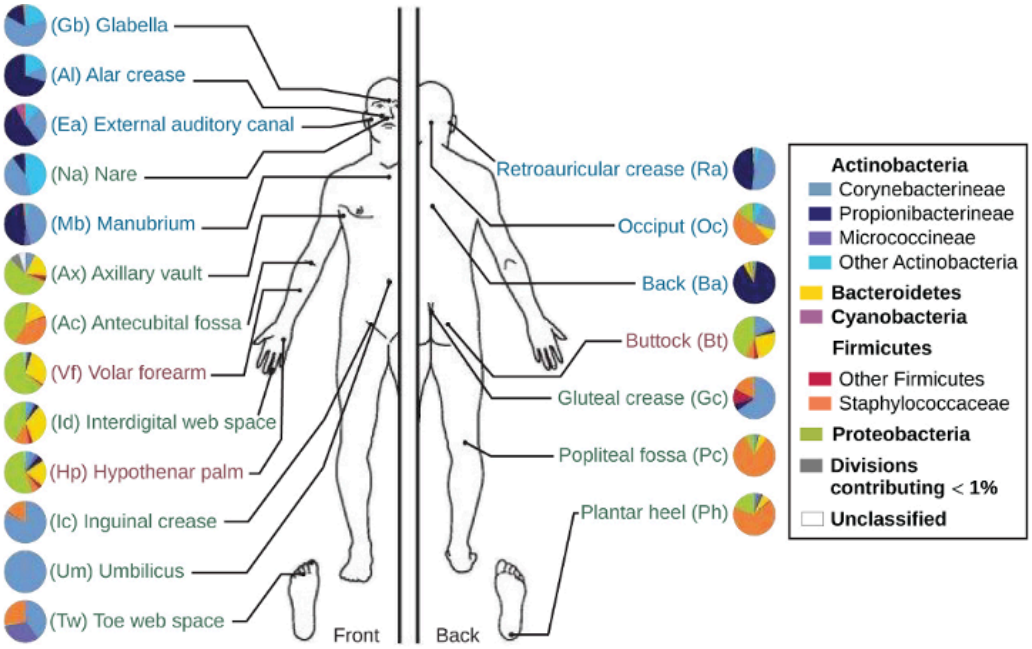


Figure 3.2: The normal microbiota varies on different regions of the skin, especially in dry versus moist areas. The figure shows the major organisms commonly found in different locations of a healthy individual's skin and external mucosa. Note that there is significant variation among individuals. *Figure description available at the end of the chapter.*

INFECTIONS OF THE SKIN

While the microbiota of the skin can play a protective role, it can also cause harm in certain cases. Often, an opportunistic pathogen residing in the skin microbiota of one individual may be transmitted to another individual more susceptible to an infection. For example, methicillin-resistant *Staphylococcus aureus* (MRSA) can often take up residence in the nares of health care workers and hospital patients; though harmless on intact, healthy skin, MRSA can cause infections if introduced into other parts of the body, as might occur during surgery or via a post-surgical incision or wound. This is one reason why clean surgical sites are essential.

Injury or damage to the skin can allow microbes to enter deeper tissues, where nutrients are more abundant and the environment is more conducive to bacterial growth. Wound infections are common after a puncture or laceration that damages the physical barrier of the skin. Microbes may infect structures in the dermis, such as hair follicles and glands, causing a localized infection, or they may reach the bloodstream, which can lead to a systemic infection.

In some cases, infectious microbes can cause a variety of rashes or lesions that differ in their physical characteristics. These rashes can be the result of inflammation reactions or direct responses to toxins produced by the microbes. Table 3.1 lists some of the medical terminology used to describe skin lesions and rashes based on their characteristics and the most common skin infections are summarized in table 3.3. Figure 3.3 and figure 3.4 illustrate some of the various types of skin lesions. It is important to note that many different diseases can lead to skin conditions of very similar appearance; generally, the terms used in the table are not exclusive to a particular type of infection or disease.

Term	Definition
abscess	localized collection of pus, often with an inflamed fibrous outer capsule
bulla (pl., bullae)	fluid-filled blister no more than 5 mm in diameter
carbuncle	deep, pus-filled abscess generally formed from multiple furuncles
crust	dried fluids from a lesion on the surface of the skin
cyst	encapsulated sac filled with fluid, semi-solid matter, or gas, typically located just below the upper layers of skin
folliculitis	a localized rash due to inflammation of hair follicles
furuncle (boil)	pus-filled abscess due to infection of a hair follicle
macules	smooth spots of discoloration on the skin
papules	small raised bumps on the skin
pseudocyst	lesion that resembles a cyst but is not lined by a defined epithelial capsule
purulent	pus-producing; suppurative
pustules	fluid- or pus-filled bumps on the skin
pyoderma	any suppurative (pus-producing) infection of the skin
suppurative	producing pus; purulent
ulcer	break in the skin with complete loss of the epidermal barrier; open sore
vesicle	small, fluid-filled lesion
wheal	swollen, inflamed skin that itches or burns, such as from an insect bite

Table 3.1: Some medical terms associated with skin lesions and rashes

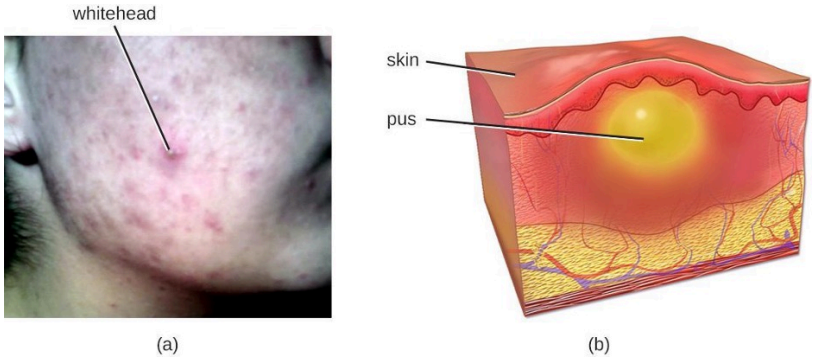


Figure 3.3: (a) Acne is a bacterial infection of the skin that manifests as a rash of inflamed hair follicles (folliculitis). The large whitehead near the center of the cheek is an infected hair follicle that has become purulent (or suppurative), leading to the formation of a furuncle. (b) An abscess is a pus-filled lesion. *Figure description available at the end of the chapter.*

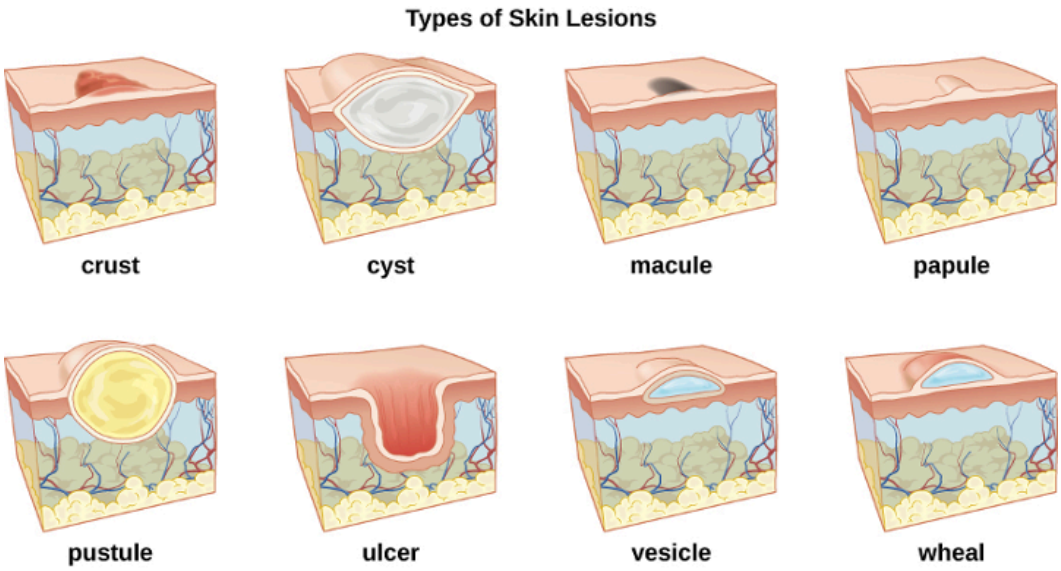


Figure 3.4: Numerous causes can lead to skin lesions of various types, some of which are very similar in appearance. *Figure description available at the end of the chapter.*

ANATOMY AND MICROBIOTA OF THE EYE

Although the eye and skin have distinct anatomy, they are both in direct contact with the external environment. An important component of the eye is the nasolacrimal drainage system, which serves as a conduit for the fluid of the eye, called tears. Tears flow from the external eye to the nasal cavity by the lacrimal apparatus, which is composed of the structures involved in tear production (figure 3.5). The lacrimal gland, above the eye, secretes tears to keep the eye moist. There are two small openings, one on the inside edge of the upper eyelid and one on the inside edge of the lower eyelid, near the nose. Each of these openings is called a lacrimal punctum. Together, these lacrimal puncta collect tears from the eye that are then conveyed through lacrimal ducts to a reservoir for tears called the lacrimal sac, also known as the dacryocyst or tear sac.

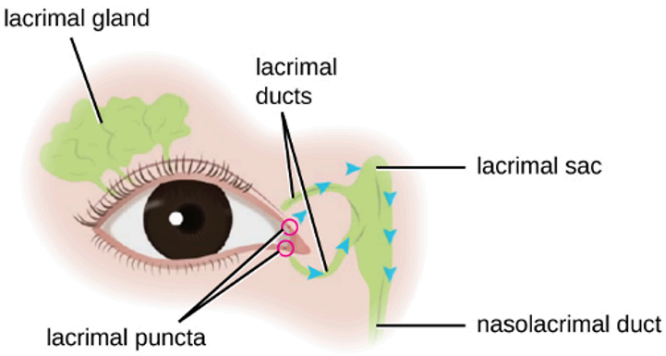


Figure 3.5: The lacrimal apparatus includes the structures of the eye associated with tear production and drainage. *Figure description available at the end of the chapter.*

From the sac, tear fluid flows via a nasolacrimal duct to the inner nose. Each nasolacrimal duct is located underneath the skin and passes through the bones of the face into the nose. Chemicals in tears, such as defensins, lactoferrin, and lysozyme, help to prevent colonization by pathogens. In addition, mucins facilitate the removal of microbes from the surface of the eye.

The surfaces of the eyeball and inner eyelid are mucous membranes called conjunctiva. The normal conjunctival microbiota has not been well characterized, but does exist. One small study (part of the Ocular Microbiome project) found twelve genera that were consistently present in the conjunctiva.[4] These microbes are thought to help defend the membranes against pathogens. However, it is still unclear which microbes may be transient and which may form a stable microbiota.[5]

Use of contact lenses can cause changes in the normal microbiota of the conjunctiva by introducing another surface into the natural anatomy of the eye. Research is currently underway to better understand how contact lenses may impact the normal microbiota and contribute to eye disease.

The watery material inside of the eyeball is called vitreous humor. Unlike the conjunctiva, it is protected from contact with the environment and is almost always sterile, with no normal microbiota (figure 3.6).

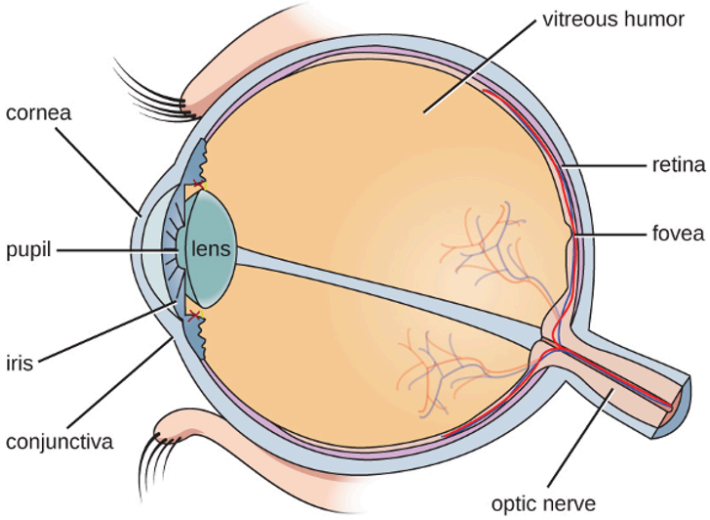


Figure 3.6: Some microbes live on the conjunctiva of the human eye, but the vitreous humor is sterile. *Figure description available at the end of the chapter.*

INFECTIONS OF THE EYE

The conjunctiva is a frequent site of infection of the eye; like other mucous membranes, it is also a common portal of entry for pathogens. Inflammation of the conjunctiva is called conjunctivitis, although it is commonly known as pinkeye because of the pink appearance in the eye. Infections of deeper structures, beneath the cornea, are less common (figure 3.7). Conjunctivitis occurs in multiple forms. It may be acute or chronic. Acute purulent conjunctivitis is associated with pus formation, while acute hemorrhagic conjunctivitis is associated with bleeding in the conjunctiva. The term blepharitis refers to an inflammation of the eyelids, while keratitis refers to an inflammation of the cornea (figure 3.7); keratoconjunctivitis is an inflammation of both the cornea and the conjunctiva, and dacryocystitis is an inflammation of the lacrimal sac that can often occur when a nasolacrimal duct is blocked.

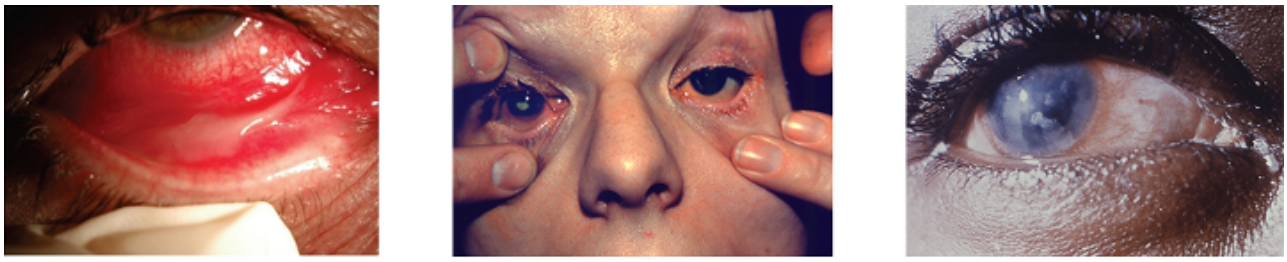


Figure 3.7: (a) Conjunctivitis is inflammation of the conjunctiva. (b) Blepharitis is inflammation of the eyelids. (c) Keratitis is inflammation of the cornea. *Figure description available at the end of the chapter.*

Infections leading to conjunctivitis, blepharitis, keratoconjunctivitis, or dacryocystitis may be caused by bacteria or viruses. Allergens, pollutants, or chemicals can also irritate the eye and cause inflammation of various structures. Viral infection is a more likely cause of conjunctivitis in cases with symptoms such as fever and watery discharge that occurs with upper respiratory infection and itchy eyes. Table 3.2 summarizes some common forms of conjunctivitis and blepharitis.

Condition	Description	Causative Agent(s)
Acute purulent conjunctivitis	Conjunctivitis with purulent discharge	Bacterial (*Haemophilus, Staphylococcus*)
Acute hemorrhagic conjunctivitis	Involves subconjunctival hemorrhages	Viral (Picornaviridae)
Acute ulcerative blepharitis	Infection involving eyelids; pustules and ulcers may develop	Bacterial (*Staphylococcal*) or viral (herpes simplex, varicella-zoster, etc.)
Follicular conjunctivitis	Inflammation of the conjunctiva with nodules (dome-shaped structures that are red at the base and pale on top)	Viral (adenovirus and others); environmental irritants
Dacryocystitis	Inflammation of the lacrimal sac often associated with a plugged nasolacrimal duct	Bacterial (*Haemophilus, Staphylococcus, Streptococcus*)
Keratitis	Inflammation of cornea	Bacterial, viral, or protozoal; environmental irritants
Keratoconjunctivitis	Inflammation of cornea and conjunctiva	Bacterial, viral (adenoviruses), or other causes (including dryness of the eye)
Nonulcerative blepharitis	Inflammation, irritation, redness of the eyelids without ulceration	Environmental irritants; allergens
Papillary conjunctivitis	Inflammation of the conjunctiva; nodules and papillae with red tops develop	Environmental irritants; allergens

Table 3.2: Types of conjunctivitis and blepharitis

3.2 BACTERIAL INFECTIONS OF THE SKIN AND EYES

Despite the skin's protective functions, infections are common. Gram-positive *Staphylococcus* spp. and *Streptococcus* spp. are responsible for many of the most common skin infections. However, many skin conditions are not strictly associated with a single pathogen. Opportunistic pathogens of many types may infect skin wounds, and individual cases with identical symptoms may result from different pathogens or combinations of pathogens.

In this section, we will examine some of the most important bacterial infections of the skin and eyes and discuss how biofilms can contribute to and exacerbate such infections. Key features of bacterial skin and eye infections are also summarized in tables 3.3 and 3.4.

STAPHYLOCOCCAL INFECTIONS OF THE SKIN

Staphylococcus species are commonly found on the skin, with *S. epidermidis* and *S. hominis* being prevalent in the normal microbiota. *S. aureus* is also commonly found in the nasal passages and on healthy skin, but pathogenic strains are often the cause of a broad range of infections of the skin and other body systems.

S. aureus is quite contagious. It is spread easily through skin-to-skin contact, and because many people are chronic nasal carriers (asymptomatic individuals who carry *S. aureus* in their nares), the bacteria can easily be transferred from the nose to the hands and then to fomites or other individuals. Because it is so contagious, *S. aureus* is prevalent in most community settings. This prevalence is particularly problematic in hospitals, where antibiotic-resistant strains of the bacteria may be present, and where immunocompromised patients may be more susceptible to infection. Resistant strains include methicillin-resistant *S. aureus* (MRSA), which can be acquired through healthcare settings (hospital-acquired MRSA, or HA-MRSA) or in the community (community-acquired MRSA, or CA-MRSA). Hospital patients often arrive at healthcare facilities already colonized with antibiotic-resistant strains of *S. aureus* that can be transferred to healthcare providers and other patients. Some hospitals have attempted to detect these individuals in order to institute prophylactic measures, but they have had mixed success.

When a staphylococcal infection develops, choice of medication is important. As discussed above, many staphylococci (such as MRSA) are resistant to some or many antibiotics. Thus, antibiotic sensitivity is measured to identify the most suitable antibiotic. However, even before receiving the results of sensitivity analysis, suspected *S. aureus* infections are often initially treated with drugs known to be effective against MRSA, such as trimethoprim-sulfamethoxazole (TMP/SMZ), clindamycin, a tetracycline (doxycycline or minocycline), or linezolid.

The pathogenicity of staphylococcal infections is often enhanced by characteristic chemicals secreted by some strains. Staphylococcal virulence factors include hemolysins called staphylolysin, which are cytotoxic for many types of cells, including skin cells and white blood cells. Virulent strains of *S. aureus* are also coagulase-positive, meaning they produce coagulase, a plasma-clotting protein that is involved in abscess formation. They may also produce leukocidins, which kill white blood cells and can contribute to the production of pus and Protein A, which inhibits phagocytosis by binding to the constant region of antibodies. Some virulent strains of *S. aureus* also produce other toxins, such as toxic shock syndrome toxin-1 (see section 2.14).

To confirm the causative agent of a suspected staphylococcal skin infection, samples from the wound are cultured. Under the microscope, gram-positive *Staphylococcus* species have cellular arrangements that form grape-like clusters; when grown on blood agar, colonies have a unique pigmentation ranging from opaque white to cream. A catalase test is used to distinguish *Staphylococcus* from *Streptococcus*, which is also a genus of gram-positive cocci and a common cause of skin infections. *Staphylococcus* species are catalase-positive while *Streptococcus* species are catalase-negative.

Other tests are performed on samples from the wound in order to distinguish coagulase-positive species of *Staphylococcus* (CoPS) such as *S. aureus* from common coagulase-negative species (CoNS) such as *S. epidermidis*. Although CoNS are less likely than CoPS to cause human disease, they can cause infections when they enter the body, as can sometimes occur via catheters, indwelling medical devices, and wounds. Passive agglutination testing can be used to distinguish CoPS from CoNS. If the sample is coagulase-positive, the sample is generally presumed to contain *S. aureus*. Additional genetic testing would be necessary to identify the particular strain of *S. aureus.*

Another way to distinguish CoPS from CoNS is by culturing the sample on mannitol salt agar (MSA). *Staphylococcus* species readily grow on this medium because they are tolerant of the high concentration of sodium chloride (7.5% NaCl). However, CoPS such as *S. aureus* ferment mannitol (which will be evident on a MSA plate). Conversely, CoNS such as *S. epidermidis* do not ferment mannitol but can be distinguished by the fermentation of other sugars such as lactose, malonate, and raffinose (figure 3.8).

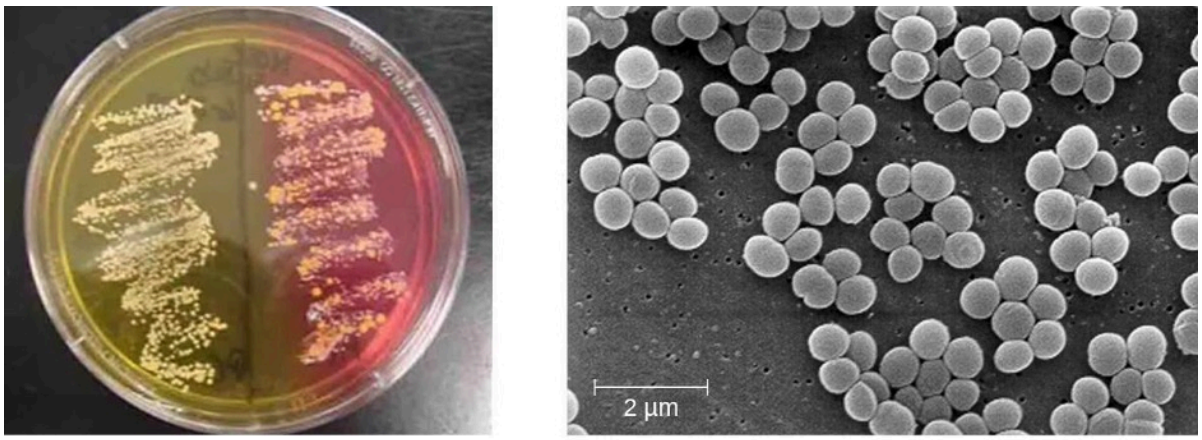


Figure 3.8: (a) A mannitol salt agar plate is used to distinguish different species of staphylococci. In this plate, S. aureus is on the left and S. epidermidis is in the right. Because S. aureus is capable of fermenting mannitol, it produces acids that cause the color to change to yellow. (b) This scanning electron micrograph shows the characteristic grapelike clusters of S. aureus. *Figure description available at the end of the chapter.*

Superficial Staphylococcal Infections

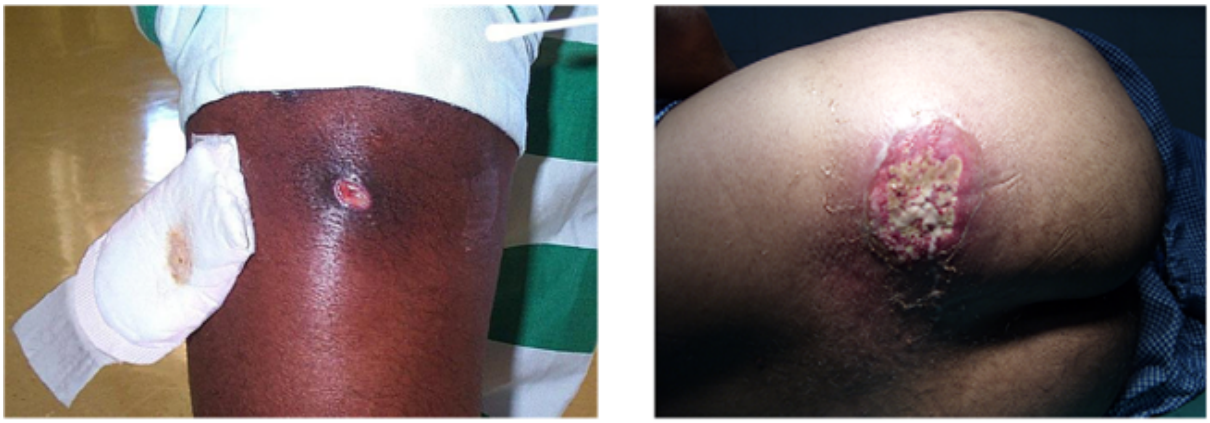
S. aureus is often associated with pyoderma, skin infections that are purulent. Pus formation occurs because many strains of *S. aureus* produce leukocidins, which kill white blood cells. These purulent skin infections may initially manifest as folliculitis, but can lead to furuncles or deeper abscesses called carbuncles.

Folliculitis generally presents as bumps and pimples that may be itchy, red, and/or pus-filled. In some cases, folliculitis is self-limiting, but if it continues for more than a few days, worsens, or returns repeatedly, it may require medical treatment. Sweat, skin injuries, ingrown hairs, tight clothing, irritation from shaving, and skin conditions can all contribute to folliculitis. Avoidance of tight clothing and skin irritation can help to prevent infection, but topical antibiotics (and sometimes other treatments) may also help. Folliculitis can be identified by skin inspection; treatment is generally started without first culturing and identifying the causative agent.

In contrast, furuncles (boils) are deeper infections (figure 3.9). They are most common in those individuals (especially young adults and teenagers) who play contact sports, share athletic equipment, have poor nutrition, live in close quarters, or have weakened immune systems. Good hygiene and skin care can often help to prevent furuncles from becoming more infective, and they generally resolve on their own. However, if furuncles spread, increase in number or size, or lead to systemic symptoms such as fever and chills, then medical care is needed. They may sometimes need to be drained (at which time the pathogens can be cultured) and treated with antibiotics.

When multiple boils develop into a deeper lesion, it is called a carbuncle (figure 3.9). Because carbuncles are deeper, they are more commonly associated with systemic symptoms and a general feeling of illness. Larger, recurrent, or worsening carbuncles require medical treatment, as do those associated with signs of illness such as fever. Carbuncles generally need to be drained and treated with antibiotics. While carbuncles are relatively easy to identify visually, culturing and laboratory analysis of the wound may be recommended for some infections because antibiotic resistance is relatively common.

Proper hygiene is important to prevent these types of skin infections or to prevent the progression of existing infections.



(a) (b)

Figure 3.9: Furuncles (boils) and carbuncles are infections of the skin often caused by Staphylococcus bacteria. (a) A furuncle contains pus and exhibits swelling. (b) A carbuncle is a pus-filled lesion that is typically deeper than the furuncle. It often forms from multiple furuncles. *Figure description available at the end of the chapter.*

Staphylococcal scalded skin syndrome (SSSS) is another superficial infection caused by *S. aureus* that is most commonly seen in young children, especially infants. Bacterial exotoxins first produce erythema (redness of the skin) and then severe peeling of the skin, as might occur after scalding (figure 3.10). SSSS is diagnosed by examining characteristics of the skin (which may rub off easily), using blood tests to check for elevated white blood cell counts, culturing, and other methods. Intravenous antibiotics and fluid therapy are used as treatment.

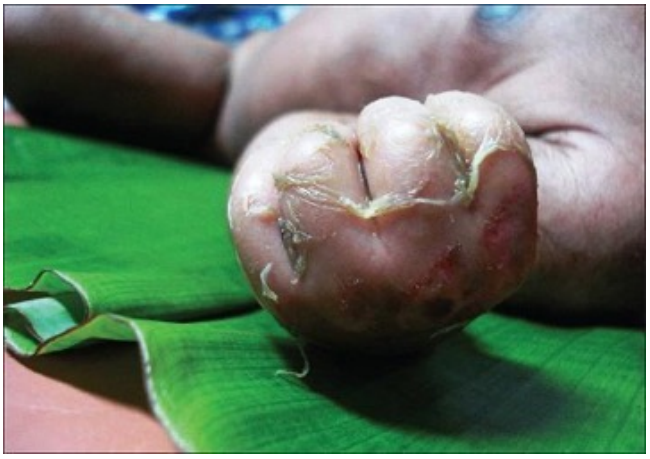


Figure 3.10: A newborn with staphylococcal scalded skin syndrome (SSSS), which results in large regions of peeling, dead skin. *Figure description available at the end of the chapter.*

Impetigo

The skin infection impetigo causes the formation of vesicles, pustules, and possibly bullae, often around the nose and mouth. Bullae are large, fluid-filled blisters that measure at least 5 mm in diameter. Impetigo can be diagnosed as either nonbullous or bullous. In nonbullous impetigo, vesicles and pustules rupture and become encrusted sores. Typically the crust is yellowish, often with exudate draining from the base of the lesion. In bullous impetigo, the bullae fill and rupture, resulting in larger, draining, encrusted lesions (figure 3.11).

Especially common in children, impetigo is particularly concerning because it is highly contagious. Impetigo can be caused by *S. aureus* alone, by *Streptococcus pyogenes* alone, or by coinfection of *S. aureus* and *S. pyogenes*. Impetigo is often diagnosed through observation of its characteristic appearance, although culture and susceptibility testing may also be used.

Topical or oral antibiotic treatment is typically effective in treating most cases of impetigo. However, cases caused by *S. pyogenes* can lead to serious sequelae (pathological conditions resulting from infection, disease, injury, therapy, or other trauma) such as acute glomerulonephritis (AGN), which is severe inflammation in the kidneys.

Nosocomial S. epidermidis Infections

Though not as virulent as *S. aureus*, the staphylococcus *S. epidermidis* can cause serious opportunistic infections. Such infections usually occur only in hospital settings. *S. epidermidis* is usually a harmless resident of the normal skin microbiota. However, healthcare workers can inadvertently transfer *S. epidermidis* to medical devices that are inserted into the body, such as catheters, prostheses, and indwelling medical devices. Once it has bypassed the skin barrier, *S. epidermidis* can cause infections inside the body that can be difficult to treat. Like *S. aureus*, *S. epidermidis* is resistant to many antibiotics, and localized infections can become systemic if not treated quickly. To reduce the risk of nosocomial (hospital-acquired) *S. epidermidis*, health-care workers must follow strict procedures for handling and sterilizing medical devices before and during surgical procedures.

Figure 3.11: Impetigo is characterized by vesicles, pustules, or bullae that rupture, producing encrusted sores. *Figure description available at the end of the chapter.*

STREPTOCOCCAL INFECTIONS OF THE SKIN

Streptococcus are gram-positive cocci with a microscopic morphology that resembles chains of bacteria. Colonies are typically small (1–2 mm in diameter), translucent, entire edge, with a slightly raised elevation that can be either nonhemolytic, alpha-hemolytic, or beta-hemolytic when grown on blood agar (figure 3.12). Additionally, they are facultative anaerobes that are catalase-negative.

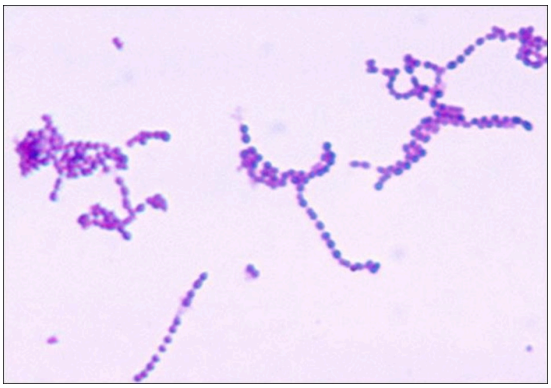


Figure 3.12: Streptococcus pyogenes forms chains of cocci. *Figure description available at the end of the chapter.*

The genus *Streptococcus* includes important pathogens that are categorized in serological Lancefield groups based on the distinguishing characteristics of their surface carbohydrates. The most clinically important streptococcal species in humans is *S. pyogenes*, also known as group A streptococcus (GAS). *S. pyogenes* produces a variety of extracellular enzymes, including streptolysins O and S, hyaluronidase, and streptokinase. These enzymes can aid in transmission and contribute to the inflammatory response.[6] *S. pyogenes* also produces a capsule and M protein, a streptococcal cell wall protein. These virulence factors help the bacteria to avoid phagocytosis while provoking a substantial immune response that contributes to symptoms associated with streptococcal infections.

S. pyogenes causes a wide variety of diseases not only in the skin, but in other organ systems as well. Examples of diseases elsewhere in the body include pharyngitis and scarlet fever.

Cellulitis, Erysipelas, and Erythema Nodosum

Common streptococcal conditions of the skin include cellulitis, erysipelas, and erythema nodosum. An infection that develops in the dermis or hypodermis can cause cellulitis, which presents as a reddened area of the skin that is warm to the touch and painful. The causative agent is often *S. pyogenes*, which may breach the epidermis through a cut or abrasion, although cellulitis may also be caused by staphylococci. *S. pyogenes* can also cause

erysipelas, a condition that presents as a large, intensely inflamed patch of skin involving the dermis (often on the legs or face). These infections can be suppurative, which results in a bullous form of erysipelas. Streptococcal and other pathogens may also cause a condition called erythema nodosum, characterized by inflammation in the subcutaneous fat cells of the hypodermis. It sometimes results from a streptococcal infection, though other pathogens can also cause the condition. It is not suppurative, but leads to red nodules on the skin, most frequently on the shins (figure 3.13).

In general, streptococcal infections are best treated through identification of the specific pathogen followed by treatment based upon that particular pathogen's susceptibility to different antibiotics. Many immunological tests, including agglutination reactions and ELISAs, can be used to detect streptococci. Penicillin is commonly prescribed for treatment of cellulitis and erysipelas because resistance is not widespread in streptococci at this time. In most patients, erythema nodosum is self-limiting and is not treated with antimicrobial drugs. Recommended treatments may include nonsteroidal anti-inflammatory drugs (NSAIDs), cool wet compresses, elevation, and bed rest.

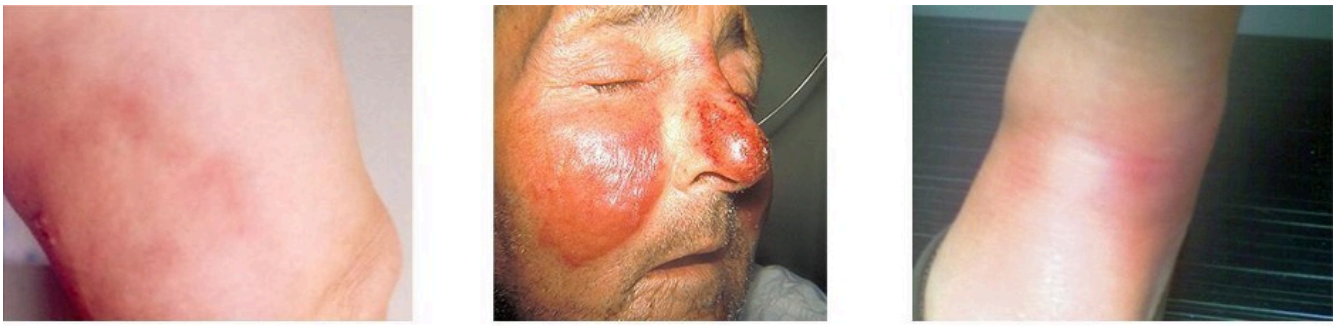


Figure 3.13: S. pyogenes can cause a variety of skin conditions once it breaches the skin barrier through a cut or wound. (a) Cellulitis presents as a painful, red rash. (b) Erysipelas presents as a raised rash, usually with clear borders. (c) Erythema nodosum is characterized by red lumps or nodules, typically on the lower legs. *Figure description available at the end of the chapter.*

Necrotizing Fasciitis

Streptococcal infections that start in the skin can sometimes spread elsewhere, resulting in a rare but potentially life-threatening condition called necrotizing fasciitis, sometimes referred to as flesh-eating bacterial syndrome. *S. pyogenes* is one of several species that can cause this rare but potentially-fatal condition; others include *Klebsiella*, *Clostridium*, *Escherichia coli*, *S. aureus*, and *Aeromonas hydrophila*.

Necrotizing fasciitis occurs when the fascia, a thin layer of connective tissue between the skin and muscle, becomes infected. Severe invasive necrotizing fasciitis due to *Streptococcus pyogenes* occurs when virulence factors that are responsible for adhesion and invasion overcome host defenses. *S. pyogenes* invasions allow bacterial cells to adhere to tissues and establish infection. Bacterial proteases unique to *S. pyogenes* aggressively infiltrate and destroy host tissues, inactivate complement, and prevent neutrophil migration to the site of infection. The infection and resulting tissue death can spread very rapidly, as large areas of skin become detached and die. Treatment generally requires debridement (surgical removal of dead or infected tissue) or amputation of infected limbs to stop the spread of the infection; surgical treatment is supplemented with intravenous antibiotics and other therapies (figure 3.14).

Necrotizing fasciitis does not always originate from a skin infection; in some cases there is no known portal of entry. Some studies have suggested that experiencing a blunt force trauma can increase the risk of developing streptococcal necrotizing fasciitis.[7]

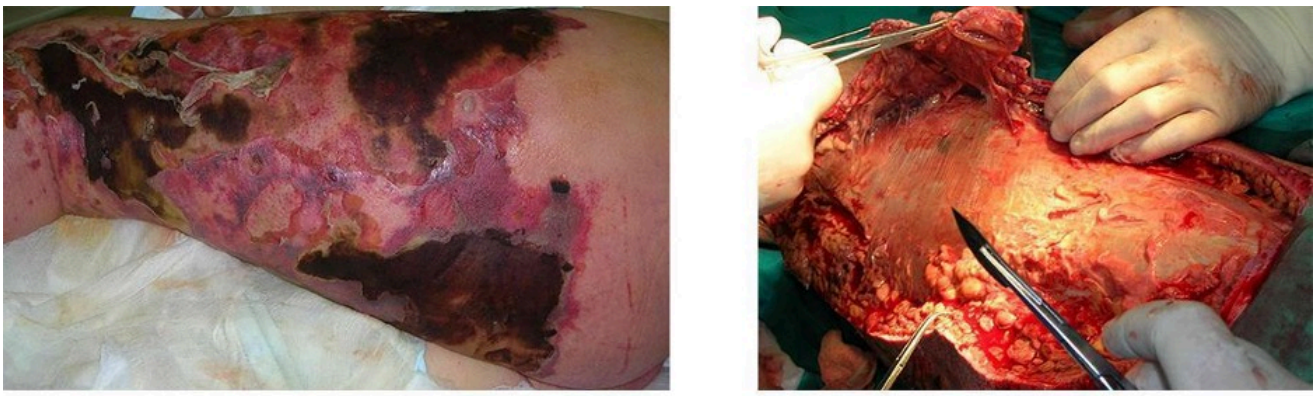


Figure 3.14: (a) The left leg of this patient shows the clinical features of necrotizing fasciitis. (b) The same patient's leg is surgically debrided to remove the infection. *Figure description available at the end of the chapter.*

PSEUDOMONAS INFECTIONS OF THE SKIN

Another important skin pathogen is *Pseudomonas aeruginosa*, a gram-negative, oxidase-positive, aerobic bacillus that is commonly found in water and soil as well as on human skin. *P. aeruginosa* is a common cause of opportunistic infections of wounds and burns. It can also cause hot tub rash, a condition characterized by folliculitis that frequently afflicts users of pools and hot tubs. *P. aeruginosa* is also the cause of otitis externa (swimmer's ear), an infection of the ear canal that causes itching, redness, and discomfort, and can progress to fever, pain, and swelling (figure 3.15).

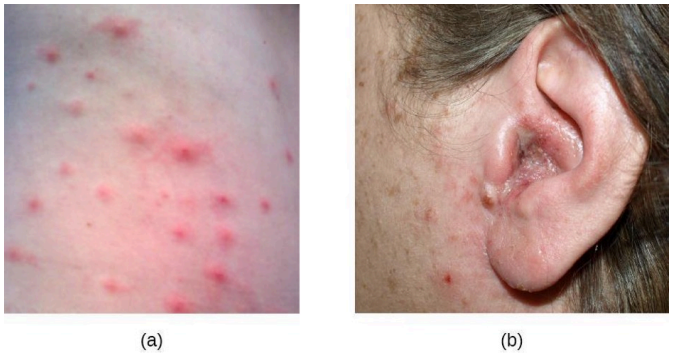


Figure 3.15: (a) Hot tub folliculitis presents as an itchy red rash. It is typically caused by P. aeruginosa, a bacterium that thrives in wet, warm environments such as hot tubs. (b) Otitis externa (swimmer's ear) may also be caused by P. aeruginosa or other bacteria commonly found in water. Inflammation of the outer ear and ear canal can lead to painful swelling. *Figure description available at the end of the chapter.*

Wounds infected with *P. aeruginosa* have a distinctive odor resembling grape soda or fresh corn tortillas. This odor is caused by the 2-aminoacetophenone that is used by *P. aeruginosa* in quorum sensing and contributes to its pathogenicity. Wounds infected with certain strains of *P. aeruginosa* also produce a blue-green pus due to the pigments pyocyanin and pyoverdin, which also contribute to its virulence. Pyocyanin and pyoverdin are siderophores that help *P. aeruginosa* survive in low-iron environments by enhancing iron uptake. *P. aeruginosa* also produces several other virulence factors, including phospholipase C (a hemolysin capable of breaking down red blood cells), exoenzyme S (involved in adherence to epithelial cells), and exotoxin A (capable of causing tissue necrosis). Other virulence factors include a slime that allows the bacterium to avoid being phagocytized, fimbriae for adherence, and proteases that cause tissue damage. *P. aeruginosa* can be detected through the use of cetrimide agar, which is selective for *Pseudomonas* species (figure 3.16).

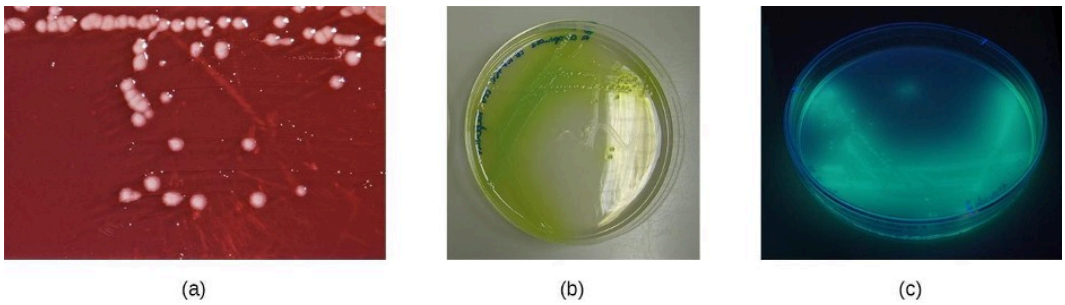


Figure 3.16: (a) These P. aeruginosa colonies are growing on xylose lysine sodium deoxycholate (XLD) agar. (b) Pseudomonas spp. can produce a variety of blue-green pigments. (c) Pseudomonas spp. may produce fluorescein, which fluoresces green under ultraviolet light under the right conditions. *Figure description available at the end of the chapter.*

Pseudomonas spp. tend to be resistant to most antibiotics. They often produce β-lactamases, may have mutations affecting porins (small cell wall channels) that limit antibiotic uptake, and may pump some antibiotics out of the cell, contributing to this resistance. Polymyxin B and gentamicin are effective, as are some fluoroquinolones. Otitis externa is typically treated with ear drops containing acetic acid, antibacterials, and/or steroids to reduce inflammation; ear drops may also include antifungals because fungi can sometimes cause or contribute to otitis externa. Wound infections caused by *Pseudomonas* spp. may be treated with topical antibiofilm agents that disrupt the formation of biofilms.

ACNE

One of the most ubiquitous skin conditions is acne. Acne afflicts nearly 80% of teenagers and young adults, but it can be found in individuals of all ages. Higher incidence among adolescents is due to hormonal changes that can result in overproduction of sebum.

Acne occurs when hair follicles become clogged by shed skin cells and sebum, causing non-inflammatory lesions called comedones. Comedones (singular "comedo") can take the form of whitehead and blackhead pimples. Whiteheads are covered by skin, whereas blackhead pimples are not; the black color occurs when lipids in the clogged follicle become exposed to the air and oxidize (figure 3.17).

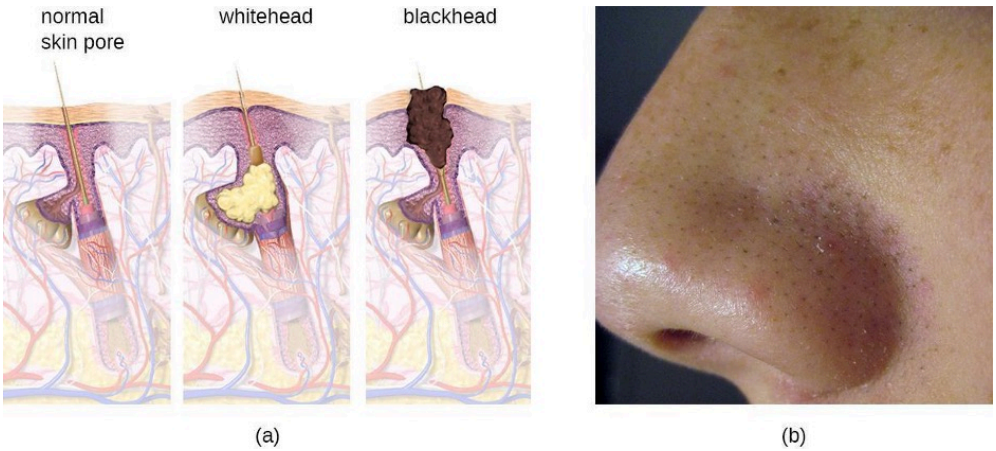


Figure 3.17: (a) Acne is characterized by whitehead and blackhead comedones that result from clogged hair follicles. (b) Blackheads, visible as black spots on the skin, have a dark appearance due to the oxidation of lipids in sebum via exposure to the air. *Figure description available at the end of the chapter.*

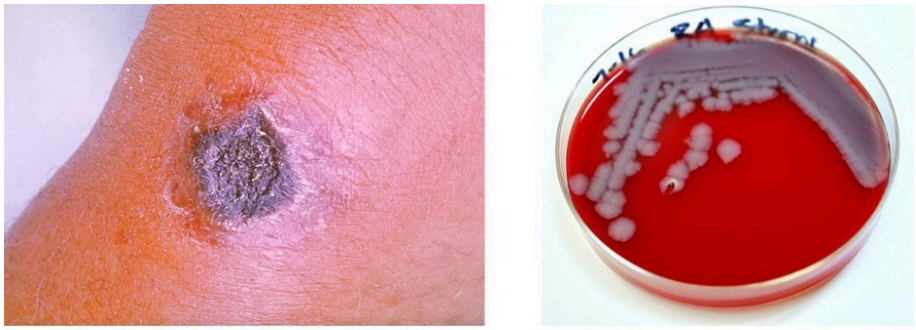
Often comedones lead to infection by *Propionibacterium acnes*, a gram-positive, non-spore-forming, aerotolerant anaerobic bacillus found on skin that consumes components of sebum. *P. acnes* secretes enzymes that damage the hair follicle, causing inflammatory lesions that may include papules, pustules, nodules, or pseudocysts, depending on their size and severity.

Treatment of acne depends on the severity of the case. There are multiple ways to grade acne severity, but three levels are usually considered based on the number of comedones, the number of inflammatory lesions, and the types of lesions. Mild acne is treated with topical agents that may include salicylic acid (which helps to remove old skin cells) or retinoids (which have multiple mechanisms, including the reduction of inflammation). Moderate acne may be treated with antibiotics (erythromycin, clindamycin), acne creams (e.g., benzoyl peroxide), and hormones. Severe acne may require treatment using strong medications such as isotretinoin (a retinoid that reduces oil buildup, among other effects, but that also has serious side effects such as photosensitivity). Other treatments, such as phototherapy and laser therapy to kill bacteria and possibly reduce oil production, are also sometimes used.

ANTHRAX

The zoonotic disease anthrax is caused by *Bacillus anthracis*, a gram-positive, endospore-forming, facultative anaerobe. Anthrax mainly affects animals such as sheep, goats, cattle, and deer, but can be found in humans as well. Sometimes called wool sorter's disease, it is often transmitted to humans through contact with infected animals or animal products, such as wool or hides. However, exposure to *B. anthracis* can occur by other means, as the endospores are widespread in soils and can survive for long periods of time, sometimes for hundreds of years.

The vast majority of anthrax cases (95–99%) occur when anthrax endospores enter the body through abrasions of the skin.[8] This form of the disease is called cutaneous anthrax. It is characterized by the formation of a nodule on the skin; the cells within the nodule die, forming a black eschar, a mass of dead skin tissue (figure 3.18). The localized infection can eventually lead to bacteremia and septicemia. If untreated, cutaneous anthrax can cause death in 20% of patients.[9] Once in the skin tissues, *B. anthracis* endospores germinate and produce a capsule, which prevents the bacteria from being phagocytized, and two binary exotoxins that cause edema and tissue damage. The first of the two exotoxins consists of a combination of protective antigen (PA) and an enzymatic lethal factor (LF), forming lethal toxin (LeTX). The second consists of protective antigen (PA) and an edema factor (EF), forming edema toxin (EdTX).



(a) (b)

Figure 3.18: (a) Cutaneous anthrax is an infection of the skin by B. anthracis, which produces tissue-damaging exotoxins. Dead tissues accumulating in this nodule have produced a small black eschar. (b) Colonies of B. anthracis grown on sheep's blood agar. *Figure description available at the end of the chapter.*

Less commonly, anthrax infections can be initiated through other portals of entry such as the digestive tract (gastrointestinal anthrax) or respiratory tract (pulmonary anthrax or inhalation anthrax). Typically, cases of noncutaneous anthrax are more difficult to treat than the cutaneous form. The mortality rate for gastrointestinal anthrax can be up to 40%, even with treatment. Inhalation anthrax, which occurs when anthrax spores are inhaled, initially causes influenza-like symptoms, but mortality rates are approximately 45% in treated individuals and 85% in those not treated. A relatively new form of the disease, injection anthrax, has been reported in Europe in intravenous drug users; it occurs when drugs are contaminated with *B. anthracis*. Patients with injection anthrax show signs and symptoms of severe soft tissue infection that differ clinically from cutaneous anthrax. This often delays diagnosis and treatment, and leads to a high mortality rate.[10]

B. anthracis colonies on blood agar have a rough texture and serrated edges that eventually form an undulating band (figure 3.18). Broad spectrum antibiotics such as penicillin, erythromycin, and tetracycline are often effective treatments.

Unfortunately, *B. anthracis* has been used as a biological weapon and remains on the United Nations' list of potential agents of bioterrorism.[11] Over a period of several months in 2001, a number of letters were mailed to members of the news media and the United States Congress. As a result, 11 individuals developed cutaneous anthrax and another 11 developed inhalation anthrax. Those infected included recipients of the letters, postal workers, and two other individuals. Five of those infected with pulmonary anthrax died. The anthrax spores had been carefully prepared to aerosolize, showing that the perpetrator had a high level of expertise in microbiology.[12]

A vaccine is available to protect individuals from anthrax. However, unlike most routine vaccines, the current anthrax vaccine is unique in both its formulation and the protocols dictating who receives it.[13] The vaccine is administered through five intramuscular injections over a period of 18 months, followed by annual boosters. The US Food and Drug Administration (FDA) has only approved administration of the vaccine prior to exposure for at-risk adults, such as individuals who work with anthrax in a laboratory, some individuals who handle animals or animal products (e.g., some veterinarians), and some members of the United States military. The vaccine protects against cutaneous and inhalation anthrax using cell-free filtrates of microaerophilic cultures of an avirulent, nonencapsulated strain of *B. anthracis*.[14] The FDA has not approved the vaccine for routine use *after* exposure to anthrax, but if there were ever an anthrax emergency in the United States, patients could be given anthrax vaccine after exposure to help prevent disease.

Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs
Acne	*Propionibacterium acnes*	Comedones (whiteheads, blackheads); papules, pustules, nodules, or pseudocysts	Not transmissible; clogged pores become infected by normal skin microbiota (*P. acnes*)	Erythromycin, clindamycin
Anthrax (cutaneous)	*Bacillus anthracis*	Eschar at site of infection; may lead to septicemia and can be fatal	Entry of *B. anthracis* endospores through cut or abrasion	Penicillin, erythromycin, or tetracycline
Cellulitis	*Streptococcus pyogenes*	Localized inflammation of dermis and hypodermis; skin red, warm, and painful to the touch	Entry of *S. pyogenes* through cut or abrasion	Oral or intravenous antibiotics (e.g., penicillin)

Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs
Erysipelas	*S. pyogenes*	Inflamed, swollen patch of skin, often on face; may be suppurative	Entry of *S. pyogenes* through cut or abrasion	Oral or intravenous antibiotics (e.g., penicillin)
Erythema nodosum	*S. pyogenes*	Small red nodules, often on shins	Associated with other streptococcal infection	None or anti-inflammatory drugs for severe cases
Impetigo	*Staphylococcus aureus, S. pyogenes*	Vesicles, pustules, and sometimes bullae around nose and mouth	Highly contagious, especially via contact	Topical or oral antibiotics.
Necrotizing fasciitis	*S. pyogenes, Klebsiella, Clostridium,* others	Infection of fascia and rapidly spreading tissue death; can lead to septic shock and death	Entry of bacteria through cut or abrasion	Intravenous broad-spectrum antibiotics
Otitis externa	*Pseudomonas aeruginosa*	Itching, redness, discomfort of ear canal, progressing to fever, pain, swelling	*P. aeruginosa* enters ear canal via pool or other water	Acidic ear drops with antibiotics, antifungals, steroids
Staphylococcal scalded skin syndrome (SSSS)	*S. aureus*	Erythema and severe peeling of skin	Infection of skin and mucous membranes, especially in children	Intravenous antibiotics, fluid therapy
Wound infections	*P. aeruginosa,* others	Formation of biofilm in or on wound	Exposure of wound to microbes in environment; poor wound hygiene	Polymyxin B, gentamicin, fluoroquinolones, topical anti-biofilm agents.

Table 3.3: Bacterial infections of the skin

BACTERIAL CONJUNCTIVITIS

Like the skin, the surface of the eye comes in contact with the outside world and is somewhat prone to infection by bacteria in the environment. Bacterial conjunctivitis (pinkeye) is a condition characterized by inflammation of the conjunctiva, often accompanied by a discharge of sticky fluid (described as acute purulent conjunctivitis) (figure 3.19). Conjunctivitis can affect one eye or both, and it usually does not affect vision permanently. Bacterial conjunctivitis is most commonly caused by *Haemophilus influenzae*, but can also be caused by other species such as *Moraxella catarrhalis*, *S. pneumoniae,* and *S. aureus*. The causative agent may be identified using bacterial cultures, Gram stain, and diagnostic biochemical, antigenic, or nucleic acid profile tests of the isolated pathogen. Bacterial conjunctivitis is very contagious, being transmitted via secretions from infected individuals, but it is also self-limiting. Bacterial conjunctivitis usually resolves in a few days, but topical antibiotics are sometimes prescribed. Because this condition is so contagious, medical attention is recommended whenever it is suspected. Indi-

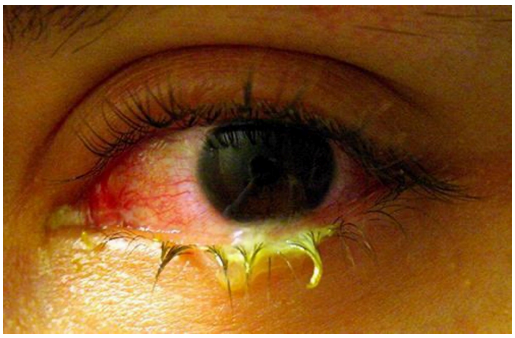


Figure 3.19: Acute, purulent, bacterial conjunctivitis causes swelling and redness in the conjunctiva, the membrane lining the whites of the eyes and the inner eyelids. It is often accompanied by a yellow, green, or white discharge, which can dry and become encrusted on the eyelashes. *Figure description available at the end of the chapter.*

viduals who use contact lenses should discontinue their use when conjunctivitis is suspected. Certain symptoms, such as blurred vision, eye pain, and light sensitivity, can be associated with serious conditions and require medical attention.

Neonatal Conjunctivitis

Newborns whose mothers have certain sexually transmitted infections are at risk of contracting ophthalmia neonatorum or inclusion conjunctivitis, which are two forms of neonatal conjunctivitis contracted through exposure to pathogens during passage through the birth canal. Gonococcal ophthalmia neonatorum is caused by *Neisseria gonorrhoeae*, the bacterium that causes the STD gonorrhea (figure 3.20). Inclusion (chlamydial) conjunctivitis is caused by *Chlamydia trachomatis*, the anaerobic, obligate, intracellular parasite that causes the STD chlamydia.

To prevent gonococcal ophthalmia neonatorum, silver nitrate ointments were once routinely applied to all infants' eyes shortly after birth; however, it is now more common to apply antibacterial creams or drops, such as erythromycin. Most hospitals are required by law to provide this preventative treatment to all infants, because conjunctivitis caused by *N. gonorrhoeae*, *C. trachomatis,* or other bacteria acquired during a vaginal delivery can have serious complications. If untreated, the infection can spread to the cornea, resulting in ulceration or perforation that can cause vision loss or even permanent blindness. As such, neonatal conjunctivitis is treated aggressively with oral or intravenous antibiotics to stop the spread of the infection. Causative agents of inclusion conjunctivitis may be identified using bacterial cultures, Gram stain, and diagnostic biochemical, antigenic, or nucleic acid profile tests.

TRACHOMA

Trachoma, or granular conjunctivitis, is a common cause of preventable blindness that is rare in the United States but widespread in developing countries, especially in Africa and Asia. The condition is caused by the same species that causes neonatal inclusion conjunctivitis in infants, *Chlamydia trachomatis*. *C. trachomatis* can be transmitted easily through fomites such as contaminated towels, bed linens, and clothing and also by direct contact with infected individuals. *C. trachomatis* can also be spread by flies that transfer infected mucous containing *C. trachomatis* from one human to another.

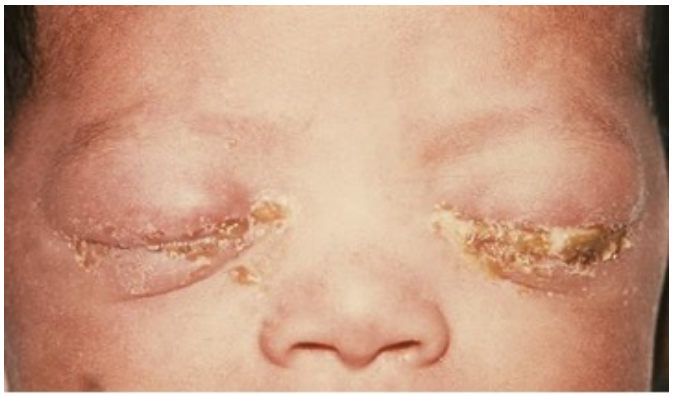


Figure 3.20: A newborn suffering from gonococcal ophthalmia neonatorum. Left untreated, purulent discharge can scar the cornea, causing loss of vision or permanent blindness. *Figure description available at the end of the chapter.*

Infection by *C. trachomatis* causes chronic conjunctivitis, which leads to the formation of necrotic follicles and scarring in the upper eyelid. The scars turn the eyelashes inward (a condition known as trichiasis) and mechanical abrasion of the cornea leads to blindness (figure 3.21). Antibiotics such as azithromycin are effective in treating trachoma, and outcomes are good when the disease is treated promptly. In areas where this disease is common, large public health efforts are focused on reducing transmission by teaching people how to avoid the risks of the infection.

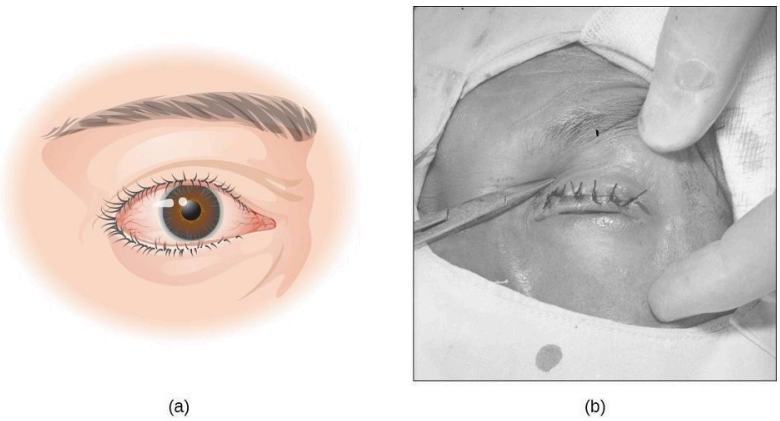


Figure 3.21: (a) If trachoma is not treated early with antibiotics, scarring on the eyelid can lead to trichiasis, a condition in which the eyelashes turn inward. (b) Trichiasis leads to blindness if not corrected by surgery, as shown here. *Figure description available at the end of the chapter.*

BACTERIAL KERATITIS

Keratitis can have many causes, but bacterial keratitis is most frequently caused by *Staphylococcus epidermidis* and/or *Pseudomonas aeruginosa*. Contact lens users are particularly at risk for such an infection because *S. epidermidis* and *P. aeruginosa* both adhere well to the surface of the lenses. Risk of infection can be greatly reduced by proper care of contact lenses and avoiding wearing lenses overnight. Because the infection can quickly lead to blindness, prompt and aggressive treatment with antibiotics is important. The causative agent may be identified using bacterial cultures, Gram stain, and diagnostic biochemical, antigenic, or nucleic acid profile tests of the isolated pathogen.

BIOFILMS AND INFECTIONS OF THE SKIN AND EYES

When treating bacterial infections of the skin and eyes, it is important to consider that few such infections can be attributed to a single pathogen. While biofilms may develop in other parts of the body, they are especially relevant to skin infections (such as those caused by *S. aureus* or *P. aeruginosa*) because of their prevalence in chronic skin wounds. Biofilms develop when bacteria (and sometimes fungi) attach to a surface and produce extracellular polymeric substances (EPS) in which cells of multiple organisms may be embedded. When a biofilm develops on a wound, it may interfere with the natural healing process as well as diagnosis and treatment.

Because biofilms vary in composition and are difficult to replicate in the lab, they are still not thoroughly understood. The extracellular matrix of a biofilm consists of polymers such as polysaccharides, extracellular DNA, proteins, and lipids, but the exact makeup varies. The organisms living within the extracellular matrix may include familiar pathogens as well as other bacteria that do not grow well in cultures (such as numerous obligate anaerobes). This presents challenges when culturing samples from infections that involve a biofilm. Because only some species grow *in vitro*, the culture may contain only a subset of the bacterial species involved in the infection.

Biofilms confer many advantages to the resident bacteria. For example, biofilms can facilitate attachment to surfaces on or in the host organism (such as wounds), inhibit phagocytosis, prevent the invasion of neutrophils, and sequester host antibodies. Additionally, biofilms can provide a level of antibiotic resistance not found in the isolated cells and colonies that are typical of laboratory cultures. The extracellular matrix provides a physical

barrier to antibiotics, shielding the target cells from exposure. Moreover, cells within a biofilm may differentiate to create subpopulations of dormant cells called persister cells. Nutrient limitations deep within a biofilm add another level of resistance, as stress responses can slow metabolism and increase drug resistance.

Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs
Acute bacterial conjunctivitis	*Haemophilus influenza*	Inflammation of conjunctiva with purulent discharge	Exposure to secretions from infected individuals	Broad-spectrum topical antibiotics
Bacterial keratitis	*Staphylococcus epidermidis, Pseudomonas aeruginosa*	Redness and irritation of eye, blurred vision, sensitivity to light; progressive corneal scarring, which can lead to blindness	Exposure to pathogens on contaminated contact lenses	Antibiotic eye drops (e.g., with fluoroquinolones)
Neonatal conjunctivitis	*Chlamydia trachomatis, Neisseria gonorrhoeae*	Inflammation of conjunctiva, purulent discharge, scarring and perforation of cornea; may lead to blindness	Neonate exposed to pathogens in birth canal of a pregnant person with chlamydia or gonorrhea	Erythromycin
Trachoma (granular conjunctivitis)	*C. trachomatis*	Chronic conjunctivitis, trichiasis, scarring, blindness	Contact with infected individuals or contaminated fomites; transmission by eye-seeking flies	Azithromycin

Table 3.4: Bacterial infections of the eye

3.3 VIRAL INFECTIONS OF THE SKIN AND EYES

Until recently, it was thought that the normal microbiota of the body consisted primarily of bacteria and some fungi. However, in addition to bacteria, the skin is colonized by viruses, and recent studies suggest that the viral families Papillomaviridae, Polyomaviridae and Circoviridae also contribute to the normal skin microbiota. However, some viruses associated with skin are pathogenic, and these viruses can cause diseases with a wide variety of presentations; common viral infections are summarized in table 3.5.

Numerous types of viral infections cause rashes or lesions on the skin; however, in many cases these skin conditions result from infections that originate in other body systems. In this section, we will limit the discussion to viral skin infections that use the skin as a portal of entry. Later chapters will discuss viral infections such as chickenpox, measles, and rubella—diseases that cause skin rashes but invade the body through portals of entry other than the skin.

PAPILLOMAS

Papillomas (warts) are the expression of common skin infections by human papillomavirus (HPV) and are transmitted by direct contact. There are many types of HPV, and they lead to a variety of different presentations, such as common warts, plantar warts, flat warts, and filiform warts. HPV can also cause sexually-transmitted genital warts. Vaccination is available for some strains of HPV.

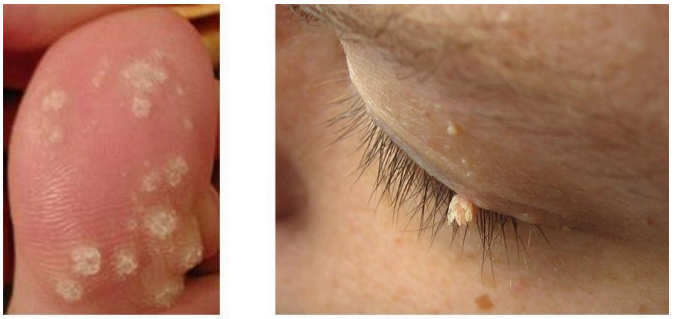


Figure 3.22: Warts can vary in shape and in location. (a) Multiple plantar warts have grown on this toe. (b) A filiform wart has grown on this eyelid. Figure description available at the end of the chapter.

Common warts tend to develop on fingers, the backs of hands, and around nails in areas with broken skin. In contrast, plantar warts (also called foot warts) develop on the sole of the foot and can grow inwards, causing pain and pressure during walking. Flat warts can develop anywhere on the body, are often numerous, and are relatively smooth and small compared with other wart types. Filiform warts are long, threadlike warts that grow quickly.

In some cases, the immune system may be strong enough to prevent warts from forming or to eradicate established warts. However, treatment of established warts is typically required. There are many available treatments for warts, and their effectiveness varies. Common warts can be frozen off with liquid nitrogen. Topical applications of salicylic acid may also be effective. Other options are electrosurgery (burning), curettage (cutting), excision, painting with cantharidin (which causes the wart to die so it can more easily be removed), laser treatments, treatment with bleomycin, chemical peels, and immunotherapy (figure 3.22).

ORAL HERPES

Another common skin virus is herpes simplex virus (HSV). HSV has historically been divided into two types, HSV-1 and HSV-2. HSV-1 is typically transmitted by direct oral contact between individuals, and is usually associated with oral herpes. HSV-2 is usually transmitted sexually and is typically associated with genital herpes. However, both HSV-1 and HSV-2 are capable of infecting any mucous membrane, and the incidence of genital HSV-1 and oral HSV-2 infections has been increasing in recent years. In this section, we will limit our discussion to infections caused by HSV-1; HSV-2 and genital herpes will be discussed in section 7.3.

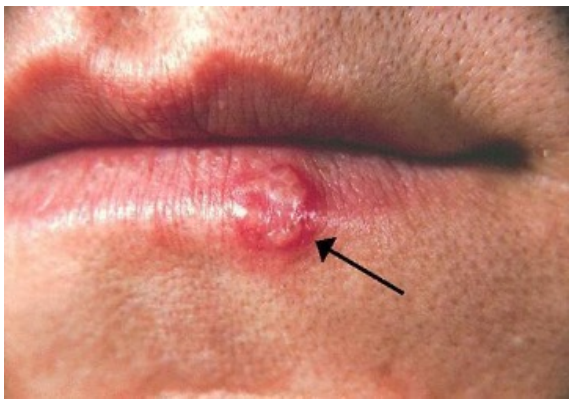


Figure 3.23: This cold sore was caused by HSV-1. Figure description available at the end of the chapter.

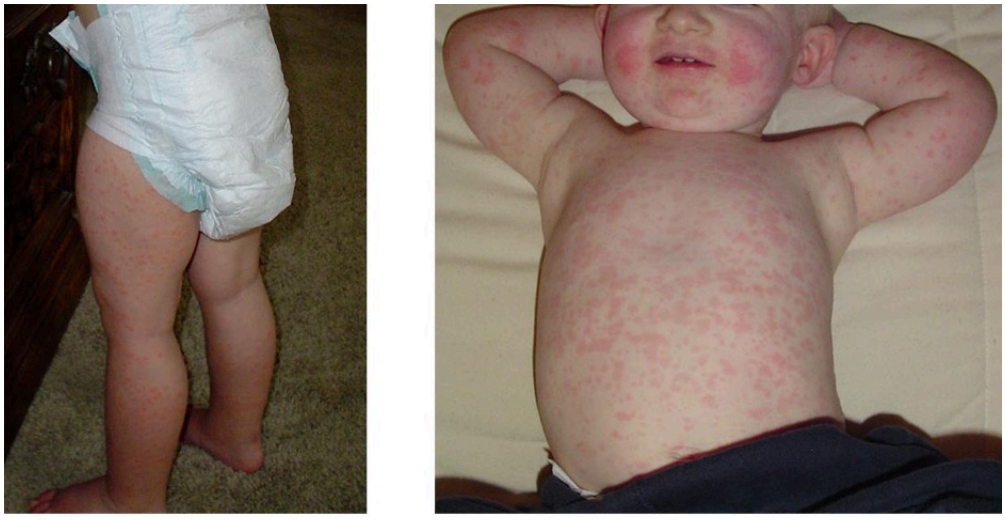
Infection by HSV-1 commonly manifests as cold sores or fever blisters, usually on or around the lips (figure 3.23). HSV-1 is highly contagious, with some studies suggesting that up to 65% of the US population is infected; however, many infected individuals are asymptomatic.[15] Moreover, the virus can be latent for long periods, residing in the trigeminal nerve ganglia between recurring bouts of symptoms. Recurrence can be triggered by stress or environmental conditions (systemic or affecting the skin). When lesions are present, they may blister, break open, and crust. The virus can be spread through direct contact, even when a patient is asymptomatic.

While the lips, mouth, and face are the most common sites for HSV-1 infections, lesions can spread to other areas of the body. Wrestlers and other athletes involved in contact sports may develop lesions on the neck, shoulders, and trunk. This condition is often called herpes gladiatorum. Herpes lesions that develop on the fingers are often called herpetic whitlow.

HSV-1 infections are commonly diagnosed from their appearance, although laboratory testing can confirm the diagnosis. There is no cure, but antiviral medications such as acyclovir, penciclovir, famciclovir, and valacyclovir are used to reduce symptoms and risk of transmission. Topical medications, such as creams with *n*-docosanol and penciclovir, can also be used to reduce symptoms such as itching, burning, and tingling.

ROSEOLA AND FIFTH DISEASE

The viral diseases roseola and fifth disease are somewhat similar in terms of their presentation, but they are caused by different viruses. Roseola, sometimes called roseola infantum or exanthem subitum ("sudden rash"), is a mild viral infection usually caused by human herpesvirus-6 (HHV-6) and occasionally by HHV-7. It is spread via direct contact with the saliva or respiratory secretions of an infected individual, often through droplet aerosols. Roseola is very common in children, with symptoms including a runny nose, a sore throat, and a cough, along with (or followed by) a high fever (39.4 °C). About three to five days after the fever subsides, a rash may begin to appear on the chest and abdomen. The rash, which does not cause discomfort, initially forms characteristic macules that are flat or papules that are firm and slightly raised; some macules or papules may be surrounded by a white ring. The rash may eventually spread to the neck and arms, and sometimes continues to spread to the face and legs. The diagnosis is generally made based upon observation of the symptoms. However, it is possible to perform serological tests to confirm the diagnosis. While treatment may be recommended to control the fever, the disease usually resolves without treatment within a week after the fever develops. For individuals at particular risk, such as those who are immunocompromised, the antiviral medication ganciclovir may be used.



(a) (b)

Figure 3.24: (a) Roseola, a mild viral infection common in young children, generally begins with symptoms similar to a cold, followed by a pink, patchy rash that starts on the trunk and spreads outward. (b) Fifth disease exhibits similar symptoms in children, except for the distinctive "slapped cheek" rash that originates on the face. *Figure description available at the end of the chapter.*

Fifth disease (also known as erythema infectiosum) is another common, highly contagious illness that causes a distinct rash that is critical to diagnosis. Fifth disease is caused by parvovirus B19, and is transmitted by contact with respiratory secretions from an infected individual. Infection is more common in children than adults. While approximately 20% of individuals will be asymptomatic during infection,[16] others will exhibit cold-like symptoms (headache, fever, and upset stomach) during the early stages when the illness is most infectious. Several days later, a distinct red facial rash appears, often called "slapped cheek" rash (figure 3.24). Within a few days, a second rash may appear on the arms, legs, chest, back, or buttocks. The rash may come and go for several weeks, but usually disappears within seven to twenty-one days, gradually becoming lacy in appearance as it recedes.

In children, the disease usually resolves on its own without medical treatment beyond symptom relief as needed. Adults may experience different and possibly more serious symptoms. Many adults with fifth disease do not develop any rash, but may experience joint pain and swelling that lasts several weeks or months. Immunocompromised individuals can develop severe anemia and may need blood transfusions or immune globulin injections. While the rash is the most important component of diagnosis (especially in children), the symptoms of fifth disease are not always consistent. Serological testing can be conducted for confirmation.

VIRAL CONJUNCTIVITIS

Like bacterial conjunctivitis, viral infections of the eye can cause inflammation of the conjunctiva and discharge from the eye. However, viral conjunctivitis tends to produce a discharge that is more watery than the thick discharge associated with bacterial conjunctivitis. The infection is contagious and can easily spread from one eye to the other or to other individuals through contact with eye discharge.

Viral conjunctivitis is commonly associated with colds caused by adenoviruses; however, other viruses can also cause conjunctivitis. If the causative agent is uncertain, eye discharge can be tested to aid in diagnosis. Antibiotic treatment of viral conjunctivitis is ineffective, and symptoms usually resolve without treatment within a week or two.

Herpes Keratitis

Herpes infections caused by HSV-1 can sometimes spread to the eye from other areas of the body, which may result in keratoconjunctivitis. This condition, generally called herpes keratitis or herpetic keratitis, affects the conjunctiva and cornea, causing irritation, excess tears, and sensitivity to light. Deep lesions in the cornea may eventually form, leading to blindness. Because keratitis can have numerous causes, laboratory testing is necessary to confirm the diagnosis when HSV-1 is suspected; once confirmed, antiviral medications may be prescribed.

Disease	Pathogen	Signs and Symptoms	Tranmission	Antimicrobial Drugs
Fifth disease	Parvovirus B19	May have initial cold-like symptoms; "slapped cheek" rash	Highly contagious via respiratory secretions of infected individuals	None
Herpes keratitis	Herpes simplex virus 1 (HSV-1)	Inflammation of conjunctiva and cornea; irritation, excess tears, sensitivity to light; lesions in cornea leading to blindness	Direct eye contact with discharge from herpes lesions elsewhere in the body or from another infected individual	Acyclovir, ganciclovir, famiclovir, valacyclovir
Oral herpes	Herpes simplex virus 1 (HSV-1)	May cause initial systemic symptoms; cold sores	Highly contagious via direct contact with infected individuals	Acyclovir, penciclovir, famiclovir, valacyclovir
Papillomas	Human papillomavirus (HPV)	Common warts, plantar warts, flat warts, filiform warts, and others	Contact with infected individuals	Topical salicylic acid, cantharidin

Disease	Pathogen	Signs and Symptoms	Tranmission	Antimicrobial Drugs
Roseola (roseola infantum, exanthem subitum)	Human herpesvirus 6 (HHV-6), human herpesvirus 7 (HHV-7)	Initial cold-like symptoms with high fever, followed by a macular rash three to five days later	Spread by viral and respiratory secretions of infected individuals	Typically none; ganciclovir for immunocompromised patients
Viral conjunctivitis	Adenoviruses and others	Inflammation of the conjunctiva; watery, nonpurulent discharge	Associated with common cold; contagious via contact with eye discharge	None

Table 3.5: Viral infections of the skin and eyes

3.4 MYCOSES OF THE SKIN

Many fungal infections of the skin involve fungi that are found in the normal skin microbiota. Some of these fungi can cause infection when they gain entry through a wound; others mainly cause opportunistic infections in immunocompromised patients. Other fungal pathogens primarily cause infection in unusually moist environments that promote fungal growth; for example, sweaty shoes, communal showers, and locker rooms provide excellent breeding grounds that promote the growth and transmission of fungal pathogens.

Fungal infections, also called mycoses, can be divided into classes based on their invasiveness. Mycoses that cause superficial infections of the epidermis, hair, and nails, are called cutaneous mycoses. Mycoses that penetrate the epidermis and the dermis to infect deeper tissues are called subcutaneous mycoses. Mycoses that spread throughout the body are called systemic mycoses; common skin mycoses are summaries in table 3.7.

TINEAS

A group of cutaneous mycoses called tineas are caused by dermatophytes, fungal molds that require keratin, a protein found in skin, hair, and nails, for growth. There are three genera of dermatophytes, all of which can cause cutaneous mycoses: *Trichophyton*, *Epidermophyton*, and *Microsporum*. Tineas on most areas of the body are generally called ringworm, but tineas in specific locations may have distinctive names and symptoms (see table 3.6 and figure 3.25). Keep in mind that these names—even though they are Latinized—refer to locations on the body, not causative organisms. Tineas can be caused by different dermatophytes in most areas of the body.

Common tineas	Location
Tinea corporis (ringworm)	Body
Tinea capitis (ringworm)	Scalp
Tinea pedis (athlete's foot)	Feet
Tinea barbae (barber's itch)	Beard
Tinea cruris (jock itch)	Groin
Tinea unguium (onychomycosis)	Toenails, fingernails

Table 3.6: Some common tineas and location on the body

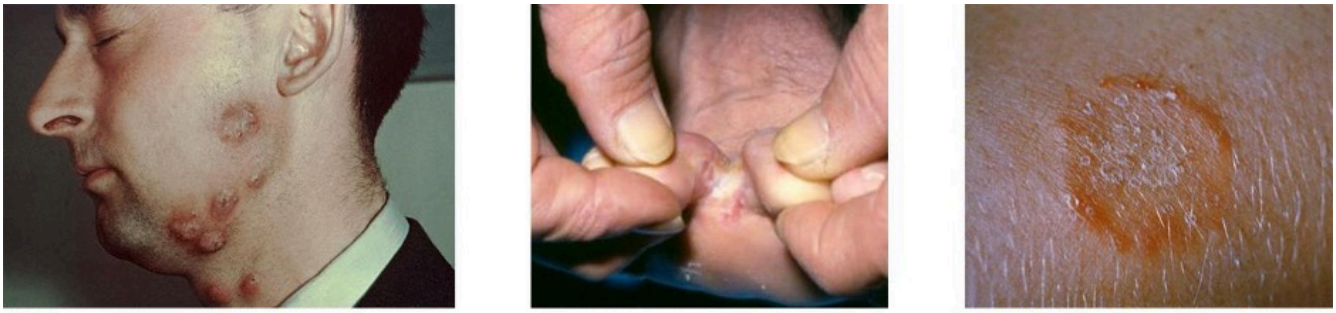


Figure 3.25: Tineas are superficial cutaneous mycoses and are common. (a) Tinea barbae (barber's itch) occurs on the lower face. (b) Tinea pedis (athlete's foot) occurs on the feet, causing itching, burning, and dry, cracked skin between the toes. (c) A close-up view of tinea corporis (ringworm) caused by Trichophyton mentagrophytes. Used under fair use. *Figure description available at the end of the chapter.*

Dermatophytes are commonly found in the environment and in soils and are frequently transferred to the skin via contact with other humans and animals. Fungal spores can also spread on hair. Many dermatophytes grow well in moist, dark environments. For example, tinea pedis (athlete's foot) commonly spreads in public showers, and the causative fungi grow well in the dark, moist confines of sweaty shoes and socks. Likewise, tinea cruris (jock itch) often spreads in communal living environments and thrives in warm, moist undergarments.

Tineas on the body (tinea corporis) often produce lesions that grow radially and heal towards the center. This causes the formation of a red ring, leading to the misleading name of ringworm.

Several approaches may be used to diagnose tineas. A Wood's lamp (also called a black lamp) with a wavelength of 365 nm is often used. When directed on a tinea, the ultraviolet light emitted from the Wood's lamp causes the fungal elements (spores and hyphae) to fluoresce. Direct microscopic evaluation of specimens from skin scrapings, hair, or nails can also be used to detect fungi. Generally, these specimens are prepared in a wet mount using a potassium hydroxide solution (10%–20% aqueous KOH), which dissolves the keratin in hair, nails, and skin cells to allow for visualization of the hyphae and fungal spores. The specimens may be grown on Sabouraud dextrose CC (chloramphenicol/cycloheximide), a selective agar that supports dermatophyte growth while inhibiting the growth of bacteria and saprophytic fungi (figure 3.26). Macroscopic colony morphology is often used to initially identify the genus of the dermatophyte; identification can be further confirmed by visualizing the microscopic morphology using either a slide culture or a sticky tape prep stained with lactophenol cotton blue.

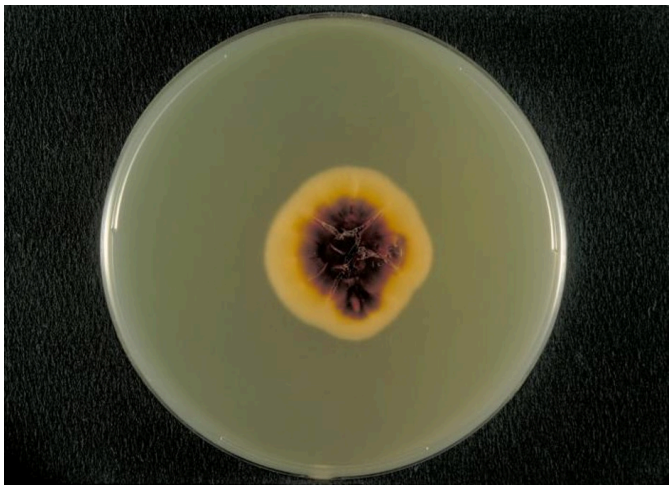


Figure 3.26: To diagnose tineas, the dermatophytes may be grown on a Sabouraud dextrose CC agar plate. This culture contains a strain of Trichophyton rubrum, one of the most common causes of tineas on various parts of the body. *Figure description available at the end of the chapter.*

Various antifungal treatments can be effective against tineas. Allylamine ointments that include terbinafine are commonly used; miconazole and clotrimazole are also available for topical treatment, and griseofulvin is used orally.

CUTANEOUS ASPERGILLOSIS

Another cause of cutaneous mycoses is *Aspergillus*, a genus consisting of molds of many different species, some of which cause a condition called aspergillosis. Primary cutaneous aspergillosis, in which the infection begins in the skin, is rare but does occur. More common is secondary cutaneous aspergillosis, in which the infection begins in the respiratory system and disseminates systemically. Both primary and secondary cutaneous aspergillosis result in distinctive eschars that form at the site or sites of infection (figure 3.27). Pulmonary aspergillosis will be discussed more thoroughly in section 5.4.

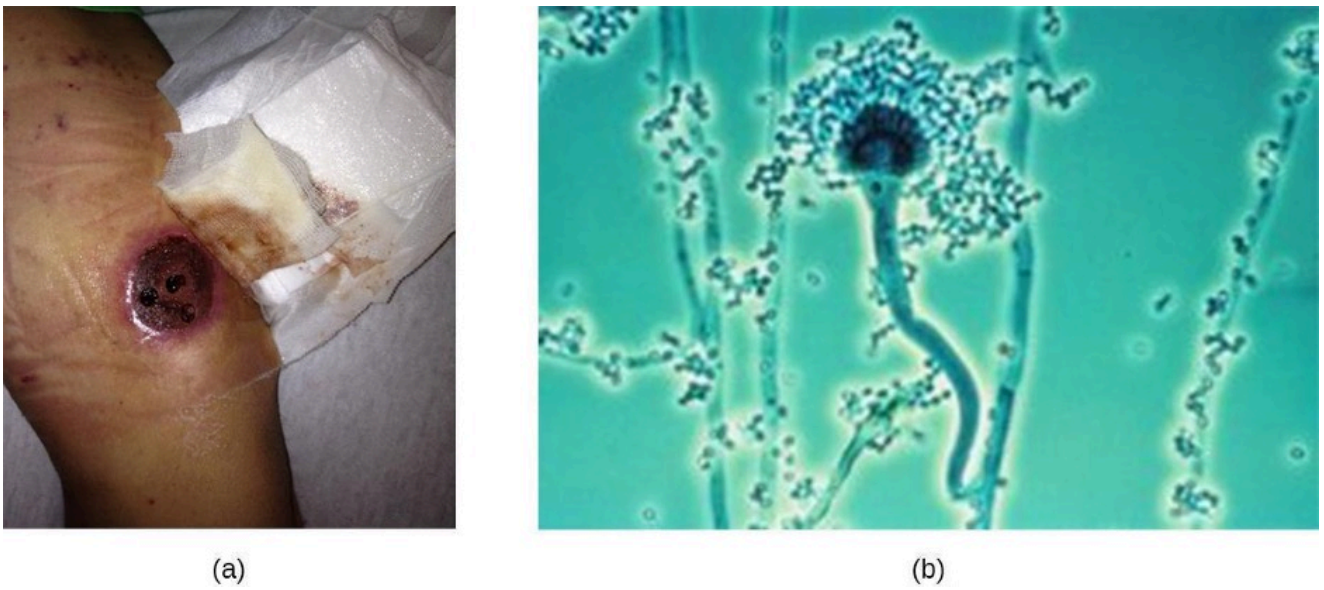


Figure 3.27: (a) Eschar on a patient with secondary cutaneous aspergillosis. (b) Micrograph showing a conidiophore of Aspergillus.
Figure description available at the end of the chapter.

Primary cutaneous aspergillosis usually occurs at the site of an injury and is most often caused by *Aspergillus fumigatus* or *Aspergillus flavus*. It is usually reported in patients who have had an injury while working in an agricultural or outdoor environment. However, opportunistic infections can also occur in healthcare settings, often at the site of intravenous catheters, venipuncture wounds, or in association with burns, surgical wounds, or occlusive dressing. After candidiasis, aspergillosis is the second most common hospital-acquired fungal infection and often occurs in immunocompromised patients, who are more vulnerable to opportunistic infections.

Cutaneous aspergillosis is diagnosed using patient history, culturing, histopathology using a skin biopsy. Treatment involves the use of antifungal medications such as voriconazole (preferred for invasive aspergillosis), itraconazole, and amphotericin B if itraconazole is not effective. For immunosuppressed individuals or burn patients, medication may be used and surgical or immunotherapy treatments may be needed.

CANDIDIASIS OF THE SKIN AND NAILS

Candida albicans and other yeasts in the genus *Candida* can cause skin infections referred to as cutaneous candidiasis. *Candida* spp. are sometimes responsible for intertrigo, a general term for a rash that occurs in a skin fold, or other localized rashes on the skin. *Candida* can also infect the nails, causing them to become yellow and harden (figure 3.28).

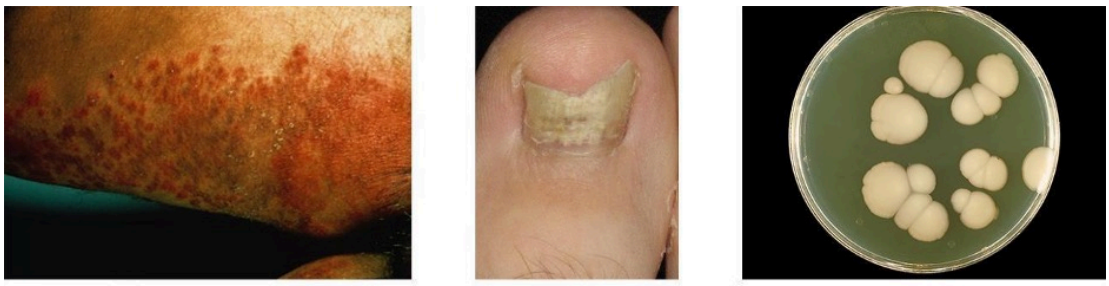


Figure 3.28: (a) This red, itchy rash is the result of cutaneous candidiasis, an opportunistic infection of the skin caused by the yeast Candida albicans. (b) Fungal infections of the nail (tinea unguium) can be caused by dermatophytes or Candida spp. The nail becomes yellow, brittle, and prone to breaking. This condition is relatively common among adults. (c) C. albicans growing on Sabouraud dextrose agar. *Figure description available at the end of the chapter.*

Candidiasis of the skin and nails is diagnosed through clinical observation and through culture, Gram stain, and KOH wet mounts. Susceptibility testing for antifungal agents can also be done. Cutaneous candidiasis can be treated with topical or systemic azole antifungal medications. Because candidiasis can become invasive, patients suffering from HIV/AIDS, cancer, or other conditions that compromise the immune system may benefit from preventive treatment. Azoles, such as clotrimazole, econazole, fluconazole, ketoconazole, and miconazole as well as the antifungal agents nystatin, terbinafine, and naftifine may be used for treatment. Long-term treatment with medications such as itraconazole or ketoconazole may be used for chronic infections. Repeat infections often occur, but this risk can be reduced by carefully following treatment recommendations, avoiding excessive moisture, maintaining good health, practicing good hygiene, and having appropriate clothing (including footwear).

Candida also causes infections in other parts of the body besides the skin. These include vaginal yeast infections (see section 7.5) and oral thrush (see section 4.2).

SPOROTRICHOSIS

Whereas cutaneous mycoses are superficial, subcutaneous mycoses can spread from the skin to deeper tissues. In temperate regions, the most common subcutaneous mycosis is a condition called sporotrichosis, caused by the fungus *Sporothrix schenkii* and commonly known as **rose gardener's disease**, or **rose thorn disease**. Sporotrichosis is often contracted after working with soil, plants, or timber, as the fungus can gain entry through a small wound such as a thorn-prick or splinter. Sporotrichosis can generally be avoided by wearing gloves and protective clothing while gardening and promptly cleaning and disinfecting any wounds sustained during outdoor activities.

Sporothrix infections initially present as small ulcers in the skin, but the fungus can spread to the lymphatic system and sometimes beyond. When the infection spreads, nodules appear, become necrotic, and may ulcerate. As more lymph nodes become affected, abscesses and ulceration may develop over a larger area (often on one arm or hand). In severe cases, the infection may spread more widely throughout the body, although this is relatively uncommon.

Sporothrix infection can be diagnosed based upon histologic examination of the affected tissue. Its macroscopic morphology can be observed by culturing the mold on potato dextrose agar, and its microscopic morphology can be observed by staining a slide culture with lactophenol cotton blue. Treatment with itraconazole is generally recommended.

Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs
Aspergillosis (cutaneous)	*Aspergillus fumigatus, Aspergillus flavus*	Distinctive eschars at site(s) of infection	Entry via wound (primary cutaneous aspergillosis) or via the respiratory system (secondary cutaneous aspergillosis); commonly a hospital-acquired infection	Itraconazole, voriconazole, amphotericin B
Candidiasis (cutaneous)	*Candida albicans*	Intertrigo, localized rash, yellowing of nails	Opportunistic infections in immunocompromised patients	Azoles
Sporotrichosis (rose gardener's disease)	*Sporothrix schenkii*	Subcutaneous ulcers and abscesses; may spread to a large area, e.g., hand or arm	Entry via thorn prick or other wound	Itraconazole
Tineas	*Trichophyton* spp., *Epidermophyton* spp., *Microsporum* spp.	Itchy, ring-like lesions (ringworm) at sites of infection	Contact with dermatophytic fungi, especially in warm, moist environments conducive to fungal growth	Terbinafine, miconazole, clotrimazole, griseofulvin

Table 3.7: Mycoses of the skin

3.5 PROTOZOAN AND HELMINTHIC INFECTIONS OF THE SKIN AND EYES

Many parasitic protozoans and helminths use the skin or eyes as a portal of entry. Some may physically burrow into the skin or the mucosa of the eye; others breach the skin barrier by means of an insect bite. Still others take advantage of a wound to bypass the skin barrier and enter the body, much like other opportunistic pathogens. Although many parasites enter the body through the skin, in this chapter we will limit our discussion to those for which the skin or eyes are the primary site of infection. Parasites that enter through the skin but travel to a different site of infection will be covered in other chapters. In addition, we will limit our discussion to microscopic parasitic infections of the skin and eyes. Macroscopic parasites such as lice, scabies, mites, and ticks are beyond the scope of this text.

ACANTHAMOEBA INFECTIONS

Acanthamoeba is a genus of free-living protozoan amoebae that are common in soils and unchlorinated bodies of freshwater. (This is one reason why some swimming pools are treated with chlorine.) The genus contains a few parasitic species, some of which can cause infections of the eyes, skin, and nervous system. Such infections can sometimes travel and affect other body systems. Skin infections may manifest as abscesses, ulcers, and nodules. When acanthamoebae infect the eye, causing inflammation of the cornea, the condition is called *Acanthamoeba* keratitis. Figure 3.29 illustrates the *Acanthamoeba* life cycle and various modes of infection.

While *Acanthamoeba* keratitis is initially mild, it can lead to severe corneal damage, vision impairment, or even blindness if left untreated. Similar to eye infections involving *P. aeruginosa, Acanthamoeba* poses a much greater risk to wearers of contact lenses because the amoeba can thrive in the space between contact lenses and the cornea. Prevention through proper contact lens care is important. Lenses should always be properly disinfected prior to use, and should never be worn while swimming or using a hot tub.

Acanthamoeba can also enter the body through other pathways, including skin wounds and the respiratory tract. It usually does not cause disease except in immunocompromised individuals; however, in rare cases, the infection can spread to the nervous system, resulting in a usually fatal condition called granulomatous amoebic encephalitis (GAE). Disseminated infections, lesions, and *Acanthamoeba* keratitis can be diagnosed by observing symptoms and examining patient samples under the microscope to view the parasite. Skin biopsies may be used.

Acanthamoeba keratitis is difficult to treat, and prompt treatment is necessary to prevent the condition from progressing. The condition generally requires three to four weeks of intensive treatment to resolve. Common treatments include topical antiseptics (e.g., polyhexamethylene biguanide, chlorhexidine, or both), sometimes with painkillers or corticosteroids (although the latter are controversial because they suppress the immune system, which can worsen the infection). Azoles are sometimes prescribed as well. Advanced cases of keratitis may require a corneal transplant to prevent blindness.

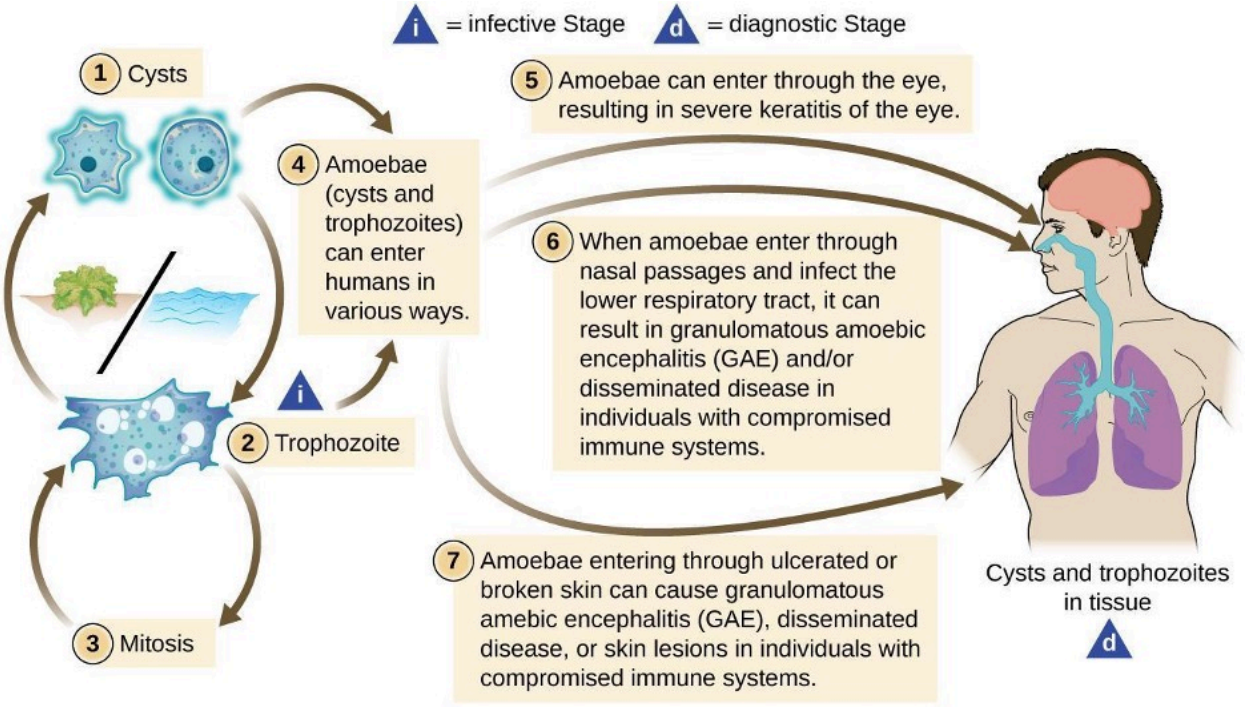


Figure 3.29: Acanthamoeba spp. are waterborne parasites very common in unchlorinated aqueous environments. As shown in this life cycle, Acanthamoeba cysts and trophozoites are both capable of entering the body through various routes, causing infections of the eye, skin, and central nervous system. *Figure description available at the end of the chapter.*

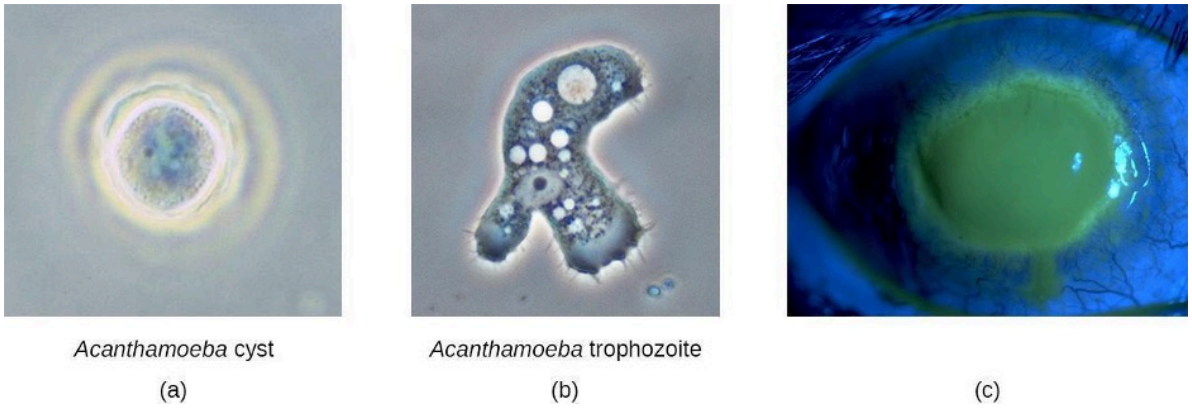


Figure 3.30: (a) An Acanthamoeba cyst. (b) An Acanthamoeba trophozoite. (c) The eye of a patient with Acanthamoeba keratitis. The fluorescent color, which is due to sodium fluorescein application, highlights significant damage to the cornea and vascularization of the surrounding conjunctiva. *Figure description available at the end of the chapter.*

LOIASIS

The helminth *Loa loa*, also known as the African eye worm, is a nematode that can cause loiasis, a disease endemic to West and Central Africa (figure 3.31 and table 3.8). The disease does not occur outside that region except when carried by travelers. There is evidence that individual genetic differences affect susceptibility to developing loiasis after infection by the *Loa loa* worm. Even in areas in which *Loa loa* worms are common, the disease is generally found in less than 30% of the population.[17] It has been suggested that travelers who spend time in the region may be somewhat more susceptible to developing symptoms than the native population, and the presentation of infection may differ.[18]

The parasite is spread by deer flies (genus *Chrysops*), which can ingest the larvae from an infected human via a blood meal (figure 3.31 and table 3.8). When the deer fly bites other humans, it deposits the larvae into their bloodstreams. After about five months in the human body, some larvae develop into adult worms, which can grow to several centimeters in length and live for years in the subcutaneous tissue of the host.

The name "eye worm" alludes to the visible migration of worms across the conjunctiva of the eye. Adult worms live in the subcutaneous tissues and can travel at about 1 cm per hour. They can often be observed when migrating through the eye, and sometimes under the skin; in fact, this is generally how the disease is diagnosed. It is also possible to test for antibodies, but the presence of antibodies does not necessarily indicate a current infection; it only means that the individual was exposed at some time. Some patients are asymptomatic, but in others the migrating worms can cause fever and areas of allergic inflammation known as **Calabar swellings**. Worms migrating through the conjunctiva can cause temporary eye pain and itching, but generally there is no lasting damage to the eye. Some patients experience a range of other symptoms, such as widespread itching, hives, and joint and muscle pain.

Worms can be surgically removed from the eye or the skin, but this treatment only relieves discomfort; it does not cure the infection, which involves many worms. The preferred treatment is diethylcarbamazine, but this medication produces severe side effects in some individuals, such as brain inflammation and possible death in patients with heavy infections. Albendazole is also sometimes used if diethylcarbamazine is not appropriate or not successful. If left untreated for many years, loiasis can damage the kidneys, heart, and lungs, though these symptoms are rare.

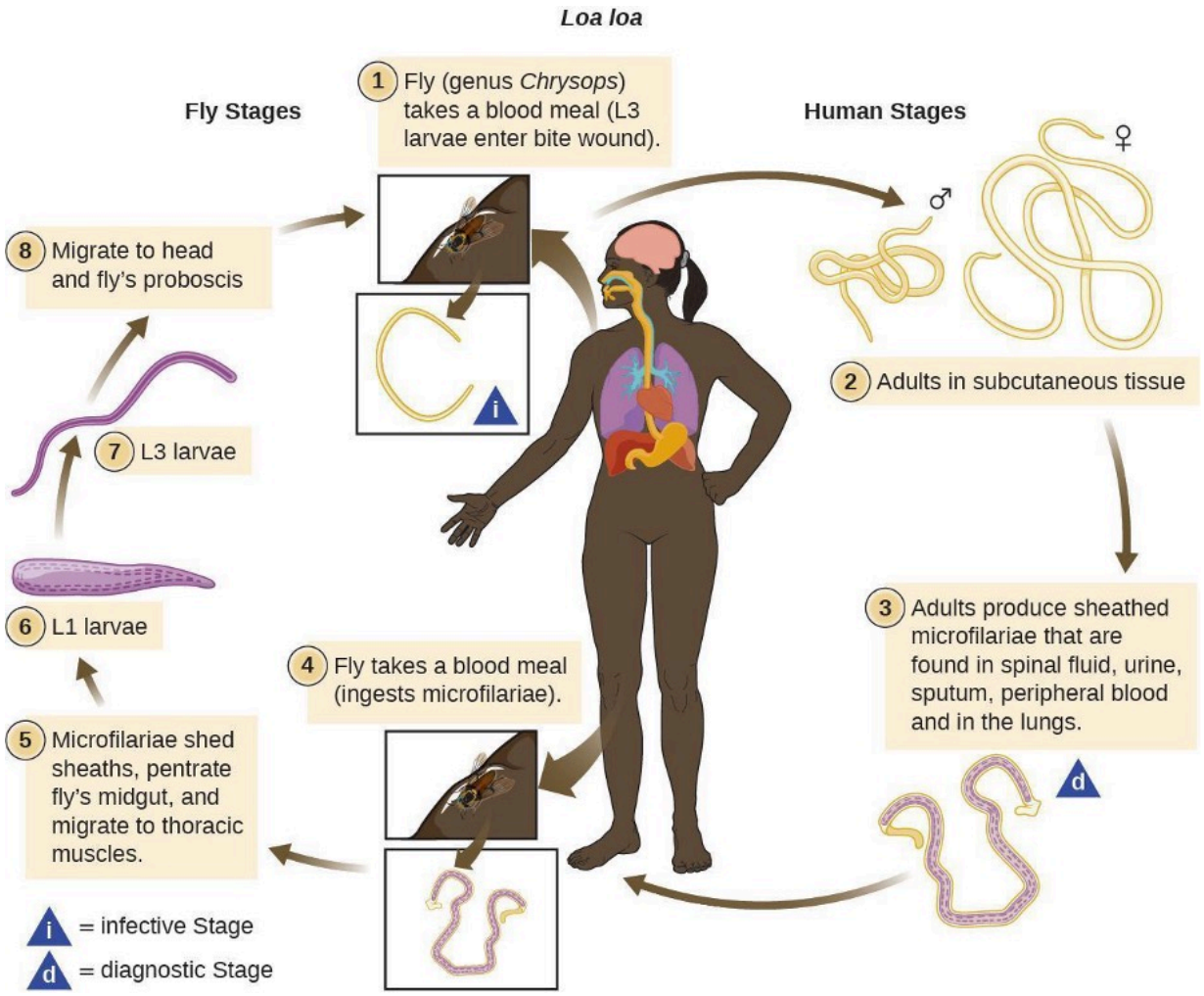


Figure 3.31: This Loa loa worm, measuring about 55 mm long, was extracted from the conjunctiva of a patient with loiasis. The Loa loa has a complex life cycle. Biting deer flies native to the rain forests of Central and West Africa transmit the larvae between humans. *Figure description available at the end of the chapter.*

Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs
Acanthamoeba keratis	*Acanthamoeba*	Inflammation and damage to cornea; vision impairment or blindness	Exposure to pathogens in contaminated water or on contact lenses	Polyhexamethylene biguanide, chlorhexidine, azoles
Loiasis	*Loa loa*	Recurring fever and localized Calabar swelling, itching, and skin or eye pain during subcutaneous migration of worms	Larvae transmitted between humans by deerfly vector	Diethylcarbamazine, albendazole

Table 3.8: Parasitic skin and eye infections

SUMMARY

The following is a summary of the material covered throughout the chapter. It summarizes key aspects from each section and the pathogens included.

BACTERIAL INFECTIONS OF THE SKIN AND EYES

- *Staphylococcus* and *Streptococcus* cause many different types of skin infections, a large number of which occur when bacteria breach the skin barrier through a cut or wound.
- *S. aureus* are frequently associated with purulent skin infections that manifest as **folliculitis**, **furuncles**, or **carbuncles**. *S. aureus* is also a leading cause of staphylococcal scalded skin syndrome (SSSS).
- *S. aureus* is generally drug resistant, and current MRSA strains are resistant to a wide range of antibiotics.
- Community-acquired and hospital-acquired staphyloccocal infections are an ongoing problem, because many people are asymptomatic carriers.
- **Group A streptococci (GAS)**, *S. pyogenes*, is often responsible for cases of **cellulitis**, **erysipelas**, and **erythema nosodum**. GAS are also one of many possible causes of **necrotizing fasciitis**.
- *P. aeruginosa* is often responsible for infections of the skin and eyes, including wound and burn infections, **hot tub rash**, **otitis externa**, and bacterial **keratitis**.
- **Acne** is a common skin condition that can become more inflammatory when *Propionibacterium acnes* infects hair follicles and pores that are clogged with dead skin cells and sebum.
- Cutaneous **anthrax** occurs when *Bacillus anthracis* breaches the skin barrier. The infection results in a localized black **eschar** on skin. Anthrax can be fatal if *B. anthracis* spreads to the bloodstream.
- Common bacterial **conjunctivitis** is often caused by *Haemophilus influenzae* and usually resolves on its own in a few days. More serious forms of conjunctivitis include gonococcal **ophthalmia neonatorum**, **inclusion conjunctivitis** (chlamydial), and **trachoma**, all of which can lead to blindness if untreated.
- **Keratitis** is frequently caused by *Staphylococcus epidermidis* and/or *Pseudomonas aeruginosa*, especially among contact lens users and can lead to blindness.
- Biofilms complicate the treatment of wound and eye infections, because pathogens living in biofilms can be difficult to treat and eliminate.

Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs
Acne	*Propionibacterium acnes*	Comedones (whiteheads, blackheads); papules, pustules, nodules, or pseudocysts	Not transmissible; clogged pores become infected by normal skin microbiota (*P. acnes*)	Erythromycin, clindamycin

Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs
Anthrax (cutaneous)	*Bacillus anthracis*	Eschar at site of infection; may lead to septicemia and can be fatal	Entry of *B. anthracis* endospores through cut or abrasion	Penicillin, erythromycin, or tetracycline
Cellulitis	*Streptococcus pyogenes*	Localized inflammation of dermis and hypodermis; skin red, warm, and painful to the touch	Entry of *S. pyogenes* through cut or abrasion	Oral or intravenous antibiotics (e.g., penicillin)
Erysipelas	*S. pyogenes*	Inflamed, swollen patch of skin, often on face; may be suppurative	Entry of *S. pyogenes* through cut or abrasion	Oral or intravenous antibiotics (e.g., penicillin)
Erythema nodosum	*S. pyogenes*	Small red nodules, often on shins	Associated with other streptococcal infection	None or anti-inflammatory drugs for severe cases
Impetigo	*Staphylococcus aureus, S. pyogenes*	Vesicles, pustules, and sometimes bullae around nose and mouth	Highly contagious, especially via contact	Topical or oral antibiotics.
Necrotizing fasciitis	*S. pyogenes, Klebsiella, Clostridium,* others	Infection of fascia and rapidly spreading tissue death; can lead to septic shock and death	Entry of bacteria through cut or abrasion	Intravenous broad-spectrum antibiotics
Otitis externa	*Pseudomonas aeruginosa*	Itching, redness, discomfort of ear canal, progressing to fever, pain, swelling	*P. aeruginosa* enters ear canal via pool or other water	Acidic ear drops with antibiotics, antifungals, steroids
Staphylococcal scalded skin syndrome (SSSS)	*S. aureus*	Erythema and severe peeling of skin	Infection of skin and mucous membranes, especially in children	Intravenous antibiotics, fluid therapy
Wound infections	*P. aeruginosa,* others	Formation of biofilm in or on wound	Exposure of wound to microbes in environment; poor wound hygiene	Polymyxin B, gentamicin, fluoroquinolones, topical anti-biofilm agents.

Table 3.9: Bacterial infections of the skin

Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs
Acute bacterial conjunctivitis	*Haemophilus influenza*	Inflammation of conjunctiva with purulent discharge	Exposure to secretions from infected individuals	Broad-spectrum topical antibiotics
Bacterial keratitis	*Staphylococcus epidermidis, Pseudomonas aeruginosa*	Redness and irritation of eye, blurred vision, sensitivity to light; progressive corneal scarring, which can lead to blindness	Exposure to pathogens on contaminated contact lenses	Antibiotic eye drops (e.g., with fluoroquinolones)
Neonatal conjunctivitis	*Chlamydia trachomatis, Neisseria gonorrhoeae*	Inflammation of conjunctiva, purulent discharge, scarring and perforation of cornea; may lead to blindness	Neonate exposed to pathogens in birth canal of a pregnant person with chlamydia or gonorrhea	Erythromycin
Trachoma (granular conjunctivitis)	*C. trachomatis*	Chronic conjunctivitis, trichiasis, scarring, blindness	Contact with infected individuals or contaminated fomites; transmission by eye-seeking flies	Azithromycin

Table 3.10: Bacterial infections of the eyes

VIRAL INFECTIONS OF THE SKIN AND EYES

- **Papillomas** (warts) are caused by human papillomaviruses.
- **Herpes simplex virus** (especially HSV-1) mainly causes **oral herpes**, but lesions can appear on other areas of the skin and mucous membranes.
- **Roseola** and **fifth disease** are common viral illnesses that cause skin rashes; roseola is caused by HHV-6 and HHV-7 while fifth disease is caused by parvovirus 19.
- **Viral conjunctivitis** is often caused by adenoviruses and may be associated with the common cold. **Herpes keratitis** is caused by herpesviruses that spread to the eye.

Disease	Pathogen	Signs and Symptoms	Tranmission	Antimicrobial Drugs
Fifth disease	Parvovirus B19	May have initial cold-like symptoms; “slapped cheek” rash	Highly contagious via respiratory secretions of infected individuals	None

Disease	Pathogen	Signs and Symptoms	Tranmission	Antimicrobial Drugs
Herpes keratitis	Herpes simplex virus 1 (HSV-1)	Inflammation of conjunctiva and cornea; irritation, excess tears, sensitivity to light; lesions in cornea leading to blindness	Direct eye contact with discharge from herpes lesions elsewhere in the body or from another infected individual	Acyclovir, ganciclovir, famiclovir, valacyclovir
Oral herpes	Herpes simplex virus 1 (HSV-1)	May cause initial systemic symptoms; cold sores	Highly contagious via direct contact with infected individuals	Acyclovir, penciclovir, famiclovir, valacyclovir
Papillomas	Human papillomavirus (HPV)	Common warts, plantar warts, flat warts, filiform warts, and others	Contact with infected individuals	Topical salicylic acid, cantharidin
Roseola (roseola infantum, exanthem subitum)	Human herpesvirus 6 (HHV-6), human herpesvirus 7 (HHV-7)	Initial cold-like symptoms with high fever, followed by a macular rash three to five days later	Spread by viral and respiratory secretions of infected individuals	Typically none; ganciclovir for immunocompromise d patients
Viral conjunctivitis	Adenoviruses and others	Inflammation of the conjunctiva; watery, nonpurulent discharge	Associated with common cold; contagious via contact with eye discharge	None

Table 3.11: Viral infections of the skin and eyes

MYCOSES OF THE SKIN

- **Mycoses** can be **cutaneous**, **subcutaneous**, or **systemic.**
- Common cutaneous mycoses include **tineas** caused by **dermatophytes** of the genera *Trichophyton*, *Epidermophyton*, and *Microsporum.* **Tinea corporis** is called **ringworm**. Tineas on other parts of the body have names associated with the affected body part.
- **Aspergillosis** is a fungal disease caused by molds of the genus *Aspergillus*. Primary cutaneous aspergillosis enters through a break in the skin, such as the site of an injury or a surgical wound; it is a common hospital-acquired infection. In secondary cutaneous aspergillosis, the fungus enters via the respiratory system and disseminates systemically, manifesting in lesions on the skin.
- The most common subcutaneous mycosis is **sporotrichosis** (rose gardener's disease), caused by *Sporothrix schenkii.*
- Yeasts of the genus *Candida* can cause opportunistic infections of the skin called **candidiasis**, producing **intertrigo**, localized rashes, or yellowing of the nails.

Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs
Aspergillosis (cutaneous)	*Aspergillus fumigatus, Aspergillus flavus*	Distinctive eschars at site(s) of infection	Entry via wound (primary cutaneous aspergillosis) or via the respiratory system (secondary cutaneous aspergillosis); commonly a hospital-acquired infection	Itraconazole, voriconazole, amphotericin B
Candidiasis (cutaneous)	*Candida albicans*	Intertrigo, localized rash, yellowing of nails	Opportunistic infections in immunocompromise d patients	Azoles
Sporotrichosis (rose gardener's disease)	*Sporothrix schenkii*	Subcutaneous ulcers and abscesses; may spread to a large area, e.g., hand or arm	Entry via thorn prick or other wound	Itraconazole
Tineas	*Trichophyton* spp., *Epidermophyton* spp., *Microsporum* spp.	Itchy, ring-like lesions (ringworm) at sites of infection	Contact with dermatophytic fungi, especially in warm, moist environments conducive to fungal growth	Terbinafine, miconazole, clotrimazole, griseofulvin

Table 3.12: Mycoses of the skin

PROTOZOAN AND HELMINTHIC INFECTIONS OF THE SKIN AND EYES

- The protozoan *Acanthamoeba* and the helminth *Loa loa* are two parasites that can breach the skin barrier, leading to infections of the skin and eyes.
- ***Acanthamoeba* keratitis** is a parasitic infection of the eye that often results from improper disinfection of contact lenses or swimming while wearing contact lenses.
- **Loiasis**, or eye worm, is a disease endemic to Africa that is caused by parasitic worms that infect the subcutaneous tissue of the skin and eyes. It is transmitted by deer fly vectors.

Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs
Acanthamoeba keratis	*Acanthamoeba*	Inflammation and damage to cornea; vision impairment or blindness	Exposure to pathogens in contaminated water or on contact lenses	Polyhexamethylene biguanide, chlorhexidine, azoles

Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs
Loiasis	*Loa loa*	Recurring fever and localized Calabar swelling, itching, and skin or eye pain during subcutaneous migration of worms	Larvae transmitted between humans by deerfly vector	Diethylcarbamazine, albendazole

Table 3.13: Parasitic skin and eye infections

Figure Descriptions

Figure 3.1: a) A micrograph of a large light pink region labeled dermis, a thinner dark pink region on top of that labeled epidermis, and a thin region of clear cells. The division between the dermis and epidermis is wavy; with areas where one projects into the other. B) A diagram of skin. The top layer is dark and is labeled epidermis. The next layer is lighter and much thicker; this is the dermis. Inside the dermis are vase-shaped hair follicles with hairs projecting out of the skin. Next to the hair follicle is a smaller vase-shape labeled sebaceous gland; this empties into the space of the hair follicle. There are also coiled shapes labeled receptor and a variety of long tubes labeled: nerve, lymph vessel and blood vessels. A coiled blob is labeled sweat gland; this leads to a tube that opens at the surface called a sweat pore. Below the dermis is a yellow bubbly-looking layer labeled fatty tissue; this is the hypodermis.

Figure 3.2: A diagram showing different regions of the body. Each region has a pie chart that shows which bacteria are most prevalent. The most common bacterium in each region: Glabella (corynebacterineae), Alar Crease (propionibacterineae), External auditory canal (propionibacterineae), Nare (other actinobacteria), manubrium (propionibacterineae), Axillary vault (proteobacteria), antecubital fossa (proteobacteria), Volar forearm (proteobacteria), interdigital web space (proteobacteria), hypothenar palm (proteobacteria), inguinal crease (corynebacterineae), umbilicus (corynebacterineae), toe web space (corynebacterineae, , propionibacterineae, and staphylococcaceae), reticular crease (propionibacterineae), occiput (staphylococcaceae, back (propionibacterineae), buttock (proteobacteria), gluteal crease (corynebacterineae), popliteal fossa (staphylococcaceae), plantar heel (staphylococcaceae). Second part of the image shows that different subjects have different bacterial percentages and that these percentages change over time.

Figure 3.3: a) Acne (labeled whitehead) on a person's cheek. B) A drawing of skin with a yellow bubble labeled pus. This is below a raised region on the skin.

Figure 3.4: A table labeled types of skin lesions. Crust is shown as a raised region on the surface of the skin. Cyst is shown as a large white sphere in the upper layers of the skin. Macule is shown as a dark mark on the surface. Papule is shown as a raised bubble on the surface. Pusture is shown as a large yellow sphere in the upper layers of the skin. Ulcer is a large cavity in the skin. Vesicle is a small blue bubble in the upper regions of the skin. Wheal is a small blue bubble on the surface of the skin.

Figure 3.5: Diagram of an eye. Above the eye is the lacrimal gland. At the point nearest the nose is the punctums and tubes leading to the lacrimal sac and nasolacrimal duct.

Figure 3.6: A cross section of the eye. The large spherical center is the vitreous humor. The layer surrounding this is the retina. A projection out of the back of the eye is the optic nerve. A region on the retina just above the optic nerve is the fovea. At the front of the eye is the lens. In front of this is a space labeled pupil. The colored region around the pupil is the iris. The cornea is the covering in front of the iris and pupil. The conjunctiva is a mucous membrane on the eye.

Figure 3.7: a) Photo of an eyelid being pulled back to show a red eye. B) A photo of inflamed eyelids. C) A photo of an eye with a cloudy cornea.

Figure 3.8: a) An agar plate with 2 regions of growth. One region has a yellow background, the other has a pink background. B) A micrograph of clusters of round cells. Each cell is just under 1 μm in diameter.

Figure 3.9: a) a photo of a small inflamed region with a white center. b) A large lesion with white and red.

Figure 3.10: Skin peeling off a hand.

Figure 3.11: Red bumps on the skin above the mouth.

Figure 3.12: A micrograph of small purple circles chained together in clumps.

Figure 3.13: a) a red rash. B) swollen, red regions on the cheeks and nose. C) red lumps on the skin.

Figure 3.14: a) skin with large black, grey and red regions. b) Cut skin in surgery.

Figure 3.15: a) photo of acne b) photo of swollen ear.

Figure 3.16: a) A red plate with white colonies. B) A clear plate with green colonies; the green extends past the colony. C) a dark plate with glowing colonies.

Figure 3.17: a) diagram of blackhead formation. A normal pore in the skin becomes filled with material forming a whitehead. Darker material forms a blackhead. B) blackheads on a nose.

Figure 3.18: a) A black nodule on skin. b) A red plate with grey colonies.

Figure 3.19: Eye with yellow discharge.

Figure 3.20: Swollen eyes with discharge.

Figure 3.21: a) eye with turned in eyelids. B) photo of eye surgery.

Figure 3.22: a) photo of warts on a toe. B) photo of wart on an eye.

Figure 3.23: Photo of cold sore on a lip.

Figure 3.24: a) photo of red patches on an infant's legs. B) photo of red spots on a child's trunk.

Figure 3.25: a) large red bumps on a cheek and neck. B) white crusty skin on a foot. C) an orange ring on skin.

Figure 3.26: A photo of a large black, fuzzy colony.

Figure 3.27: a) photo of a large, round, dark area on their leg. B) many thick strands and small dots. One of the strands ends in a sphere with long chains of dots around the top part of the structure.

Figure 3.28: A) a dark, lumpy rash. B) a broken, yellow nail. C) large, white, fuzzy colonies on a plate.

Figure 3.29: Live cycle of Acanthamoeba. In the water the cyst becomes a trophozoite. This then undergoes mitosis to form more trophozoites. Trophozoites can also become cysts. The amebae (cysts and trophozoites) can enter humans in various ways. Amoebae can enter through the eye, resulting in severe keratitis of the eye. When amoebae enter through nasal passages and infect the lower respiratory tract, it can result in granulomatous amebic encephalitis (GAE) and/or disseminated disease in individuals with compromised immune systems. Amoebae entering through ulcerated or broken skin can cause granulomatous amebic encephalitis (GAE), disseminated disease, or skin lesions in individuals with compromised immune systems.

Figure 3.30: a) an acanthamoeba cyst is shown. b) an acanthamoeba trophozoite micrograph is shown. c) a photo of an eye with a fluorescent cornea is shown.

Figure 3.31: The first part of the image is a photograph of an eye with a visible worm inside of it and a photo of a close-up of the worm. The second image is an illustrated chart showing the Life cycle of Lao lao. Fly (genus Chrysops) takes a blood meal (L3 larvae enter the bite wound). Adults grow into long worms in the subcutaneous tissue. Adults produce sheathed microfilariae that are found in spinal fluid, urine, sputum, peripheral blood, and

in the lungs. Another fly take a blood meal and ingests microfilariae. The microfilariae shed sheaths, penetrate fly's midgut, and migrate to thoracic muscles. The L1 larvae forms and becomes an L3 larvae which migrates to the head and fly's proboscis. The fly is now ready to infect another person.

Figure References

Figure 3.1: A micrograph of a section through human skin shows the epidermis and dermis. Left: Modification of work by Kibad. Public Domain. https://commons.wikimedia.org/wiki/File:Normal_Epidermis_and_Dermis_with_Intradermal_Nevus_10x.JPG. Right: Modification of work by National Cancer Institute. Public Domain. https://commons.wikimedia.org/wiki/File:Anatomy_The_Skin_-_NCI_Visuals_Online.jpg

Figure 3.2: The normal microbiota varies on different regions of the skin, especially in dry versus moist areas. Modification of work by National Human Genome Research Institute. Public domain.

Figure 3.3: Acne is a bacterial infection of the skin that manifests as a rash of inflamed hair follicles (folliculitis). Left: by Modification of work by Diariodaj. Public Domain. https://commons.wikimedia.org/wiki/File:Teenager-with-acne.jpg. Right: Modification of work by (c) BruceBlaus. Blausen.com staff (2014). "Medical gallery of Blausen Medical 2014". WikiJournal of Medicine 1 (2). DOI:10.15347/wjm/2014.010. ISSN 2002-4436. https://commons.wikimedia.org/wiki/File:Blausen_0007_Abscess.png

Figure 3.4: Numerous causes can lead to skin lesions of various types, some of which are very similar in appearance. Modification of work (c) Bruce Blaus. CC BY 4.0.

Figure 3.5: The lacrimal apparatus includes the structures of the eye associated with tear production and drainage. Modification of work (c) Evidence Based Medical Educator Inc./YouTube. CC BY 4.0.

Figure 3.6: Some microbes live on the conjunctiva of the human eye, but the vitreous humor is sterile. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 3.7: Left: Conjunctivitis is inflammation of the conjunctiva. modification of Figure 4 from Lopez-Prats MJ, Sanz Marco E, Hidalgo-Mora JJ, Garcia-Delpech S, Diaz-Llopis M. 2010. "Bleeding Follicular Conjunctivitis due to Influenza H1N1 Virus". Journal of Ophthalmology. https://doi.org/10.1155/2010/423672. CC BY 3.0. Middle and Right: Modification of work by Centers for Disease Control and Prevention. Public Domain.

Figure 3.8: (a) A mannitol salt agar plate is used to distinguish different species of staphylococci. Left: modification of work by "ScienceProfOnline"/YouTube. Public domain. Right: modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 3.9: Furuncles (boils) and carbuncles are infections of the skin often caused by Staphylococcus bacteria. Left: Public Health Image Library / CDC; Public Domain. Right: modification of work by Drvgaikwad. CC BY SA 3.0 Unported. https://commons.wikimedia.org/wiki/File:Carbuncle_on_buttok.JPG

Figure 3.10: A newborn with staphylococcal scalded skin syndrome (SSSS), which results in large regions of peeling, dead skin. Modification of Figure 2 in Jeyakumari, D; Gopal, R; Eswaran, M1; MaheshKumar, C1. Staphylococcal Scalded Skin Syndrome in a Newborn. Journal of Global Infectious Diseases 1(1):p 45-47, Jan–Jun 2009. | DOI: 10.4103/0974-777X.52981. CC BY 4.0.

Figure 3.11 Impetigo is characterized by vesicles, pustules, or bullae that rupture, producing encrusted sores. Modification of work by FDA. Public domain.

Figure 3.12: Streptococcus pyogenes forms chains of cocci. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 3.13: S. pyogenes can cause a variety of skin conditions once it breaches the skin barrier through a cut or wound. Left: (c) modification Figure 1 in Bassukas, I.D., Gaitanis, G., Zioga, A. et al. Febrile "migrating" eosinophilic cellulitis with hepatosplenomegaly: adult toxocariasis – a case report. Cases Journal 1, 356 (2008). https://doi.org/10.1186/1757-1626-1-356. CC BY 2.0. Middle: Modification of work by Centers for Disease Control and Prevention. Public Domain. Right: Modification of Figure 1 in Dean, C., Crow, W. Painful red nodules in female patient with recent travel history: a case report. Cases Journal 2, 8248 (2009). https://doi.org/10.4076/1757-1626-2-8248. CC BY 2.0.

Figure 3.14: The left leg of this patient shows the clinical features of necrotizing fasciitis. Figures 1 and 2 in Smuszkiewicz, P., Trojanowska, I. & Tomczak, H. Late diagnosed necrotizing fasciitis as a cause of multiorgan dysfunction syndrome: A case report. Cases Journal 1, 125 (2008). https://doi.org/10.1186/1757-1626-1-125. CC BY 2.0.

Figure 3.15: Hot tub folliculitis presents as an itchy red rash. Left: Lsupellmel. Public Domain. https://commons.wikimedia.org/wiki/File:HotTubFolliculitis.jpg. Right: Modification of work (c) Klaus D. Peter. CC BY 3.0 Germany. https://commons.wikimedia.org/wiki/File:Otitis_externa.jpg

Figure 3.16: These P. aeruginosa colonies are growing on xylose lysine sodium deoxycholate (XLD) agar. Left: modification of work by Centers for Disease Control and Prevention. Public Domain. Middle and Right: Public Domain.

Figure 3.17: Acne is characterized by whitehead and blackhead comedones that result from clogged hair follicles. Left: Modification of work (c) Blausen.com staff (2014). "Medical gallery of Blausen Medical 2014". WikiJournal of Medicine 1 (2). DOI:10.15347/wjm/2014.010. ISSN 2002-4436. CC BY 3.0 Unported. https://commons.wikimedia.org/wiki/File:Blausen_0811_SkinPores.png. Right: By Elecbullet. Public Domain. https://commons.wikimedia.org/wiki/File:Blackheads.JPG

Figure 3.18: (a) Cutaneous anthrax is an infection of the skin by B. anthracis, which produces tissue-damaging exotoxins. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 3.19: Acute, purulent, bacterial conjunctivitis causes swelling and redness in the conjunctiva, the membrane lining the whites of the eyes and the inner eyelids. (c) "Tanalai. CC BY 3.0. https://commons.wikimedia.org/wiki/File:Swollen_eye_with_conjunctivitis.jpg

Figure 3.20: A newborn suffering from gonococcal ophthalmia neonatorum. Centers for Disease Control and Prevention. Public domain.

Figure 3.21: If trachoma is not treated early with antibiotics, scarring on the eyelid can lead to trichiasis, a condition in which the eyelashes turn inward. Left: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology. Right: Modification of work (c) Otis Historical Archives National Museum of Health & Medicine. CC BY 2.0. https://commons.wikimedia.org/wiki/File:Entropion_and_trichiasis_secondary_to_trachoma_A44-652-11.jpg

Figure 3.22: Warts can vary in shape and in location. Credit a: Raimar. Public Domain. https://commons.wikimedia.org/wiki/File:Dornwarzen.jpg. Credit b: Schweintechnik. Public Domain. https://commons.wikimedia.org/wiki/File:Wart_filiform_eyelid.jpg

Figure 3.23: This cold sore was caused by HSV-1. Centers for Disease Control and Prevention. Public domain.

Figure 3.24: Roseola. Credit a: Modified from work of M Davis. Public Domain. https://commons.wikimedia.org/wiki/File:Roseola_on_a_21-month-old_girl.jpg. Credit b: Modified from work of Andrew Kerr. Public Domain. https://en.wikipedia.org/wiki/File:Fifth_disease.jpg

Figure 3.25: Tineas are superficial cutaneous mycoses and are common. Left, Right: Modification of work by Centers for Disease Control and Prevention. Public Domain. Middle: Figure 1 from Al Hasan, M., Fitzgerald, S.M., Saoudian, M. et al. Dermatology for the practicing allergist: Tinea pedis and its complications. Clin Mol Allergy 2, 5 (2004). Fair Use. https://doi.org/10.1186/1476-7961-2-5

Figure 3.26: To diagnose tineas, the dermatophytes may be grown on a Sabouraud dextrose CC agar plate. Centers for Disease Control and Prevention. Public domain.

Figure 3.27 Eschar on a patient with secondary cutaneous aspergillosis. Left: Figure 1 in Santiago, Mónica, Martinez, José Hernán, Palermo, Coromoto, Figueroa, Carlos, Torres, Oberto, Trinidad, Rafael, Gonzalez, Eva, Miranda, Maria de Lourdes, Garcia, Miosotis, Villamarzo, Guillermo, Rapidly Growing Thyroid Mass in an Immunocompromised Young Male Adult, Case Reports in Endocrinology, 2013, 290843, 4 pages, 2013. https://doi.org/10.1155/2013/290843. CC BY 3.0. Right: Modification of work by U.S. Department of Health and Human Services. Public Domain.

Figure 3.28: This red, itchy rash is the result of cutaneous candidiasis, an opportunistic infection of the skin caused by the yeast Candida albicans. Left: modification of work by U.S. Department of Veterans Affairs. Public Domain. Middle: Medguy. https://commons.wikimedia.org/wiki/File:Toefungus.jpg. Public Domain. Right: modification of work by Centers for Disease Control and Prevention.

Figure 3.29: Acanthamoeba spp. are waterborne parasites very common in unchlorinated aqueous environments. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 3.30: An Acanthamoeba cyst. An Acanthamoeba trophozoite. Left: modification of work by Centers for Disease Control and Prevention. Public Domain. Middle, Right: Modification Figure 1, 3 from Lorenzo-Morales J, Khan NA, Walochnik J. An update on Acanthamoeba keratitis: diagnosis, pathogenesis and treatment. Parasite. 2015;22:10. https://pmc.ncbi.nlm.nih.gov/articles/PMC4330640. CC BY 4.0.

Figure 3.31: This Loa loa worm, measuring about 55 mm long, was extracted from the conjunctiva of a patient with loiasis. Modification of work by Centers for Disease Control and Prevention. Public domain.

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CHAPTER 4.

SYSTEMIC INFECTIONS OF THE ORAL CAVITY AND GI

4.1 INTRODUCTION TO THE ANATOMY AND NORMAL MICROBIOTA OF THE DIGESTIVE SYSTEM

The digestive system contains normal microbiota, including archaea, bacteria, fungi, protists, and even viruses. Because this microbiota is important for normal functioning of the digestive system, alterations to the microbiota by antibiotics or diet can be harmful. Additionally, the introduction of pathogens to the GI tract can cause infections and diseases. In this section, we will review the microbiota found in a healthy digestive tract and the general signs and symptoms associated with oral and GI infections.

NORMAL MICROBIOTA OF THE ORAL CAVITY

Microbes such as bacteria and archaea are abundant in the mouth and coat all of the surfaces of the oral cavity. However, different structures, such as the teeth or cheeks, host unique communities of both aerobic and anaerobic microbes. Some factors appear to work against making the mouth hospitable to certain microbes. For example, chewing allows microbes to mix better with saliva so they can be swallowed or spit out more easily. Saliva also contains enzymes, including lysozyme, which can damage microbial cells. Recall that lysozyme is part of the first line of defense in the innate immune system and cleaves the β-(1,4) glycosidic linkages between N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) in bacterial peptidoglycan (see section 1.4). Additionally, fluids containing immunoglobulins and phagocytic cells are produced in the gingival spaces. Despite all of these chemical and mechanical activities, the mouth supports a large microbial community.

NORMAL MICROBIOTA OF THE GI TRACT

The environment of the GI tract is diverse, serving two purposes: digestion and immunity. The stomach is an extremely acidic environment (pH 1.5–3.5) due to the gastric juices that break down food and kill many ingested microbes; this helps prevent infection from food or waterborne pathogens. The environment in the small intestine is rich in mono and disaccharides as well as amino acids making it able to support microbial communities. Microorganisms present in the small intestine can include lactobacilli, diphtheroids, and the fungus *Candida*. On the other hand, the large intestine (colon) contains a diverse and abundant microbiota that is important for normal function. These microbes include Bacteroidetes (especially the genera *Bacteroides* and *Prevotella*) and *Firmicutes* (especially members of the genus *Clostridium*). Methanogenic archaea and some fungi are also present, among many other species of bacteria. These microbes all aid in digestion and contribute to the production of feces, the waste excreted from the digestive tract, and flatus, the gas produced from microbial fermentation of undigested food. They can also produce valuable nutrients. For example, lactic acid bacteria, such as bifidobacteria, can synthesize vitamins, such as vitamin B12, folate, and riboflavin, that humans cannot synthesize themselves. *E. coli* found in the intestine can also break down food and help the body produce vitamin K, which is important for blood coagulation.

The GI tract has several other methods of reducing the risk of infection by pathogens. One example of this is the presence of Peyer's patches. Within the ileum, aggregates of underlying lymphoid tissue (figure 4.1) detect pathogens in the intestines via microfold (M) cells, which transfer antigens from the lumen of the intestine to the lymphocytes on Peyer's patches to induce an immune response. The Peyer's patches then secrete IgA and other pathogen-specific antibodies into the intestinal lumen to help keep intestinal microbes at safe levels. Goblet cells, which are modified simple columnar epithelial cells, also line the GI tract (figure 4.2). Goblet cells secrete a gel-forming mucin, which is the major component of mucus. The production of a protective layer of mucus helps reduce the risk of pathogens reaching deeper tissues.

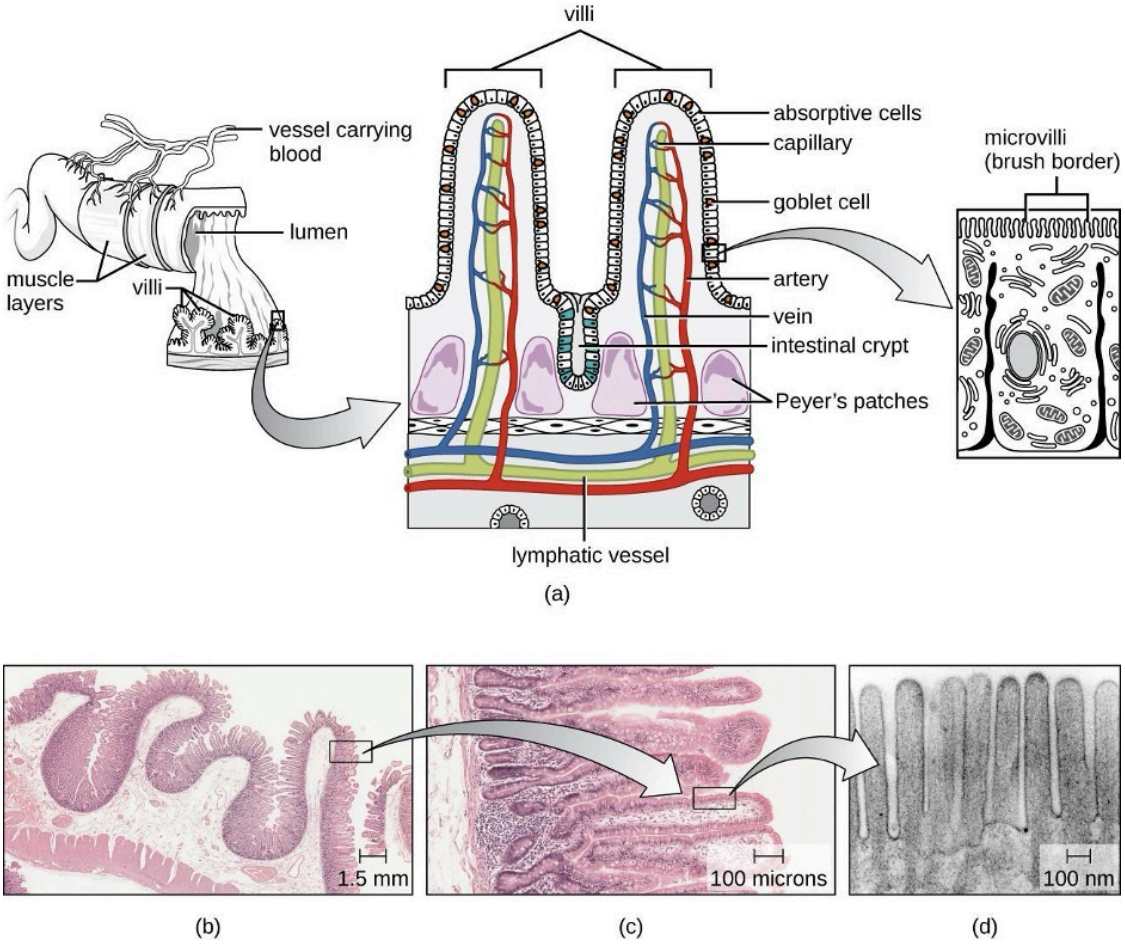

Figure 4.1: (a) The structure of the wall of the small intestine allows for the majority of nutrient absorption in the body. (b) Villi are folds in the surface of the small intestine. Microvilli are cytoplasmic extensions on individual cells that increase the surface area for absorption. (c) A light micrograph shows the shape of the villi. (d) An electron micrograph shows the shape of the microvilli. *Figure description available at the end of the chapter.*

The constant movement of materials through the gastrointestinal tract also helps to move transient pathogens out of the body. In fact, feces are composed of approximately 25% microbes, 25% sloughed epithelial cells, 25% mucus, and 25% digested or undigested food. Finally, the normal microbiota provides an additional barrier to infection via a variety of mechanisms. For example, these organisms outcompete potential pathogens for space and nutrients within the intestine. This is known as competitive exclusion. Members of the microbiota may also secrete protein toxins known as bacteriocins that are able to bind to specific receptors on the surface of susceptible bacteria.

GENERAL SIGNS AND SYMPTOMS OF ORAL AND GI DISEASE

Despite numerous defense mechanisms that protect against infection, all parts of the digestive tract can become sites of infection or intoxication. The term food poisoning is sometimes used as a catch-all for GI infections and intoxications, but not all forms of GI disease originate with foodborne pathogens or toxins.

In the mouth, fermentation by anaerobic microbes produces acids that damage the teeth and gums. This can lead to tooth decay, cavities, and periodontal disease, a condition characterized by chronic inflammation and erosion of the gums. Additionally, some pathogens can cause infections of the mucosa, glands, and other structures in the mouth, resulting in inflammation, sores, cankers, and other lesions. An open sore in the mouth or GI tract is typically called an ulcer.

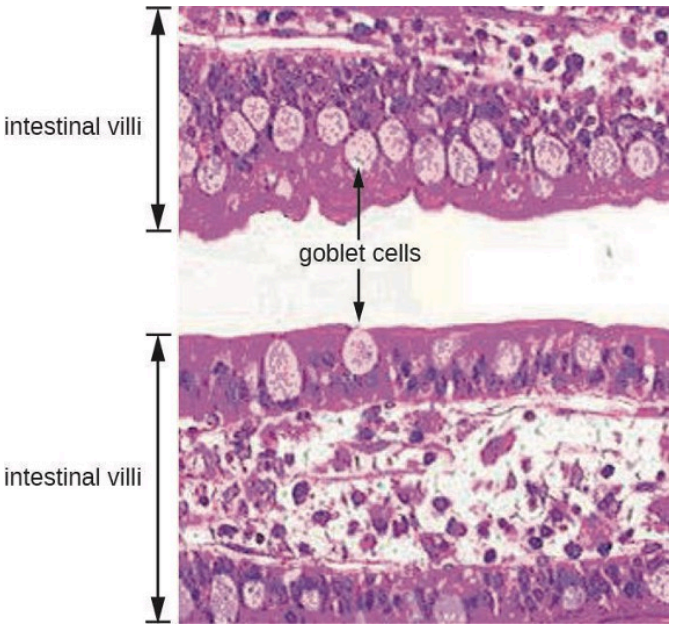

Figure 4.2: A magnified image of intestinal villi in the GI tract shows goblet cells. These cells are important in producing a protective layer of mucus. *Figure description available at the end of the chapter.*

Infections and intoxications of the lower GI tract often produce symptoms such as nausea, vomiting, diarrhea, aches, and fever. In some cases, vomiting and diarrhea may cause severe dehydration and other complications that can become serious or fatal. Various clinical terms are used to describe gastrointestinal symptoms. For example, gastritis is an inflammation of the stomach lining that results in swelling, and enteritis refers to inflammation of the intestinal mucosa. When the inflammation involves both the stomach lining and the intestinal lining, the condition is called gastroenteritis. Inflammation of the liver is called hepatitis. Inflammation of the colon, called colitis, commonly occurs in cases of food intoxication. Because an inflamed colon does not reabsorb water as effectively as it normally does, stools become watery, causing diarrhea. Damage to the epithelial cells of the colon can also cause bleeding and excess mucus to appear in watery stools, a condition called dysentery.

4.2 MICROBIAL DISEASES OF THE MOUTH AND ORAL CAVITY

Despite the presence of saliva and the mechanical forces of chewing and eating, some microbes thrive in the mouth. These microbes can cause damage to the teeth and can cause infections that have the potential to spread beyond the mouth and sometimes throughout the body.

DENTAL CARIES

Cavities of the teeth, known clinically as dental caries, are microbial lesions that cause damage to the teeth. Over time, the lesion can grow through the outer enamel layer to infect the underlying dentin or even the innermost pulp. If dental caries are not treated, the infection can become an abscess that spreads to the deeper tissues of the teeth, near the roots, or to the bloodstream.

Tooth decay results from the metabolic activity of microbes that live on the teeth. A layer of proteins and carbohydrates forms when clean teeth come into contact with saliva. Microbes are attracted to this food source and form a biofilm called plaque. The most important cariogenic species in these biofilms is *Streptococcus mutans*. When sucrose, a disaccharide sugar from food, is broken down by bacteria in the mouth, glucose and fructose are produced. The glucose is used to make dextran, which is part of the extracellular matrix of the biofilm. Fructose is fermented, producing organic acids such as lactic acid. These acids dissolve the minerals of the tooth, including enamel, even though it is the hardest material in the body. The acids work even more quickly on exposed dentin (figure 4.3). Over time, the plaque biofilm can become thick and eventually calcify. When a heavy plaque deposit becomes hardened in this way, it is called tartar or dental calculus (figure 4.4). These substantial plaque biofilms can include a variety of bacterial species, including *Streptococcus* and *Actinomyces* species.

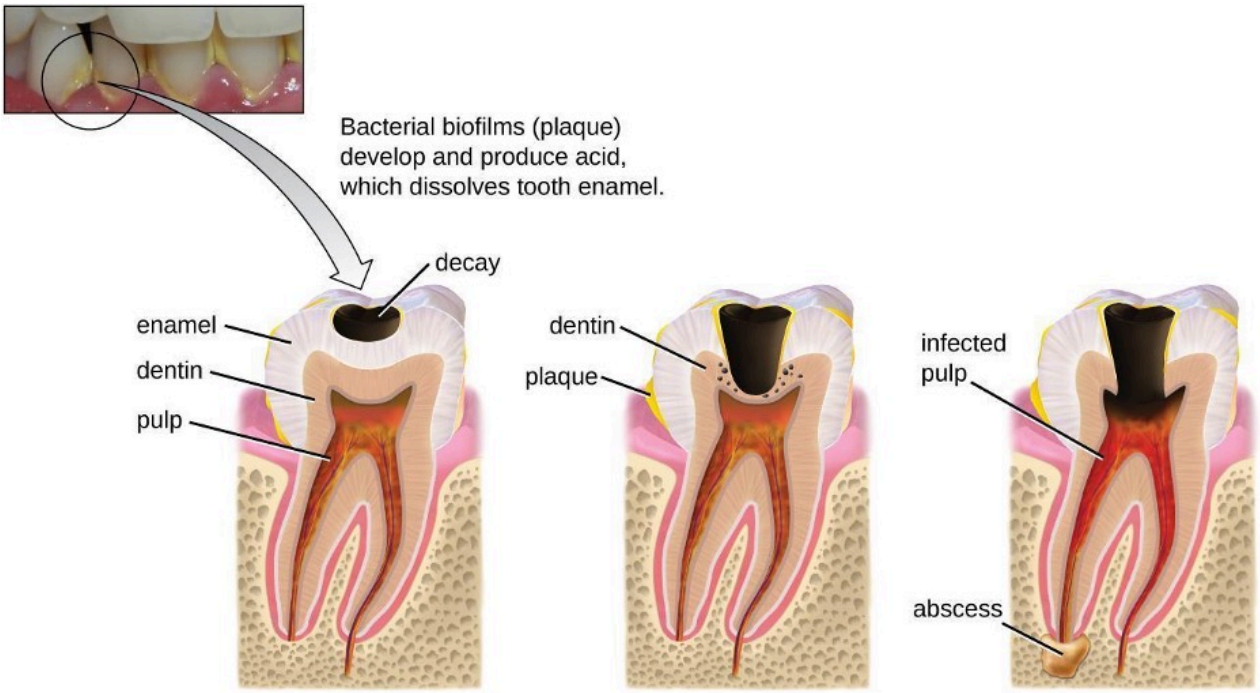

Figure 4.3: Tooth decay occurs in stages. When bacterial biofilms (plaque) develop on teeth, the acids produced gradually dissolve the enamel, followed by the dentin. Eventually, if left untreated, the lesion may reach the pulp and cause an abscess. *Figure description available at the end of the chapter.*

Some tooth decay is visible from the outside, but it is not always possible to see all decay or the extent of the decay. X-ray imaging is used to produce radiographs that can be studied to look for deeper decay and damage to the root or bone (figure 4.4). If not detected, the decay can reach the pulp or even spread to the bloodstream. Painful abscesses can develop.

To prevent tooth decay, prophylactic treatment and good hygiene are important. Regular tooth brushing and flossing physically removes microbes and combats microbial growth and biofilm formation. Toothpaste contains fluoride, which becomes incorporated into the hydroxyapatite of tooth enamel, protecting it against acidity caused by fermentation of mouth microbiota. Fluoride is also bacteriostatic, thus slowing enamel degradation. Antiseptic mouthwashes commonly contain plant-derived phenolics like thymol and eucalyptol and/or heavy metals like zinc chloride. Phenolics tend to be stable and persistent on surfaces, and they act through denaturing proteins and disrupting membranes.

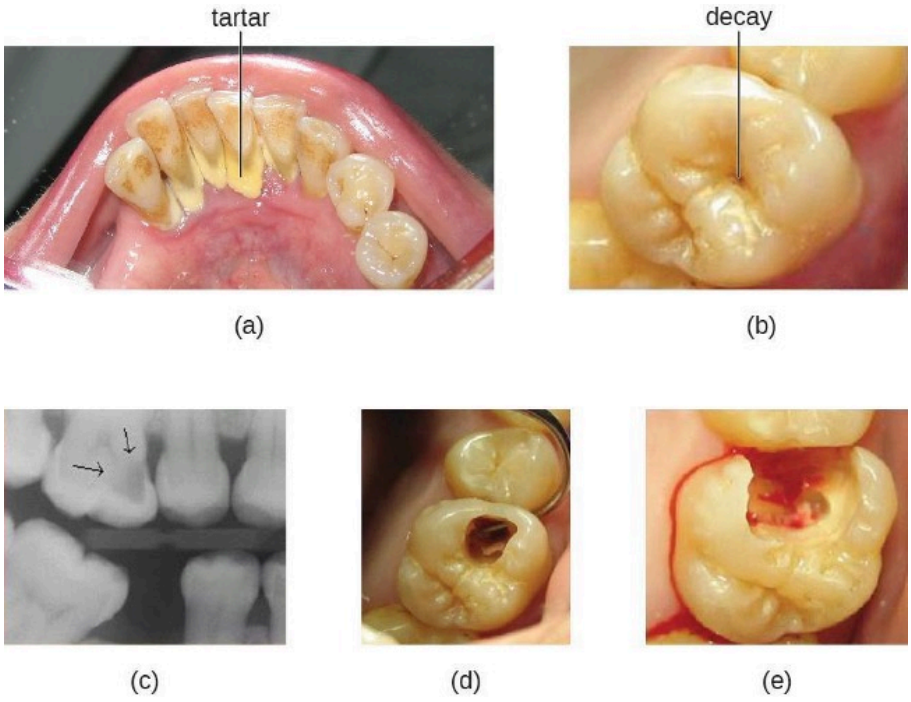

Figure 4.4: (a) Tartar (dental calculus) is visible at the bases of these teeth. The darker deposits higher on the crowns are staining. (b) This tooth shows only a small amount of visible decay. (c) An X-ray of the same tooth shows that there is a dark area representing more decay inside the tooth. (d) Removal of a portion of the crown reveals the area of damage. (e) All of the cavity must be removed before filling. *Figure description available at the end of the chapter.*

Regular dental cleanings allow for the detection of decay at early stages and the removal of tartar. They may also help to draw attention to other concerns, such as damage to the enamel from acidic drinks. Reducing sugar consumption may help prevent damage that results from the microbial fermentation of sugars. Additionally, sugarless candies or gum with sugar alcohols (such as xylitol) can reduce the production of acids because these are fermented to nonacidic compounds (although excess consumption may lead to gastrointestinal distress). Fluoride treatment or ingesting fluoridated water strengthens the minerals in teeth and reduces the incidence of dental caries.

If caries develop, prompt treatment prevents worsening. Smaller areas of decay can be drilled to remove affected tissue and then filled. If the pulp is affected, then a root canal may be needed to completely remove the infected tissues to avoid continued spread of the infection, which could lead to painful abscesses.

PERIODONTAL DISEASE

Periodontal disease is the result of infections that lead to inflammation and tissue damage in the structures surrounding the teeth. The progression from mild to severe periodontal disease is generally reversible and preventable with good oral hygiene.

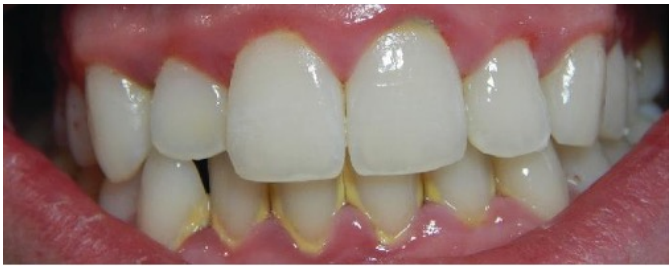

Figure 4.5: Redness and irritation of the gums are evidence of gingivitis. *Figure description available at the end of the chapter.*

Inflammation of the gums that can lead to irritation and bleeding is called gingivitis. When plaque accumulates on the teeth, bacteria colonize the gingival space. As this space becomes increasingly blocked, the environment becomes anaerobic. This allows a wide variety of microbes to colonize, including *Porphyromonas*, *Streptococcus*, and *Actinomyces*. The bacterial products, which include lipopolysaccharide (LPS), proteases, lipoteichoic acids, and others, cause inflammation and gum damage (figure 4.5). It is possible that methanogenic archaeans (including *Methanobrevibacter oralis* and other *Methanobrevibacter* species) also contribute to disease progression as some species have been identified in patients with periodontal disease, but this has proven difficult to study.[123]

Gingivitis is diagnosed by visual inspection, including measuring pockets in the gums, and X-rays, and is usually treated using good dental hygiene and professional dental cleaning, with antibiotics reserved for severe cases.

Over time, chronic gingivitis can develop into the more serious condition of periodontitis (figure 4.6). When this happens, the gums recede and expose parts of the tooth below the crown. This newly exposed area is relatively unprotected, so bacteria can grow on it and spread underneath the enamel of the crown and cause cavities. Bacteria in the gingival space can also erode the cementum, which helps to hold the teeth in place. If not treated, erosion of cementum can lead to the movement or loss of teeth. The bones of the jaw can even erode if the infection spreads. This condition can be associated with bleeding and halitosis (bad breath). Cleaning and appropriate dental hygiene may be sufficient to treat periodontitis. However, in cases of severe periodontitis, an antibiotic may be given. Antibiotics may be given in pill form or applied directly to the gum (local treatment). Antibiotics given can include tetracycline, doxycycline, macrolides or β-lactams. Because periodontitis can be caused by a mix of microbes, a combination of antibiotics may be given.

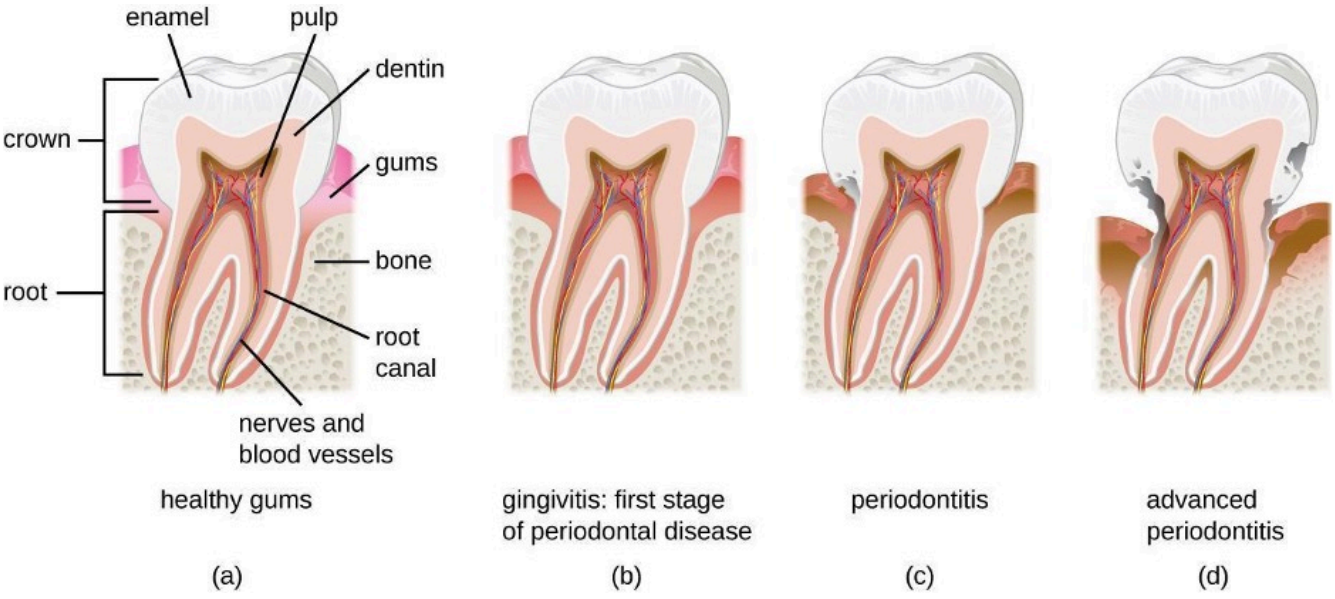

Figure 4.6: (a) Healthy gums hold the teeth firmly and do not bleed. (b) Gingivitis is the first stage of periodontal disease. Microbial infection causes gums to become inflamed and irritated, with occasional bleeding. (c) In periodontitis, gums recede and expose parts of the tooth normally covered. (d) In advanced periodontitis, the infection spreads to ligaments and bone tissue supporting the teeth. Tooth loss may occur, or teeth may need to be surgically removed. *Figure description available at the end of the chapter.*

Trench Mouth

When certain bacteria, such as *Prevotella intermedia*, *Fusobacterium* species, and *Treponema vicentii*, are involved and periodontal disease progresses, acute necrotizing ulcerative gingivitis or trench mouth, also called Vincent's disease, can develop. This is severe periodontitis characterized by erosion of the gums, ulcers, substantial pain with chewing, and halitosis (figure 4.7) that can be diagnosed by visual examination and X-rays. In countries with good medical and dental care, it is most common in individuals with weakened immune systems, such as patients with AIDS. In addition to cleaning and pain medication, patients may be prescribed antibiotics such as amoxicillin, amoxicillin clavulanate, clindamycin, or doxycycline.

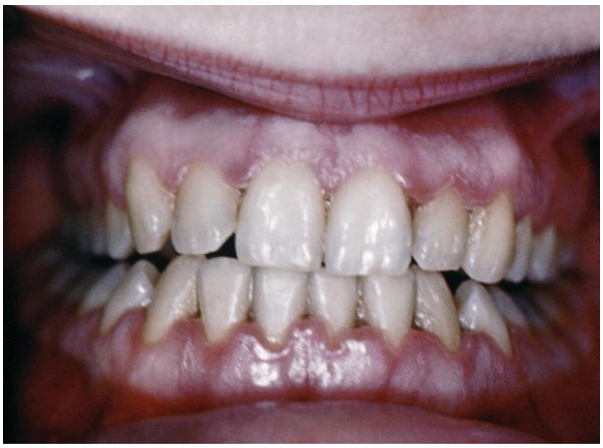

Figure 4.7: These inflamed, eroded gums are an example of a mild case of acute necrotizing ulcerative gingivitis, also known as trench mouth. Figure description available at the end of the chapter.

ORAL INFECTIONS

As noted earlier, normal oral microbiota can cause dental and periodontal infections. However, there are a number of other infections that can manifest in the oral cavity when other microbes are present. Common oral infections are summarized in table 4.1.

Herpetic Gingivostomatitis

As described in section 3.2, infections by herpes simplex virus type 1 (HSV-1) frequently manifest as oral herpes, also called acute herpes labialis and characterized by cold sores on the lips, mouth, or gums. HSV-1 can also cause acute herpetic gingivostomatitis, a condition that results in ulcers of the mucous membranes inside the mouth (figure 4.8). Herpetic gingivostomatitis is normally self-limiting except in immunocompromised patients. Like oral herpes, the infection is generally diagnosed through clinical examination, but cultures or biopsies may be obtained if other signs or symptoms suggest the possibility of a different causative agent. If treatment is needed, mouthwashes or antiviral medications such as acyclovir, famciclovir, or valacyclovir may be used.

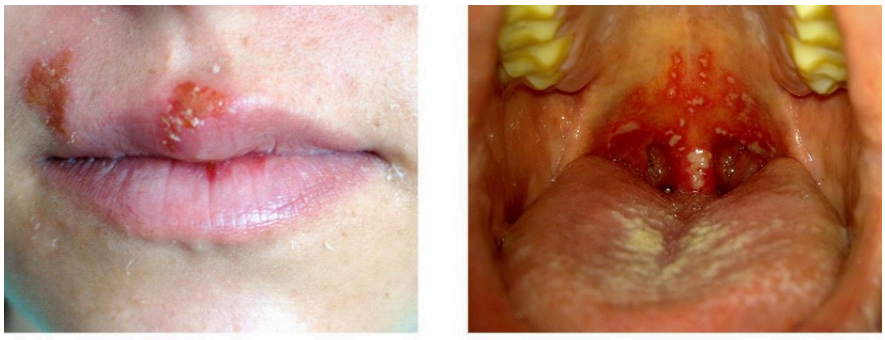

Figure 4.8: (a) This cold sore is caused by infection with herpes simplex virus type 1 (HSV-1). (b) HSV-1 can also cause acute herpetic gingivostomatitis. Figure description available at the end of the chapter.

Oral Thrush

The yeast *Candida* is part of the normal human microbiota, but overgrowths, especially of *Candida albicans*, can lead to infections in several parts of the body. When *Candida* infection develops in the oral cavity, it is called oral thrush. Oral thrush is most common in infants because they do not yet have well developed immune systems and have not acquired the robust normal microbiota that keeps *Candida* in check in adults. Oral thrush is also common in immunodeficient patients and is a common infection in patients with AIDS.

Oral thrush is characterized by the appearance of white patches and pseudomembranes in the mouth (figure 4.9) and can be associated with bleeding. The infection may be treated topically with nystatin or clotrimazole oral suspensions, although systemic treatment is sometimes needed. In serious cases, systemic azoles such as fluconazole or itraconazole (for strains resistant to fluconazole), may be used. Amphotericin B can also be used if the infection is severe or if the *Candida* species is azole-resistant.

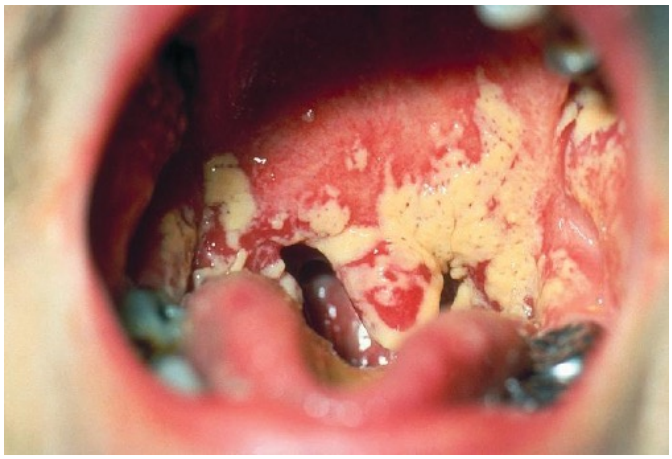

Figure 4.9: Overgrowth of Candida in the mouth is called thrush. It often appears as white patches. *Figure description available at the end of the chapter.*

Mumps

The viral disease mumps is an infection of the parotid glands, the largest of the three pairs of salivary glands (figure 4.10). The causative agent is mumps virus (MuV), a paramyxovirus with an envelope that has hemagglutinin and neuraminidase spikes. A fusion protein located on the surface of the envelope helps to fuse the viral envelope to the host cell plasma membrane.

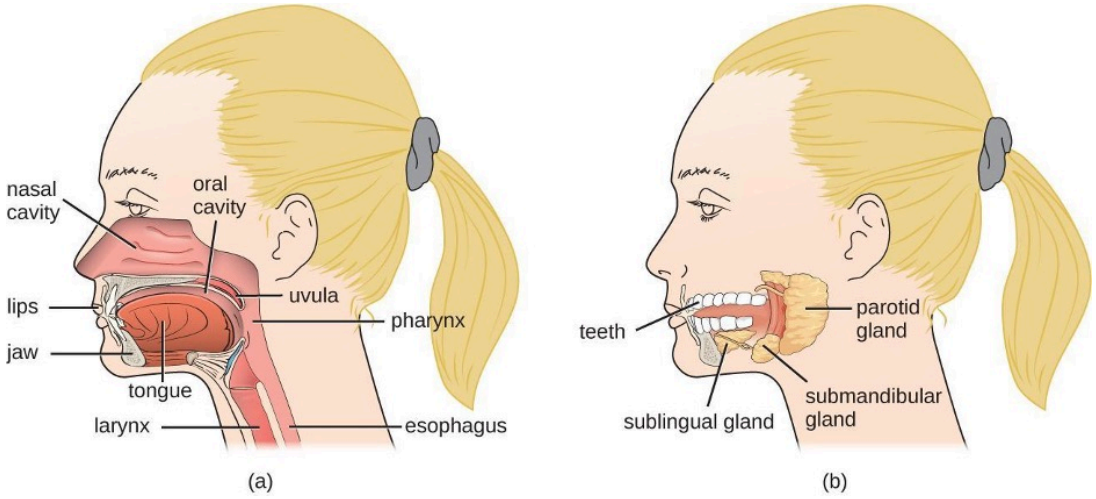

Figure 4.10: (a) When food enters the mouth, digestion begins. (b) Salivary glands are accessory digestive organs. *Figure description available at the end of the chapter.*

Mumps virus is transmitted through respiratory droplets or through contact with contaminated saliva, making it quite contagious so that it can lead easily to epidemics. It causes fever, muscle pain, headache, pain with chewing, loss of appetite, fatigue, and weakness. There is swelling of the salivary glands and associated pain (figure 4.11). The virus can enter the bloodstream (viremia), allowing it to spread to the organs and the central nervous system. The infection ranges from subclinical cases to cases with serious complications, such as encephalitis, meningitis, and deafness. Inflammation of the pancreas, testes, ovaries, and breasts may also occur and cause permanent damage to those organs; despite these complications, a mumps infection rarely causes sterility.

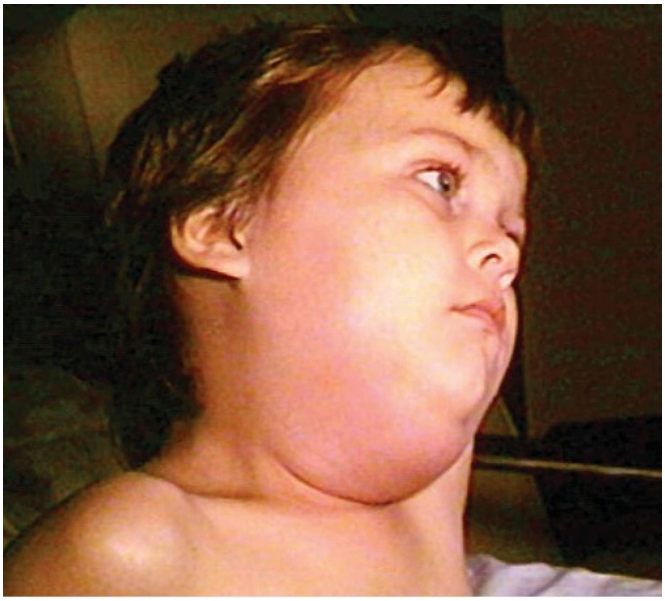

Figure 4.11: This child shows the characteristic parotid swelling associated with mumps. *Figure description available at the end of the chapter.*

Mumps can be recognized based on clinical signs and symptoms, and a diagnosis can be confirmed with laboratory testing. The virus can be identified using culture or molecular techniques such as RT-PCR. Serologic tests are also available, especially enzyme immunoassays that detect antibodies. There is no specific treatment for mumps, so supportive therapies are used. The most effective way to avoid infection is through vaccination. Although mumps used to be a common childhood disease, it is now rare in the United States due to vaccination with the measles, mumps, and rubella (MMR) vaccine.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Dental caries	*Streptococcus mutans*	Discoloration, softening, cavities in teeth	Non-transmissible; caused by bacteria of the normal oral microbiota	Visual examinations, X-rays	Oral antiseptics (e.g., Listerine)
Gingivitis and periodontitis	*Porphyromonas, Streptococcus, Actinomyces*	Inflammation and erosion of gums, bleeding, halitosis; erosion of cementum leading to tooth loss in advanced infections	Non-transmissible; caused by bacteria of the normal oral microbiota	Visual examination, X-rays, measuring pockets in gums	Tetracycline, doxycycline, macrolides or beta-lactams. Mixture of antibiotics may be given
Herpetic gingivostomatitis	Herpes simplex virus type 1 (HSV-1)	Lesions in mucous membranes of mouth	Contact with saliva or lesions of an infected person	Culture or biopsy	Acyclovir, famcyclovir, valacyclovir

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Mumps	Mumps virus (a paramyxovirus)	Swelling of parotid glands, fever, headache, muscle pain, weakness, fatigue, loss of appetite, pain while chewing; in serious cases, encephalitis, meningitis, and inflammation of testes, ovaries, and breasts	Contact with saliva or respiratory droplets of an infected person	Virus culture or serologic tests for antibodies, enzyme immunoassay, RT-PCR	None for treatment; MMR vaccine for prevention
Oral thrush	*Candida albicans*, other *Candida* spp.	White patches and pseudomembranes in mouth, may cause bleeding	Nontransmissible; caused by overgrowth of Candida spp. in the normal oral microbiota; primarily affects infants and the immunocompromised	Microscopic analysis of oral samples	Clotrimazole, nystatin, fluconazole, or itraconazole; amphotericin B in severe cases
Trench mouth (acute necrotizing ulcerative gingivitis)	*Prevotella intermedia Fusobacterium* species, *Treponema vincentii*, others	Erosion of gums, ulcers, substantial pain with chewing, halitosis	Nontransmissible; caused by members of the normal oral microbiota	Visual examinations, X-rays	Amoxicillin, amoxicillin clavulanate, clindamycin, or doxycycline
Tonsilitis	*Streptococcus pyogenes (group A Streptococcus)*	Sore throat, fever, swollen tonsils, ear pain	Respiratory droplets or direct contact	Throat culture or Rapid strep test	Penicillin or amoxicillin

Table 4.1: Oral infections

4.3 BACTERIAL INFECTIONS OF THE GASTROINTESTINAL TRACT

A wide range of gastrointestinal diseases are caused by bacterial contamination of food (table 4.3). Recall that foodborne disease can arise from either infection or intoxication. In both cases, bacterial toxins are typically responsible for producing disease signs and symptoms. The distinction lies in where the toxins are produced. In an infection, the microbial agent is ingested, colonizes the gut, and then produces toxins that damage host cells. In an intoxication, bacteria produce toxins in the food before it is ingested. In either case, the toxins cause damage to the cells lining the gastrointestinal tract, typically the colon. This leads to the common signs and symptoms of diarrhea or watery stool and abdominal cramps, or the more severe dysentery. Symptoms of foodborne diseases also often include nausea and vomiting, which are mechanisms the body uses to expel the toxic materials.

Most bacterial gastrointestinal illness is short-lived and self-limiting; however, loss of fluids due to severe diarrheal illness can lead to dehydration that can, in some cases, be fatal without proper treatment. Oral rehydration therapy with electrolyte solutions is an essential aspect of treatment for most patients with GI disease, especially in children and infants.

STAPHYLOCOCCAL FOOD POISONING

Staphylococcal food poisoning is one form of food intoxication. When *Staphylococcus aureus* grows in food, it may produce enterotoxins that, when ingested, can cause symptoms such as nausea, diarrhea, cramping, and vomiting within one to six hours. In some severe cases, it may cause headache, dehydration, and changes in blood pressure and heart rate. Signs and symptoms resolve within 24 to 48 hours. *S. aureus* is often associated with a variety of raw or undercooked and cooked foods including meat (e.g., canned meat, ham, and sausages) and dairy products (e.g., cheeses, milk, and butter). It is also commonly found on hands and can be transmitted to prepared foods through poor hygiene, including poor handwashing and the use of contaminated food preparation surfaces, such as cutting boards. The greatest risk is for food left at a temperature below 60 °C (140 °F), which allows the bacteria to grow. Cooked foods should generally be reheated to at least 60 °C (140 °F) for safety and most raw meats should be cooked to even higher internal temperatures (figure 4.12).

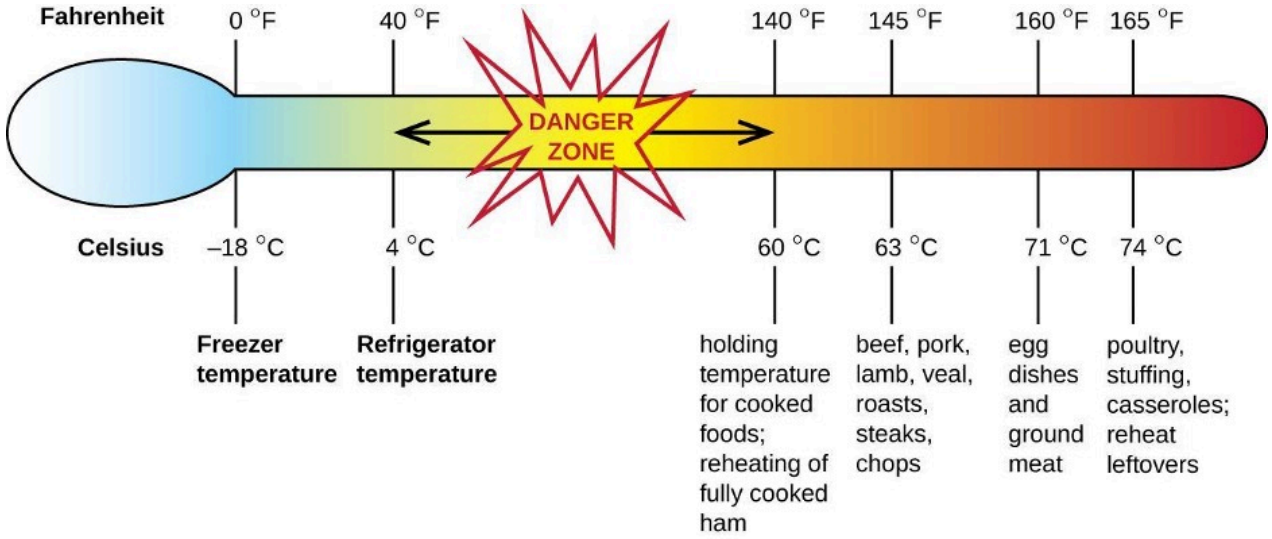

Figure 4.12: This figure indicates safe internal temperatures associated with the refrigeration, cooking, and reheating of different foods. Temperatures above refrigeration and below the minimum cooking temperature may allow for microbial growth, increasing the likelihood of foodborne disease. *Figure description available at the end of the chapter.*

There are at least 21 *Staphylococcal* enterotoxins and *Staphylococcal* enterotoxin-like toxins that can cause food intoxication. The enterotoxins are proteins that are resistant to low pH, allowing them to pass through the stomach. They are heat stable and are not destroyed by boiling at 100 °C. Even though the bacterium itself may be killed, the enterotoxins alone can cause vomiting and diarrhea, although the mechanisms are not fully understood. At least some of the symptoms may be caused by the enterotoxin functioning as a superantigen and provoking a strong immune response by activating T cell proliferation.

The rapid onset of signs and symptoms helps to diagnose this foodborne illness. Because the bacterium does not need to be present for the toxin to cause symptoms, diagnosis is confirmed by identifying the toxin in a food sample or in biological specimens (feces or vomitus) from the patient. Serological techniques, including ELISA, can also be used to identify the toxin in food samples.

The condition generally resolves relatively quickly, within 24 hours, without treatment. In some cases, supportive treatment in a hospital may be needed.

SHIGELLOSIS (BACILLARY DYSENTERY)

When gastrointestinal illness is associated with the rod-shaped, gram-negative bacterium *Shigella*, it is called bacillary dysentery, or shigellosis. Infections can be caused by *S. dysenteriae*, *S. flexneri*, *S. boydii*, and/or *S. sonnei* that colonize the GI tract. Shigellosis can be spread from hand to mouth or through contaminated food and water. Most commonly, it is transmitted through the fecal-oral route.

Shigella bacteria invade intestinal epithelial cells. When taken into a phagosome, they can escape and then live within the cytoplasm of the cell or move to adjacent cells. As the organisms multiply, the M cells of the Peyer's patches in the intestine may become ulcerated and cause loss of fluid. Stomach cramps, fever, and watery diarrhea that may also contain pus, mucus, and/or blood often develop. More severe cases may result in ulceration of the mucosa, dehydration, and rectal bleeding. Additionally, patients may later develop hemolytic uremic syndrome (HUS), a serious condition in which damaged blood cells build up in the kidneys and may cause kidney failure, or reactive arthritis, a condition in which arthritis develops in multiple joints following infection. Patients may also develop chronic post-infection irritable bowel syndrome (IBS).

S. dysenteriae type 1 is able to produce Shiga toxin, which targets the endothelial cells of small blood vessels in the small and large intestine by binding to a glycosphingolipid. Once inside the endothelial cells, the toxin targets the large ribosomal subunit, thus affecting protein synthesis of these cells. Hemorrhaging and lesions in the colon can result. The toxin can target the kidney's glomerulus, the blood vessels where filtration of blood in the kidney begins, thus resulting in HUS.

Stool samples, which should be processed promptly, are analyzed using serological or molecular techniques. One common method is to perform immunoassays for *S. dysenteriae.* (Other methods that can be used to identify *Shigella* include API test strips, Enterotube systems, or PCR testing. The presence of white blood cells and blood in fecal samples occurs in about 70% of patients[4] (figure 4.13). Severe cases may require antibiotics such as ciprofloxacin and azithromycin, but these must be carefully prescribed because resistance is increasingly common.

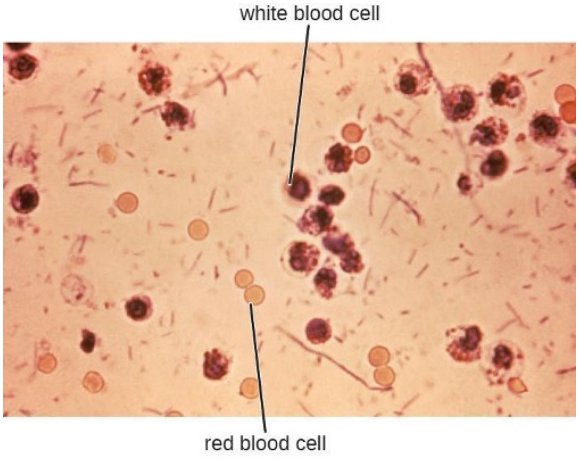

Figure 4.13: Red and white blood cells can be seen in this micrograph of a stool sample from a patient with shigellosis. *Figure description available at the end of the chapter.*

SALMONELLOSIS

Salmonella gastroenteritis, also called salmonellosis, is caused by the rod-shaped, gram-negative bacterium *Salmonella*. Two species, *S. enterica* and *S. bongori*, cause disease in humans, but *S. enterica* is the most common. The most common serotypes of *S. enterica* are Enteritidis and Typhi. We will discuss typhoid fever caused by serotypes Typhi and Paratyphi A separately. Here, we will focus on salmonellosis caused by other serotypes.

Salmonella is a part of the normal intestinal microbiota of many individuals. However, salmonellosis is caused by exogenous agents, and infection can occur depending on the serotype, size of the inoculum, and overall health of the host. Infection is caused by ingestion of contaminated food, handling of eggshells, or exposure to certain animals. *Salmonella* is part of poultry's normal microbiota, so exposure to raw eggs and raw poultry can increase the risk of infection. Handwashing and cooking foods thoroughly greatly reduce the risk of transmission. *Salmonella* bacteria can survive freezing for extended periods but cannot survive high temperatures.

Once the bacteria are ingested, they multiply within the intestines and penetrate the epithelial mucosal cells via M cells where they continue to grow (figure 4.14). They trigger inflammatory processes and the hypersecretion of fluids. Once inside the body, they can persist inside the phagosomes of macrophages. *Salmonella* can cross the epithelial cell membrane and enter the bloodstream and lymphatic system. Some strains of *Salmonella* also produce an enterotoxin that can cause an intoxication.

Infected individuals develop fever, nausea, abdominal cramps, vomiting, headache, and diarrhea. These signs and symptoms generally last a few days to a week. According to the Centers for Disease Control and Prevention (CDC), there are 1,000,000 cases annually, with 380 deaths each year.[5] However, because the disease is usually self-limiting, many cases are not reported to doctors and the overall incidence may be underreported. Diagnosis involves culture followed by serotyping and DNA fingerprinting if needed. Positive results are reported to the CDC. When an unusual serotype is detected, samples are sent to the CDC for further analysis. Serotyping is important for determining treatment. Oral rehydration therapy is commonly used. Antibiotics are only recommended for serious cases. When antibiotics are needed, as in immunocompromised patients, fluoroquinolones, third-generation cephalosporins, and ampicillin are recommended. Antibiotic resistance is a serious concern.

TYPHOID FEVER

Certain serotypes of *S. enterica*, primarily serotype Typhi (*S. typhi*) but also Paratyphi, cause a more severe type of salmonellosis called typhoid fever. This serious illness, which has an untreated mortality rate of 10%, causes high fever, body aches, headache, nausea, lethargy, and a possible rash.

Some individuals carry *S. typhi* without presenting signs or symptoms (known as asymptomatic carriers) and continually shed them through their feces. These carriers often have the bacteria in the gallbladder or intestinal epithelium. Individuals consuming food or water contaminated with these feces can become infected.

S. typhi penetrate the intestinal mucosa, grow within the macrophages, and are transported through the body, most notably to the liver and gallbladder. Eventually, the macrophages lyse, releasing *S. typhi* into the bloodstream and lymphatic system. Mortality can result from ulceration and perforation of the intestine. A wide range of complications, such as pneumonia and jaundice, can occur with disseminated disease.

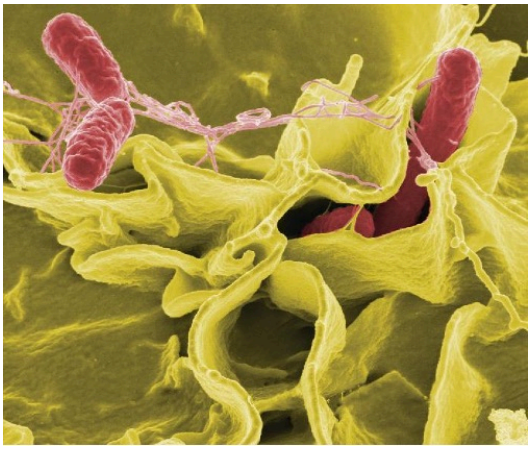

Figure 4.14: Salmonella entering an intestinal epithelial cell by reorganizing the host cell's cytoskeleton via the trigger mechanism. *Figure description available at the end of the chapter.*

S. typhi have *Salmonella* pathogenicity islands (SPIs) that contain the genes for many of their virulence factors. Two examples of important typhoid toxins are the Vi antigen, which encodes for capsule production, and chimeric A2B5 toxin, which causes many of the signs and symptoms of the acute phase of typhoid fever.

Clinical examination and culture are used to make the diagnosis. The bacteria can be cultured from feces, urine, blood, or bone marrow. Serology, including ELISA, is used to identify the most pathogenic strains, but confirmation with DNA testing or culture is needed. A PCR test can also be used, but is not widely available.

The recommended antibiotic treatment involves fluoroquinolones, ceftriaxone, and azithromycin. Individuals must be extremely careful to avoid infecting others during treatment. Typhoid fever can be prevented through vaccination for individuals traveling to parts of the world where it is common.

E. COLI INFECTIONS

The gram-negative rod *Escherichia coli* is a common member of the normal microbiota of the colon. Although the vast majority of *E. coli* strains are helpful commensal bacteria, some can be pathogenic and may cause dangerous diarrheal disease. The pathogenic strains have additional virulence factors such as type 1 fimbriae that promote colonization of the colon or may produce toxins (see section 2.14). These virulence factors are acquired through horizontal gene transfer.

Extraintestinal disease can result if the bacteria spread from the gastrointestinal tract. Although these bacteria can be spread from person to person, they are often acquired through contaminated food or water. There are six recognized pathogenic groups of *E. coli*, but we will focus here on the four that are most commonly transmitted through food and water.

Enterotoxigenic *E. coli* (ETEC), also known as traveler's diarrhea, causes diarrheal illness and is common in less developed countries. In Mexico, ETEC infection is called Montezuma's Revenge. Following ingestion of contaminated food or water, infected individuals develop watery diarrhea, abdominal cramps, malaise (a feeling of being unwell), and a low fever. ETEC produces a heat-stable enterotoxin similar to cholera toxin, and adhesins called colonization factors that help the bacteria to attach to the intestinal wall. Some strains of ETEC also produce heat-labile toxins. The disease is usually relatively mild and self-limiting. Diagnosis involves culturing and PCR. If needed, antibiotic treatment with fluoroquinolones, doxycycline, rifaximin, and trimethoprim-sulfamethoxazole (TMP/SMZ) may shorten infection duration. However, antibiotic resistance is a problem.

Enteroinvasive *E. coli* (EIEC) is very similar to shigellosis, including its pathogenesis of intracellular invasion into intestinal epithelial tissue. This bacterium carries a large plasmid that is involved in epithelial cell penetration. The illness is usually self-limiting, with symptoms including watery diarrhea, chills, cramps, malaise, fever, and dysentery. Culturing and PCR testing can be used for diagnosis. Antibiotic treatment is not recommended, so supportive therapy is used if needed.

Enteropathogenic *E. coli* (EPEC) can cause potentially fatal diarrhea, especially in infants and those in less developed countries. Fever, vomiting, and diarrhea can lead to severe dehydration. These *E. coli* inject a protein (Tir) that attaches to the surface of the intestinal epithelial cells and triggers rearrangement of host cell actin from microvilli to pedestals. Tir also happens to be the receptor for Intimin, a surface protein produced by EPEC, thereby allowing *E. coli* to "sit" on the pedestal. The genes necessary for this pedestal formation are encoded on the locus of the enterocyte effacement (LEE) pathogenicity island. As with ETEC, diagnosis involves culturing and PCR. Treatment is similar to that for ETEC.

The most dangerous strains are enterohemorrhagic *E. coli* (EHEC), which are the strains capable of causing epidemics. In particular, the strain O157:H7 has been responsible for several recent outbreaks. Recall that the O and H refer to surface antigens that contribute to pathogenicity and trigger a host immune response ("O" refers to the O-side chain of the lipopolysaccharide and the "H" refers to the flagella). Similar to EPEC, EHEC also forms pedestals. EHEC also produces a Shiga-like toxin. Because the genome of this bacterium has been sequenced, it is known that the Shiga toxin genes were most likely acquired through transduction (horizontal gene transfer). The Shiga toxin genes originated from *Shigella dysenteriae*. Prophage from a bacteriophage that previously infected *Shigella* integrated into the chromosome of *E. coli*. The Shiga-like toxin is often called verotoxin.

EHEC can cause disease ranging from relatively mild to life-threatening. Symptoms include bloody diarrhea with severe cramping, but no fever. Although it is often self-limiting, it can lead to hemorrhagic colitis and profuse bleeding. One possible complication is HUS. Diagnosis involves culture, often using MacConkey with sorbitol agar to differentiate between *E. coli* O157:H7, which does not ferment sorbitol, and other less virulent strains of *E. coli* that can ferment sorbitol.

Serological typing or PCR testing also can be used, as well as genetic testing for Shiga toxin. To distinguish EPEC from EHEC, because they both form pedestals on intestinal epithelial cells, it is necessary to test for genes encoding for both the Shiga-like toxin and for the LEE. Both EPEC and EHEC have LEE, but EPEC lacks the gene for Shiga toxin. Antibiotic therapy is not recommended and may worsen HUS because of the toxins released when the bacteria are killed, so supportive therapies must be used. Table 4.2 summarizes the characteristics of the four most common pathogenic groups.

Group	Virulence Factors and Genes	Signs and Symptoms	Diagnostic Tests	Treatment
Enterotoxigenic *E. coli* (ETEC)	Heat stable enterotoxin similar to cholera toxin	Relatively mild, watery diarrhea	Culturing, PCR	Self-limiting; if needed, fluoroquinolones, doxycycline, rifaximin, TMP/SMZ; antibiotic resistance is a problem
Enteroinvasive *E. coli* (EIEC)	*Inv* (invasive plasmid) genes	Relatively mild, watery diarrhea; dysentery or inflammatory colitis may occur	Culturing, PCR; testing for *inv* gene; additional assays to distinguish from *Shigella*	Supportive therapy only; antibiotics not recommended
Enteropathogenic *E. coli* (EPEC)	Locus of enterocyte effacement (LEE) pathogenicity island	Severe fever, vomiting, nonbloody diarrhea, dehydration; potentially fatal	Culturing, PCR; detection of LEE lacking Shiga-like toxin genes	Self-limiting; if needed, fluoroquinolones, doxycycline, rifaximin (TMP/SMZ); antibiotic resistance is a problem
Enterohemorrhagic *E. coli* (EHEC)	Verotoxin	May be mild or very severe; bloody diarrhea; may result in HUS	Culturing; plate on MacConkey agar with sorbitol agar as it does not ferment sorbitol; PCR detection of LEE containing Shiga-like toxin genes	Antibiotics are not recommended due to the risk of HUS

Table 4.2: Some pathogenic groups of E. coli

CHOLERA AND OTHER VIBRIOS

The gastrointestinal disease cholera is a serious infection often associated with poor sanitation, especially following natural disasters, because it is spread through contaminated water and food that has not been heated to temperatures high enough to kill the bacteria. It is caused by *Vibrio cholerae* serotype O1, a gram-negative, flagellated bacterium in the shape of a curved rod (vibrio). According to the CDC, cholera causes an estimated 3 to 5 million cases and 100,000 deaths each year.[6]

Because *V. cholerae* is killed by stomach acid, relatively large doses are needed for a few microbial cells to survive to reach the intestines and cause infection. The motile cells travel through the mucous layer of the intestines, where they attach to epithelial cells and release cholera enterotoxin. The toxin is an A-B toxin with activity through adenylate cyclase (see section 2.14). Within the intestinal cell, cyclic AMP (cAMP) levels increase, which

activates a chloride channel and results in the release of ions into the intestinal lumen. This increase in osmotic pressure in the lumen leads to water entering the lumen as well. As the water and electrolytes leave the body, it causes rapid dehydration and electrolyte imbalance. Diarrhea is so profuse that it is often called "rice water stool," and patients are placed on cots with a hole in them to monitor the fluid loss (figure 4.15).

Cholera is diagnosed by taking a stool sample and culturing for *Vibrio*. The bacteria are oxidase positive and show non-lactose fermentation on MacConkey agar. Gram-negative lactose fermenters will produce red colonies while non-fermenters will produce white/colorless colonies. Gram-positive bacteria will not grow on MacConkey. Lactose fermentation is commonly used for pathogen identification because the normal microbiota generally ferments lactose while pathogens do not. *V. cholerae* may also be cultured on thiosulfate citrate bile salts sucrose (TCBS) agar, a selective and differential media for *Vibrio* spp., which produce a distinct yellow colony.

Cholera may be self-limiting and treatment involves rehydration and electrolyte replenishment. Although antibiotics are not typically needed, they can be used for severe or disseminated disease. Tetracyclines are recommended, but doxycycline, erythromycin, norfloxacin, ciprofloxacin, and TMP/SMZ may be used. Recent evidence suggests that azithromycin is also a good first-line antibiotic. Good sanitation—including appropriate sewage treatment, clean supplies for cooking, and purified drinking water—is important to prevent infection (figure 4.15).

Figure 4.15: (a) Outbreaks of cholera often occur in areas with poor sanitation or after natural disasters that compromise sanitation infrastructure. (b) At a cholera treatment center in Haiti, patients are receiving intravenous fluids to combat the dehydrating effects of this disease. They often lie on a cot with a hole in it and a bucket underneath to allow for monitoring of fluid loss. (c) This scanning electron micrograph shows Vibrio cholera. *Figure description available at the end of the chapter.*

V. cholera is not the only *Vibrio* species that can cause disease. *V. parahemolyticus* is associated with consumption of contaminated seafood and causes gastrointestinal illness with signs and symptoms such as watery diarrhea, nausea, fever, chills, and abdominal cramps. The bacteria produce a heat-stable hemolysin, leading to dysentery and possible disseminated disease. It also sometimes causes wound infections. *V. parahemolyticus* is diagnosed using cultures from blood, stool, or a wound. As with *V. cholera*, selective medium (especially TCBS agar) works well. Tetracycline and ciprofloxacin can be used to treat severe cases, but antibiotics generally are not needed.

Vibrio vulnificus is found in warm seawater and, unlike *V. cholerae*, is not associated with poor sanitary conditions. The bacteria can be found in raw seafood, and ingestion causes gastrointestinal illness. It can also be acquired by individuals with open skin wounds who are exposed to water with high concentrations of the pathogen. In some cases, the infection spreads to the bloodstream and causes septicemia. Skin infection can lead to edema, ecchymosis (discoloration of skin due to bleeding), and abscesses. Patients with underlying disease have a high fatality rate of about 50%. It is of particular concern for individuals with chronic liver disease or who are otherwise immunodeficient because a healthy immune system can often prevent infection from developing.

V. vulnificus is diagnosed by culturing for the pathogen from stool samples, blood samples, or skin abscesses. Adult patients are treated with doxycycline combined with a third generation cephalosporin or with fluoroquinolones, and children are treated with TMP/SMZ.

CAMPYLOBACTER JEJUNI GASTROENTERITIS

Campylobacter is a genus of gram-negative, spiral or curved bacteria. They may have one or two flagella. *Campylobacter jejuni* gastroenteritis, a form of campylobacteriosis, is a widespread illness that is caused by *Campylobacter jejuni*. The primary route of transmission is through poultry that becomes contaminated during slaughter. Handling of the raw chicken in turn contaminates cooking surfaces, utensils, and other foods. Unpasteurized milk or contaminated water are also potential vehicles of transmission. In most cases, the illness is self-limiting and includes fever, diarrhea, cramps, vomiting, and sometimes dysentery. More serious signs and symptoms, such as bacteremia, meningitis, pancreatitis, cholecystitis, and hepatitis, sometimes occur. It has also been associated with autoimmune conditions such as Guillain-Barré syndrome, a neurological disease that occurs after some infections and results in temporary paralysis. HUS following infection can also occur. The virulence in many strains is the result of hemolysin production and the presence of *Campylobacter* cytolethal distending toxin (CDT), a powerful deoxyribonuclease (DNase) that irreversibly damages the host cell DNA.

Diagnosis involves culture under special conditions, such as elevated temperature, low oxygen tension, and often medium supplemented with antimicrobial agents. These bacteria should be cultured on selective medium (such as Campy CV, charcoal selective medium, or cefoperazone charcoal deoxycholate agar) and incubated under microaerophilic conditions for at least 72 hours at 42 °C. Antibiotic treatment is not usually needed, but erythromycin or ciprofloxacin may be used.

PEPTIC ULCERS

The gram-negative bacterium *Helicobacter pylori* is able to tolerate the acidic environment of the human stomach and has been shown to be a major cause of peptic ulcers, which are ulcers of the stomach or duodenum. The bacterium is also associated with increased risk of stomach cancer (figure 4.16). According to the CDC, approximately two-thirds of the population is infected with *H. pylori,* but less than 20% have a risk of developing ulcers or stomach cancer. *H. pylori* is found in approximately 80% of stomach ulcers and in over 90% of duodenal ulcers.[7]

H. pylori colonizes epithelial cells in the stomach using pili for adhesion. These bacteria produce urease, which stimulates an immune response and creates ammonia that neutralizes stomach acids to provide a more hospitable microenvironment. The infection damages the cells of the stomach lining, including those that normally produce the protective mucus that serves as a barrier between the tissue and stomach acid. As a result, inflammation (gastritis) occurs and ulcers may slowly develop. Ulcer formation can also be caused by toxin activity. It has been reported that 50% of clinical isolates of *H. pylori* have detectable levels of exotoxin activity *in vitro*.[8] This toxin, VacA, induces vacuole formation in host cells. VacA has no primary sequence homology with other bacterial toxins, and in a mouse model, there is a correlation between the presence of the toxin gene, the activity of the toxin, and gastric epithelial tissue damage.

Signs and symptoms include nausea, lack of appetite, bloating, burping, and weight loss. Bleeding ulcers may produce dark stools. If no treatment is provided, the ulcers can become deeper, more tissues can be involved, and stomach perforation can occur. Because perforation allows digestive enzymes and acid to leak into the body, it is a very serious condition.

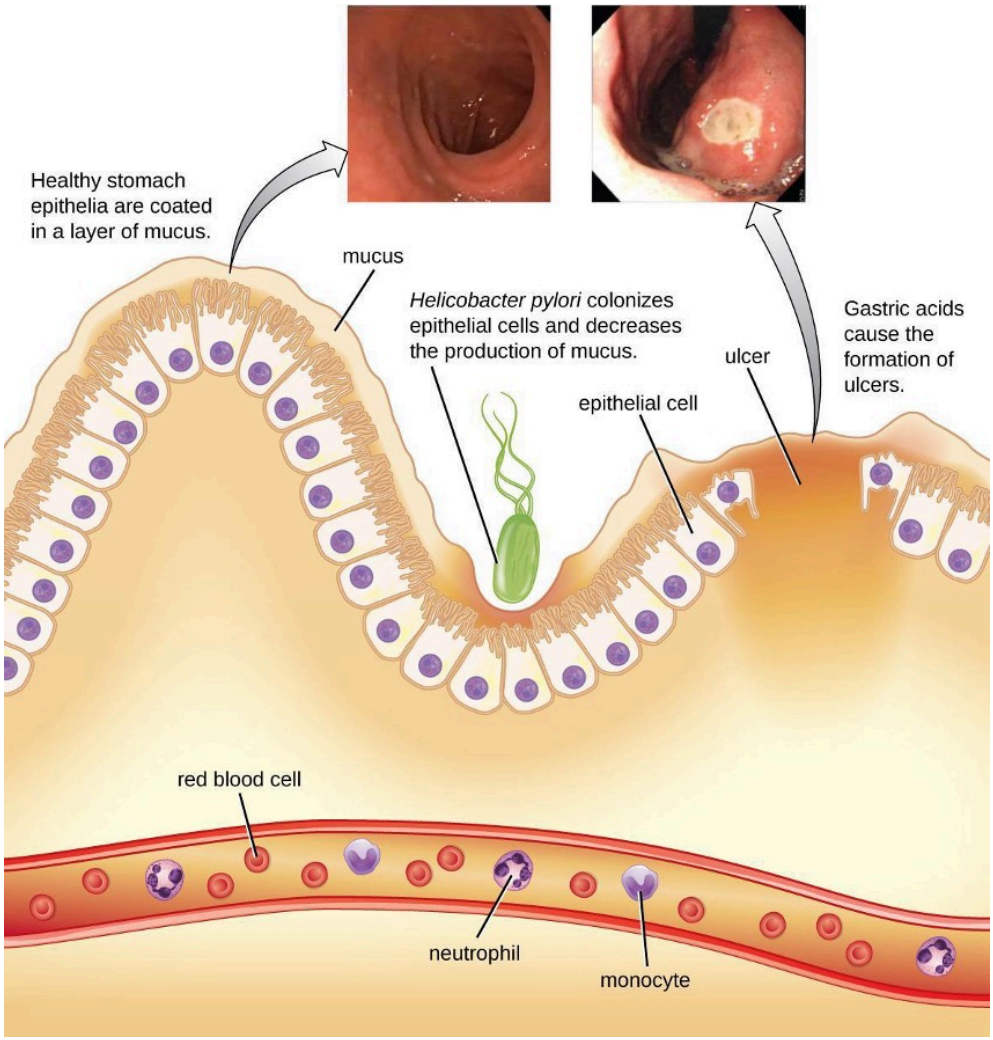

Figure 4.16: Helicobacter infection decreases mucus production and causes peptic ulcers. *Figure description available at the end of the chapter.*

To diagnose *H. pylori* infection, multiple methods are available. In a breath test, the patient swallows radiolabeled urea. If *H. pylori* is present, the bacteria will produce urease to break down the urea. This reaction produces radiolabeled carbon dioxide that can be detected in the patient's breath. Blood testing can also be used to detect antibodies to *H. pylori*. The bacteria themselves can be detected using either a stool test or a stomach wall biopsy.

Antibiotics can be used to treat the infection. However, unique to *H. pylori,* the recommendation from the US Food and Drug Administration is to use a triple therapy. The current protocols are 10 days of treatment with omeprazole, amoxicillin, and clarithromycin (OAC); 14 days of treatment with bismuth subsalicylate, metronidazole, and tetracycline (BMT); or 10 or 14 days of treatment with lansoprazole, amoxicillin, and clarithromycin (LAC). Omeprazole, bismuth subsalicylate, and lansoprazole are not antibiotics but are instead used to decrease acid levels because *H. pylori* prefers acidic environments.

Although treatment is often valuable, there are also risks to *H. pylori* eradication. Infection with *H. pylori* may actually protect against some cancers, such as esophageal adenocarcinoma and gastroesophageal reflux disease.[9,10]

CLOSTRIDIUM PERFRINGENS GASTROENTERITIS

Clostridium perfringens gastroenteritis is a generally mild foodborne disease that is associated with undercooked meats and other foods. *C. perfringens* is a gram-positive, rod-shaped, endospore-forming anaerobic bacterium that is tolerant of high and low temperatures. At high temperatures, the bacteria can form endospores that will germinate rapidly in foods or within the intestine. Food poisoning by type A strains is common. This strain always produces an enterotoxin, sometimes also present in other strains, that causes the clinical symptoms of cramps and diarrhea. A more severe form of the illness, called pig-bel or enteritis necroticans, causes hemorrhaging, pain, vomiting, and bloating. Gangrene of the intestines may result. This form has a high mortality rate but is rare in the United States.

Diagnosis involves detecting the *C. perfringens* toxin in stool samples using either molecular biology techniques (PCR detection of the toxin gene) or immunology techniques (ELISA). The bacteria itself may also be detected in foods or in fecal samples. Treatment includes rehydration therapy, electrolyte replacement, and intravenous fluids. Antibiotics are not recommended because they can damage the balance of the microbiota in the gut, and there are concerns about antibiotic resistance. The illness can be prevented through proper handling and cooking of foods, including prompt refrigeration at sufficiently low temperatures and cooking food to a sufficiently high temperature.

CLOSTRIDIUM DIFFICILE

Clostridium difficile is a gram-positive rod that can be a commensal bacterium as part of the normal microbiota of healthy individuals. When the normal microbiota is disrupted by long-term antibiotic use, it can allow the overgrowth of this bacterium, resulting in antibiotic-associated diarrhea caused by *C. difficile*. Antibiotic-associated diarrhea can also be considered a nosocomial disease. Patients at the greatest risk of *C. difficile* infection are those who are immunocompromised, have been in health-care settings for extended periods, are older, have recently taken antibiotics, have had gastrointestinal procedures done, or use proton pump inhibitors, which reduce stomach acidity and allow proliferation of *C. difficile*. Because this species can form endospores, it can survive for extended periods of time in the environment under harsh conditions and is a considerable concern in health-care settings.

This bacterium produces two toxins, *Clostridium difficile* toxin A (TcdA) and *Clostridium difficile* toxin B (TcdB). These toxins inactivate small GTP-binding proteins, resulting in actin condensation and cell rounding, followed by cell death. Infections begin with focal necrosis, then ulceration with exudate and can progress to pseudomembranous colitis, which involves inflammation of the colon and the development of a pseudomembrane of fibrin containing dead epithelial cells and leukocytes (figure 4.17). Watery diarrhea, dehydration, fever, loss of appetite, and abdominal pain can result. Perforation of the colon can occur, leading to septicemia, shock, and death. *C. difficile* is also associated with necrotizing enterocolitis in premature babies and neutropenic enterocolitis associated with cancer therapies.

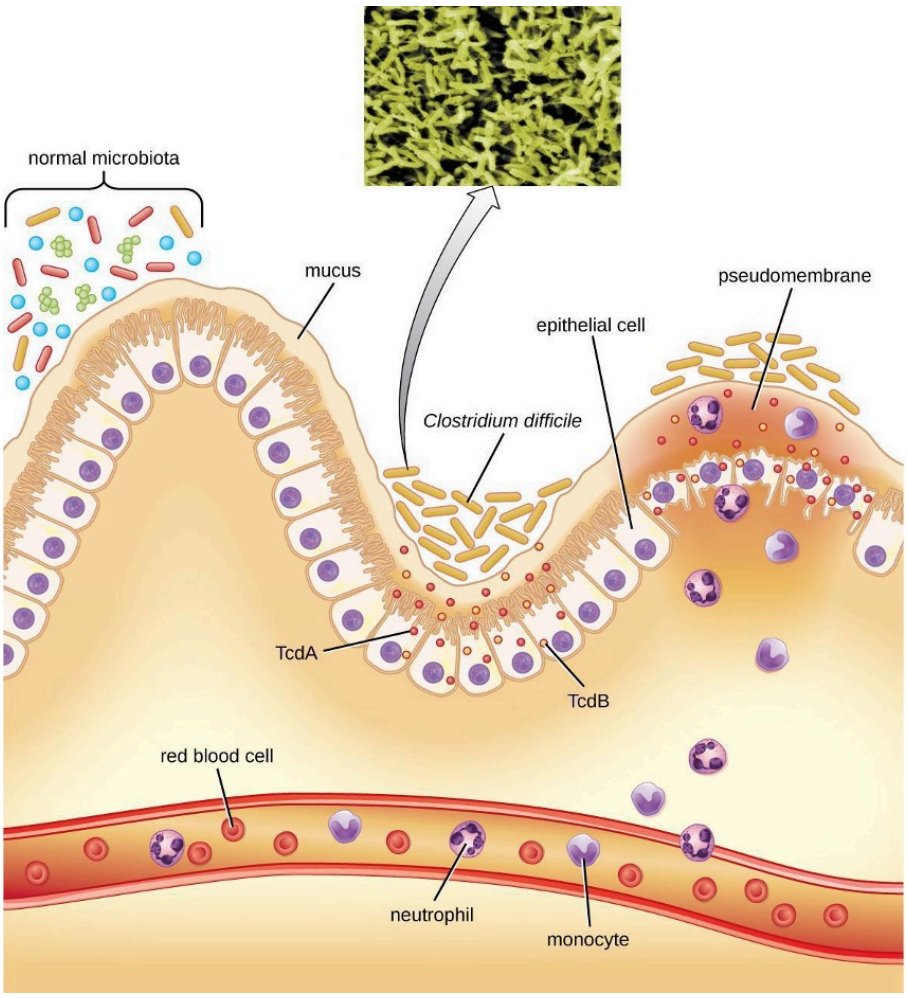

Figure 4.17: Clostridium difficile is able to colonize the mucous membrane of the colon when the normal microbiota is disrupted. The toxins TcdA and TcdB trigger an immune response, with neutrophils and monocytes migrating from the bloodstream to the site of infection. Over time, inflammation and dead cells contribute to the development of a pseudomembrane. *Figure description available at the end of the chapter.*

Diagnosis is made by considering the patient history (such as exposure to antibiotics), clinical presentation, imaging, endoscopy, lab tests, and other available data. Detecting the toxin in stool samples is used to confirm diagnosis. Although culture is preferred, it is rarely practical in clinical practice because the bacterium is an obligate anaerobe. Nucleic acid amplification tests, including PCR, are considered preferable to ELISA testing for molecular analysis.

The first step of conventional treatment is to stop antibiotic use, and then to provide supportive therapy with electrolyte replacement and fluids. Metronidazole is the preferred treatment if the *C. difficile* diagnosis has been confirmed. Vancomycin can also be used, but it should be reserved for patients for whom metronidazole was ineffective or who meet other criteria (e.g., under 10 years of age, pregnant, or allergic to metronidazole).

A newer approach to treatment, known as a fecal transplant, focuses on restoring the microbiota of the gut in order to combat the infection. In this procedure, a healthy individual donates a stool sample, which is mixed with saline and transplanted to the recipient via colonoscopy, endoscopy, sigmoidoscopy, or enema. It has been reported that this procedure has greater than 90% success in resolving *C. difficile* infections.[11]

FOODBORNE ILLNESS DUE TO *BACILLUS CEREUS*

Bacillus cereus, commonly found in soil, is a gram-positive endospore-forming bacterium that can sometimes cause foodborne illness. *B. cereus* endospores can survive cooking and produce enterotoxins in food after it has been heated; illnesses often occur after eating rice and other prepared foods left at room temperature for too long. The signs and symptoms appear within a few hours of ingestion and include nausea, pain, and abdominal cramps. *B. cereus* produces two toxins: one causing diarrhea, and the other causing vomiting. More severe signs and symptoms can sometimes develop.

Diagnosis can be accomplished by isolating bacteria from stool samples or vomitus and uneaten infected food. Treatment involves rehydration and supportive therapy. Antibiotics are not typically needed, as the illness is usually relatively mild and is due to toxin activity.

FOODBORNE ILLNESS DUE TO *YERSINIA*

The genus *Yersinia* is best known for *Yersinia pestis*, a gram-negative rod that causes the plague. However, *Y. enterocolitica* and *Y. pseudotuberculosis* can cause gastroenteritis. The infection is generally transmitted through the fecal-oral route, with ingestion of food or water that has been contaminated by feces. Intoxication can also result because of the activity of its endotoxin and exotoxins (enterotoxin and cytotoxin necrotizing factor). The illness is normally relatively mild and self-limiting. However, severe diarrhea and dysentery can develop in infants. In adults, the infection can spread and cause complications such as reactive arthritis, thyroid disorders, endocarditis, glomerulonephritis, eye inflammation, and/or erythema nodosum. Bacteremia may develop in rare cases.

Diagnosis is generally made by detecting the bacteria in stool samples. Samples may also be obtained from other tissues or body fluids. Treatment is usually supportive, including rehydration, without antibiotics. If bacteremia or other systemic disease is present, then antibiotics such as fluoroquinolones, aminoglycosides, doxycycline, and trimethoprim-sulfamethoxazole may be used. Recovery can take up to two weeks.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Bacillus cereus infection	*Bacillus cereus*	Nausea, pain, abdominal cramps, diarrhea or vomiting	Ingestion of contaminated rice or meat, even after cooking	Testing stool sample, vomitus, or uneaten food for presence of bacteria	None
Campylobacter jejuni gastroenteritis	*Campylobacter jejuni*	Fever, diarrhea, cramps, vomiting, and sometimes dysentery; sometimes more severe organ or autoimmune effects	Ingestion of unpasteurized milk, undercooked chicken, or contaminated water	Culture on selective medium with elevated temperature and low oxygen concentration	Generally none; erythromycin or ciprofloxacin if necessary
Cholera	*Vibrio cholera*	Severe diarrhea and fluid loss, potentially leading to shock, renal failure, and death	Ingestion of contaminated water or food	Culture on selective medium (TCBS agar); distinguished as oxidase positive with fermentative metabolisms	Generally none; tetracyclines, azithromycin, others if necessary

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Clostridium difficile infection	*Clostridium difficile*	Pseudomembranous colitis, watery diarrhea, fever, abdominal pain, loss of appetite, dehydration; in severe cases, perforation of the colon, septicemia, shock, and death	Overgrowth of *C. difficile* in the normal microbiota due to antibiotic use; hospital-acquired infections in immunocompromised patients	Detection of toxin in stool, nucleic acid amplification tests (e.g., PCR)	Discontinuation of previous antibiotic treatment; metronidazole or vancomycin
Clostridium perfringens gastroenteritis	*Clostridium perfringens* (especially type A)	Mild cramps and diarrhea in most cases; in rare cases, hemorrhaging, vomiting, intestinal gangrene, and death	Ingestion of undercooked meats containing *C. perfringens* endospores	Detection of toxin or bacteria in stool or uneaten food	None
E. coli infection	ETEC, EPEC, EIEC, EHEC	Watery diarrhea, dysentery, cramps, malaise, fever, chills, dehydration; in EHEC, possible severe complications such as hematolytic uremic syndrome	Ingestion of contaminated food or water	Tissue culture, immunochemical assays, PCR, gene probes	Not recommended for EIEC and EHEC; fluoroquinolones, doxycycline, rifaximin, and TMP/SMZ possible for ETEC and EPEC
Peptic ulcers	*Helicobacter pylori*	Nausea, bloating, burping, lack of appetite, weight loss, perforation of stomach, blood in stools	Normal flora, can also be acquired via saliva, Fecal-oral route via contaminated food and water	Breath test, detection of antibodies in blood, detection of bacteria in stool sample or stomach biopsy	Amoxicillin, clarithromycin metronidazole, tetracycline, lansoprazole; antacids may also be given in combination with antibiotics
Salmonellosis	*Salmonella enterica*, serotype Enteritides	Fever, nausea, vomiting, abdominal cramps, headache, diarrhea; can be fatal in infants	Ingestion of contaminated food, handling of eggshells or contaminated animals	Culturing, serotyping and DNA fingerprinting	Not generally recommended; fluoroquinolones, ampicillin, others for immunocompromised patients
Shigella dysentery	*Shigella dysenteriae, S. flexneri, S. boydii,* and *S. sonnei*	Abdominal cramps, fever, diarrhea, dysentery; possible complications: reactive arthritis and hemolytic uremic syndrome	Fecal-oral route via contaminated food and water	Testing of stool samples for presence of blood and leukocytes; culturing, PCR, immunoassay for *S. dysenteriae*	Ciprofloxacin, azithromycin

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Staphylococcal food poisoning	*Staphylococcus aureus*	Rapid-onset nausea, diarrhea, vomiting lasting 24–48 hours; possible dehydration and change in blood pressure and heart rate	Ingestion of raw or undercooked meat or dairy products contaminated with staphylococcal enterotoxins	ELISA to detect enterotoxins in uneaten food, stool, or vomitus	None
Typhoid fever	*S. enterica*, subtypes Typhi or Paratyphi	Aches, headaches, nausea, lethargy, diarrhea or constipation, possible rash; lethal perforation of intestine can occur	Fecal-oral route; may be spread by asymptomatic carriers	Culture of blood, stool, or bone marrow, serologic tests; PCR tests when available	Fluoroquinolones, ceftriaxone, azithromycin; preventive vaccine available
Yersinia infection	*Yersinia enterocolitica, Y. pseudotuberculosis*	Generally mild diarrhea and abdominal cramps; in some cases, bacteremia can occur, leading to severe complications	Fecal-oral route, typically via contaminated food or water	Testing stool samples, tissues, body fluids	Generally none; fluoroquinolones, aminoglycosides, others for systemic infections

Table 4.3: Bacterial infections of the GI tract

4.4 VIRAL INFECTIONS OF THE GASTROINTESTINAL TRACT

In the developing world, acute viral gastroenteritis is devastating and a leading cause of death for children.[12] Worldwide, diarrhea is the second leading cause of mortality for children under age five, and 70% of childhood gastroenteritis is viral.[13] In this section, we will discuss rotaviruses and other, less common viruses that can also cause gastrointestinal illnesses.

GASTROENTERITIS CAUSED BY ROTAVIRUSES

Rotaviruses are double-stranded RNA viruses in the family Reoviridae. They are responsible for common diarrheal illness, although prevention through vaccination is becoming more common. The virus is primarily spread by the fecal-oral route (figure 4.18).

These viruses are widespread in children, especially in day-care centers. The CDC estimates that 95% of children in the United States have had at least one rotavirus infection by the time they reach age five.[14] Due to the memory of the body's immune system, adults who come into contact with rotavirus will not contract the infection or, if they do, are asymptomatic. The elderly, however, are vulnerable to rotavirus infection due to weakening of the immune system with age, so infections can spread through nursing homes and similar facilities. In these cases, the infection may be transmitted from a family member who may have subclinical or clinical disease. The virus can also be transmitted from contaminated surfaces on which it can survive for some time.

Infected individuals exhibit fever, vomiting, and diarrhea. The virus can survive in the stomach following a meal, but is normally found in the small intestines, particularly the epithelial cells on the villi. Infection can cause food intolerance, especially with respect to lactose. The illness generally appears after an incubation period of about two days and lasts for approximately one week (three to eight days). Without supportive treatment, the illness can cause severe fluid loss, dehydration, and even death. Even with milder illness, repeated infections can potentially lead to malnutrition, especially in developing countries, where rotavirus infection is common due to poor sanitation and lack of access to clean drinking water. Patients (especially children) who are malnourished after an episode of diarrhea are more susceptible to future diarrheal illness, increasing their risk of death from rotavirus infection.

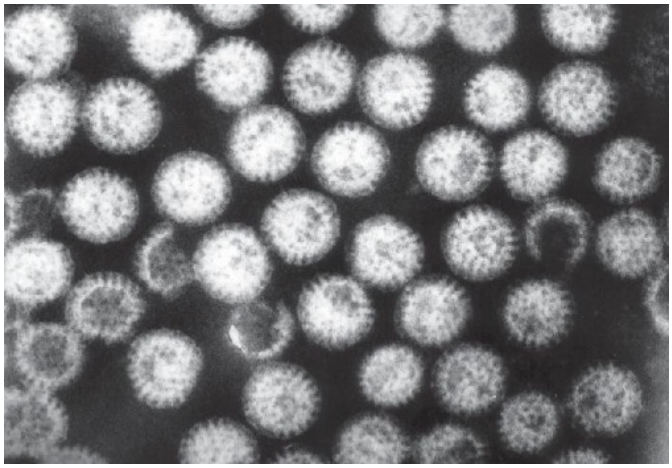

Figure 4.18: Rotaviruses in a fecal sample are visualized using electron microscopy. *Figure description available at the end of the chapter.*

The most common clinical tool for diagnosis is enzyme immunoassay, which detects the virus from fecal samples. Latex agglutination assays are also used. Additionally, the virus can be detected using electron microscopy and RT-PCR.

Treatment is supportive with oral rehydration therapy. Preventive vaccination is also available. In the United States, rotavirus vaccines are part of the standard vaccine schedule and administration follows the guidelines of the World Health Organization (WHO). The WHO recommends that all infants worldwide receive the rotavirus vaccine, the first dose between six and 15 weeks of age and the second before 32 weeks.[15]

GASTROENTERITIS CAUSED BY NOROVIRUSES

Noroviruses, commonly identified as Norwalk viruses, are caliciviruses (RNA viruses) . Several strains can cause gastroenteritis. There are millions of cases a year, predominately in infants, young children, and the elderly. These viruses are easily transmitted and highly contagious. They are known for causing widespread infections in groups of people in confined spaces, such as on cruise ships. The viruses can be transmitted through direct contact, through touching contaminated surfaces, and through contaminated food. Because the virus is not killed by disinfectants used at standard concentrations for killing bacteria, the risk of transmission remains high, even after cleaning.

The signs and symptoms of norovirus infection are similar to those for rotavirus, with watery diarrhea, mild cramps, and fever. Additionally, these viruses sometimes cause projectile vomiting. The illness is usually relatively mild, develops 12 to 48 hours after exposure, and clears within a couple of days without treatment. However, dehydration may occur.

Norovirus can be detected using PCR or enzyme immunoassay (EIA) testing. RT-qPCR is the preferred approach as EIA is insufficiently sensitive. If EIA is used for rapid testing, diagnosis should be confirmed using PCR. No medications are available, but the illness is usually self-limiting. Rehydration therapy and electrolyte replacement may be used. Good hygiene, hand washing, and careful food preparation reduce the risk of infection.

GASTROENTERITIS CAUSED BY ASTROVIRUSES

Astroviruses are single-stranded RNA viruses (family Astroviridae) that can cause severe gastroenteritis, especially in infants and children (table 4.4). Signs and symptoms include diarrhea, nausea, vomiting, fever, abdominal pain, headache, and malaise. The viruses are transmitted through the fecal-oral route (contaminated food or water). For diagnosis, stool samples are analyzed. Testing may involve enzyme immunoassays and immune electron microscopy. Treatment involves supportive rehydration and electrolyte replacement if needed.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Vaccine
Astrovirus gastroenteritis	Astroviruses	Fever, headache, abdominal pain, malaise, diarrhea, vomiting	Fecal-oral route, contaminated food or water	Enzyme immunoassays, immune electron microscopy	None
Norovirus gastroenteritis	Noroviruses	Fever, diarrhea, projectile vomiting, dehydration; generally self-limiting within two days	Highly contagious via direct contact or contact with contaminated food or fomites	Rapid enzyme immunoassay confirmed with RT-qPCR	None
Rotavirus gastroenteritis	Rotaviruses	Fever, diarrhea, vomiting, severe dehydration; recurring infections can lead to malnutrition and death	Fecal-oral route; children and elderly most susceptible	Enzyme immunoassay of stool sample, latex agglutination assays, RT-PCR	Preventive vaccine recommended for infants

Table 4.4: Viral causes of gastroenteritis

HEPATITIS

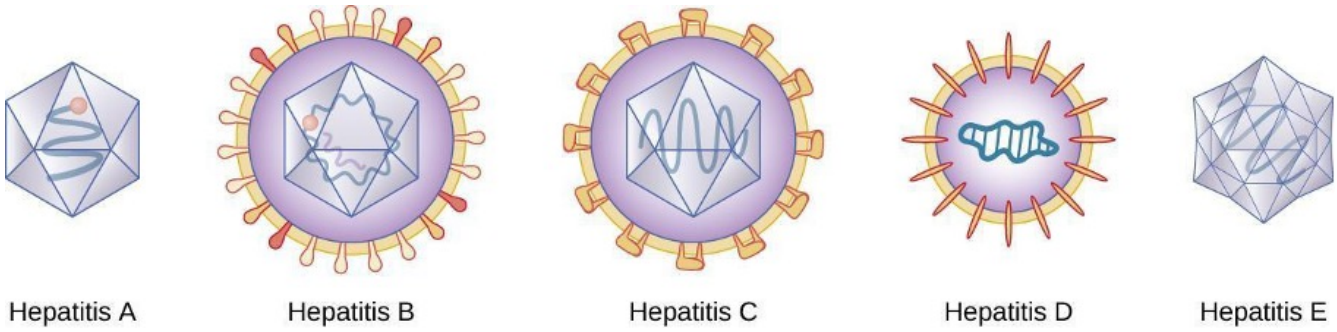

Figure 4.19: Five main types of viruses cause hepatitis. HAV is a non-enveloped ssRNA(+) virus and is a member of the picornavirus family (Baltimore Group IV). HBV is a dsDNA enveloped virus, replicates using reverse transcriptase, and is a member of the hepadnavirus family (Baltimore Group VII). HCV is an enveloped ssRNA(+) virus and is a member of the flavivirus family (Baltimore Group IV). HDV is an enveloped ssRNA(–) that is circular (Baltimore Group V). This virus can only propagate in the presence of HBV. HEV is a non-enveloped ssRNA(+) virus and a member of the Hepeviridae family (Baltimore Group IV). *Figure description available at the end of the chapter.*

Hepatitis is a general term meaning inflammation of the liver, which can have a variety of causes. In some cases, the cause is viral infection. There are five main hepatitis viruses that are clinically significant: hepatitis viruses

A (HAV), B (HBV), C (HCV), D, (HDV) and E (HEV) (figure 4.19). Note that other viruses, such as Epstein-Barr virus (EBV), yellow fever, and cytomegalovirus (CMV) can also cause hepatitis and are discussed in section 6.3.

Although the five hepatitis viruses differ (table 4.5), they can cause some similar signs and symptoms because they all have an affinity for hepatocytes (liver cells). HAV and HEV can be contracted through ingestion while HBV, HCV, and HDV are transmitted by parenteral contact. It is possible for individuals to become long term or chronic carriers of hepatitis viruses.

The virus enters the blood (viremia), spreading to the spleen, the kidneys, and the liver. During viral replication, the virus infects hepatocytes. The inflammation is caused by the hepatocytes replicating and releasing more hepatitis virus. Signs and symptoms include malaise, anorexia, loss of appetite, dark urine, pain in the upper right quadrant of the abdomen, vomiting, nausea, diarrhea, joint pain, and gray stool. Additionally, when the liver is diseased or injured, it is unable to break down hemoglobin effectively, and bilirubin can build up in the body, giving the skin and mucous membranes a yellowish color, a condition called jaundice (figure 4.20). In severe cases, death from liver necrosis may occur.

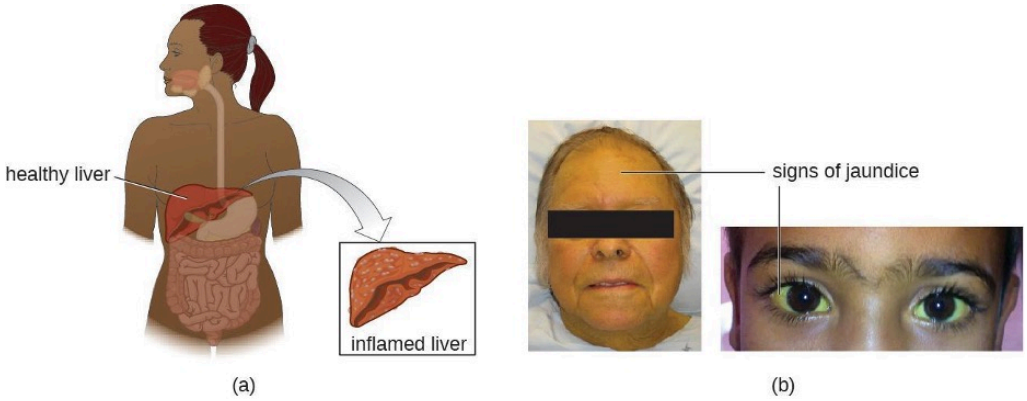

Figure 4.20: (a) Hepatitis is inflammation of the liver resulting from a variety of root causes. It can cause jaundice. (b) Jaundice is characterized by yellowing of the skin, mucous membranes, and sclera of the eyes. *Figure description available at the end of the chapter.*

Despite having many similarities, each of the hepatitis viruses has its own unique characteristics. HAV is generally transmitted through the fecal-oral route, close personal contact, or exposure to contaminated water or food. Hepatitis A can develop after an incubation period of 15 to 50 days (the mean is 30). It is normally mild or even asymptomatic and is usually self-limiting within weeks to months. A more severe form, fulminant hepatitis, rarely occurs but has a high fatality rate of 70–80%. Vaccination is available and is recommended especially for children (between ages one and two), those traveling to countries with higher risk, those with liver disease and certain other conditions, and drug users.

Although HBV is associated with similar signs and symptoms, transmission and outcomes differ. This virus has a mean incubation period of 120 days and is generally associated with exposure to infectious blood or body fluids such as semen or saliva. Exposure can occur through skin puncture, across the placenta, or through mucosal contact, but it is not spread through casual contact such as hugging, hand holding, sneezing, or coughing, or even through breastfeeding or kissing. Risk of infection is greatest for those who use intravenous drugs or who have sexual contact with an infected individual. Health-care workers are also at risk from needle sticks and other injuries when treating infected patients. The infection can become chronic and may progress to cirrhosis or liver failure. It is also associated with liver cancer. Chronic infections are associated with the highest mortality rates and are more common in infants. Approximately 90% of infected infants become chronic carriers, compared with only 6–10% of infected adults.[16] Vaccination is available and is recommended for children as

part of the standard vaccination schedule (one dose at birth and the second by 18 months of age) and for adults at greater risk (e.g., those with certain diseases, intravenous drug users, and those who have sex with multiple partners). Health-care agencies are required to offer the HBV vaccine to all workers who have occupational exposure to blood and/or other infectious materials.

HCV is often undiagnosed and therefore may be more widespread than is documented. It has a mean incubation period of 45 days and is transmitted through contact with infected blood. Although some cases are asymptomatic and/or resolve spontaneously, 75%–85% of infected individuals become chronic carriers. Nearly all cases result from parenteral transmission often associated with IV drug use or transfusions. The risk is greatest for individuals with past or current history of intravenous drug use or who have had sexual contact with infected individuals. It has also been spread through contaminated blood products and can even be transmitted through contaminated personal products such as toothbrushes and razors. New medications have recently been developed that show great effectiveness in treating HCV and that are tailored to the specific genotype causing the infection.

HDV is uncommon in the United States and only occurs in individuals who are already infected with HBV, which it requires for replication. Therefore, vaccination against HBV is also protective against HDV infection. HDV is transmitted through contact with infected blood.

HEV infections are also rare in the United States but many individuals have a positive antibody titer for HEV. The virus is most commonly spread by the fecal-oral route through food and/or water contamination, or person-to-person contact, depending on the genotype of the virus, which varies by location. There are four genotypes that differ somewhat in their mode of transmission, distribution, and other factors (for example, two are zoonotic and two are not, and only one causes chronic infection). Genotypes three and four are only transmitted through food, while genotypes one and two are also transmitted through water and fecal-oral routes. Genotype one is the only type transmitted person-to-person and is the most common cause of HEV outbreaks. Consumption of undercooked meat, especially deer or pork, and shellfish can lead to infection. Genotypes three and four are zoonoses, so they can be transmitted from infected animals that are consumed. Pregnant women are at particular risk. This disease is usually self-limiting within two weeks and does not appear to cause chronic infection.

General laboratory testing for hepatitis begins with blood testing to examine liver function (figure 4.21). When the liver is not functioning normally, the blood will contain elevated levels of alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), direct bilirubin, total bilirubin, serum albumin, serum total protein, and calculated globulin, albumin/globulin (A/G) ratio. Some of these are included in a complete metabolic panel (CMP), which may first suggest a possible liver problem and indicate the need for more comprehensive testing. A hepatitis virus serological test panel can be used to detect antibodies for hepatitis viruses A, B, C, and sometimes D. Additionally, other immunological and genomic tests are available.

Specific treatments other than supportive therapy, rest, and fluids are often not available for hepatitis virus infection, except for HCV, which is often self-limited. Immunoglobulins can be used prophylactically following possible exposure. Medications are also used, including interferon alpha 2b and antivirals (e.g., lamivudine, entecavir, adefovir, and telbivudine) for chronic infections. Hepatitis C can be treated with interferon (as monotherapy or combined with other treatments), protease inhibitors, and other antivirals (e.g., the polymerase inhibitor sofosbuvir). Combination treatments are commonly used. Antiviral and immunosuppressive medications may be used for chronic cases of HEV. In severe cases, liver transplants may be necessary. Additionally, vaccines are available to prevent infection with HAV and HBV. The HAV vaccine is also protective against HEV. The HBV vaccine is also protective against HDV. There is no vaccine against HCV.

Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs	Vaccines
Hepatitis A	Hepatitisvirus A (HAV)	Usually asymptomatic or mild and self-limiting within one to two weeks to a few months, sometimes longer but not, chronic; in rare cases leads to serious or fatal fulminant hepatitis	Contaminated food, water, objects, and person to person	None	Vaccine recommended for one year olds and high-risk adults
Hepatitis B	Hepatitisvirus B (HBV)	Similar to Hepatitis A, but may progress to cirrhosis and liver failure; associated with liver cancer	Contact with infected body fluids (blood, semen, saliva), e.g., via IV drug use, sexual transmission, health-care workers treating infected patients	Interferon, entecavir, tenofovir, lamivudine, adefovir	Vaccine recommended for infants and high-risk adults
Hepatitis C	Hepatitisvirus C (HCV)	Often asymptomatic, with 75%–85% chronic carriers; may progress to cirrhosis and liver failure; associated with liver cancer	Contact with infected body fluids, e.g., via IV drug use, transfusions, sexual transmission	Depends on genotype and on whether cirrhosis is present; interferons, new treatment such as simeprevir plus sofosbuvir, ombitasvir/ paritaprevir/ ritonavir and dasabuvir	None available
Hepatitis D	Hepatitisvirus D (HDV)	Similar to Hepatitis B; usually self-limiting within one to two weeks but can become chronic or fulminant in rare cases	Contact with infected blood; infections can only occur in patients already infected with hepatitis B	None	Hepatitis B vaccine protects against HDV
Hepatitis E	Hepatitisvirus E (HEV)	Generally asymptomatic or mild and self-limiting; typically does not cause chronic disease	Fecal-oral route, often in contaminated water or undercooked meat; most common in developing countries	Supportive treatment; usually self-limiting, but some strains can become chronic; antiviral and immunosuppressive possible for chronic cases	Vaccine available in China only

Table 4.5: Viral forms of hepatitis

4.5 PROTOZOAN INFECTIONS OF THE GASTROINTESTINAL TRACT

Like other microbes, protozoa are abundant in natural microbiota but can also be associated with significant illness. Gastrointestinal diseases caused by protozoa are generally associated with exposure to contaminated food and water, meaning that those without access to good sanitation are at greatest risk. Even in developed countries, infections can occur and these microbes have sometimes caused significant outbreaks from contamination of public water supplies. Table 4.6 summarizes the protozoan infections of the GI tract.

GIARDIASIS

Also called backpacker's diarrhea or beaver fever, giardiasis is a common disease in the United States caused by the flagellated protist *Giardia lamblia*, also known as *Giardia intestinalis* or *Giardia duodenalis* (figure 4.21). To establish infection, *G. lamblia* uses a large adhesive disk to attach to the intestinal mucosa. The disk is composed of microtubules. During adhesion, the flagella of *G. lamblia* move in a manner that draws fluid out from under the disk, resulting in an area of lower pressure that promotes its adhesion to the intestinal epithelial cells. Due to its attachment, *Giardia* also blocks absorption of nutrients, including fats.

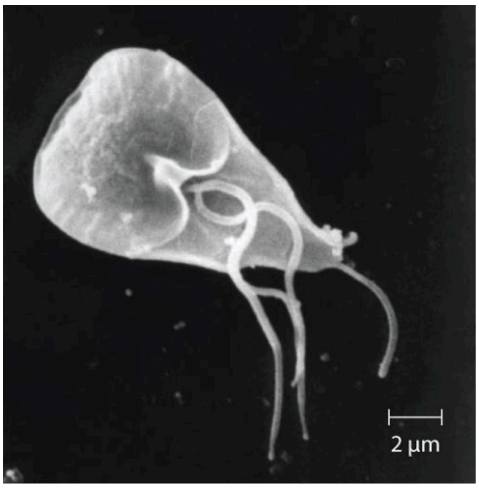

Figure 4.21: Giardia lamblia, an intestinal protozoan parasite that infects humans and other mammals, causing severe diarrhea. Figure description available at the end of the chapter.

Transmission occurs through contaminated food or water or directly from person to person. Children in day-care centers are at risk due to their tendency to put items into their mouths that may be contaminated. Large outbreaks may occur if a public water supply becomes contaminated. *Giardia* have a resistant cyst stage in their life cycle that is able to survive cold temperatures and the chlorination treatment typically used for drinking water in municipal reservoirs. As a result, municipal water must be filtered to trap and remove these cysts. Once consumed by the host, *Giardia* develops into the active trophozoite.

Infected individuals may be asymptomatic or have gastrointestinal signs and symptoms, sometimes accompanied by weight loss. Common symptoms, which appear one to three weeks after exposure, include diarrhea, nausea, stomach cramps, gas, greasy stool (because fat absorption is being blocked), and possible dehydration. The parasite remains in the colon and does not cause systemic infection. Signs and symptoms generally clear within two to six weeks. Chronic infections may develop and are often resistant to treatment. These are associated with weight loss, episodic diarrhea, and malabsorption syndrome due to the blocked nutrient absorption.

Diagnosis may be made using observation under the microscope. A stool ova and parasite (O&P) exam involves direct examination of a stool sample for the presence of cysts and trophozoites; it can be used to distinguish common parasitic intestinal infections. ELISA and other immunoassay tests, including commercial direct fluorescence antibody kits, are also used. The most common treatments use metronidazole as the first-line choice, followed by tinidazole. If the infection becomes chronic, the parasites may become resistant to medications.

CRYPTOSPORIDIOSIS

Another protozoan intestinal illness is cryptosporidiosis, which is usually caused by *Cryptosporidium parvum* or *C. hominis* (figure 4.22). These pathogens are commonly found in animals and can be spread in feces from mice, birds, and farm animals. Contaminated water and food are most commonly responsible for transmission. The protozoan can also be transmitted through human contact with infected animals or their feces.

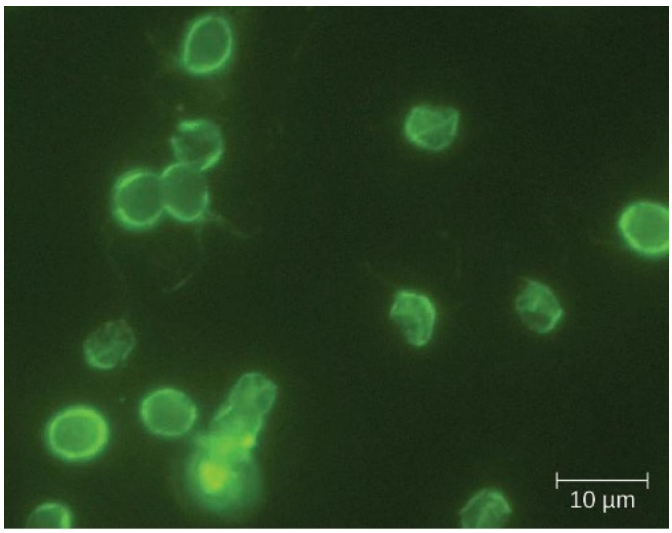

Figure 4.22: Immunofluorescent staining allows for visualization of Cryptosporidium spp. *Figure description available at the end of the chapter.*

In the United States, outbreaks of cryptosporidiosis generally occur through contamination of the public water supply or contaminated water at water parks, swimming pools, and day-care centers. The risk is greatest in areas with poor sanitation, making the disease more common in developing countries.

Signs and symptoms include watery diarrhea, nausea, vomiting, cramps, fever, dehydration, and weight loss. The illness is generally self-limiting within a month. However, immunocompromised patients, such as those with HIV/AIDS, are at particular risk of severe illness or death.

Diagnosis involves direct examination of stool samples, often over multiple days. As with giardiasis, a stool O&P exam may be helpful. Acid fast staining is often used. Enzyme immunoassays and molecular analysis (PCR) are available.

The first line of treatment is typically oral rehydration therapy. Medications are sometimes used to treat the associated diarrhea. The broad-range anti-parasitic drug nitazoxanide can be used to treat cryptosporidiosis. Other anti-parasitic drugs that can be used include azithromycin and paromomycin.

AMOEBIASIS (AMEBIASIS)

The protozoan parasite *Entamoeba histolytica* causes amoebiasis, which is known as amoebic dysentery in severe cases. *E. histolytica* is generally transmitted through water or food that has fecal contamination. The disease is most widespread in the developing world and is one of the leading causes of mortality from parasitic disease worldwide. Disease can be caused by as few as 10 cysts being transmitted.

Signs and symptoms range from nonexistent to mild diarrhea to severe amoebic dysentery. Severe infection causes the abdomen to become distended and may be associated with fever. The parasite may live in the colon without causing signs or symptoms or may invade the mucosa to cause colitis. In some cases, the disease spreads to the spleen, brain, genitourinary tract, or lungs. In particular, it may spread to the liver and cause an abscess. When a liver abscess develops, fever, nausea, liver tenderness, weight loss, and pain in the right abdominal quadrant may occur. Chronic infection may occur and is associated with intermittent diarrhea, mucus, pain, flatulence, and weight loss.

Direct examination of fecal specimens may be used for diagnosis. As with cryptosporidiosis, samples are often examined on multiple days. A stool O&P exam of fecal or biopsy specimens may be helpful. Immunoassay, serology, biopsy, molecular, and antibody detection tests are available. Enzyme immunoassay may not distinguish current from past illness. Magnetic resonance imaging (MRI) can be used to detect any liver abscesses. The first line of treatment is metronidazole or tinidazole, followed by diloxanide furoate, iodoquinol, or paromomycin to eliminate the cysts that remain.

CYCLOSPORIASIS

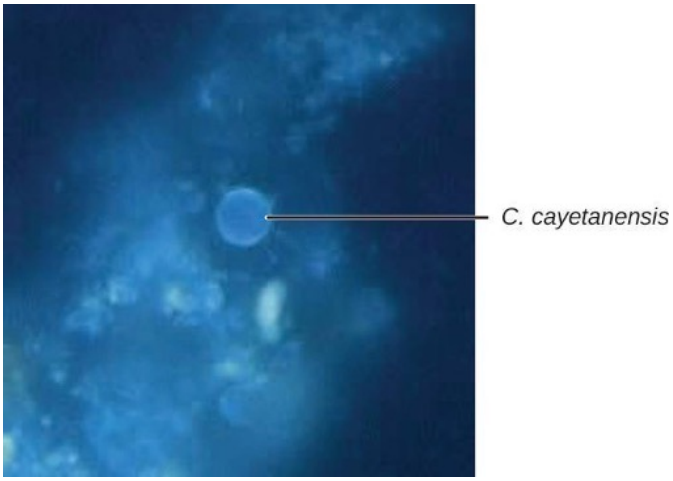

Figure 4.23: Cyclospora cayetanensis are autofluorescent under ultraviolet light. Figure description available at the end of the chapter.

The intestinal disease cyclosporiasis is caused by the protozoan *Cyclospora cayetanensis*. It is endemic to tropical and subtropical regions and therefore uncommon in the United States, although there have been outbreaks associated with contaminated produce imported from regions where the protozoan is more common.

This protist is transmitted through contaminated food and water and reaches the lining of the small intestine, where it causes infection. Signs and symptoms begin within seven to ten days after ingestion. Based on limited data, it appears to be seasonal in ways that differ regionally and that are poorly understood.[17]

Some individuals do not develop signs or symptoms. Those who do may exhibit explosive and watery diarrhea, fever, nausea, vomiting, cramps, loss of appetite, fatigue, and bloating. These symptoms may last for months without treatment. Trimethoprim-sulfamethoxazole is the recommended treatment.

Microscopic examination is used for diagnosis. A stool O&P examination may be helpful. The oocysts have a distinctive blue halo when viewed using ultraviolet fluorescence microscopy (figure 4.23).

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Amoebiasis (amoebic dysentery)	*Entamoeba histolytica*	From mild diarrhea to severe dysentery and colitis; may cause abscess on the liver	Fecal-oral route; ingestion of cysts from fecally contaminated water, food, or hands	Stool O&P exam, enzyme immunoassay	Metronidazole, tinidazole, diloxanide furoate, iodoquinol, paromomycin

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Cryptosporidiosis	*Cryptosporidium parvum, Cryptosporidium hominis*	Watery diarrhea, nausea, vomiting, cramps, fever, dehydration, and weight loss	Contact with feces of infected mice, birds, farm animals; ingestion of contaminated food or water; exposure to contaminated water while swimming or bathing	Stool O&P exam, enzyme immunoassay, PCR	Nitazoxanide, azithromycin, and paromomycin
Cyclosporiasis	*Cyclospora cayetanensis*	Explosive diarrhea, fever, nausea, vomiting, cramps, loss of appetite, fatigue, bloating	Ingestion of contaminated food or water	Stool O&P exam using ultraviolet fluorescence microscopy	Trimethoprim-sulf methoxazole
Giardiasis	*Giardia lamblia*	Diarrhea, nausea, stomach cramps, gas, greasy stool, dehydration if severe; sometimes malabsorption syndrome	Contact with infected individual or contaminated fomites; ingestion of contaminated food or water	Stool O&P exam; ELISA, direct fluorescence antibody assays	Metronidazole, tinidazole

Table 4.6: Protozoan infections of the GI tract

4.6 HELMINTHIC INFECTIONS OF THE GASTROINTESTINAL TRACT

Helminths are widespread intestinal parasites. These parasites can be divided into three common groups: round-bodied worms (also described as nematodes), flat-bodied worms that are segmented (also described as cestodes), and flat-bodied worms that are non-segmented (also described as trematodes). The nematodes include roundworms, pinworms, hookworms, and whipworms. Cestodes include beef, pork, and fish tapeworms. Trematodes are collectively called flukes and more uniquely identified with the body site where the adult flukes are located. Although infection can have serious consequences, many of these parasites are so well adapted to the human host that there is little obvious disease. Helminthic infections of the GI tract are summarized in tables 4.7 and 4.8.

ASCARIASIS

Infections caused by the large nematode roundworm *Ascaris lumbricoides*, a soil-transmitted helminth, are called ascariasis. Over 800 million to 1 billion people are estimated to be infected worldwide.[18] Infections are most common in warmer climates and at warmer times of year. At present, infections are uncommon in the United States. The eggs of the worms are transmitted through contaminated food and water. This may happen if food is grown in contaminated soil, including when manure is used as fertilizer.

When an individual consumes embryonated eggs (those with a developing embryo), the eggs travel to the intestine and the larvae are able to hatch. *Ascaris* is able to produce proteases that allow for penetration and degradation of host tissue. The juvenile worms can then enter the circulatory system and migrate to the lungs where they enter the alveoli (air sacs). From here they crawl to the pharynx and then follow the gut lumen to return

to the small intestine, where they mature into adult roundworms. Females in the host will produce and release eggs that leave the host via feces. In some cases, the worms can block ducts such as those of the pancreas or gallbladder.

The infection is commonly asymptomatic. When signs and symptoms are present, they include shortness of breath, cough, nausea, diarrhea, blood in the stool, abdominal pain, weight loss, and fatigue. The roundworms may be visible in the stool. In severe cases, children with substantial infections may experience intestinal blockage.

The eggs can be identified by microscopic examination of the stool (figure 4.24). In some cases, the worms themselves may be identified if coughed up or excreted in stool. They can also sometimes be identified by X-rays, ultrasounds, or MRIs.

Ascariasis is self-limiting, but can last one to two years because the worms can inhibit the body's inflammatory response through glycan gimmickry. The first line of treatment is mebendazole or albendazole. In some severe cases, surgery may be required.

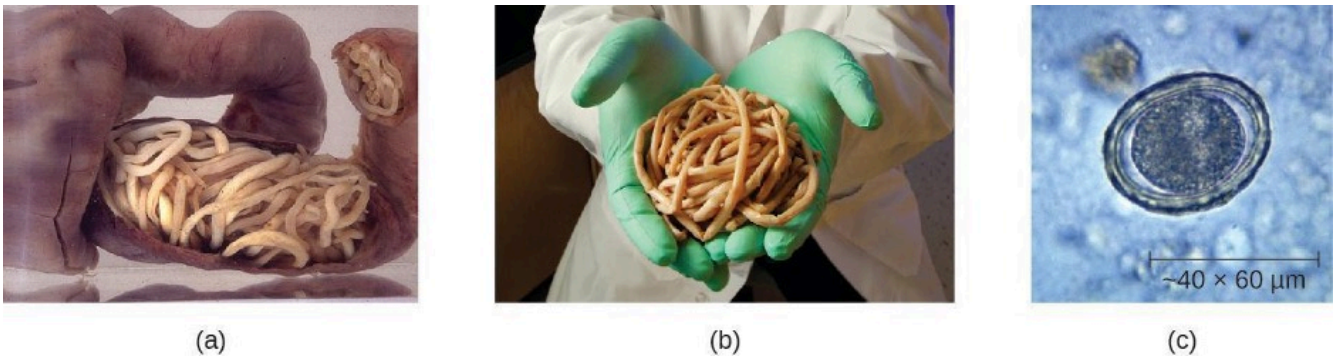

Figure 4.24: (a) Adult Ascaris lumbricoides roundworms can cause intestinal blockage. (b) This mass of A. lumbricoides worms was excreted by a child. (c) A micrograph of a fertilized egg of A. lumbricoides. Fertilized eggs can be distinguished from unfertilized eggs because they are round rather than elongated and have a thicker cell wall. *Figure description available at the end of the chapter.*

HOOKWORM

Two species of nematode worms are associated with hookworm infection. Both species are found in the Americas, Africa, and Asia. *Necator americanus* is found predominantly in the United States and Australia. Another species, *Ancylostoma duodenale*, is found in southern Europe, North Africa, the Middle East, and Asia.

The eggs of these species develop into larvae in soil contaminated by dog or cat feces. These larvae can penetrate the skin. After traveling through the venous circulation, they reach the lungs. When they are coughed up, they are then swallowed and can enter the intestine and develop into mature adults. At this stage, they attach to the wall of the intestine, where they feed on blood and can potentially cause anemia. Signs and symptoms include cough, an itchy rash, loss of appetite, abdominal pain, and diarrhea. In children, hookworms can affect physical and cognitive growth.

Some hookworm species, such as *Ancylostoma braziliense* that is commonly found in animals such as cats and dogs, can penetrate human skin and migrate, causing cutaneous larva migrans, a skin disease caused by the larvae of hookworms. As they move across the skin, in the subcutaneous tissue, pruritic tracks appear (figure 4.25).

The infection is diagnosed using microscopic examination of the stool, allowing for observation of eggs in the feces. Medications such as albendazole, mebendazole, and pyrantel pamoate are used as needed to treat systemic

infection. In addition to systemic medication for symptoms associated with cutaneous larva migrans, topical thiabendazole is applied to the affected areas.

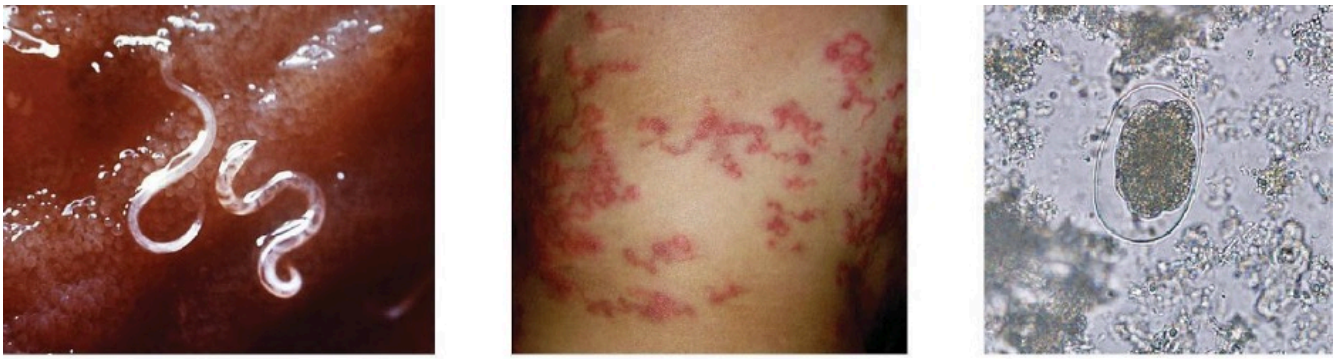

Figure 4.25: (a) This animal hookworm, Ancylostoma caninum, is attached to the intestinal wall. (b) The tracks of hookworms are visible in this individual with cutaneous larva migrans. (c) This micrograph shows the microscopic egg of a hookworm. *Figure description available at the end of the chapter.*

STRONGYLOIDIASIS

Strongyloidiasis is generally caused by *Strongyloides stercoralis*, a soil-transmitted helminth with both free-living and parasitic forms. In the parasitic form, the larvae of these nematodes generally penetrate the body through the skin, especially through bare feet, although transmission through organ transplantation or at facilities like day-care centers can also occur. When excreted in the stool, larvae can become free-living adults rather than developing into the parasitic form. These free-living worms reproduce, laying eggs that hatch into larvae that can develop into the parasitic form. In the parasitic life cycle, infective larvae enter the skin, generally through the feet. The larvae reach the circulatory system, which allows them to travel to the alveolar spaces of the lungs. They are transported to the pharynx where, like many other helminths, the infected patient coughs them up and swallows them again so that they return to the intestine. Once they reach the intestine, females live in the epithelium and produce eggs that develop asexually, unlike the free-living forms, which use sexual reproduction. The larvae may be excreted in the stool or can reinfect the host by entering the tissue of the intestines and skin around the anus, which can lead to chronic infections.

The condition is generally asymptomatic, although severe symptoms can develop after treatment with corticosteroids for asthma or chronic obstructive pulmonary disease, or following other forms of immunosuppression. When the immune system is suppressed, the rate of autoinfection increases, and huge amounts of larvae migrate to organs throughout the body.

Signs and symptoms are generally nonspecific. The condition can cause a rash at the site of skin entry, cough (dry or with blood), fever, nausea, difficulty breathing, bloating, pain, heartburn, and, rarely, arthritis as well as cardiac or kidney complications. Disseminated strongyloidiasis or hyperinfection is a life-threatening form of the disease that can occur, usually following immunosuppression such as that caused by glucocorticoid treatment (most commonly), with other immunosuppressive medications, with HIV infection, or with malnutrition.

As with other helminths, direct examination of the stool is important in diagnosis. Ideally, this should be continued over seven days. Serological testing, including antigen testing, is also available. These can be limited by cross-reactions with other similar parasites and by the inability to distinguish current from resolved infection. Ivermectin is the preferred treatment, with albendazole as a secondary option.

PINWORMS (ENTEROBIASIS)

Enterobius vermicularis, commonly called pinworms, are tiny (2–13 mm) nematodes that cause enterobiasis. Of all helminthic infections, enterobiasis is the most common in the United States, affecting as many as one-third of American children.[19] Although the signs and symptoms are generally mild, patients may experience abdominal pain and insomnia from itching of the perianal region, which frequently occurs at night when worms leave the anus to lay eggs. The itching contributes to transmission, as the disease is transmitted through the fecal-oral route. When an infected individual scratches the anal area, eggs may get under the fingernails and later be deposited near the individual's mouth, causing reinfection, or on fomites, where they can be transferred to new hosts. After being ingested, the larvae hatch within the small intestine and then take up residence in the colon and develop into adults. From the colon, the female adult exits the body at night to lay eggs (figure 4.26).

Infection is diagnosed in any of three ways. First, because the worms emerge at night to lay eggs, it is possible to inspect the perianal region for worms while an individual is asleep. An alternative is to use transparent tape to remove eggs from the area around the anus first thing in the morning for three days to yield eggs for microscopic examination. Finally, it may be possible to detect eggs through examination of samples from under the fingernails, where eggs may lodge due to scratching. Once diagnosis has been made, mebendazole, albendazole, and pyrantel pamoate are effective for treatment.

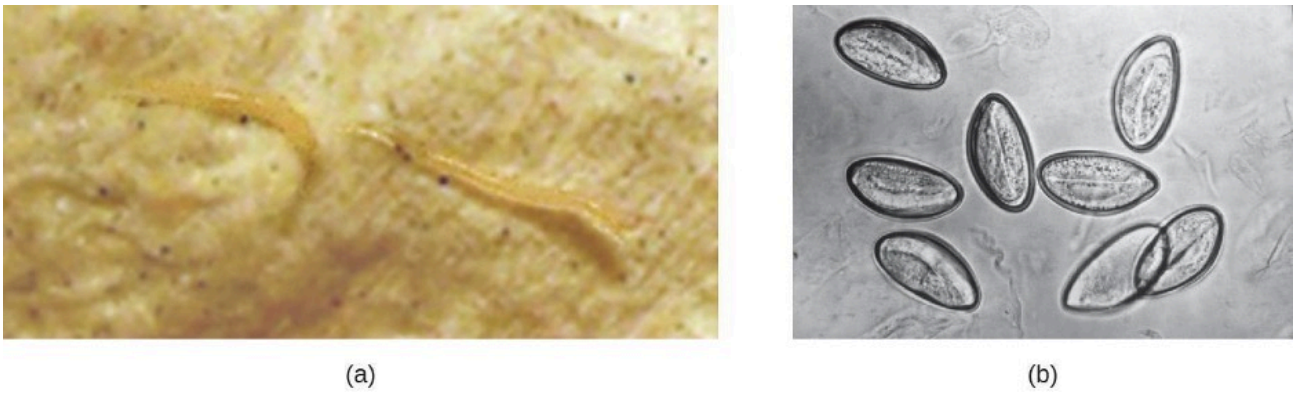

Figure 4.26: (a) E. vermicularis are tiny nematodes commonly called pinworms. (b) This micrograph shows pinworm eggs. *Figure description available at the end of the chapter.*

TRICHURIASIS

The nematode whipworm *Trichuris trichiura* is a parasite that is transmitted by ingestion from soil-contaminated hands or food and causes trichuriasis. Infection is most common in warm environments, especially when there is poor sanitation and greater risk of fecal contamination of soil, or when food is grown in soil using manure as a fertilizer. The signs and symptoms may be minimal or nonexistent. When a substantial infection develops, signs and symptoms include painful, frequent diarrhea that may contain mucus and blood. It is possible for the infection to cause rectal prolapse, a condition in which a portion of the rectum becomes detached from the inside of the body and protrudes from the anus (figure 4.27). Severely infected children may experience reduced growth and their cognitive development may be affected.

When fertilized eggs are ingested, they travel to the intestine and the larvae emerge, taking up residence in the walls of the colon and cecum. They attach themselves with part of their bodies embedded in the mucosa. The larvae mature and live in the cecum and ascending colon. After 60 to 70 days, females begin to lay 3000 to 20,000 eggs per day.

Diagnosis involves examination of the feces for the presence of eggs. It may be necessary to use concentration techniques and to collect specimens on multiple days. Following diagnosis, the infection may be treated with mebendazole, albendazole, or ivermectin.

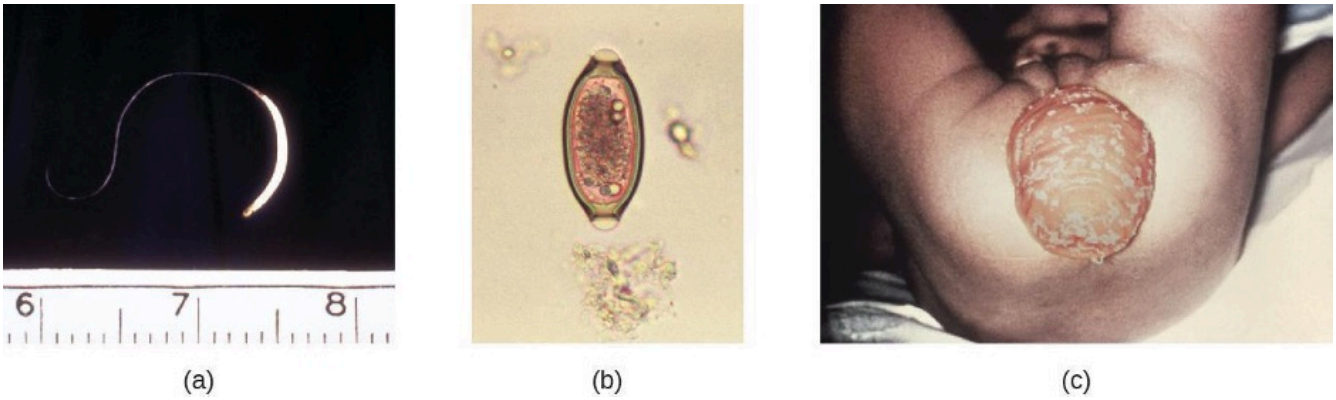

Figure 4.27: (a) This adult female Trichuris whipworm is a soil-transmitted parasite. (b) Trichuris trichiura eggs are ingested and travel to the intestines where the larvae emerge and take up residence. (c) Rectal prolapse is a condition that can result from whipworm infections. It occurs when the rectum loses its attachment to the internal body structure and protrudes from the anus. *Figure description available at the end of the chapter.*

TRICHINOSIS

Trichinosis (trichenellosis) develops following consumption of food that contains *Trichinella spiralis* (most commonly) or other *Trichinella* species. These microscopic nematode worms are most commonly transmitted in meat, especially pork, that has not been cooked thoroughly. *T. spiralis* larvae in meat emerge from cysts when exposed to acid and pepsin in the stomach. They develop into mature adults within the large intestine. The larvae produced in the large intestine are able to migrate into the muscles mechanically via the stylet of the parasite, forming cysts. Muscle proteins are reduced in abundance or undetectable in cells that contain *Trichinella* (nurse cells). Animals that ingest the cysts from other animals can later develop infection (figure 4.28).

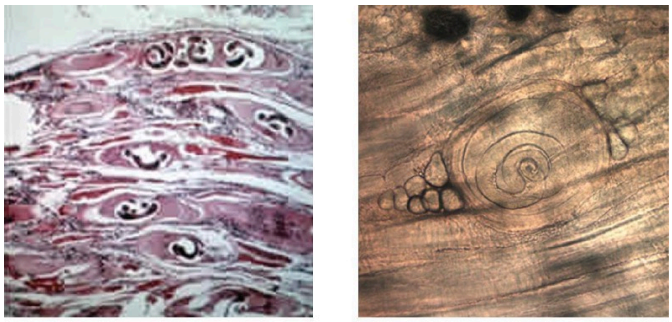

Figure 4.28: (a) This image shows larvae of T. spiralis within muscle. (b) In meat, the larvae have a characteristic coiled appearance, as seen in this partially digested larva in bear meat. *Figure description available at the end of the chapter.*

Although infection may be asymptomatic, symptomatic infections begin within a day or two of consuming the nematodes. Abdominal symptoms arise first and can include diarrhea, constipation, and abdominal pain. Other possible symptoms include headache, light sensitivity, muscle pain, fever, cough, chills, and conjunctivitis. More severe symptoms affecting motor coordination, breathing, and the heart sometimes occur. It may take months for the symptoms to resolve, and the condition is occasionally fatal. Mild cases may be mistaken for influenza or similar conditions.

Infection is diagnosed using clinical history, muscle biopsy to look for larvae, and serological testing, including immunoassays. Enzyme immunoassay is the most common test. It is difficult to effectively treat larvae that have formed cysts in the muscle, although medications may help. It is best to begin treatment as soon as possible

because medications such as mebendazole and albendazole are effective in killing only the adult worms in the intestine. Steroids may be used to reduce inflammation if larvae are in the muscles.

TAPEWORMS (TAENIASIS)

Taeniasis is a tapeworm infection, generally caused by pork (*Taenia solium*), beef (*Taenia saginata*), and Asian (*Taenia asiatica*) tapeworms found in undercooked meat. Consumption of raw or undercooked fish, including contaminated sushi, can also result in infection from the fish tapeworm (*Diphyllobothrium latum*). Tapeworms are flatworms (cestodes) with multiple body segments and a head called a scolex that attaches to the intestinal wall (figure 4.29). Tapeworms can become quite large, reaching 4 to 8 meters long (figure 4.30). Figure 4.30 illustrates the life cycle of a tapeworm.

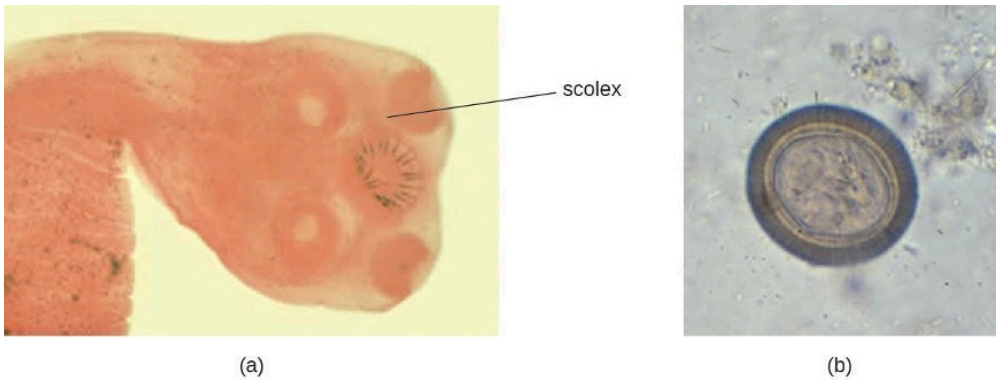

Figure 4.29: (a) An adult tapeworm uses the scolex to attach to the intestinal wall. (b) The egg of a pork tapeworm (Taenia solium) is visible in this micrograph. *Figure description available at the end of the chapter.*

Tapeworms attached to the intestinal wall produce eggs that are excreted in feces. After ingestion by animals, the eggs hatch and the larvae emerge. They may take up residence in the intestine, but can sometimes move to other tissues, especially muscle or brain tissue. When *T. solium* larvae form cysts in tissue, the condition is called cysticercosis. This occurs through ingestion of eggs via the fecal-oral route, not through consumption of undercooked meat. It can develop in the muscles, eye (ophthalmic cysticercosis), or brain (neurocysticercosis).

Infections may be asymptomatic or they may cause mild gastrointestinal symptoms such as epigastric discomfort, nausea, diarrhea, flatulence, or hunger pains. It is also common to find visible tapeworm segments passed in the stool. In cases of cysticercosis, symptoms differ depending upon where the cysts become established. Neurocysticercosis can have severe, life-threatening consequences and is associated with headaches and seizures because of the presence of the tapeworm larvae encysted in the brain. Cysts in muscles may be asymptomatic, or they may be painful.

To diagnose these conditions, microscopic analysis of stool samples from three separate days is generally recommended. Eggs or body segments, called proglottids, may be visible in these samples. Molecular methods have been developed but are not yet widely available. Imaging, such as CT and MRI, may be used to detect cysts. Praziquantel or niclosamide are used for treatment.

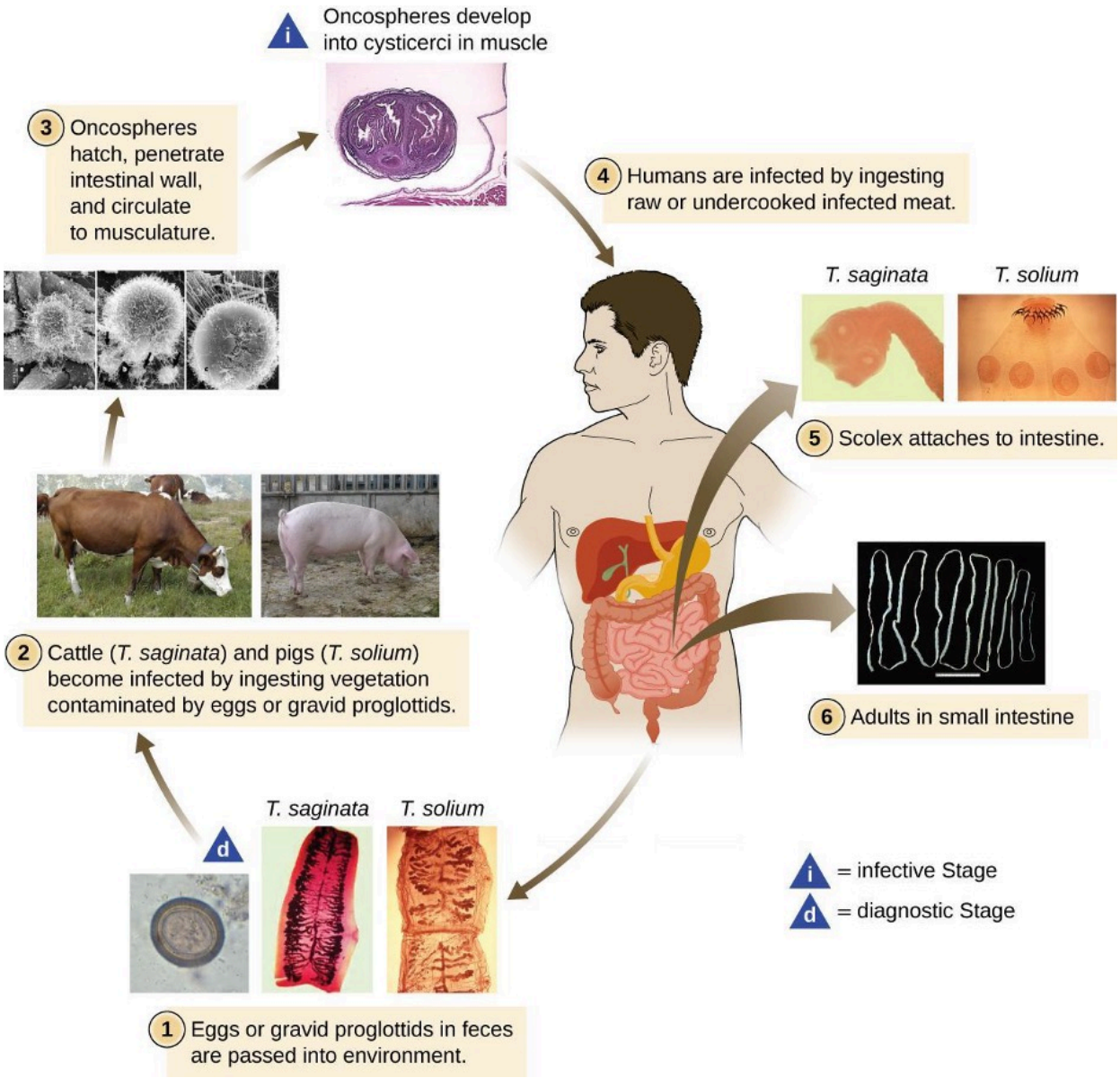

Figure 4.30: Life cycle of a tapeworm. *Figure description available at the end of the chapter.*

HYDATID DISEASE

Another cestode, *Echinococcus granulosus*, causes a serious infection known as hydatid disease (cystic echinococcosis). *E. granulosus* is found in dogs (the definitive host), as well as several intermediate hosts (sheep, pigs, goats, cattle). The cestodes are transmitted through eggs in the feces from infected animals, which can be an occupational hazard for individuals who work in agriculture.

Once ingested, *E. granulosus* eggs hatch in the small intestine and release the larvae. The larvae invade the intestinal wall to gain access to the circulatory system. They form hydatid cysts in internal organs, especially in the lungs and liver, that grow slowly and are often undetected until they become large. If the cysts burst, a severe allergic reaction (anaphylaxis) may occur.

Cysts present in the liver can cause enlargement of the liver, nausea, vomiting, right epigastric pain, pain in the right upper quadrant, and possible allergic signs and symptoms. Cysts in the lungs can lead to alveolar disease. Abdominal pain, weight loss, pain, and malaise may occur, and inflammatory processes develop.

E. granulosus can be detected through imaging (ultrasonography, CT, MRI) that shows the cysts. Serologic tests, including ELISA and indirect hemagglutination tests, are used. Cystic disease is most effectively treated with surgery to remove cysts, but other treatments are also available, including chemotherapy with anti-helminthic drugs (albendazole or mebendazole).

FLUKES

Flukes are flatworms that have a leaflike appearance. They are a type of trematode worm, and multiple species are associated with disease in humans. The most common are liver flukes and intestinal flukes (figure 4.31).

Figure 4.31: (a) A liver fluke infects the bile ducts. (b) An intestinal fluke infects the intestines. *Figure description available at the end of the chapter.*

Liver Flukes

The liver flukes are several species of trematodes that cause disease by interfering with the bile duct. Fascioliasis is caused by *Fasciola hepatica* and *Fasciola gigantica* in contaminated raw or undercooked aquatic plants (e.g., watercress). In *Fasciola* infection, adult flukes develop in the bile duct and release eggs into the feces. Clonorchiasis is caused by *Clonorchis sinensis* in contaminated freshwater fish. Other flukes, such as *Opisthorchis viverrini* (found in fish) and *Opisthorchis felineus* (found in freshwater snails), also cause infections. Liver flukes spend part of their life cycle in freshwater snails, which serve as an intermediate host. Humans are typically infected after eating aquatic plants contaminated by the infective larvae after they have left the snail. Once they reach the human intestine, they migrate back to the bile duct, where they mature. The life cycle is similar for the other infectious liver flukes (see figure 4.32).

When *Fasciola* flukes cause acute infection, signs and symptoms include nausea, vomiting, abdominal pain, rash, fever, malaise, and breathing difficulties. If the infection becomes chronic, with adult flukes living in the bile duct, then cholangitis, cirrhosis, pancreatitis, cholecystitis, and gallstones may develop. Symptoms are similar for infections by other liver flukes. Cholangiocarcinoma can occur from *C. sinensis* infection. The *Opisthorchis* species can also be associated with cancer development.

Diagnosis is accomplished using patient history and examination of samples from feces or other samples (such as vomitus). Because the eggs may appear similar, immunoassay techniques are available that can help distinguish species. The preferred treatment for fascioliasis is triclabendazole. *C. sinensis* and *Opisthorchis* spp. infections are treated with praziquantel or albendazole.

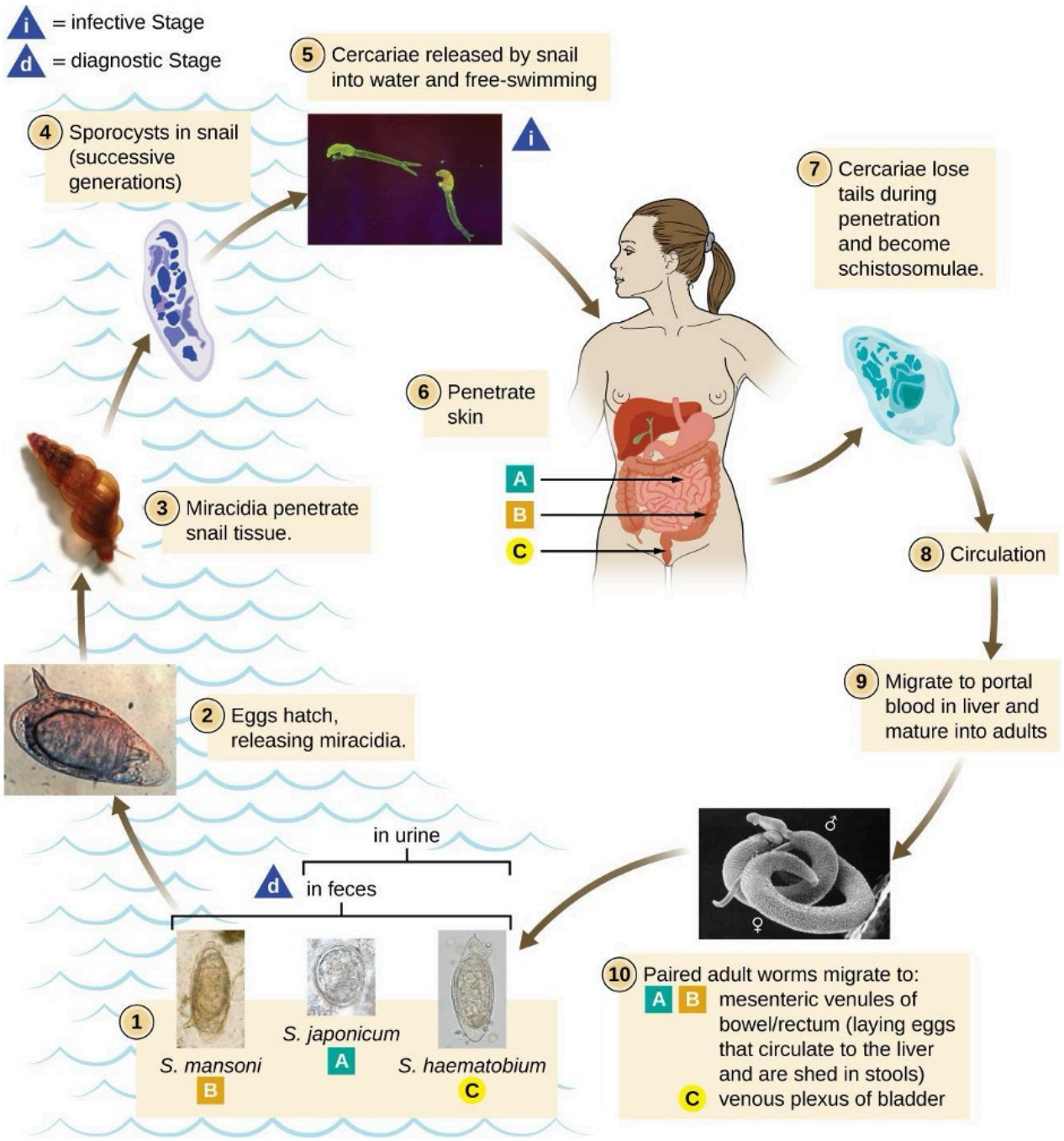

Figure 4.32: The life cycle of Schistosoma spp. includes several species of water snails, which serve as secondary hosts. The parasite is transmitted to humans through contact with contaminated water and takes up residence in the veins of the digestive system. Eggs escape the host in the urine or feces and infect a snail to complete the life cycle. *Figure description available at the end of the chapter.*

Intestinal Flukes

The intestinal flukes are trematodes that develop in the intestines. Many, such as *Fasciolopsis buski*, which causes fasciolopsiasis, are closely related to liver flukes. Intestinal flukes are ingested from contaminated aquatic plants that have not been properly cooked. When the cysts are consumed, the larvae emerge in the duodenum and develop into adults while attached to the intestinal epithelium. The eggs are released in stool.

Intestinal fluke infection is often asymptomatic, but some cases may involve mild diarrhea and abdominal pain. More severe symptoms such as vomiting, nausea, allergic reactions, and anemia can sometimes occur, and high parasite loads may sometimes lead to intestinal obstructions.

Diagnosis is the same as with liver flukes: examination of feces or other samples and immunoassay. Praziquantel is used to treat infections caused by intestinal flukes.

Disease	Causative Agent(s)	Mode of Transmission	Laboratory Tests	Symptoms	Treatments
Ascariasis	*Ascaris lumbricoides*	Eggs in fecally contaminated food or water	Microscopic examination of the stool, imaging	Shortness of breath, cough, nausea, diarrhea, blood in stool, abdominal pain, weight loss, fatigue	Self-limiting within 1 to 2 years; albendazole and mebendazole if needed
Hookworm	*Necator americanus, Ancyclostoma doudenale*	Larvae in soil contaminated by dog or cat feces penetrate skin	Microscopic examination of stool (may require. a concentration procedure)	Cough, itchy rash, loss of appetite, abdominal pain, diarrhea; in children, may affect physical and cognitive growth	Albendazole and mebendazole: pyrantel pamoatemay if needed
Strongyloidiasis	*Strongyloides stercoralis*	Soil-dwelling larvae penetrate the skin, usually bare feet	Microscopic examination of stool over several days (ideally at least 7); some serologic testing available	Often asymptomatic; cough (sometimes bloody), skin rash, abdominal pain, and diarrhea; in immunosuppressed patients, may become disseminated, causing serious and potentially fatal complications	Ivermectin (preferred), albendazole
Enterobiasis (pinworm)	*Enterobius vermicularis*	Fecal-oral route	Observation of eggs or worms from anal area; examination of samples under fingernails	Itching around the anus, abdominal pain, insomnia, irritation of female genital tract	Mebendazole, albendazole, pyrantel pamoate
Trichiuriasis (whipworm)	*Trichuris trichiura*	Fecal contamination or fertilization in soil	Microscopic examination of stool	Abdominal pain, anemia, diarrhea that may be bloody	Albendazole, mebendazole, ivermectin if needed

Disease	Causative Agent(s)	Mode of Transmission	Laboratory Tests	Symptoms	Treatments
Trichinosis	*Trichinella spiralis*	Eating raw or undercooked pork or other meat of infected animal	Clinical history, muscle biopsy, serological testing, enzyme immunoassay	Diarrhea, constipation, abdominal pain, headache, cough, chills, light sensitivity. muscle pain, fever, conjunctivitis; in severe cases may affect motor coordination, breathing, heart function	Albendazole, mebendazole if needed
Taeniasis and cysticercosis	*Taenia solium, T. saginata, T. asiatica, Diphyllobo- thrium latum*	Eating raw or undercooked beef or pork from infected animal	Observation of worm segments or microscopic eggs in stool samples	Asymptomatic or mild GI distress: cysts in muscle, eye, or brain (cysticercosis); brain cysts can cause headaches, seizures, or death	Praziquantel or niclosamide
Cystic echinococcosis (hydatid disease)	*Echinococcus granulosus* (cystic)	Exposure to eggs in feces of infected dogs or livestock	Imaging; serological testing including ELISA and indirect hemagglutinin test	Cysts in lungs, liver, and other organs causing nausea, Gl distress, and weight loss; severe anaphylaxis or death if cysts burst	Surgical removal or aspiration of cysts or chemotherapy with albendazole or mebenazole
Liver fluke infections	*Fasciola hepatica, F. gigantica, Clonorchis sinensis, Opisthorchis viverrini, O. felineus*	Eating raw or undercooked aquatic plants (*Fasciola* spp.) or freshwater fish (*Clonorchis* spp.) contaminated with eggs or cysts	Microscopic examination of eggs in stool or other samples; immunoassays	Fever, malaise, anemia, abdominal symptoms. transaminitis; cholangitis, cirrhosis, pancreatitis, cholecystitis, gall stones in chronic phase	Triclabendazole (preferred) for *Fasciola* spp.; praziquantel and albendazole for *C. sinensis* and *Opisthorchis* spp.
Fasciolopiasis (intestinal fluke)	*Fasciola buski*	Eating raw or undercooked aquatic plants containing cysts	Microscopic examination of eggs in stool or other samples; immunoassays	Diarrhea, abdominal pain; in severe cases, vomiting, nausea, intestinal obstruction, anemia, allergic reactions	Praziquantel

Table 4.7: Common helminthic infections of the GI tract

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Strongyloidiasis	*Strongyloides stercoralis*	Often asymptomatic; cough (sometimes bloody), skin rash, abdominal pain, diarrhea; in immunosuppressed patients, may become disseminated, causing serious and potentially fatal complications	Soil-dwelling larvae penetrate the skin, usually bare feet	Microscopic observation of larvae in stool; serological testing for antigens	Ivermectin, albendazole
Tapeworms (taeniasis)	*Taenia solium, T. saginata, T. asiatica, Diphyllobothrium latum*	Asymptomatic or mild GI distress; cysts in muscle, eye, or brain (cysticercosis); brain cysts can cause headaches, seizures, or death	Ingestion of raw or undercooked pork or beef from infected animal	Observation of worm segments or microscopic eggs in stool; CT or MRI to detect cysts	Praziquantel, niclosamide
Trichinosis	*Trichinella spiralis*, other *Trichinella* spp.	Diarrhea, constipation, abdominal pain, headache, cough, chills, light sensitivity, muscle pain, fever, conjunctivitis; in severe cases may affect motor coordination, breathing, heart function	Ingestion of raw or undercooked pork or other meat of infected animal	Observation of cysts in muscle biopsy, enzyme immunoassay	Albendazole, mebendazole
Whipworm (trichuriasis)	*Trichuris trichiura*	Abdominal pain, anemia, diarrhea (possibly bloody), rectal prolapse	Ingestion of eggs in fecally contaminated food	Microscopic observation of eggs in stool	Albendazole, mebendazole, ivermectin

Table 4.8: Helminthic infections of the GI tract

SUMMARY

The following is a summary of the material covered throughout the chapter. It summarizes key aspects from each section and the pathogens included.

MICROBIAL DISEASES OF THE MOUTH AND ORAL CAVITY

- **Dental caries**, **tartar**, and **gingivitis** are caused by overgrowth of oral bacteria, usually *Streptococcus* and *Actinomyces* species, as a result of insufficient dental hygiene.
- Gingivitis can worsen, allowing *Porphyromonas*, *Streptococcus*, and *Actinomyces* species to spread and cause **periodontitis**. When *Prevotella intermedia*, *Fusobacterium* species, and *Treponema vicentii* are involved, it can lead to **acute necrotizing ulcerative gingivitis**.
- The herpes simplex virus type 1 can cause lesions of the mouth and throat called **herpetic gingivostomatitis.**
- Other infections of the mouth include **oral thrush**, a fungal infection caused by overgrowth of *Candida* yeast, and **mumps**, a viral infection of the salivary glands caused by the mumps virus, a paramyxovirus.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Dental caries	*Streptococcus mutans*	Discoloration, softening, cavities in teeth	Non-transmissible; caused by bacteria of the normal oral microbiota	Visual examinations, X-rays	Oral antiseptics (e.g., Listerine)
Gingivitis and periodontitis	*Porphyromonas, Streptococcus, Actinomyces*	Inflammation and erosion of gums, bleeding, halitosis; erosion of cementum leading to tooth loss in advanced infections	Non-transmissible; caused by bacteria of the normal oral microbiota	Visual examination, X-rays, measuring pockets in gums	Tetracycline, doxycycline, macrolides or beta-lactams. Mixture of antibiotics may be given
Herpetic gingivostomatitis	Herpes simplex virus type 1 (HSV-1)	Lesions in mucous membranes of mouth	Contact with saliva or lesions of an infected person	Culture or biopsy	Acyclovir, famcyclovir, valacyclovir

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Mumps	Mumps virus (a paramyxovirus)	Swelling of parotid glands, fever, headache, muscle pain, weakness, fatigue, loss of appetite, pain while chewing; in serious cases, encephalitis, meningitis, and inflammation of testes, ovaries, and breasts	Contact with saliva or respiratory droplets of an infected person	Virus culture or serologic tests for antibodies, enzyme immunoassay, RT-PCR	None for treatment; MMR vaccine for prevention
Oral thrush	*Candida albicans*, other *Candida* spp.	White patches and pseudomembranes in mouth, may cause bleeding	Nontransmissible; caused by overgrowth of Candida spp. in the normal oral microbiota; primarily affects infants and the immunocompromised	Microscopic analysis of oral samples	Clotrimazole, nystatin, fluconazole, or itraconazole; amphotericin B in severe cases
Trench mouth (acute necrotizing ulcerative gingivitis)	*Prevotella intermedia Fusobacterium* species, *Treponema vincentii*, others	Erosion of gums, ulcers, substantial pain with chewing, halitosis	Nontransmissible; caused by members of the normal oral microbiota	Visual examinations, X-rays	Amoxicillin, amoxicillin clavulanate, clindamycin, or doxycycline

Table 4.9: Oral infections

BACTERIAL INFECTIONS OF THE GASTROINTESTINAL TRACT

- Major causes of gastrointestinal illness include *Salmonella* spp., *Staphylococcus* spp., *Helicobacter pylori*, *Clostridium perfringens*, *Clostridium difficile*, *Bacillus cereus*, and *Yersinia* bacteria.
- *C. difficile* is an important cause of hospital acquired infection.
- *Vibrio cholerae* causes **cholera**, which can be a severe diarrheal illness.
- Different strains of *E. coli*, including **ETEC**, **EPEC**, **EIEC**, and **EHEC**, cause different illnesses with varying degrees of severity.
- *H. pylori* is associated with **peptic ulcers**.
- *Salmonella enterica* serotypes can cause **typhoid fever**, a more severe illness than **salmonellosis**.
- Careful antibiotic use is required to reduce the risk of causing *C. difficile* infections and when treating

antibiotic-resistant infections.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Bacillus cereus infection	*Bacillus cereus*	Nausea, pain, abdominal cramps, diarrhea or vomiting	Ingestion of contaminated rice or meat, even after cooking	Testing stool sample, vomitus, or uneaten food for presence of bacteria	None
Campylobacter jejuni gastroenteritis	*Campylobacter jejuni*	Fever, diarrhea, cramps, vomiting, and sometimes dysentery; sometimes more severe organ or autoimmune effects	Ingestion of unpasteurized milk, undercooked chicken, or contaminated water	Culture on selective medium with elevated temperature and low oxygen concentration	Generally none; erythromycin or ciprofloxacin if necessary
Cholera	*Vibrio cholera*	Severe diarrhea and fluid loss, potentially leading to shock, renal failure, and death	Ingestion of contaminated water or food	Culture on selective medium (TCBS agar); distinguished as oxidase positive with fermentative metabolisms	Generally none; tetracyclines, azithromycin, others if necessary
Clostridium difficile infection	*Clostridium difficile*	Pseudomembranous colitis, watery diarrhea, fever, abdominal pain, loss of appetite, dehydration; in severe cases, perforation of the colon, septicemia, shock, and death	Overgrowth of *C. difficile* in the normal microbiota due to antibiotic use; hospital-acquired infections in immunocompromised patients	Detection of toxin in stool, nucleic acid amplification tests (e.g., PCR)	Discontinuation of previous antibiotic treatment; metronidazole or vancomycin
Clostridium perfringens gastroenteritis	*Clostridium perfringens* (especially type A)	Mild cramps and diarrhea in most cases; in rare cases, hemorrhaging, vomiting, intestinal gangrene, and death	Ingestion of undercooked meats containing *C. perfringens* endospores	Detection of toxin or bacteria in stool or uneaten food	None

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
E. coli infection	ETEC, EPEC, EIEC, EHEC	Watery diarrhea, dysentery, cramps, malaise, fever, chills, dehydration; in EHEC, possible severe complications such as hematolytic uremic syndrome	Ingestion of contaminated food or water	Tissue culture, immunochemical assays, PCR, gene probes	Not recommended for EIEC and EHEC; fluoroquinolones, doxycycline, rifaximin, and TMP/SMZ possible for ETEC and EPEC
Peptic ulcers	*Helicobacter pylori*	Nausea, bloating, burping, lack of appetite, weight loss, perforation of stomach, blood in stools	Normal flora, can also be acquired via saliva, Fecal-oral route via contaminated food and water	Breath test, detection of antibodies in blood, detection of bacteria in stool sample or stomach biopsy	Amoxicillin, clarithromycin metronidazole, tetracycline, lansoprazole; antacids may also be given in combination with antibiotics
Salmonellosis	*Salmonella enterica*, serotype Enteritides	Fever, nausea, vomiting, abdominal cramps, headache, diarrhea; can be fatal in infants	Ingestion of contaminated food, handling of eggshells or contaminated animals	Culturing, serotyping and DNA fingerprinting	Not generally recommended; fluoroquinolones, ampicillin, others for immunocompromised patients
Shigella dysentery	*Shigella dysenteriae, S. flexneri, S. boydii,* and *S. sonnei*	Abdominal cramps, fever, diarrhea, dysentery; possible complications: reactive arthritis and hemolytic uremic syndrome	Fecal-oral route via contaminated food and water	Testing of stool samples for presence of blood and leukocytes; culturing, PCR, immunoassay for *S. dysenteriae*	Ciprofloxacin, azithromycin
Staphylococcal food poisoning	*Staphylococcus aureus*	Rapid-onset nausea, diarrhea, vomiting lasting 24–48 hours; possible dehydration and change in blood pressure and heart rate	Ingestion of raw or undercooked meat or dairy products contaminated with staphylococcal enterotoxins	ELISA to detect enterotoxins in uneaten food, stool, or vomitus	None

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Typhoid fever	*S. enterica*, subtypes Typhi or Paratyphi	Aches, headaches, nausea, lethargy, diarrhea or constipation, possible rash; lethal perforation of intestine can occur	Fecal-oral route; may be spread by asymptomatic carriers	Culture of blood, stool, or bone marrow, serologic tests; PCR tests when available	Fluoroquinolones, ceftriaxone, azithromycin; preventive vaccine available
Yersinia infection	*Yersinia enterocolitica, Y. pseudotuberculosis*	Generally mild diarrhea and abdominal cramps; in some cases, bacteremia can occur, leading to severe complications	Fecal-oral route, typically via contaminated food or water	Testing stool samples, tissues, body fluids	Generally none; fluoroquinolones, aminoglycosides, others for systemic infections

Table 4.10: Bacterial infections of the GI tract

VIRAL INFECTIONS OF THE GASTROINTESTINAL TRACT

- Common viral causes of gastroenteritis include **rotaviruses, noroviruses**, and astroviruses.
- **Hepatitis** may be caused by several unrelated viruses: **hepatitis viruses A, B, C, D, and E.**

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Vaccine
Astrovirus gastroenteritis	Astroviruses	Fever, headache, abdominal pain, malaise, diarrhea, vomiting	Fecal-oral route, contaminated food or water	Enzyme immunoassays, immune electron microscopy	None
Norovirus gastroenteritis	Noroviruses	Fever, diarrhea, projectile vomiting, dehydration; generally self-limiting within two days	Highly contagious via direct contact or contact with contaminated food or fomites	Rapid enzyme immunoassay confirmed with RT-qPCR	None

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Vaccine
Rotavirus gastroenteritis	Rotaviruses	Fever, diarrhea, vomiting, severe dehydration; recurring infections can lead to malnutrition and death	Fecal-oral route; children and elderly most susceptible	Enzyme immunoassay of stool sample, latex agglutination assays, RT-PCR	Preventive vaccine recommended for infants

Table 4.11: Viral causes of gastroenteritis

Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs	Vaccines
Hepatitis A	Hepatitisvirus A (HAV)	Usually asymptomatic or mild and self-limiting within one to two weeks to a few months, sometimes longer but not, chronic; in rare cases leads to serious or fatal fulminant hepatitis	Contaminated food, water, objects, and person to person	None	Vaccine recommended for one year olds and high-risk adults
Hepatitis B	Hepatitisvirus B (HBV)	Similar to Hepatitis A, but may progress to cirrhosis and liver failure; associated with liver cancer	Contact with infected body fluids (blood, semen, saliva), e.g., via IV drug use, sexual transmission, health-care workers treating infected patients	Interferon, entecavir, tenofovir, lamivudine, adefovir	Vaccine recommended for infants and high-risk adults

Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs	Vaccines
Hepatitis C	Hepatitisvirus C (HCV)	Often asymptomatic, with 75%–85% chronic carriers; may progress to cirrhosis and liver failure; associated with liver cancer	Contact with infected body fluids, e.g., via IV drug use, transfusions, sexual transmission	Depends on genotype and on whether cirrhosis is present; interferons, new treatment such as simeprevir plus sofosbuvir, ombitasvir/ paritaprevir/ ritonavir and dasabuvir	None available
Hepatitis D	Hepatitisvirus D (HDV)	Similar to Hepatitis B; usually self-limiting within one to two weeks but can become chronic or fulminant in rare cases	Contact with infected blood; infections can only occur in patients already infected with hepatitis B	None	Hepatitis B vaccine protects against HDV
Hepatitis E	Hepatitisvirus E (HEV)	Generally asymptomatic or mild and self-limiting; typically does not cause chronic disease	Fecal-oral route, often in contaminated water or undercooked meat; most common in developing countries	Supportive treatment; usually self-limiting, but some strains can become chronic; antiviral and immunosuppressive possible for chronic cases	Vaccine available in China only

Table 4.12: Viral forms of hepatitis

PROTOZOAN INFECTIONS OF THE GASTROINTESTINAL TRACT

- **Giardiasis**, **cryptosporidiosis**, **amoebiasis**, and **cyclosporiasis** are intestinal infections caused by protozoans.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Amoebiasis (amoebic dysentery)	*Entamoeba histolytica*	From mild diarrhea to severe dysentery and colitis; may cause abscess on the liver	Fecal-oral route; ingestion of cysts from fecally contaminated water, food, or hands	Stool O&P exam, enzyme immunoassay	Metronidazole, tinidazole, diloxanide furoate, iodoquinol, paromomycin
Cryptosporidiosis	*Cryptosporidium parvum, Cryptosporidium hominis*	Watery diarrhea, nausea, vomiting, cramps, fever, dehydration, and weight loss	Contact with feces of infected mice, birds, farm animals; ingestion of contaminated food or water; exposure to contaminated water while swimming or bathing	Stool O&P exam, enzyme immunoassay, PCR	Nitazoxanide, azithromycin, and paromomycin
Cyclosporiasis	*Cyclospora cayetanensis*	Explosive diarrhea, fever, nausea, vomiting, cramps, loss of appetite, fatigue, bloating	Ingestion of contaminated food or water	Stool O&P exam using ultraviolet fluorescence microscopy	Trimethoprim-sulfmethoxazole
Giardiasis	*Giardia lamblia*	Diarrhea, nausea, stomach cramps, gas, greasy stool, dehydration if severe; sometimes malabsorption syndrome	Contact with infected individual or contaminated fomites; ingestion of contaminated food or water	Stool O&P exam; ELISA, direct fluorescence antibody assays	Metronidazole, tinidazole

Table 4.13: Protozoan infections of the GI tract

HELMINTHIC INFECTIONS OF THE GASTROINTESTINAL TRACT

- *Ascaris lumbricoides* eggs are transmitted through contaminated food or water and hatch in the intestine. Juvenile larvae travel to the lungs and then to the pharynx where they are swallowed and returned to the intestines to mature. These nematode roundworms cause **ascariasis**.
- *Necator americanus* and *Ancylostoma doudenale* cause **hookworm infection** when larvae penetrate the skin from soil contaminated by dog or cat feces. They travel to the lungs and are then swallowed to mature in the intestines.
- *Strongyloides stercoralis* are transmitted from soil through the skin, to the lungs, and, then, to the intestine where they cause **strongyloidiasis**.

- *Enterobius vermicularis* are nematode pinworms transmitted by the fecal-oral route. After ingestion, they travel to the colon where they cause **enterobiasis**.
- *Trichuris trichiura* can be transmitted through soil or fecal contamination and cause **trichuriasis**. After ingestion, the eggs travel to the intestine where the larvae emerge and mature before attaching to the walls of the colon and cecum.
- *Trichinella* spp. is transmitted through undercooked meat. Larvae in the meat emerge from cysts and mature in the large intestine. They can migrate to the muscles and form new cysts, causing **trichinosis**.
- *Taenia* spp. and *Diphyllobothrium latum* are tapeworms transmitted through undercooked food or the fecal-oral route. *Taenia* infections cause **taeniasis**. Tapeworms use their scolex to attach to the intestinal wall. Larvae may also move to muscle or brain tissue.
- *Echinococcus granulosus* is a cestode transmitted through eggs in the feces of infected animals, especially dogs. After ingestion, eggs hatch in the small intestine, and the larvae invade the intestinal wall and travel through the circulatory system to form dangerous cysts in internal organs, causing **hydatid disease**.
- Flukes are transmitted through aquatic plants or fish. **Liver flukes** cause disease by interfering with the bile duct. **Intestinal flukes** develop in the intestines where they attach to the intestinal epithelium.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Strongyloidiasis	*Strongyloides stercoralis*	Often asymptomatic; cough (sometimes bloody), skin rash, abdominal pain, diarrhea; in immunosuppressed patients, may become disseminated, causing serious and potentially fatal complications	Soil-dwelling larvae penetrate the skin, usually bare feet	Microscopic observation of larvae in stool; serological testing for antigens	Ivermectin, albendazole
Tapeworms (taeniasis)	*Taenia solium, T. saginata, T. asiatica, Diphyllobothrium latum*	Asymptomatic or mild GI distress; cysts in muscle, eye, or brain (cysticercosis); brain cysts can cause headaches, seizures, or death	Ingestion of raw or undercooked pork or beef from infected animal	Observation of worm segments or microscopic eggs in stool; CT or MRI to detect cysts	Praziquantel, niclosamide

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Trichinosis	*Trichinella spiralis*, other *Trichinella* spp.	Diarrhea, constipation, abdominal pain, headache, cough, chills, light sensitivity, muscle pain, fever, conjunctivitis; in severe cases may affect motor coordination, breathing, heart function	Ingestion of raw or undercooked pork or other meat of infected animal	Observation of cysts in muscle biopsy, enzyme immunoassay	Albendazole, mebendazole
Whipworm (trichuriasis)	*Trichuris trichiura*	Abdominal pain, anemia, diarrhea (possibly bloody), rectal prolapse	Ingestion of eggs in fecally contaminated food	Microscopic observation of eggs in stool	Albendazole, mebendazole, ivermectin

Table 4.14: Helminthic infections of the GI tract

Figure Descriptions

Figure 4.1: The small intestines with increasing magnification. A) is a diagram and b), c), and d) are micrographs of each magnification. The micrograph of the large magnification shows a pink region on the bottom with a deeply waved darker pink region at the surface; the top of the image is clear. There are some darker patches in the bottom layer labeled Peyer's patches. The diagram shows a tube lined with three layers of muscle; blood vessels connected to the outside of the tube. A cutout of the tube shows circular folds along the diameter of the tube. These folds contain deeply lobed villi. The empty space in the tube is labeled lumen. The next layer of magnification is one of the villi. The micrograph is filled with pink layers folding back and forth. The diagram shows two folds. The surface of the fold is covered with absorptive cells and some goblet cells. Between the folds is further indent labeled intestinal crypt. Inside the folds are capillaries, arteries, and lymphatic vesicles. At the very bottom of the structure (below the blood and lymph vessels, are a few duodenal glands. The final close-up shows finger-shapes in a row on the surface of a cell. These are labeled microvilli (brush border) on the diagram.

Figure 4.2: Micrograph of intestinal villi which are 2 pink regions separated by a clear space. The surface of each pink band is darker pink than the center and the surface contains lighter pink oval cells labeled goblet cells.

Figure 4.3: A photo of teeth with yellow plaque; label reads: bacterial biofilms (plaque) develop and produce acid which dissolves tooth enamel. This leads to a diagram showing the process. The first step shows a black region labeled decay in the enamel; the dentin and pulp are not yet affected. Yellow material on the tooth and near the region of decay is labeled plaque. Next, the decay expands and is labeled abscess; this reaches the dentin layer. Finally, the abscess expands and causes an infected pulp.

Figure 4.4: A) photo of the back of teeth with severe buildup, labeled tartar. B) photo of a tooth with a dark spot labeled decay. C) micrograph of a tooth; dark regions have an arrow. D) photo of a tooth with a hole. E) photo of a tooth with a large, bleeding hole

Figure 4.5: Photo of teeth with yellowing and red inflamed gums.

Figure 4.6: Diagram of a tooth with healthy gums. The crown is the part above the gums, the root is the part below the gums. The enamel is the outer layer, inside is the dentin and inside that is the pulp which contains the root canal, nerves, and blood vessels. Below the gums is bone. Gingivitis is the first stage of periodontal disease. This is when the gums become darker red and swollen. Periodontitis the gums recede and the enamel begins to break. In advanced periodontitis the gums recede even further and the tooth degenerates past the enamel and into the dentin and pulp.

Figure 4.7: Photo of inflamed gums that have receded showing more of the teeth length.

Figure 4.8: a) photo of a cold sore (red bump) on the lip. B) bumps are present in the back of a person's mouth.\

Figure 4.9: Photo of white lumpy patches in the mouth.

Figure 4.10: a) Structures of the head and neck: lips, jaw, nasal cavity (large space behind the nose), oral cavity (space in the mouth), tongue, uvula (structure in at the back of the mouth), pharynx (tube at the back of the mouth), esophagus (the pharynx is the top part of this tube which is now called the esophagus in the throat), and the larynx (this is also continuous with the pharynx but leads to the respiratory system). B) Components of the mouth region: teeth, sublingual gland (Below the tongue), submandibular gland (at the back and to the bottom of the mouth), and the parotid gland (a large gland at the very back of the mouth).

Figure 4.11: Photo of child with a very large swelling on one side of the neck.

Figure 4.12: This figure shows a large thermometer with Fahrenheit and Celsius marks for freezer temperature, refrigerator temperature, safe holding temperature for cooked foods, and safe internal cooking temperatures for different meets and prepared meals. The figure identifies the danger zone between refrigerator temperature of 40 degrees Fahrenheit or 4 degrees Celsius and the safe holding temperature of 140 degrees Fahrenheit or 60 degrees Celsius for cooked foods. It is within this danger zone that microbial growth presents a risk for food-borne diseases.

Figure 4.13: Micrograph of small round red blood cells and larger and darker white blood cells.

Figure 4.14: Micrograph of red rod-shaped cells entering green flaky-shaped cells.

Figure 4.15: a) photo of a person getting water from a dirty waterway. B) photo of a person sleeping in a cot. C) micrograph of a rod shaped cell with a length of 1 micrometer.

Figure 4.16: A diagram showing the lining of the stomach. At the very bottom is a blood vessel with red blood cells, neutrophils, and monocytes. At the top is a wavy layer of epithelial cells covered in mucous. Healthy stomach epithelia are coated in a layer of mucous. Helicobacter pylori colonizes epithelial cells and decreases the production of mucus. Gastric acids cause the formation of ulcers. Images of a healthy lining show smooth pink regions, and an ulcer is seen as a white spot in the lining.

Figure 4.17: A diagram showing the lining of the stomach. At the very bottom is a blood vessel with red blood cells, neutrophils, and monocytes. At the top is a wavy layer of epithelial cells covered in mucous. A variety of bacteria (different shapes and colors to indicate different species) are seen on the mucus. In one region is a cluster of rod shaped cells labeled Clostridium difficile that release small dots labeled TcdA and TcdB. These create a pseudomembrane that is a swelling above destroyed epithelial cells. In response neutrophils and monocytes released.

Figure 4.18: A micrograph of circles with dots all over them.

Figure 4.19: Hepatitis A is a polyhedron with a single strand inside. Hepatitis B is a polyhedron with 2 strands inside and a layer outside with bulb-shaped studs in it. Hepatitis C is a polyhedron with a single strand inside and a layer outside that has studs rectangular studs. Hepatitis D is a sphere with a wavy circle in the center and an outer layer with oval studs. Hepatitis E is a more complex polyhedron with a single strand inside.

Figure 4.20: A) Shows an illustration comparing a healthy liver to an inflamed liver. B) A woman with yellowing eyes is shown and another with yellowing skin.

Figure 4.21: An SEM micrograph showing a triangular cell with three long, thin projections; one from the end and two from the middle of the cell. The cell is approximately 3 x 8 μm in size.

Figure 4.22: Micrograph of green glowing circles on a dark background.

Figure 4.23: Micrograph of a blue glowing sphere labeled C. cayetanensis on a black background.

Figure 4.24: a) photo of worms filling the intestines. B) photo of a large handful of worms. C) photo of a circle in a thicker circle. The outer circle is about 60 micrometers.

Figure 4.25: a) Photo of a clear worm attached to tissue. B) Photo of red lines in the skin. c) Micrograph of an oval structure.

Figure 4.26: a) photo of a small clear worm. B) micrograph of cells shaped like pointed ovals.

Figure 4.27: a) a micrograph of a worm about 2 inches in length. B) a micrograph of an oval cell. c) A photo of a large protruding sac from the anus.

Figure 4.28: a) a micrograph of worms in bubbles within muscle tissue. B) a micrograph of a coiled worm on muscle.

Figure 4.29: a) a micrograph of a worm with a round end labeled scolex. The scolex has round structures that look like suckers. B) micrograph of an oval cell with a thick wall.

Figure 4.30: Eggs or gravid proglottids from an infected individual are passed into the environment; this is the diagnostic stage. Cattle (T. saginata) and pigs (T. solium) become infected by ingesting vegetation contaminated by eggs or gravid proglottids. Oncospheres hatch, penetrating intestinal wall and circulate to musculature. The oncospheres develop into cysticerci in muscles and become infective. Humans are infected by ingesting raw or undercooked infected meat. The scolex attaches to intestine and adults are found in the small intestine.

Figure 4.31: a) an oval organism with lines in the center and a small projection at one end. B) an oval organism with lines throughout and a small projection at one end.

Figure 4.32: Schistosoma mansoni, japonicum, and haematobium are found in feces; S. japonicum and S. haematobium are also found in urine. These can be diagnosed in the water and produce eggs which hatch releasing miracidia. The miracidia penetrate snail tissues and produce sporocysts in the snail (successive generations). The Cercariae released by snail into the water are free flowing and are the infective stage which can penetrate skin. S. mansoni travels to the large intestines, S. japonicum travels to the small intestines, and S. haematobium travels to the rectum. The cercariae lose their tails during penetration and become schistosomula. These enter circulation and migrate to portal blood in liver and mature into adults. The paired adult worms migrate to the mesenteric venules of the bowels/rectum (laying eggs that circulate to the liver and are shed in stools) – for S. mansoni and S. Japonicum. S. haematobium migrates to the venous plexus of the bladder.

Figure References

Figure 4.1: The structure of the wall of the small intestine allows for the majority of nutrient absorption in the body. Top: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology. Bottom: (c) 2012. Regents of University of Michigan Medical School. Redistribution authorized with attribution.

Figure 4.2: A magnified image of intestinal villi in the GI tract shows goblet cells. Modified from Gobletcell.jpg. Permission granted. https://commons.wikimedia.org/wiki/File:Gobletcell.jpg

Figure 4.3: Tooth decay occurs in stages. Photo: Onetimeuseaccount. CC0/Public Domain. https://commons.wikimedia.org/wiki/File:Gingivitis-before.JPG; Illustration: Modification by Rice University of free-to-use illustration. credit: Blausen.com staff (2014). "Medical gallery of Blausen Medical 2014". WikiJournal of Medicine 1 (2). DOI:10.15347/wjm/2014.010. ISSN 2002-4436. https://commons.wikimedia.org/wiki/File:Blausen_0864_ToothDecay.png

Figure 4.4: Tartar (dental calculus) is visible at the bases of these teeth. Credit a. Modification of work by DRosenbach. Public Domain. https://commons.wikimedia.org/wiki/File:MandibularAnteriorCalculus.JPG; b,c,d,e: Modification of ToothMontage3 by DRosenback. CC BY 3.0 Unported. https://commons.wikimedia.org/wiki/File:ToothMontage3.jpg

Figure 4.5: Redness and irritation of the gums are evidence of gingivitis. By Onetimeuseaccount. CC0/Public Domain. https://commons.wikimedia.org/wiki/File:Gingivitis-before.JPG

Figure 4.6: Healthy gums hold the teeth firmly and do not bleed. Modification of free-to-use illustration by Rice University. credit: Blausen.com staff (2014). "Medical gallery of Blausen Medical 2014". WikiJournal of Medicine 1 (2). DOI:10.15347/wjm/2014.010. ISSN 2002-4436. https://commons.wikimedia.org/wiki/File:Blausen_0864_ToothDecay.png

Figure 4.7: These inflamed, eroded gums are an example of a mild case of acute necrotizing ulcerative gingivitis, also known as trench mouth. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 4.8: This cold sore is caused by infection with herpes simplex virus type 1 (HSV-1). HSV-1 can also cause acute herpetic gingivostomatitis. Left: Jojo. Public Domain. https://commons.wikimedia.org/wiki/File:Herpes_labialis_-_opryszczka_wargowa.jpg. Right: Modification of work (c) Klaus D. Peter. CC BY 4.0. https://commons.wikimedia.org/wiki/File:Stomatitis_herpetica.jpg

Figure 4.9: Overgrowth of Candida in the mouth is called thrush. It often appears as white patches. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 4.10 (a) When food enters the mouth, digestion begins. Modification of work by National Cancer Institute. Public domain.

Figure 4.11: This child shows the characteristic parotid swelling associated with mumps. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 4.12 This figure indicates safe internal temperatures associated with the refrigeration, cooking, and reheating of different foods. Modification of work by USDA. Public domain.

Figure 4.13: Red and white blood cells can be seen in this micrograph of a stool sample from a patient with shigellosis. Centers for Disease Control and Prevention. Public Domain. https://commons.wikimedia.org/wiki/File:Shigella_stool.jpg

Figure 4.14: Salmonella entering an intestinal epithelial cell by reorganizing the host cell's cytoskeleton via the trigger mechanism. Modification of work by National Institutes for Health. Public Domain.

Figure 4.15: (a) Outbreaks of cholera often occur in areas with poor sanitation or after natural disasters that compromise sanitation infrastructure. Left, Middle: modification of work by Centers for Disease Control and Prevention. Public domain. Right: modification of work by Janice Carr, Centers for Disease Control and Prevention. Public domain.

Figure 4.16: Helicobacter infection decreases mucus production and causes peptic ulcers. Top Left: Modification of work (c) Santhosh Thomas/YouTube. CC BY 4.0. Top Right: Figure 1B from Moriya, M., Uehara, A., Okumura, T. et al. Stress-induced hemorrhagic gastric ulcer after successful Helicobacter pylori eradication: two case reports. J Med Case Reports 5, 252 (2011). https://doi.org/10.1186/1752-1947-5-252. CC BY 2.0. Bottom: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology

Figure 4.17: Clostridium difficile is able to colonize the mucous membrane of the colon when the normal microbiota is disrupted. Top: modification of work by Janice Carr, Centers for Disease Control and Prevention. Public domain. Bottom: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 4.18: Rotaviruses in a fecal sample are visualized using electron microscopy. (c) Dr. Graham Beards. CC BY 4.0. https://commons.wikimedia.org/wiki/File:Multiple_rotavirus_particles.jpg

Figure 4.19: Five main types of viruses cause hepatitis. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 4.20: Hepatitis is inflammation of the liver resulting from a variety of root causes. Left: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology. Middle: Modification of work by (c) James Heilman, MD. CC BY 3.0 Unported. https://commons.wikimedia.org/wiki/File:Jaundice08.jpg. Right: Modification of work (c) Sab3el3eish. CC BY 3.0 Unported. https://commons.wikimedia.org/wiki/File:Jaundice.jpg

Figure 4.21: Giardia lamblia, an intestinal protozoan parasite that infects humans and other mammals, causing severe diarrhea. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 4.22: Immunofluorescent staining allows for visualization of Cryptosporidium spp. Modification of work by EPA/H.D.A. Lindquist. Public domain.

Figure 4.23: Cyclospora cayetanensis are autofluorescent under ultraviolet light. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 4.24: Adult Ascaris lumbricoides roundworms can cause intestinal blockage. Left: Modification of work (c) South African Medical Research Council. CC BY 4.0. https://commons.wikimedia.org/wiki/File:Piece_of_intestine,_blocked_by_worms_(16424898321).jpg. Middle: modification of work by James Gathany, Centers for Disease Control and Prevention. Public Domain. Right: modification of work by Centers for Disease Control and Prevention. Public Domain.

Figure 4.25: This animal hookworm, Ancylostoma caninum. Left,Right: modification of work by Centers for Disease Control and Prevention. Public Domain. Middle: modification of work by WeisSagung Public Domain. https://commons.wikimedia.org/wiki/File:Larva_Migrans_Cutanea.jpg

Figure 4.26: E. vermicularis are tiny nematodes commonly called pinworms. By Erich gasboy. Public Domain. https://commons.wikimedia.org/wiki/File:Threadworm.jpg. (b) Centers for Disease Control. Public Domain. http://www.publicdomainfiles.com/show_file.php?id=13539970213828

Figure 4.27: (a) This adult female Trichuris whipworm is a soil-transmitted parasite. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 4.28: This image shows larvae of T. spiralis within muscle. modification of works by Centers for Disease Control and Prevention. Public Domain. Image a: https://commons.wikimedia.org/wiki/File:Trichinella_larvaeD.jpg Image b: https://commons.wikimedia.org/wiki/File:Trichinella_HBb.jpg

Figure 4.29: (a) An adult tapeworm uses the scolex to attach to the intestinal wall. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 4.30: Life cycle of a tapeworm. Illustration: Modification of work by Centers for Disease Control and Prevention. CC BY 4.0. Step 1, 2, 4, 5, 6 Images: Public Domain. Step 3 Images: (c) American Society of Microbiology. Redistribution authorized with attribution.

Figure 4.31: A liver fluke infects the bile ducts. Left: Figure 2 in Shafiei R, Sarkari B, Sadjjadi SM, Mowlavi GR, and Moshfe A. (2014) "Molecular and Morphological Characterization of Fasciola spp. Isolated from Different Host Species in a Newly Emerging Focus of Human Fascioliasis in Iran" Veterinary Medicine International. https://doi.org/10.1155/2014/405740. CC BY 3.0. Right: Centers for Disease Control and Prevention – Georgia Division of Public Health. Public Domain. https://commons.wikimedia.org/wiki/File:Fasciolopsis_buski_adult_GA.jpg

Figure 4.32: The life cycle of Schistosoma spp. includes several species of water snails, which serve as secondary hosts. Illustration (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology. Modification of work by Centers for Disease Control and Prevention. Public Domain. https://www.cdc.gov/dpdx/schistosomiasis/modules/Schistomes_LifeCycle_lg.jpg. Step 3 photo: modification of Figure 1 in (c) Lewis FA, Liang Y-s, Raghavan N, Knight M (2008) The NIH-NIAID Schistosomiasis Resource Center. PLoS Negl Trop Dis 2(7): e267. https://doi.org/10.1371/journal.pntd.0000267. CC BY.

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CHAPTER 5.

SYSTEMIC INFECTIONS OF THE RESPIRATORY TRACT

5.1 ANATOMY AND NORMAL MICROBIOTA OF THE RESPIRATORY TRACT

The respiratory system can be conceptually divided into upper and lower regions at the point of the epiglottis, the structure that seals off the lower respiratory system from the pharynx during swallowing (figure 5.1).

ANATOMY OF THE UPPER RESPIRATORY SYSTEM

The upper respiratory system is in direct contact with the external environment. The nares (or nostrils) are the external openings of the nose that lead back into the nasal cavity, a large air-filled space behind the nares. These anatomical sites constitute the primary opening and first section of the respiratory tract, respectively. The nasal cavity is lined with hairs that trap large particles, like dust and pollen, and prevent their access to deeper tissues. The nasal cavity is also lined with a mucous membrane and Bowman's glands that produce mucus to help trap particles and microorganisms for removal. The nasal cavity is connected to several other air-filled spaces. The sinuses, a set of four, paired small cavities in the skull, communicate with the nasal cavity through a series of small openings. The nasopharynx is part of the upper throat extending from the posterior nasal cavity. The nasopharynx carries air inhaled through the nose. The middle ear is connected to the nasopharynx through the eustachian tube. The middle ear is separated from the outer ear by the tympanic membrane, or ear drum. And finally, the lacrimal glands drain to the nasal cavity through the nasolacrimal ducts (tear ducts). The open connections between these sites allow microorganisms to move from the nasal cavity to the sinuses, middle ears (and back), and down into the lower respiratory tract from the nasopharynx.

The oral cavity is a secondary opening for the respiratory tract. The oral and nasal cavities connect through the fauces to the pharynx, or throat. The pharynx can be divided into three regions: the nasopharynx, the oropharynx, and the laryngopharynx. Air inhaled through the mouth does not pass through the nasopharynx; it proceeds first through the oropharynx and then through the laryngopharynx. The palatine tonsils, which consist of lymphoid tissue, are located within the oropharynx. The laryngopharynx, the last portion of the pharynx, connects to the larynx, which contains the vocal fold (figure 5.1).

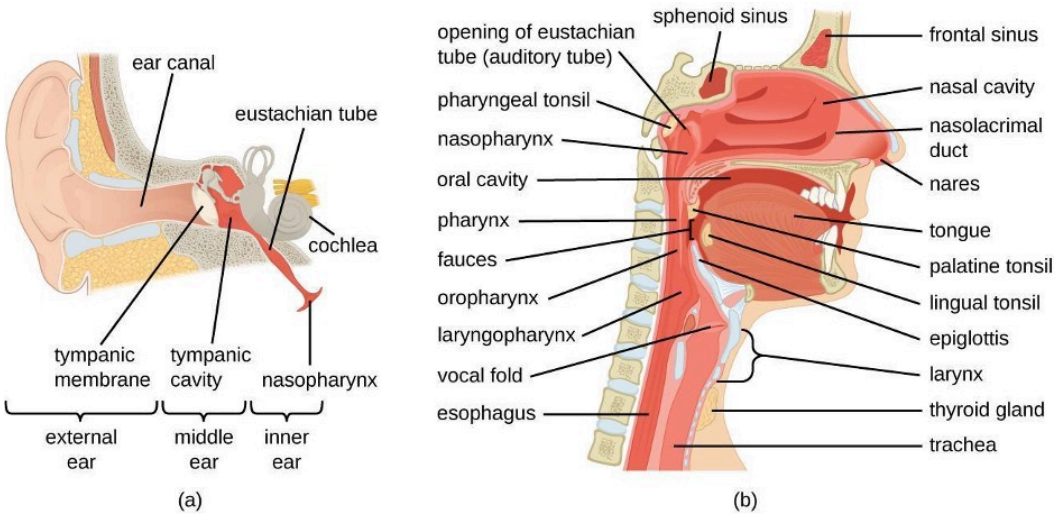


Figure 5.1: (a) The ear is connected to the upper respiratory tract by the eustachian tube, which opens to the nasopharynx. (b) The structures of the upper respiratory tract. *Figure description available at the end of the chapter.*

ANATOMY OF THE LOWER RESPIRATORY SYSTEM

The lower respiratory system begins below the epiglottis in the larynx or voice box (figure 5.2). The trachea, or windpipe, is a cartilaginous tube extending from the larynx that provides an unobstructed path for air to reach the lungs. The trachea bifurcates into the left and right bronchi as it reaches the lungs. These paths branch repeatedly to form smaller and more extensive networks of tubes, the bronchioles. The terminal bronchioles formed in this tree-like network end in cul-de-sacs called the alveoli. These structures are surrounded by capillary networks and are the site of gas exchange in the respiratory system. Human lungs contain on the order of 400,000,000 alveoli. The outer surface of the lungs is protected with a double-layered pleural membrane. This structure protects the lungs and provides lubrication to permit the lungs to move easily during respiration.

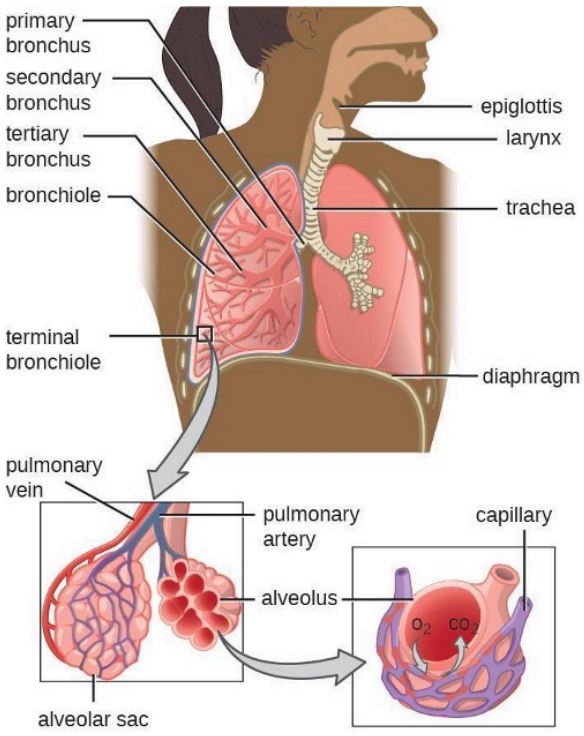


Figure 5.2: The structures of the lower respiratory tract are identified in this illustration. *Figure description available at the end of the chapter.*

DEFENSES OF THE RESPIRATORY SYSTEM

The inner lining of the respiratory system consists of mucous membranes (figure 5.3) and is protected by multiple immune defenses. The goblet cells within the respiratory epithelium secrete a layer of sticky mucus. The viscosity and acidity of this secretion inhibits microbial attachment to the underlying cells. In addition, the respiratory tract contains ciliated epithelial cells. The beating cilia dislodge and propel the mucus, and any trapped microbes, upward to the

epiglottis, where they will be swallowed. Elimination of microbes in this manner is referred to as the mucociliary escalator effect and is an important mechanism that prevents inhaled microorganisms from migrating further into the lower respiratory tract.

The upper respiratory system is under constant surveillance by mucosa-associated lymphoid tissue (MALT), including the adenoids and tonsils. Other mucosal defenses include secreted antibodies (IgA), lysozyme, surfactant, and antimicrobial peptides called defensins. Meanwhile, the lower respiratory tract is protected by alveolar macrophages. These phagocytes efficiently kill any microbes that manage to evade the other defenses. The combined action of these factors renders the lower respiratory tract nearly devoid of colonized microbes.

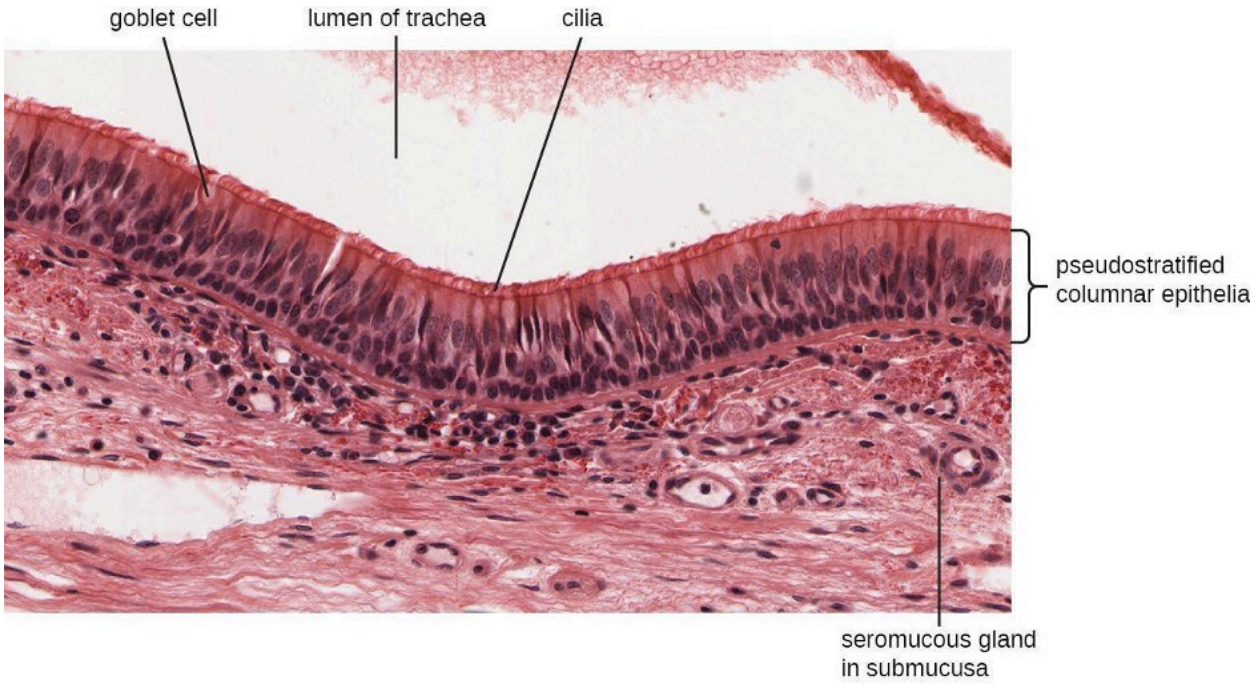


Figure 5.3: This micrograph shows the structure of the mucous membrane of the respiratory tract. *Figure description available at the end of the chapter.*

NORMAL MICROBIOTA OF THE RESPIRATORY SYSTEM

The upper respiratory tract contains an abundant and diverse microbiota. The nasal passages and sinuses are primarily colonized by members of the Firmicutes, Actinobacteria, and Proteobacteria. The most common bacteria identified include *Staphylococcus epidermidis*, viridans group streptococci (VGS), *Corynebacterium* spp. (diphtheroids), *Propionibacterium* spp., and *Haemophilus* spp. The oropharynx includes many of the same isolates as the nose and sinuses, with the addition of variable numbers of bacteria like species of *Prevotella*, *Fusobacterium*, *Moraxella*, and *Eikenella*, as well as some *Candida* fungal isolates. In addition, many healthy humans asymptomatically carry potential pathogens in the upper respiratory tract. As much as 20% of the population carry *Staphylococcus aureus* in their nostrils.[1] The pharynx, too, can be colonized with pathogenic strains of *Streptococcus*, *Haemophilus*, and *Neisseria*.

The lower respiratory tract, by contrast, is scantily populated with microbes. Of the organisms identified in the lower respiratory tract, species of *Pseudomonas*, *Streptococcus*, *Prevotella*, *Fusobacterium*, and *Veillonella* are the most common. It is not clear at this time if these small populations of bacteria constitute a normal microbiota or if they are transients.

Many members of the respiratory system's normal microbiota are opportunistic pathogens. To proliferate and cause host damage, they first must overcome the immune defenses of respiratory tissues. Many mucosal pathogens produce virulence factors such as adhesins that mediate attachment to host epithelial cells, or polysaccharide capsules that allow microbes to evade phagocytosis. The endotoxins of gram-negative bacteria can stimulate a strong inflammatory response that damages respiratory cells. Other pathogens produce exotoxins, and still others have the ability to survive within the host cells. Once an infection of the respiratory tract is established, it tends to impair the mucociliary escalator, limiting the body's ability to expel the invading microbes. This process makes it easier for pathogens to multiply and spread.

Vaccines have been developed for many of the most serious bacterial and viral pathogens. Several of the most important respiratory pathogens and their vaccines, if available, are summarized in table 5.1. Components of these vaccines will be explained later in the chapter.

Disease	Pathogen	Available Vaccine(s)*
Chickenpox/shingles	Varicella-zoster virus	Varicella (chickenpox) vaccine, herpes zoster (shingles) vaccine
Common cold	Rhinovirus	None
Diphtheria	*Corynebacterium diphtheriae*	DtaP, Tdap, DT,Td, DTP
Epiglottitis, otitis media	*Haemophilus influenzae*	Hib
Influenza	Influenza viruses	Inactivated, FluMist
Measles	Measles virus	MMR
Pertussis	*Bordetella pertussis*	DTaP, Tdap
Pneumonia	*Streptococcus pneumoniae*	Pneumococcal conjugate vaccine (PCV13), pneumococcal polysaccharide vaccine (PPSV23)
Rubella (German measles)	Rubella virus	MMR
Severe acute respiratory syndrome (SARS)	SARS-associated coronavirus (SARS-CoV)	None
Tuberculosis	*Mycobacterium tuberculosis*	BCG
*Full names of vaccines listed in table: Haemophilus influenzae type B (Hib); Diphtheria, tetanus, and acellular pertussis (DtaP); tetanus, diphtheria, and acellular pertussis (Tdap); diphtheria and tetanus (DT); tetanus and diphtheria (Td); diphtheria, pertussis, and tetanus (DTP); Bacillus Calmette-Guérin; Measles, mumps, rubella (MMR)		

Table 5.1: Some important respiratory diseases and vaccines

SIGNS AND SYMPTOMS OF RESPIRATORY INFECTION

Microbial diseases of the respiratory system (table 5.2) typically result in an acute inflammatory response. These infections can be grouped by the location affected and have names ending in "itis", which literally means *inflammation of*. For instance, rhinitis is an inflammation of the nasal cavities, often characteristic of the common cold. Rhinitis may also be associated with hay fever allergies or other irritants. Inflammation of the sinuses is called sinusitis. Inflammation of the ear is called otitis. Otitis media is an inflammation of the middle ear. A variety of microbes can cause pharyngitis, commonly known as a sore throat. An inflammation of the larynx is called laryngitis. The resulting inflammation may interfere with vocal cord function, causing voice loss. When tonsils are inflamed, it is called tonsillitis. Chronic cases of tonsillitis may be treated surgically with tonsillectomy. More rarely, the epiglottis can be infected, a condition called epiglottitis. In the lower respiratory system,

the inflammation of the bronchial tubes results in bronchitis. Most serious of all is pneumonia, in which the alveoli in the lungs are infected and become inflamed. Pus and edema accumulate and fill the alveoli with fluids (called consolidations). This reduces the lungs' ability to exchange gasses and often results in a productive cough expelling phlegm and mucus. Cases of pneumonia can range from mild to life-threatening, and remain an important cause of mortality in the very young and very old (table 5.3).

5.2 BACTERIAL INFECTIONS OF THE RESPIRATORY TRACT

The respiratory tract can be infected by a variety of bacteria, both gram positive and gram negative. Although the diseases that they cause may range from mild to severe, in most cases, the microbes remain localized within the respiratory system. Fortunately, most of these infections also respond well to antibiotic therapy.

STREPTOCOCCAL INFECTIONS

A common upper respiratory infection, streptococcal pharyngitis (strep throat) is caused by *Streptococcus pyogenes*. This gram-positive bacterium appears as chains of cocci, as seen in figure 5.4. Rebecca Lancefield (1895-1981) serologically classified streptococci in the 1930s using carbohydrate antigens from the bacterial cell walls. *S. pyogenes* is the sole member of the Lancefield group A streptococci and is often referred to as GAS, or group A strep.

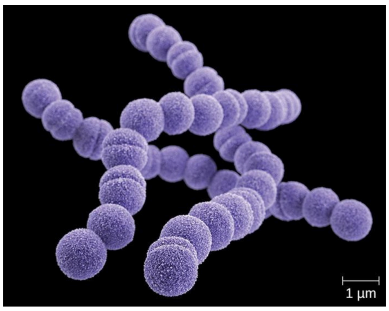
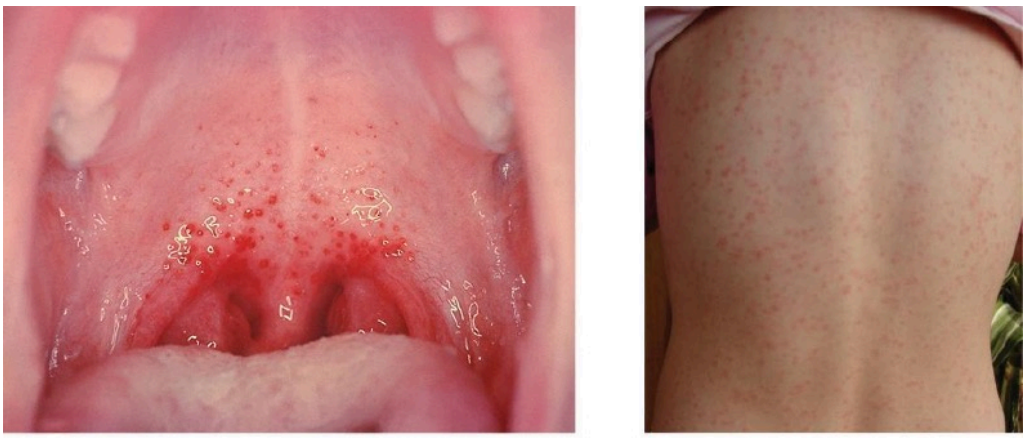


Figure 5.4: This scanning electron micrograph of Streptococcus pyogenes shows the characteristic cellular phenotype resembling chains of cocci. *Figure description available at the end of the chapter.*

Similar to streptococcal infections of the skin, the mucosal membranes of the pharynx are damaged by the release of a variety of exoenzymes and exotoxins by this extracellular pathogen. Many strains of *S. pyogenes* can degrade connective tissues by using hyaluronidase, collagenase and streptokinase. Streptokinase activates plasmin, which leads to degradation of fibrin and, in turn, dissolution of blood clots, which assists in the spread of the pathogen. Released toxins include streptolysins that can destroy red and white blood cells. The classic signs of streptococcal pharyngitis are a fever higher than 38 °C (100.4 °F); intense pharyngeal pain; erythema associated with pharyngeal inflammation; and swollen, dark-red palatine tonsils, often dotted with patches of pus; and petechiae (microcapillary hemorrhages) on the soft or hard palate (roof of the mouth) (figure 5.5). The submandibular lymph nodes beneath the angle of the jaw are also often swollen during strep throat.

Some strains of group A streptococci produce erythrogenic toxin. This exotoxin is encoded by a temperate bacteriophage (bacterial virus) and is an example of phage conversion (see section 2.11). The toxin attacks the plasma membranes of capillary endothelial cells and leads to scarlet fever (or scarlatina), a disseminated fine red rash on the skin, and strawberry tongue, a red rash on the tongue (figure 5.5). Severe cases may even lead to streptococcal toxic shock syndrome (STSS), which results from massive superantigen production that leads to septic shock and death.

S. pyogenes can be easily spread by direct contact or droplet transmission through coughing and sneezing. The disease can be diagnosed quickly using a rapid enzyme immunoassay for the group A antigen. However, due to a significant rate of false-negative results (up to 30%[2]), culture identification is still the gold standard to confirm pharyngitis due to *S. pyogenes*. *S. pyogenes* can be identified as a catalase-negative, beta hemolytic bacterium that is susceptible to 0.04 units of bacitracin. Antibiotic resistance is limited for this bacterium, so most β-lactams remain effective; oral amoxicillin and intramuscular penicillin G are those most commonly prescribed.



(a) (b)

Figure 5.5: Streptococcal infections of the respiratory tract may cause localized pharyngitis or systemic signs and symptoms. (a) The characteristic appearance of strep throat: bright red arches of inflammation with the presence of dark-red spots (petechiae). (b) Scarlet fever presents as a rash on the skin. *Figure description available at the end of the chapter.*

Sequelae of S. pyogenes Infections

One reason strep throat infections are aggressively treated with antibiotics is because they can lead to serious sequelae, later clinical consequences of a primary infection. It is estimated that 1%–3% of untreated *S. pyogenes* infections can be followed by nonsuppurative (without the production of pus) sequelae that develop 1–3 weeks after the acute infection has resolved. Two such sequelae are acute rheumatic fever and acute glomerulonephritis.

Acute rheumatic fever can follow pharyngitis caused by specific rheumatogenic strains of *S. pyogenes* (strains 1, 3, 5, 6, and 18). Although the exact mechanism responsible for this sequela remains unclear, molecular mimicry between the M protein of rheumatogenic strains of *S. pyogenes* and heart tissue is thought to initiate the autoimmune attack. The most serious and lethal clinical manifestation of rheumatic fever is damage to and inflammation of the heart (carditis). Acute glomerulonephritis also results from an immune response to streptococcal antigens following pharyngitis and cutaneous infections. Acute glomerulonephritis develops within 6–10 days after pharyngitis, but can take up to 21 days after a cutaneous infection. Similar to acute rheumatic fever, there are strong associations between specific nephritogenic strains of *S. pyogenes* and acute glomerulonephritis, and evidence suggests a role for antigen mimicry and autoimmunity. However, the primary mechanism of acute glomerulonephritis appears to be the formation of immune complexes between *S. pyogenes* antigens and antibodies, and their deposition between endothelial cells of the glomeruli of kidney. Inflammatory response against the immune complexes leads to damage and inflammation of the glomeruli (glomerulonephritis).

ACUTE OTITIS MEDIA

An infection of the middle ear is called acute otitis media (AOM), but often it is simply referred to as an earache. The condition is most common between ages 3 months and 3 years. In the United States, AOM is the second-leading cause of visits to pediatricians by children younger than age 5 years, and it is the leading indication for antibiotic prescription.[3]

AOM is characterized by the formation and accumulation of pus in the middle ear. Unable to drain, the pus builds up, resulting in moderate to severe bulging of the tympanic membrane and otalgia (ear pain). Inflamma-

tion resulting from the infection leads to swelling of the eustachian tubes, and may also lead to fever, nausea, vomiting, and diarrhea, particularly in infants. Infants and toddlers who cannot yet speak may exhibit nonverbal signs suggesting AOM, such as holding, tugging, or rubbing of the ear, as well as uncharacteristic crying or distress in response to the pain.

AOM can be caused by a variety of bacteria. Among neonates, *S. pneumoniae* is the most common cause of AOM, but *Escherichia coli*, *Enterococcus* spp., and group B *Streptococcus* species can also be involved. In older infants and children younger than 14 years old, the most common bacterial causes are *S. pneumoniae*, *Haemophilus influenzae*, or *Moraxella catarrhalis*. Among *S. pneumoniae* infections, encapsulated strains are frequent causes of AOM. By contrast, the strains of *H. influenzae* and *M. cattarhalis* that are responsible for AOM do not possess a capsule. Rather than direct tissue damage by these pathogens, bacterial components such as lipopolysaccharide (LPS) in gram-negative pathogens induce an inflammatory response that causes swelling, pus, and tissue damage within the middle ear (figure 5.6).

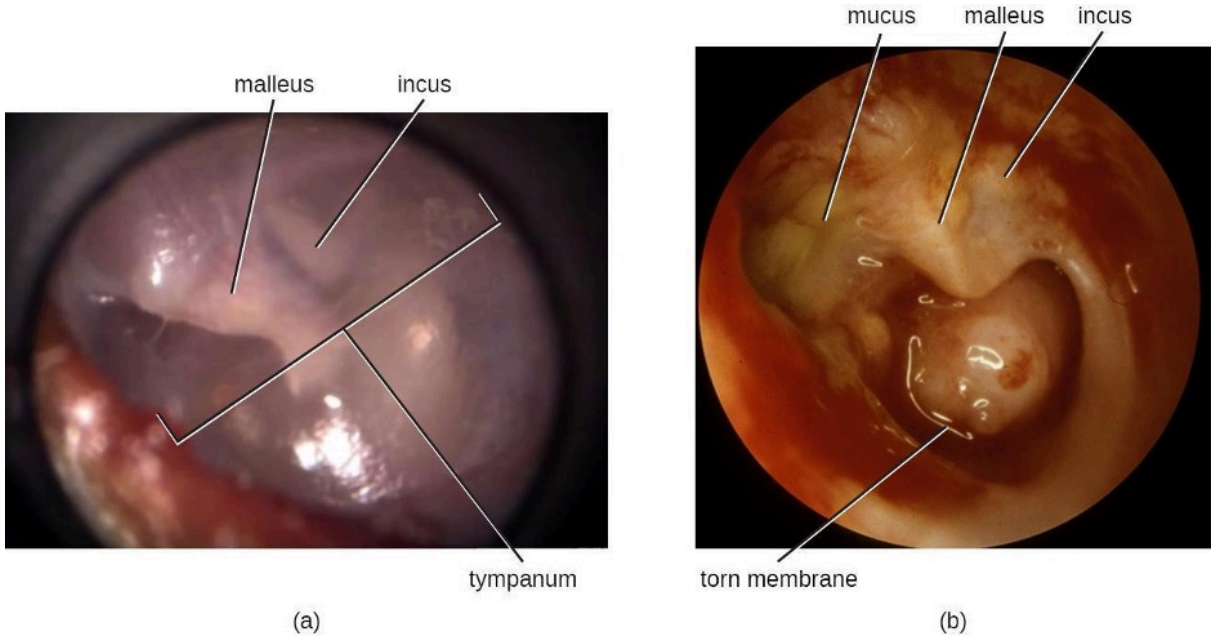


Figure 5.6: (a) A healthy tympanic membrane; the middle ear bones can be seen behind the membrane. (b) An ear with chronic inflammation that has resulted in a torn membrane, erosion of the inner ear bones, and mucus buildup. *Figure description available at the end of the chapter.*

Any blockage of the eustachian tubes, with or without infection, can cause fluid to become trapped and accumulate in the middle ear. This is referred to as otitis media with effusion (OME). The accumulated fluid offers an excellent reservoir for microbial growth. Consequently, secondary bacterial infections often ensue. This can lead to recurring and chronic earaches, which are especially common in young children. The higher incidence in children can be attributed to many factors. Children have more upper respiratory infections, in general, and their eustachian tubes are also shorter and drain at a shallower angle. Young children also tend to spend more time lying down than adults, which facilitates drainage from the nasopharynx through the eustachian tube and into the middle ear. Bottle feeding while lying down enhances this risk because the sucking action on the bottle causes negative pressure to build up within the eustachian tube, promoting the movement of fluid and bacteria from the nasopharynx.

Diagnosis is typically made based on clinical signs and symptoms, without laboratory testing to determine the specific causative agent. Antibiotics are frequently prescribed for the treatment of AOM. High-dose amoxicillin is the first-line drug, but with increasing resistance concerns, macrolides and cephalosporins may also be used.

The pneumococcal conjugate vaccine (PCV13) contains serotypes that are important causes of AOM, and vaccination has been shown to decrease the incidence of AOM. Vaccination against influenza has also been shown to decrease the risk for AOM, likely because viral infections like influenza predispose patients to secondary infections with *S. pneumoniae*. Although there is a conjugate vaccine available for the invasive serotype B of *H. influenzae*, this vaccine does not impact the incidence of *H. influenzae* AOM. Because unencapsulated strains of *H. influenzae* and *M. catarrhalis* are involved in AOM, vaccines against bacterial cellular factors other than capsules will need to be developed.

Bacterial Rhinosinusitis

The microbial community of the nasopharynx is extremely diverse and harbors many opportunistic pathogens, so it is perhaps not surprising that infections leading to rhinitis and sinusitis have many possible causes. These conditions often occur as secondary infections after a viral infection, which effectively compromises the immune defenses and allows the opportunistic bacteria to establish themselves. Bacterial sinusitis involves infection and inflammation within the paranasal sinuses. Because bacterial sinusitis rarely occurs without rhinitis, the preferred term is rhinosinusitis. The most common causes of bacterial rhinosinusitis are similar to those for AOM, including *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*.

DIPHTHERIA

The causative agent of diphtheria, *Corynebacterium diphtheriae*, is a club-shaped, gram-positive rod that belongs to the phylum Actinobacteria. Diphtheroids are common members of the normal nasopharyngeal microbiota. However, some strains of *C. diphtheriae* become pathogenic because of the presence of a temperate bacteriophage-encoded protein—the diphtheria toxin. Diphtheria is typically a respiratory infection of the oropharynx but can also cause impetigo-like lesions on the skin. Although the disease can affect people of all ages, it tends to be most severe in those younger than 5 years or older than 40 years. Like strep throat, diphtheria is commonly transmitted in the droplets and aerosols produced by coughing. After colonizing the throat, the bacterium remains in the oral cavity and begins producing the diphtheria toxin. This protein is an A-B toxin that blocks host-cell protein synthesis by inactivating elongation factor (EF)-2 (see section 2.14). The toxin's actions lead to the death of the host cells and an inflammatory response. An accumulation of grayish exudate consisting of dead host cells, pus, red blood cells, fibrin, and infectious bacteria results in the formation of a pseudomembrane. The pseudomembrane can cover mucous membranes of the nasal cavity, tonsils, pharynx, and larynx (figure 5.7). This is a classic sign of diphtheria. As the disease progresses, the pseudomembrane can enlarge to obstruct the fauces of the pharynx or trachea and can lead to suffocation and death. Sometimes, intubation, the placement of a breathing tube in the trachea, is required in advanced infections. If the diphtheria toxin spreads throughout the body, it can damage other tissues as well. This can include myocarditis (heart damage) and nerve damage that may impair breathing.

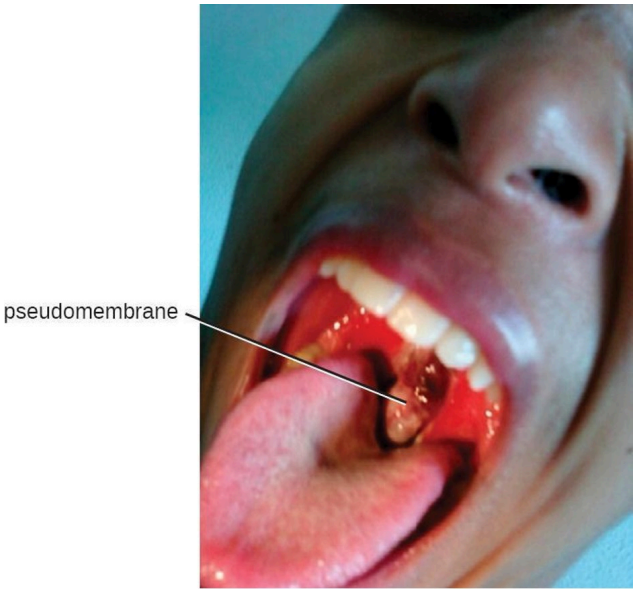


Figure 5.7: The pseudomembrane in a patient with diphtheria presents as a leathery gray patch consisting of dead cells, pus, fibrin, red blood cells, and infectious microbes. Figure description available at the end of the chapter.

The presumptive diagnosis of diphtheria is primarily based on the clinical symptoms (i.e., the pseudomembrane) and vaccination history, and is typically confirmed by identifying bacterial cultures obtained from throat swabs. The diphtheria toxin itself can be directly detected in vitro using polymerase chain reaction (PCR)-based, direct detection systems for the diphtheria *tox* gene, and immunological techniques like radial immunodiffusion or Elek's immunodiffusion test.

Broad-spectrum antibiotics like penicillin and erythromycin tend to effectively control *C. diphtheriae* infections. Regrettably, they have no effect against preformed toxins. If toxin production has already occurred in the patient, antitoxins (preformed antibodies against the toxin) are administered. Although this is effective in neutralizing the toxin, the antitoxins may lead to serum sickness because they are produced in horses (see section 1.13).

Widespread vaccination efforts have reduced the occurrence of diphtheria worldwide. There are currently four combination toxoid vaccines available that provide protection against diphtheria and other diseases: DTaP, Tdap, DT, and Td. In all cases, the letters "d," "t," and "p" stand for diphtheria, tetanus, and pertussis, respectively; the "a" stands for acellular. If capitalized, the letters indicate a full-strength dose; lowercase letters indicate reduced dosages. According to current recommendations, children should receive five doses of the DTaP vaccine in their youth and a Td booster every 10 years. Children with adverse reactions to the pertussis vaccine may be given the DT vaccine in place of the DTaP.

BACTERIAL PNEUMONIA

Pneumonia is a general term for infections of the lungs that lead to inflammation and accumulation of fluids and white blood cells in the alveoli. Pneumonia can be caused by bacteria, viruses, fungi, and other organisms (Table 5.3), although the vast majority of pneumonias are bacterial in origin. Bacterial pneumonia is a prevalent, potentially serious infection; it caused more than 50,000 deaths in the United States in 2014.[4] As the alveoli fill with fluids and white blood cells (consolidation), air exchange becomes impaired and patients experience respiratory distress (figure 5.8). In addition, pneumonia can lead to pleurisy, an infection of the pleural membrane surrounding the lungs, which can make breathing very painful. Although many different bacteria can cause pneumonia under the right circumstances, three bacterial species cause most clinical cases: *Streptococcus pneumoniae*, *H. influenzae*, and *Mycoplasma pneumoniae*. In addition to these, we will also examine some of the less common causes of pneumonia.

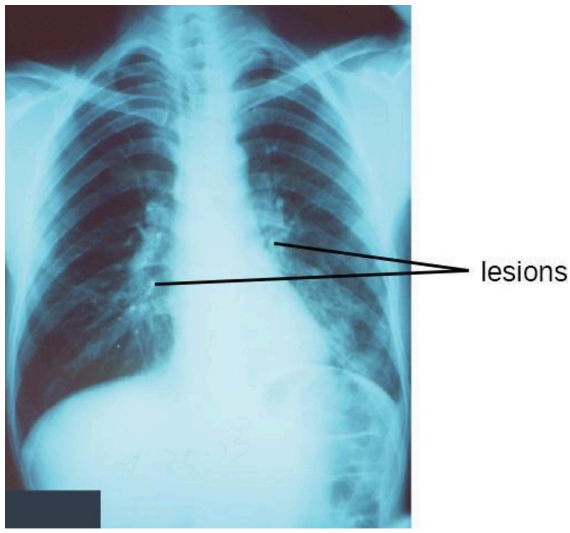


Figure 5.8: A chest radiograph of a patient with pneumonia shows the consolidations (lesions) present as opaque patches. *Figure description available at the end of the chapter.*

Pneumococcal Pneumonia

The most common cause of community-acquired bacterial pneumonia is *Streptococcus pneumoniae*. This gram-positive, alpha hemolytic streptococcus is commonly found as part of the normal microbiota of the human respiratory tract. The cells tend to be somewhat lancet-shaped and typically appear as pairs (figure 5.9). The pneumococci initially colonize the bronchioles of the lungs. Eventually, the infection spreads to the alveoli, where the microbe's polysaccharide capsule interferes with phagocytic clearance. Other virulence factors include autolysins like Lyt A, which degrade the microbial cell wall,

resulting in cell lysis and the release of cytoplasmic virulence factors. One of these factors, pneumolysin O, is important in disease progression; this pore-forming protein damages host cells, promotes bacterial adherence, and enhances pro-inflammatory cytokine production. The resulting inflammatory response causes the alveoli to fill with exudate rich in neutrophils and red blood cells. As a consequence, infected individuals develop a productive cough with bloody sputum.

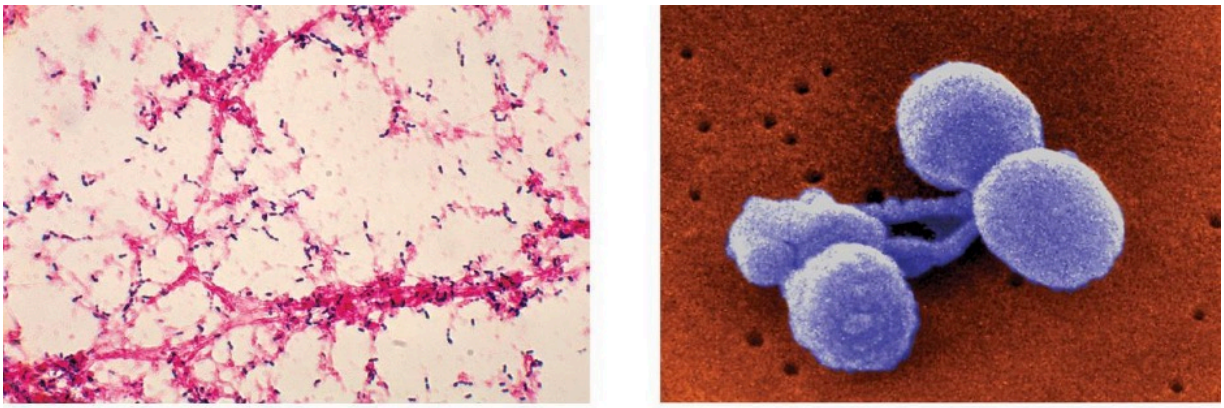


Figure 5.9: (a) This micrograph of Streptococcus pneumoniae grown from a blood culture shows the characteristic lancet-shaped diplococcal morphology. (b) A colorized scanning electron micrograph of S. pneumoniae. *Figure description available at the end of the chapter.*

Pneumococci can be presumptively identified by their distinctive gram-positive, lancet-shaped cell morphology and diplococcal arrangement. In blood agar cultures, the organism demonstrates alpha hemolytic colonies that are autolytic after 24 to 48 hours. In addition, *S. pneumoniae* is extremely sensitive to optochin and colonies are rapidly destroyed by the addition of 10% solution of sodium deoxycholate. All clinical pneumococcal isolates are serotyped using the quellung reaction with typing antisera produced by the CDC. Positive quellung reactions are considered definitive identification of pneumococci.

Antibiotics remain the mainstay treatment for pneumococci. β-Lactams like penicillin are the first-line drugs, but resistance to β-lactams is a growing problem. When β-lactam resistance is a concern, macrolides and fluoroquinolones may be prescribed. However, *S. pneumoniae* resistance to macrolides and fluoroquinolones is increasing as well, limiting the therapeutic options for some infections. There are currently two pneumococcal vaccines available: pneumococcal conjugate vaccine (PCV13) and pneumococcal polysaccharide vaccine (PPSV23). These are generally given to the most vulnerable populations of individuals: children younger than 2 years and adults older than 65 years.

Haemophilus Pneumonia

Encapsulated strains of *Haemophilus influenzae* are known for causing meningitis, but nonencapsulated strains are important causes of pneumonia. This small, gram-negative coccobacillus is found in the pharynx of the majority of healthy children; however, *Haemophilus* pneumonia is primarily seen in the elderly. Like other pathogens that cause pneumonia, *H. influenzae* is spread by droplets and aerosols produced by coughing. A fastidious organism, *H. influenzae* will only grow on media with available factor X (hemin) and factor V (NAD), like chocolate agar (figure 5.10). Serotyping must be performed to confirm the identity of *H. influenzae* isolates.

Infections of the alveoli by *H. influenzae* result in inflammation and accumulation of fluids. Increasing resistance to β-lactams, macrolides, and tetracyclines presents challenges for the treatment of *Haemophilus* pneumonia. Resistance to the fluoroquinolones is rare among isolates of *H. influenzae* but has been observed. As discussed for AOM, a vaccine directed against nonencapsulated *H. influenzae,* if developed, would provide protection against pneumonia caused by this pathogen.

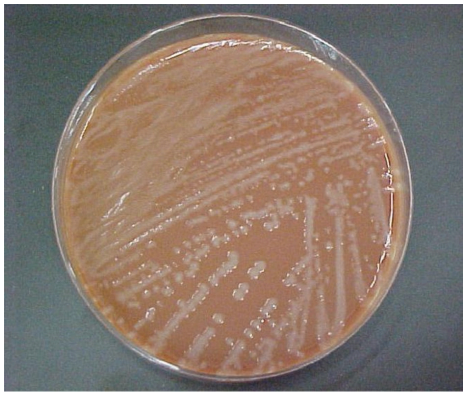


Figure 5.10: Culture of Haemophilus influenzae on a chocolate agar plate. Figure description available at the end of the chapter.

Mycoplasma Pneumonia (Walking Pneumonia)

Primary atypical pneumonia is caused by *Mycoplasma pneumoniae*. This bacterium is not part of the respiratory tract's normal microbiota and can cause epidemic disease outbreaks. Also known as walking pneumonia, *mycoplasma* pneumonia infections are common in crowded environments like college campuses and military bases. It is spread by aerosols formed when coughing or sneezing. The disease is often mild, with a low fever and persistent cough. These bacteria, which do not have cell walls, use a specialized attachment organelle to bind to ciliated cells. In the process, epithelial cells are damaged and the proper function of the cilia is hindered (figure 5.11).

Mycoplasma grows very slowly when cultured. Therefore, penicillin and thallium acetate are added to agar to prevent the overgrowth by faster-growing potential contaminants. Since *M. pneumoniae* does not have a cell wall, it is resistant to these substances. Without a cell wall, the microbial cells appear pleomorphic. *M. pneumoniae* infections tend to be self-limiting but may also respond well to macrolide antibiotic therapy. β-lactams, which target cell wall synthesis, are not indicated for treatment of infections with this pathogen.

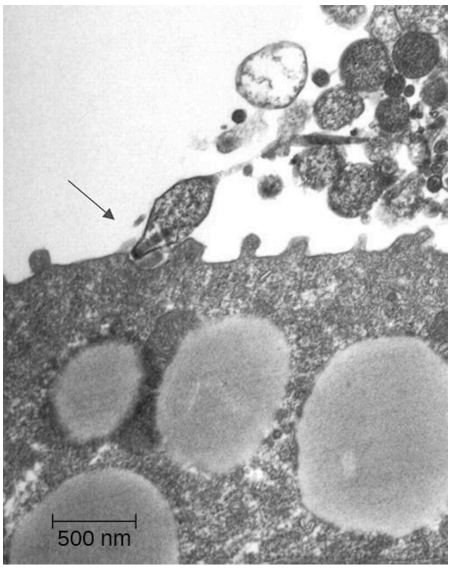


Figure 5.11: The micrograph shows Mycoplasma pneumoniae using their specialized receptors to attach to epithelial cells in the trachea of an infected hamster. Figure description available at the end of the chapter.

Chlamydial Pneumonias and Psittacosis

Chlamydial pneumonia can be caused by three different species of bacteria: *Chlamydophila pneumoniae* (formerly known as *Chlamydia pneumoniae*), *Chlamydophila psittaci* (formerly known as *Chlamydia psittaci*), and *Chlamydia trachomatis*. All three are obligate intracellular pathogens and cause mild to severe pneumonia and bronchitis. Of the three, *Chlamydophila pneumoniae* is the most common and is transmitted via respiratory droplets or aerosols. *C. psittaci* causes psittacosis, a zoonotic disease that primarily affects domesticated birds such as parakeets, turkeys, and ducks, but can be transmitted from birds to humans. Psittacosis is a relatively rare infection and is typically found in people who work with birds. *Chlamydia trachomatis,* the causative agent of the sexually transmitted disease chlamydia, can cause pneumonia in infants when the infection is passed from mother to baby during birth.

Diagnosis of chlamydia by culturing tends to be difficult and slow. Because they are intracellular pathogens, they require multiple passages through tissue culture. Recently, a variety of PCR- and serologically based tests have been developed to enable easier identification of these pathogens. Tetracycline and macrolide antibiotics are typically prescribed for treatment.

Health Care-Associated Pneumonia

A variety of opportunistic bacteria that do not typically cause respiratory disease in healthy individuals are common causes of healthcare-associated pneumonia. These include *Klebsiella pneumoniae*, *Staphylococcus aureus*, and proteobacteria such as species of *Escherichia*, *Proteus*, and *Serratia*. Patients at risk include the elderly, those who have other preexisting lung conditions, and those who are immunocompromised. In addition, patients receiving supportive therapies such as intubation, antibiotics, and immunomodulatory drugs may also be at risk because these interventions disrupt the mucociliary escalator and other pulmonary defenses. Invasive medical devices such as catheters, medical implants, and ventilators can also introduce opportunistic pneumonia-causing pathogens into the body.[5]

Pneumonia caused by *K. pneumoniae* is characterized by lung necrosis and "currant jelly sputum," so named because it consists of clumps of blood, mucus, and debris from the thick polysaccharide capsule produced by the bacterium. *K. pneumoniae* is often multidrug resistant. Aminoglycoside and cephalosporin are often prescribed but are not always effective. *Klebsiella* pneumonia is frequently fatal even when treated.

Pseudomonas Pneumonia

Pseudomonas aeruginosa is another opportunistic pathogen that can cause serious cases of bacterial pneumonia in patients with cystic fibrosis (CF) and hospitalized patients assisted with artificial ventilators. This bacterium is extremely antibiotic resistant and can produce a variety of exotoxins. Ventilator-associated pneumonia with *P. aeruginosa* is caused by contaminated equipment that causes the pathogen to be aspirated into the lungs. In patients with CF, a genetic defect in the cystic fibrosis transmembrane receptor (CFTR) leads to the accumulation of excess dried mucus in the lungs. This decreases the effectiveness of the defensins and inhibits the mucociliary escalator. *P. aeruginosa* is known to infect more than half of all patients with CF. It adapts to the conditions in the patient's lungs and begins to produce alginate, a viscous exopolysaccharide that inhibits the mucociliary escalator. Lung damage from the chronic inflammatory response that ensues is the leading cause of mortality in patients with CF.[6]

TUBERCULOSIS

Tuberculosis (TB) is one of the deadliest infectious diseases in human history. Although tuberculosis infection rates in the United States are extremely low, the CDC estimates that about one-third of the world's population is infected with *Mycobacterium tuberculosis*, the causal organism of TB. In 2021, TB affected 10.6 million persons and caused 1.6 million deaths worldwide.[7]

M. tuberculosis is an acid-fast, high G + C, gram-positive, non spore-forming rod. Its cell wall is rich in waxy mycolic acids, which make the cells impervious to polar molecules. It also causes these organisms to grow slowly. *M. tuberculosis* causes a chronic granulomatous disease that can infect any area of the body, although it is typically associated with the lungs. *M. tuberculosis* is spread by inhalation of respiratory droplets or aerosols from an infected person. The infectious dose of *M. tuberculosis* is only 10 cells.[8]

After inhalation, the bacteria enter the alveoli (figure 5.12). The cells are phagocytized by macrophages but can survive and multiply within these phagocytes because of the protection by the waxy mycolic acid in their cell walls. If not eliminated by macrophages, the infection can progress, causing an inflammatory response and an accumulation of neutrophils and macrophages in the area. Several weeks or months may pass before an immunological response is mounted by T cells and B cells. Eventually, the lesions in the alveoli become walled off, forming small round lesions called tubercles. Bacteria continue to be released into the center of the tubercles and the chronic immune response results in tissue damage and induction of apoptosis (programmed host-

cell death) in a process called liquefaction. This creates a caseous center, or air pocket, where the aerobic *M. tuberculosis* can grow and multiply. Tubercles may eventually rupture and bacterial cells can invade pulmonary capillaries; from there, bacteria can spread through the bloodstream to other organs, a condition known as miliary tuberculosis. The rupture of tubercles also facilitates transmission of the bacteria to other individuals via droplet aerosols that exit the body in coughs. Because these droplets can be very small and stay aloft for a long time, special precautions are necessary when caring for patients with TB, such as the use of face masks and negative-pressure ventilation and filtering systems.

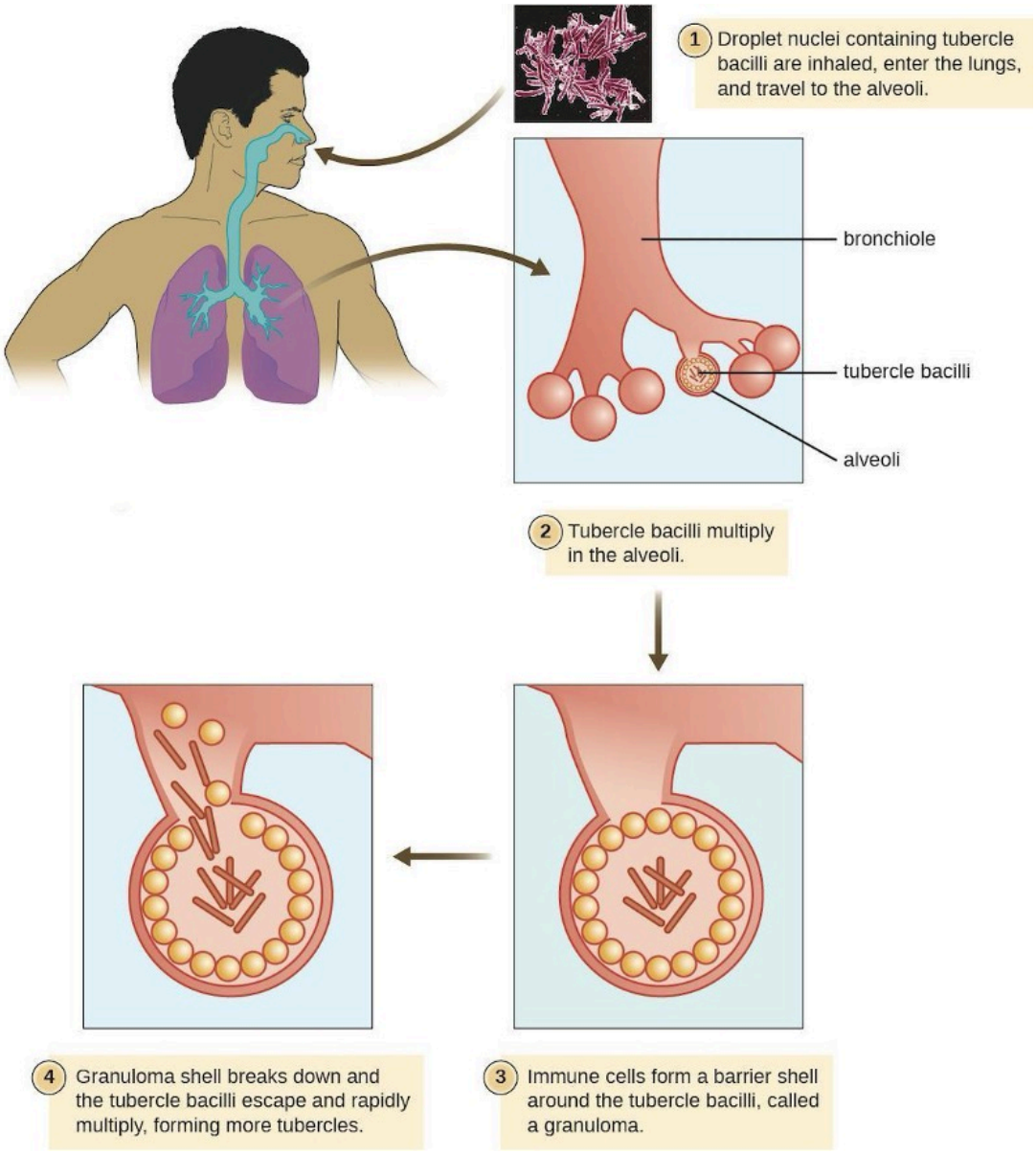


Figure 5.12: In the infectious cycle of tuberculosis, the immune response of most infected individuals (approximately 90%) results in the formation of tubercles in which the infection is walled off.[footnote]G. Kaplan et al. "Mycobacterium tuberculosis Growth at the Cavity Surface: A Microenvironment with Failed Immunity." Infection and Immunity 71 no. 12 (2003):7099–7108.[/footnote] The remainder will suffer progressive primary tuberculosis. The sequestered bacteria may be reactivated to form secondary tuberculosis in immunocompromised patients at a later time. *Figure description available at the end of the chapter.*

Eventually, most lesions heal to form calcified Ghon complexes. These structures are visible on chest radiographs and are a useful diagnostic feature. But even after the disease has apparently ended, viable bacteria remain sequestered in these locations. Release of these organisms at a later time can produce reactivation tuberculosis (or secondary TB). This is mainly observed in people with alcoholism, the elderly, or in otherwise immunocompromised individuals (figure 5.12).

Because TB is a chronic disease, chemotherapeutic treatments often continue for months or years. Multidrug resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains of *M. tuberculosis* are a growing clinical concern. These strains can arise due to misuse or mismanagement of antibiotic therapies. Therefore, it is imperative that proper multidrug protocols are used to treat these infections. Common antibiotics included in these mixtures are isoniazid, rifampin, ethambutol, and pyrazinamide.

A TB vaccine is available that is based on the so-called bacillus Calmette-Guérin (BCG) strain of *M. bovis* commonly found in cattle. In the United States, the BCG vaccine is only given to healthcare workers and members of the military who are at risk of exposure to active cases of TB. It is used more broadly worldwide. Many individuals born in other countries have been vaccinated with BCG strain. BCG is used in many countries with a high prevalence of TB, to prevent childhood tuberculous meningitis and miliary disease.

The Mantoux tuberculin skin test (figure 5.13) is regularly used in the United States to screen for potential TB exposure (see section 1.13). However, prior vaccinations with the BCG vaccine can cause false-positive results. Chest radiographs to detect Ghon complex formation are required, therefore, to confirm exposure.

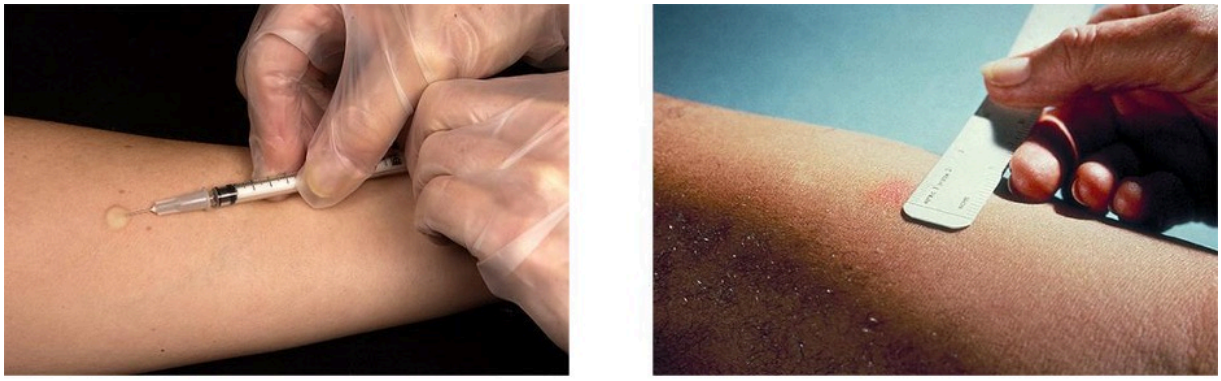


Figure 5.13: (a) The Mantoux skin test for tuberculosis involves injecting the subject with tuberculin protein derivative. The injection should initially produce a raised wheal. (b) The test should be read in 48–72 hours. A positive result is indicated by redness, swelling, or hardness; the size of the responding region is measured to determine the final result. *Figure description available at the end of the chapter.*

PERTUSSIS (WHOOPING COUGH)

The causative agent of pertussis, commonly called whooping cough, is *Bordetella pertussis*, a gram-negative coccobacillus. The disease is characterized by mucus accumulation in the lungs that leads to a long period of severe coughing. Sometimes, following a bout of coughing, a sound resembling a "whoop" is produced as air is inhaled through the inflamed and restricted airway—hence the name whooping cough. Although adults can be infected, the symptoms of this disease are most pronounced in infants and children. Pertussis is highly communicable through droplet transmission, so the uncontrollable coughing produced is an efficient means of transmitting the disease in a susceptible population.

Following inhalation, *B. pertussis* specifically attaches to epithelial cells using an adhesin, filamentous hemagglutinin. The bacteria then grow at the site of infection and cause disease symptoms through the production of exotoxins. One of the main virulence factors of this organism is an A-B exotoxin called the pertussis toxin (PT). When PT enters the host cells, it increases the cyclic adenosine monophosphate (cAMP) levels and disrupts cellular signaling. PT is known to enhance inflammatory responses involving histamine and serotonin. In addition to PT, *B. pertussis* produces a tracheal cytotoxin that damages ciliated epithelial cells and results in accumulation of mucus in the lungs. The mucus can support the colonization and growth of other microbes and, as a consequence, secondary infections are common. Together, the effects of these factors produce the cough that characterizes this infection.

A pertussis infection can be divided into three distinct stages. The initial infection, termed the catarrhal stage, is relatively mild and unremarkable. The signs and symptoms may include nasal congestion, a runny nose, sneezing, and a low-grade fever. This, however, is the stage in which *B. pertussis* is most infectious. In the paroxysmal stage, mucus accumulation leads to uncontrollable coughing spasms that can last for several minutes and frequently induce vomiting. The paroxysmal stage can last for several weeks. A long convalescence stage follows the paroxysmal stage, during which time patients experience a chronic cough that can last for up to several months. In fact, the disease is sometimes called the 100-day cough.

In infants, coughing can be forceful enough to cause fractures to the ribs, and prolonged infections can lead to death. The CDC reported 20 pertussis-related deaths in 2012,[9] but that number had declined to five by 2015.[10]

During the first 2 weeks of infection, laboratory diagnosis is best performed by culturing the organism directly from a nasopharyngeal (NP) specimen collected from the posterior nasopharynx. The NP specimen is streaked onto a Bordet-Gengou medium. The specimens must be transported to the laboratory as quickly as possible, even if transport media are used. Transport times of longer than 24 hours reduce the viability of *B. pertussis* significantly.

Within the first month of infection, *B. pertussis* can be diagnosed using PCR techniques. During the later stages of infection, pertussis-specific antibodies can be immunologically detected using an enzyme-linked immunosorbent assay (ELISA).

Pertussis is generally a self-limiting disease. Antibiotic therapy with erythromycin or tetracycline is only effective at the very earliest stages of disease. Antibiotics given later in the infection, and prophylactically to uninfected individuals, reduce the rate of transmission. Active vaccination is a better approach to control this disease. The DPT vaccine was once in common use in the United States. In that vaccine, the P component consisted of killed whole-cell *B. pertussis* preparations. Because of some adverse effects, that preparation has now been superseded by the DTaP and Tdap vaccines. In both of these new vaccines, the "aP" component is a pertussis toxoid.

Widespread vaccination has greatly reduced the number of reported cases and prevented large epidemics of pertussis. Recently, however, pertussis has begun to reemerge as a childhood disease in some states because of declining vaccination rates and an increasing population of susceptible children.

LEGIONNAIRES DISEASE

An atypical pneumonia called Legionnaires disease (also known as legionellosis) is caused by an aerobic gram-negative bacillus, *Legionella pneumophila*. This bacterium infects free-living amoebae that inhabit moist environments, and infections typically occur from human-made reservoirs such as air-conditioning cooling towers, humidifiers, misting systems, and fountains (figure 5.20). Aerosols from these reservoirs can lead to infections

of susceptible individuals, especially those suffering from chronic heart or lung disease or other conditions that weaken the immune system.

When *L. pneumophila* bacteria enter the alveoli, they are phagocytized by resident macrophages. However, *L. pneumophila* uses a secretion system to insert proteins in the endosomal membrane of the macrophage; these proteins prevent lysosomal fusion, allowing *L. pneumophila* to continue to proliferate within the phagosome. The resulting respiratory disease can range from mild to severe pneumonia, depending on the status of the host's immune defenses. Although this disease primarily affects the lungs, it can also cause fever, nausea, vomiting, confusion, and other neurological effects.

Diagnosis of Legionnaires disease is somewhat complicated. *L. pneumophila* is a fastidious bacterium and is difficult to culture. In addition, since the bacterial cells are not efficiently stained with the Gram stain, other staining techniques, such as the Warthin-Starry silver-precipitate procedure, must be used to visualize this pathogen. A rapid diagnostic test has been developed that detects the presence of *Legionella* antigen in a patient's urine; results take less than 1 hour, and the test has high selectivity and specificity (greater than 90%). Unfortunately, the test only works for one serotype of *L. pneumophila* (type 1, the serotype responsible for most infections). Consequently, isolation and identification of *L. pneumophila* from sputum remains the defining test for diagnosis.

Once diagnosed, Legionnaire disease can be effectively treated with fluoroquinolone and macrolide antibiotics. However, the disease is sometimes fatal; about 10% of patients die of complications.[11] There is currently no vaccine available.

Q FEVER

The zoonotic disease Q fever is caused by the rickettsia, *Coxiella burnetii*. The primary reservoirs for this bacterium are domesticated livestock such as cattle, sheep, and goats. The bacterium may be transmitted by ticks or through exposure to the urine, feces, milk, or amniotic fluid of an infected animal. In humans, the primary route of infection is through inhalation of contaminated farmyard aerosols. It is, therefore, largely an occupational disease of farmers. Humans are acutely sensitive to *C. burnetii*—the infective dose is estimated to be just a few cells.[12] In addition, the organism is hardy and can survive in a dry environment for an extended time. Symptoms associated with acute Q fever include high fever, headache, coughing, pneumonia, and general malaise. In a small number of patients (less than 5%[13]), the condition may become chronic, often leading to endocarditis, which may be fatal.

Diagnosing rickettsial infection by cultivation in the laboratory is both difficult and hazardous because of the easy aerosolization of the bacteria, so PCR and ELISA are commonly used. Doxycycline is the first-line drug to treat acute Q fever. In chronic Q fever, doxycycline is often paired with hydroxychloroquine.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs	Vaccine
Acute otitis media (AOM)	*Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis,* others	Earache, possible effusion; may cause fever, nausea, vomiting, diarrhea	Often a secondary infection; bacteria from respiratory tract become trapped in eustachian tube, cause infection	None	Cephalosporins, fluoroquinolones	None

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs	Vaccine
Diphtheria	*Corynebacterium diphtheria*	Pseudomembrane on throat, possibly leading to suffocation and death	Inhalation of respiratory droplets or aerosols from infected person	Identification of bacteria in throat swabs; PCR to detect diphtheria toxin in vitro	Erythromycin, penicillin, antitoxin produced in horses	DtaP, Tdap, DT, Td, DTP
Legionnaires disease	*Legionella pneumophila*	Cough, fever, muscle aches, headaches, nausea, vomiting, confusion; sometimes fatal	Inhalation of aerosols from contaminated water reservoirs	Isolation, using Warthin-Starry procedure, of bacteria in sputum	Fluoroquinolones, macrolides	None
Pertussis (whooping cough)	*Bordetella pertussis*	Severe coughing with "whoop" sound; chronic cough lasting several months; can be fatal in infants	Inhalation of respiratory droplets from infected person	Direct culture of throat swab, PCR, ELISA	Macrolides	DTaP, Tdap
Q fever	*Coxiella burnetii*	High fever, coughing, pneumonia, malaise; in chronic cases, potentially fatal endocarditis	Inhalation of aerosols of urine, feces, milk, or amniotic fluid of infected cattle, sheep, goats	PCR, ELISA	Doxycycline, hydroxychloroquine	None
Streptococcal pharyngitis, scarlet fever	*Streptococcus pyogenes*	Fever, sore throat, inflammation of pharynx and tonsils, petechiae, swollen lymph nodes; skin rash (scarlet fever), strawberry tongue	Direct contact, inhalation of respiratory droplets or aerosols from infected person	Direct culture of throat swab, rapid enzyme immunoassay	β-lactams	None
Tuberculosis	*Mycobacterium tuberculosis*	Formation of tubercles in lungs; rupture of tubercles, leading to chronic, bloody cough; healed tubercles (Ghon complexes) visible in radiographs; can be fatal	Inhalation of respiratory droplets or aerosols from infected person	Mantoux tuberculin skin test with chest radiograph to identify Ghon complexes	Isoniazid, rifampin, ethambutol, pyrazinamide	BCG

Table 5.2: Bacterial infections of the respiratory tract

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs	Vaccine
Chlamydial pneumonia	*Chlamydophila pneumoniae, C. psittaci, Chlamydia trachomatis*	Bronchitis; mild to severe respiratory distress	Inhalation of respiratory droplets or aerosols from infected person (*C. pneumoniae*); exposure to infected bird (*C. psittaci*); exposure in the birth canal (*Chlamydia trachomatis*)	Tissue culture, PCR	Tetracycline, macrolides	None
Haemophilus pneumonia	*Haemophilus influenza*	Cough, fever or low body temperature, chills, chest pain, headache, fatigue	Inhalation of respiratory droplets or aerosols from infected person or asymptomatic carrier	Culture on chocolate agar, serotyping of blood or cerebrospinal fluid samples	Cephalosporins, fluoroquinolones	Hib
Klebsiella pneumonia	*Klebsiella pneumoniae*, others	Lung necrosis, "currant jelly" sputum; often fatal	Health care associated; bacteria introduced via contaminated ventilators, intubation, or other medical equipment	Culture, PCR	Multidrug resistant; antibiotic susceptibility testing necessary	None
Mycoplasma pneumonia (walking pneumonia)	*Mycoplasma pneumoniae*	Low fever, persistent cough	Inhalation of respiratory droplets or aerosols from infected person	Culture with penicillin, thallium acetate	Macrolides	None
Pneumococcal pneumonia	*Streptococcus pneumoniae*	Productive cough, bloody sputum, fever, chills, chest pain, respiratory distress	Direct contact with respiratory secretions	Gram stain, blood agar culture with optichin and sodium deoxycholate, quellung reaction	β-lactams, macrolides, fluoroquinolones	Pneumococcal conjugate vaccine (PCV13), pneumococcal polysaccharide vaccine (PPSV23)
Pseudomonas pneumonia	*Pseudomonas aeruginosa*	Viscous fluid and chronic inflammation of lungs; often fatal	Health care associated; bacteria introduced via contaminated ventilators; also frequently affects patients with cystic fibrosis	Culture from sputum or other body fluid	Multidrug resistant; antibiotic susceptibility testing necessary	None

Table 5.3: Bacterial causes of pneumonia

5.3 VIRAL INFECTIONS OF THE RESPIRATORY TRACT

Viruses are the most frequent cause of respiratory tract infections. Unlike the bacterial pathogens, we have few effective therapies to combat viral respiratory infections. Fortunately, many of these diseases are mild and self-limiting. A few respiratory infections manifest their primary symptoms at other locations in the body. Viral infections of the respiratory tract are summarized in table 5.6.

THE COMMON COLD

The common cold is a generic term for a variety of mild viral infections of the nasal cavity. More than 200 different viruses are known to cause the common cold. The most common groups of cold viruses include rhinoviruses, coronaviruses, and adenoviruses. These infections are widely disseminated in the human population and are transmitted through direct contact and droplet transmission. Coughing and sneezing efficiently produce infectious aerosols, and rhinoviruses are known to persist on environmental surfaces for up to a week.[14]

Viral contact with the nasal mucosa or eyes can lead to infection. Rhinoviruses tend to replicate best between 33 °C (91.4 °F) and 35 °C (95 °F), somewhat below normal body temperature (37 °C [98.6 °F]). As a consequence, they tend to infect the cooler tissues of the nasal cavities. Colds are marked by an irritation of the mucosa that leads to an inflammatory response. This produces common signs and symptoms such as excess nasal secretions (runny nose), congestion, sore throat, coughing, and sneezing. The absence of high fever is typically used to differentiate common colds from other viral infections, like influenza. Some colds may progress to cause otitis media, pharyngitis, or laryngitis, and patients may also experience headaches and body aches. The disease, however, is self-limiting and typically resolves within 1–2 weeks.

There are no effective antiviral treatments for the common cold and antibacterial drugs should not be prescribed unless secondary bacterial infections have been established. Many of the viruses that cause colds are related, so immunity develops throughout life. Given the number of viruses that cause colds, however, individuals are never likely to develop immunity to all causes of the common cold.

INFLUENZA

Commonly known as the flu, influenza is a common viral disease caused by an orthomyxovirus that primarily affects the upper respiratory tract but can also extend into the lower respiratory tract. Influenza is pervasive worldwide and causes 3,000–50,000 deaths each year in the United States. The annual mortality rate can vary greatly depending on the virulence of the strain(s) responsible for seasonal epidemics.[15]

Influenza infections are most typically characterized by fever, chills, and body aches. This is followed by symptoms similar to the common cold that may last a week or more. Table 5.4 compares the signs and symptoms of influenza and the common cold.

Sign/Symptom	Common Cold	Influenza
Fever	Low (37.2 °C [99 °F])	High (39 °C [102.2 °F])
Headache	Common	Common
Aches and pains	Mild	Severe
Fatigue	Slight	Severe
Nasal congestion	Common	Rare

Sign/Symptom	Common Cold	Influenza
Sneezing	Common	Rare

Table 5.4: Comparing the common cold and influenza

In general, influenza is self-limiting. However, serious cases can lead to pneumonia and other complications that can be fatal. Such cases are more common in the very young and the elderly; however, certain strains of influenza virus (like the 1918–1919 variant discussed below) are more lethal to young adults than to the very young or old. Strains that affect young adults are believed to involve a cytokine storm—a positive feedback loop that forms between cytokine production and leukocytes. This cytokine storm produces an acute inflammatory response that leads to rapid fluid accumulation in the lungs, culminating in pulmonary failure. In such cases, the ability to mount a vigorous immune response is actually detrimental to the patient. The very young and very old are less susceptible to this effect because their immune systems are less robust.

A complication of influenza that occurs primarily in children and teenagers is Reye syndrome. This sequela causes swelling in the liver and brain, and may progress to neurological damage, coma, or death. Reye syndrome may follow other viral infections, like chickenpox, and has been associated with the use of aspirin. For this reason, the CDC and other agencies recommend that aspirin and products containing aspirin never be used to treat viral illnesses in children younger than age 19 years.[16]

The influenza virus is primarily transmitted by direct contact and inhalation of aerosols. The RNA genome of this virus exists as seven or eight segments, each coated with ribonucleoprotein and encoding one or two specific viral proteins. The influenza virus is surrounded by a lipid membrane envelope, and two of the main antigens of the influenza virus are the spike proteins hemagglutinin (H) and neuraminidase (N), as shown in figure 5.14. These spike proteins play important roles in the viral infectious cycle.

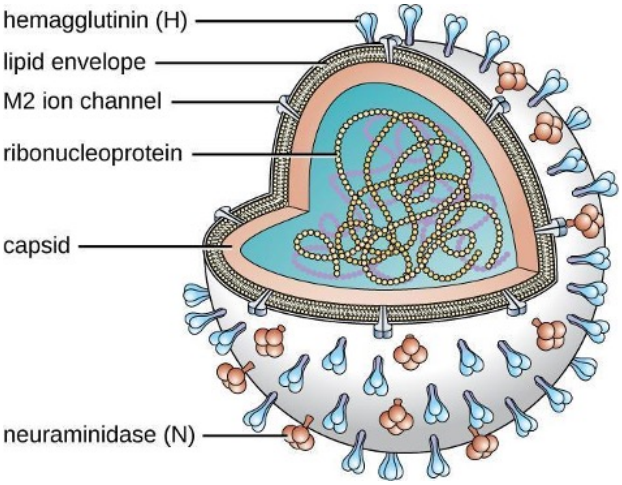


Figure 5.14: The illustration shows the structure of an influenza virus. The viral envelope is studded with copies of the proteins neuraminidase and hemagglutinin, and surrounds the individual seven or eight RNA genome segments. *Figure description available at the end of the chapter.*

Following inhalation, the influenza virus uses the hemagglutinin protein to bind to sialic acid receptors on host respiratory epithelial cells. This facilitates endocytosis of the viral particle. Once inside the host cell, the negative strand viral RNA is replicated by the viral RNA polymerase to form mRNA, which is translated by the host to produce viral proteins. Additional viral RNA molecules are transcribed to produce viral genomic RNA, which assemble with viral proteins to form mature virions. Release of the virions from the host cell is facilitated by viral neuraminidase, which cleaves sialic-acid receptors to allow progeny viruses to make a clean exit when budding from an infected cell.

There are three genetically related influenza viruses, called A, B, and C. The influenza A viruses have different subtypes based on the structure of their hemagglutinin and neuraminidase proteins. There are currently 18 known subtypes of hemagglutinin and 11 known subtypes of neuraminidase. Influenza viruses are serologically characterized by the type of H and N proteins that they possess. Of the nearly 200 different combinations of H and N, only a few, such as the H1N1 strain, are associated with human disease. The influenza viruses A, B, and C make up three of the five major groups of orthomyxoviruses. The differences between the three types of

influenza are summarized in table 5.5. The most virulent group is the influenza A viruses, which cause seasonal pandemics of influenza each year. Influenza A virus can infect a variety of animals, including pigs, horses, and even whales and dolphins. Influenza B virus is less virulent and is sometimes associated with epidemic outbreaks. Influenza C virus generally produces the mildest disease symptoms and is rarely connected with epidemics. Neither influenza B virus nor influenza C virus has significant animal reservoirs.

	Influenza A virus	Influenza B virus	Influenza C virus
Severity	Severe	Moderate	Mild
Animal reservoir	Yes	No	No
Genome segments	8	8	7
Population spread	Epidemic and pandemic	Epidemic	Sporadic
Antigenic variation	Shift/drift	Drift	Drift

Table 5.5: The three major groups of influenza viruses

Influenza virus infections elicit a strong immune response, particularly to the hemagglutinin protein, which would protect the individual if they encountered the same virus. Unfortunately, the antigenic properties of the virus change relatively rapidly, so new strains are evolving that immune systems previously challenged by influenza virus cannot recognize. When an influenza virus gains a new hemagglutinin or neuraminidase type, it is able to evade the host's immune response and be successfully transmitted, often leading to an epidemic.

There are two mechanisms by which these evolutionary changes may occur. The mechanisms of antigen drift and antigenic shift for influenza virus have been described in section 2.14. Of these two genetic processes, it is viruses produced by antigenic shift that have the potential to be extremely virulent because individuals previously infected by other strains are unlikely to produce any protective immune response against these novel variants.

The most lethal influenza pandemic in recorded history occurred from 1918 through 1919. Near the end of World War I, an antigenic shift involving the recombination of avian and human viruses is thought to have produced a new H1N1 virus. This strain rapidly spread worldwide and is commonly claimed to have killed as many as 40 million to 50 million people—more than double the number killed in the war. Although referred to as the Spanish flu, this disease is thought to have originated in the United States. Regardless of its source, the conditions of World War I greatly contributed to the spread of this disease. Crowding, poor sanitation, and rapid mobilization of large numbers of personnel and animals facilitated the dissemination of the new virus once it appeared.

Laboratory diagnosis of influenza is typically performed using a variety of RIDTs. These tests are inoculated by point-of-care personnel and give results within 15–20 minutes. Unfortunately, these tests have variable sensitivity and commonly yield false-negative results. Other tests include hemagglutination of erythrocytes (due to hemagglutinin action) or complement fixation. Patient serum antibodies against influenza viruses can also be detected in blood samples. Because influenza is a self-limiting disease, diagnosis through these more time-consuming and expensive methods is not typically used.

Three drugs that inhibit influenza neuraminidase activity are available: inhaled zanamivir, oral oseltamivir, and intravenous peramivir. If taken at the onset of symptoms, these drugs can shorten the course of the disease. These drugs are thought to impair the ability of the virus to efficiently exit infected host cells. A more effective means of controlling influenza outbreaks, though, is vaccination. Every year, new influenza vaccines are developed to be effective against the strains expected to be predominant. This is determined in February by a review

of the dominant strains around the world from a network of reporting sites; their reports are used to generate a recommendation for the vaccine combination for the following winter in the northern hemisphere. In September, a similar recommendation is made for the winter season in the southern hemisphere.[17] These recommendations are used by vaccine manufacturers to formulate each year's vaccine. In most cases, three or four viruses are selected—the two most prevalent influenza A strains and one or two influenza B strains. The chosen strains are typically cultivated in eggs and used to produce either an inactivated or a live attenuated vaccine (e.g., FluMist). For individuals 18 years or older with an allergy to egg products, a recombinant egg-free trivalent vaccine is available. Most of the influenza vaccines over the past decade have had an effectiveness of about 50%.[18]

VIRAL PNEUMONIA

Viruses cause fewer cases of pneumonia than bacteria; however, several viruses can lead to pneumonia in children and the elderly. The most common sources of viral pneumonia are adenoviruses, influenza viruses, parainfluenza viruses, and respiratory syncytial viruses. The signs and symptoms produced by these viruses can range from mild cold-like symptoms to severe cases of pneumonia, depending on the virulence of the virus strain and the strength of the host defenses of the infected individual. Occasionally, infections can result in otitis media.

Respiratory syncytial virus (RSV) infections are fairly common in infants; most people have been infected by the age of 2 years. During infection, a viral surface protein causes host cells to fuse and form multinucleated giant cells called syncytia. There are no specific antiviral therapies or vaccines available for viral pneumonia. In adults, these infections are self-limiting, resemble the common cold, and tend to resolve uneventfully within 1 or 2 weeks. Infections in infants, however, can be life-threatening. RSV is highly contagious and can be spread through respiratory droplets from coughing and sneezing. RSV can also survive for a long time on environmental surfaces and, thus, be transmitted indirectly via fomites.

SARS AND MERS

Severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) are two acute respiratory infections caused by coronaviruses. In both cases, these are thought to be zoonotic infections. Bats and civet cats are thought to have been the reservoirs for SARS; camels seem to be the reservoir for MERS.

SARS originated in southern China in the winter of 2002 and rapidly spread to 37 countries. Within about 1 year, more than 8,000 people experienced influenza-like symptoms and nearly 800 people died. The rapid spread and severity of these infections caused grave concern at the time. However, the outbreak was controlled in 2003 and no further cases of SARS have been recorded since 2004.[19] Signs and symptoms of SARS include high fever, headache, body aches, and cough, and most patients will develop pneumonia.

MERS was first reported in Saudi Arabia in 2013. Although some infected individuals will be asymptomatic or have mild cold-like symptoms, most will develop a high fever, aches, cough and a severe respiratory infection that can progress to pneumonia. As of 2015, over 1,300 people in 27 countries have been infected. About 500 people have died. There are no specific treatments for either MERS or SARS. In addition, no vaccines are currently available. Several recombinant vaccines, however, are being developed.

VIRAL RESPIRATORY DISEASES CAUSING SKIN RASHES

Measles, rubella (German measles), and chickenpox are three important viral diseases often associated with skin rashes. However, their symptoms are systemic, and because their portal of entry is the respiratory tract, they can be considered respiratory infections.

Measles (Rubeola)

The measles virus (MeV) causes the highly contagious disease measles, also known as rubeola, which is a major cause of childhood mortality worldwide. Although vaccination efforts have greatly reduced the incidence of measles in much of the world, epidemics are still common in unvaccinated populations in certain countries.[20]

The measles virus is a single-stranded, negative-strand RNA virus and, like the influenza virus, it possesses an envelope with spikes of embedded hemagglutinin. The infection is spread by direct contact with infectious secretions or inhalation of airborne droplets spread by breathing, coughing, or sneezing. Measles is initially characterized by a high fever, conjunctivitis, and a sore throat. The virus then moves systemically through the bloodstream and causes a characteristic rash. The measles rash initially forms on the face and later spreads to the extremities. The red, raised macular rash will eventually become confluent and can last for several days. At the same time, extremely high fevers (higher than 40.6 °C [105 °F]) can occur. Another diagnostic sign of measles infections is Koplik's spots, white spots that form on the inner lining of inflamed cheek tissues (figure 5.15).

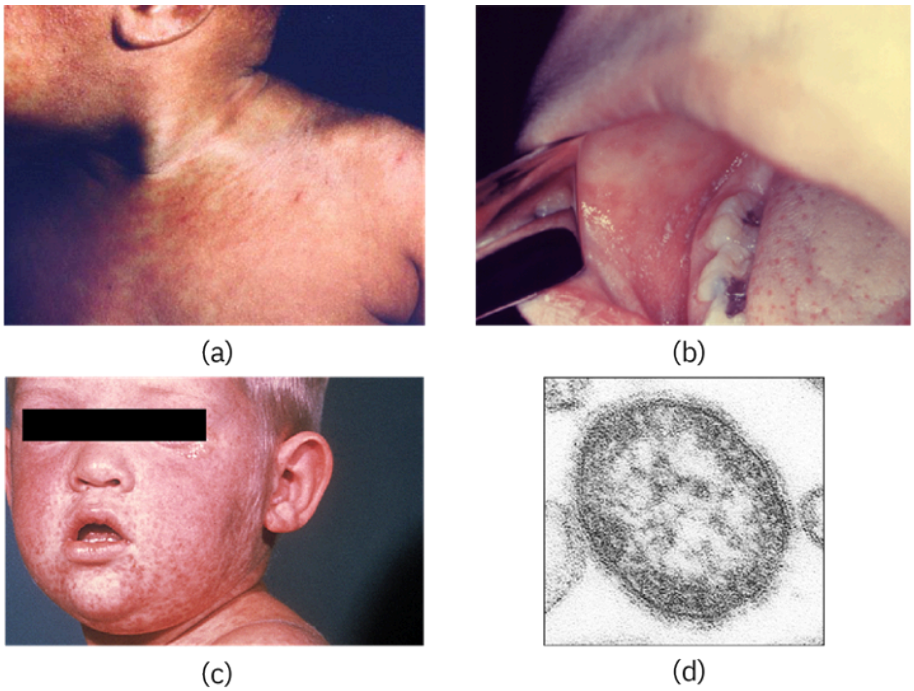


Figure 5.15: (a and b) Measles typically presents as a raised macular rash that begins on the face and spreads to the extremities. (c) Koplik's spots on the oral mucosa are also characteristic of measles. (d) A thin-section transmission electron micrograph of a measles virion. *Figure description available at the end of the chapter.*

Although measles is usually self-limiting, it can lead to pneumonia, encephalitis, and death. In addition, the inhibition of immune system cells by the measles virus predisposes patients to secondary infections. In severe infections with highly virulent strains, measles fatality rates can be as high as 10% to 15%. There were more than 145,000 measles deaths (mostly young children) worldwide in 2013.[21]

The preliminary diagnosis of measles is typically based on the appearance of the rash and Koplik's spots. Hemagglutination inhibition tests and serological tests may be used to confirm measles infections in low-prevalence settings.

There are no effective treatments for measles. Vaccination is widespread in developed countries as part of the measles, mumps, and rubella (MMR) vaccine. As a result, there are typically fewer than 200 cases of measles in the United States annually.[22] When it is seen, it is often associated with children who have not been vaccinated.

Rubella (German Measles)

Rubella, or the German measles, is a relatively mild viral disease that produces a rash somewhat like that caused by the measles, even though the two diseases are unrelated. The rubella virus is an enveloped RNA virus that can be found in the respiratory tract. It is transmitted from person to person in aerosols produced by coughing or sneezing. Nearly half of all infected people remain asymptomatic. However, the virus is shed and spread by asymptomatic carriers. Like rubeola, rubella begins with a facial rash that spreads to the extremities (figure 5.16). However, the rash is less intense, shorter lived (2–3 days), not associated with Koplik's spots, and the resulting fever is lower (101 °F [38.3 °C]).

Congenital rubella syndrome is the most severe clinical complication of the German measles. This occurs if a woman is infected with rubella during pregnancy. The rubella virus is teratogenic, meaning it can cause developmental defects if it crosses the placenta during pregnancy. There is a very high incidence of stillbirth, spontaneous abortion, or congenital birth defects if the mother is infected before 11 weeks of pregnancy and 35% if she is infected between weeks 13–16; after this time the incidence is low.[23] For this reason, prenatal screening for rubella is commonly practiced in the United States. Postnatal infections are usually self-limiting and rarely cause severe complications.

Like measles, the preliminary diagnosis of rubella is based on the patient's history, vaccination records, and the appearance of the rash. The diagnosis can be confirmed by hemagglutinin inhibition assays and a variety of other immunological techniques. There are no antiviral therapies for rubella, but an effective vaccine (MMR) is widely available. Vaccination efforts have essentially eliminated rubella in the United States; fewer than a dozen cases are reported in a typical year.

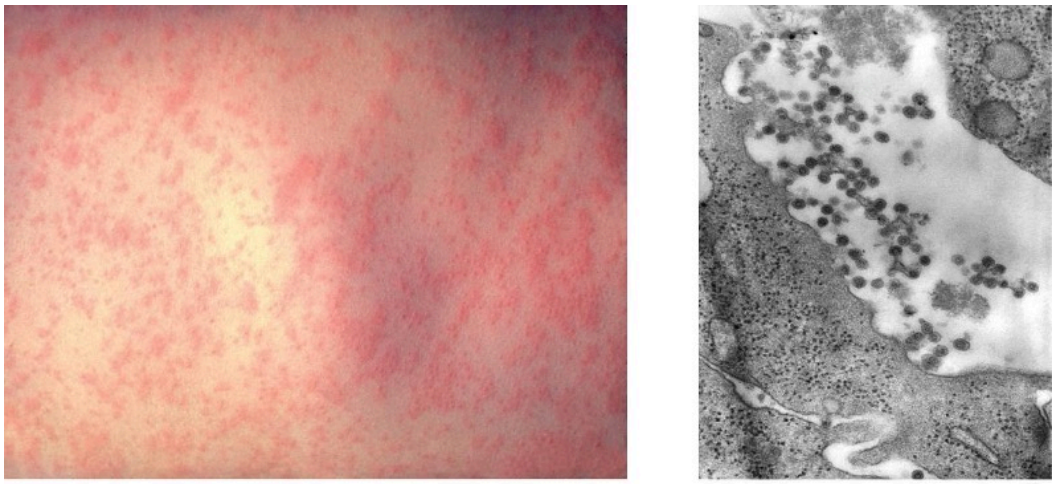


Figure 5.16: (a) This photograph shows the appearance of the German measles (rubella) rash. Note that this is less intense than the rash of measles and the lesions are not confluent. (b) This transmission electron micrograph shows rubella virus virions just budding from a host cell. *Figure description available at the end of the chapter.*

Chickenpox and Shingles

Chickenpox, also known as varicella, was once a common viral childhood disease. The causative agent of chickenpox, the varicella-zoster virus, is a member of the herpesvirus family. In children, the disease is mild and self-limiting, and is easily transmitted by direct contact or inhalation of material from the skin lesions. In adults, however, chickenpox infections can be much more severe and can lead to pneumonia and birth defects in the case of infected pregnant women. Reye syndrome, mentioned earlier in this chapter, is also a serious complication associated with chickenpox, generally in children.

Once infected, most individuals acquire a lifetime immunity to future chickenpox outbreaks. For this reason, parents once held "chickenpox parties" for their children. At these events, uninfected children were intentionally exposed to an infected individual so they would contract the disease earlier in life, when the incidence of complications is very low, rather than risk a more severe infection later.

After the initial viral exposure, chickenpox has an incubation period of about 2 weeks. The initial infection of the respiratory tract leads to viremia and eventually produces fever and chills. A pustular rash then develops on the face, progresses to the trunk, and then the extremities, although most form on the trunk (figure 5.17). Eventually, the lesions burst and form a crusty scab. Individuals with chickenpox are infectious from about 2 days before the outbreak of the rash until all the lesions have scabbed over.

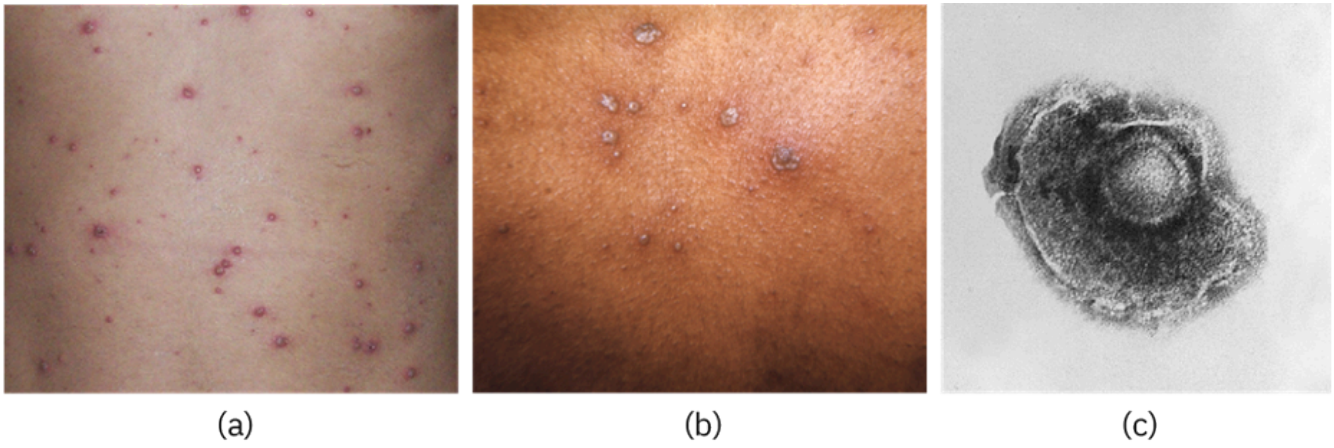


Figure 5.17: (a) and (b) The characteristic appearance of the pustular chickenpox rash is concentrated on the trunk region. (c) This transmission electron micrograph shows a viroid of human herpesvirus 3, the virus that causes chickenpox in children and shingles when it is reactivated in adults. *Figure description available at the end of the chapter.*

Like other herpesviruses, the varicella-zoster virus can become dormant in nerve cells. While the pustular vesicles are developing, the virus moves along sensory nerves to the dorsal ganglia in the spinal cord. Once there, the varicella-zoster virus can remain latent for decades. These dormant viruses may be reactivated later in life by a variety of stimuli, including stress, aging, and immunosuppression. Once reactivated, the virus moves along sensory nerves to the skin of the face or trunk. This results in the production of the painful lesions in a condition known as shingles (figure 5.18). These symptoms generally last for 2–6 weeks, and may recur more than once. Postherpetic neuralgia, pain signals sent from damaged nerves long after the other symptoms have subsided, is also possible. In addition, the virus can spread to other organs in immunocompromised individuals. A person with shingles lesions can transmit the virus to a nonimmune contact, and the newly infected individual would develop chickenpox as the primary infection. Shingles cannot be transmitted from one person to another.

The primary diagnosis of chickenpox in children is mainly based on the presentation of a pustular rash of the trunk. Serological and PCR-based tests are available to confirm the initial diagnosis. Treatment for chickenpox

infections in children is usually not required. In patients with shingles, acyclovir treatment can often reduce the severity and length of symptoms, and diminish the risk of postherpetic neuralgia. An effective vaccine is now available for chickenpox. A vaccine is also available for adults older than 60 years who were infected with chickenpox in their youth. This vaccine reduces the likelihood of a shingles outbreak by boosting the immune defenses that are keeping the latent infection in check and preventing reactivation.

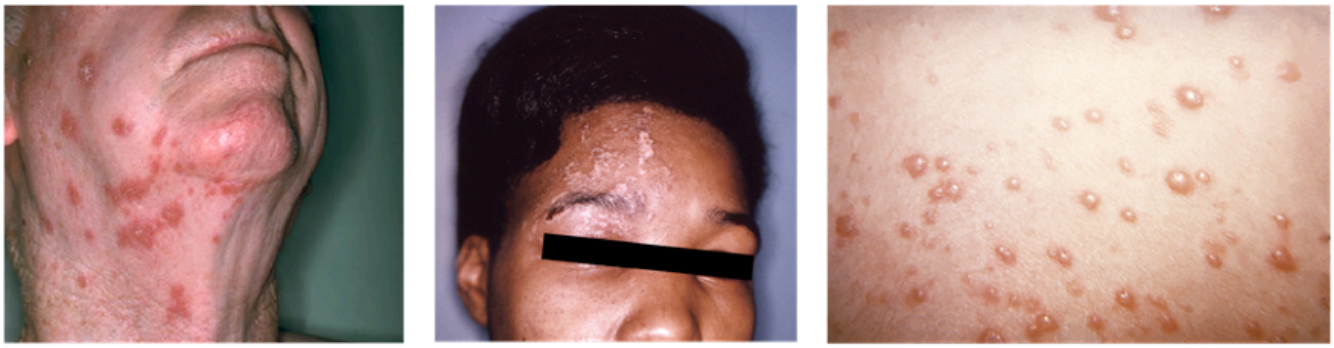


Figure 5.18: (a and b) An individual suffering from shingles. (c) The rash is formed because of the reactivation of a varicella-zoster infection that was initially contracted in childhood. *Figure description available at the end of the chapter.*

Disease	Pathogen	Signs and Symptoms	Transmission	Vaccine
Chickenpox (varicella)	Varicella-zoster virus	In children, fever, chills, pustular rash of lesions that burst and form crusty scabs; in adults, more severe symptoms and complications (e.g., pneumonia)	Highly contagious via contact with aerosols, particles, or droplets from infected individual's blisters or respiratory secretions	Chickenpox vaccine.
Common cold	Rhinoviruses, adenoviruses, coronaviruses, others	Runny nose, congestion, sore throat, sneezing, headaches and muscle aches; may lead to otitis media, pharyngitis, laryngitis	Highly contagious via contact with respiratory secretions or inhalation of droplets or aerosols	None
Influenza	Influenza viruses A, B, C	Fever, chills, headaches, body aches, fatigue; may lead to pneumonia or complications such as Reye syndrome. Highly virulent strains may cause lethal complications	Highly contagious between humans via contact with respiratory secretions or inhalation of droplets or aerosols. Influenza A virus can be transmitted from animal reservoirs	Vaccines developed yearly against most prevalent strains
Measles	Measles virus (MeV)	High fever, conjunctivitis, sore throat, macular rash becoming confluent, Koplik's spots on oral mucosa; in severe cases, can lead to fatal pneumonia or encephalitis, especially in children	Highly contagious via contact with respiratory secretions, skin rash, or eye secretions of infected individual	MMR

Disease	Pathogen	Signs and Symptoms	Transmission	Vaccine
MERS	Middle East respiratory syndrome coronavirus (MERS-CoV)	Fever, cough, shortness of breath; in some cases, complications such as pneumonia and kidney failure; can be fatal	Contact with respiratory secretions or inhalation of droplets or aerosols	None
Rubella (German measles)	Rubella virus	Facial rash spreading to extremities, followed by low-grade fever, headache, conjunctivitis, cough, runny nose, swollen lymph nodes; congenital rubella may cause birth defects, miscarriage, or stillbirth	Contagious via inhalation of droplets or aerosols from infected person or asymptomatic carrier; ransplacental infection from a pregnant person to fetus	MMR
SARS	SARS-associated coronavrius (SARS-CoV)	High fever, headache, body aches, dry cough, pneumonia; can be fatal	Contact with respiratory secretions or inhalation of droplets or aerosols	None
Shingles	Varicella-zoster virus	Painful lesions on face or trunk lasting several weeks; may cause postherpetic neuralgia (chronic pain) or spread to organs in severe cases	Nontransmissible; occurs when dormant virus is reactivated, generally many years after initial chickenpox infection	Shingles vaccine
Viral pneumonia	Adenoviruses, influenza viruses, parainfluenza viruses, respiratory syncytial viruses, others	From mild cold-like symptoms to severe pneumonia; in infants, RSV infections may be life-threatening	Highly contagious via contact with respiratory secretions or inhalation of droplets or aerosols	None

Table 5.6: Viral infections of the respiratory tract

5.4 RESPIRATORY MYCOSES

Fungal pathogens are ubiquitous in the environment. Serological studies have demonstrated that most people have been exposed to fungal respiratory pathogens during their lives. Yet symptomatic infections by these microbes are rare in healthy individuals. This demonstrates the efficacy of the defenses of our respiratory system. In this section, we will examine some of the fungi that can cause respiratory infections (summarized in table 5.7).

HISTOPLASMOSIS

Histoplasmosis is a fungal disease of the respiratory system and most commonly occurs in the Mississippi Valley of the United States and in parts of Central and South America, Africa, Asia, and Australia. The causative agent, *Histoplasma capsulatum*, is a dimorphic fungus. This microbe grows as a filamentous mold in the environment but occurs as a budding yeast during human infections. The primary reservoir for this pathogen is soil, particularly in locations rich in bat or bird feces.

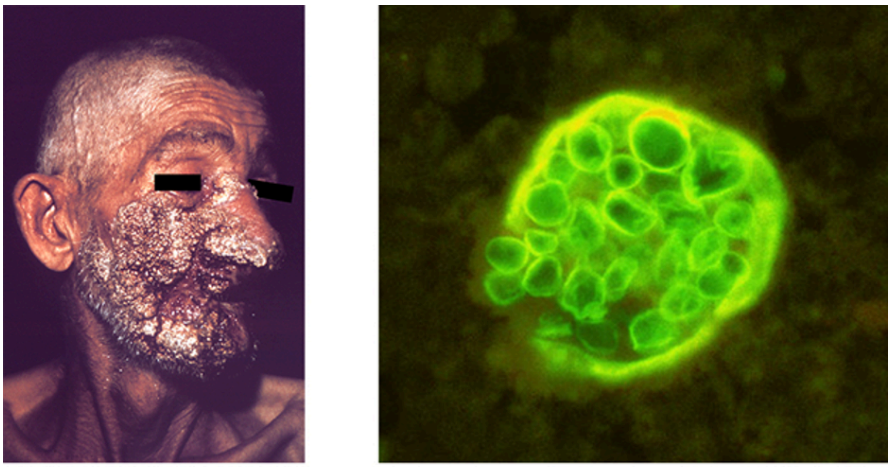
Histoplasmosis is acquired by inhaling microconidia spores in the air; this disease is not transmitted from human to human. The incidence of histoplasmosis exposure is high in endemic areas, with 60%–90% of the population having anti-Histoplasma antibodies, depending on location;[24] however, relatively few individuals exposed to the fungus actually experience symptoms. Those most likely to be affected are the very young, the elderly, and immunocompromised people.

In many ways, the course of this disease is similar to that of tuberculosis. Following inhalation, the spores enter the lungs and are phagocytized by alveolar macrophages. The fungal cells then survive and multiply within these phagocytes (see figure 2.62). Focal infections cause the formation of granulomatous lesions, which can lead to calcifications that resemble the Ghon complexes of tuberculosis, even in asymptomatic cases. Also like tuberculosis, histoplasmosis can become chronic and reactivation can occur, along with dissemination to other areas of the body (e.g., the liver or spleen).

Signs and symptoms of pulmonary histoplasmosis include fever, headache, and weakness with some chest discomfort. The initial diagnosis is often based on chest radiographs and cultures grown on fungal selective media like Sabouraud's dextrose agar. Direct fluorescent antibody staining and Giemsa staining can also be used to detect this pathogen. In addition, serological tests including a complement fixation assay and histoplasmin sensitivity can be used to confirm the diagnosis. In most cases, these infections are self-limiting and antifungal therapy is not required. However, in disseminated disease, the antifungal agents amphotericin B and ketoconazole are effective; itraconazole may be effective in immunocompromised patients, in whom the disease can be more serious.

COCCIDIOIDOMYCOSIS

Infection by the dimorphic fungus Coccidioides immitis causes coccidioidomycosis. Because the microbe is endemic to the San Joaquin Valley of California, the disease is sometimes referred to as Valley fever. A related species that causes similar infections is found in semi-arid and arid regions of the southwestern United States, Mexico, and Central and South America.[25]



(a) (b)

Figure 5.19: (a) This patient has extensive facial lesions due to a disseminated Coccidioides infection. (b) This fluorescent micrograph depicts a spherule of C. immitis containing endospores. *Figure description available at the end of the chapter.*

Like histoplasmosis, coccidioidomycosis is acquired by inhaling fungal spores—in this case, arthrospores formed by hyphal fragmentation. Once in the body, the fungus differentiates into spherules that are filled with endospores. Most *C. immitis* infections are asymptomatic and self-limiting. However, the infection can be very serious for immunocompromised patients. The endospores may be transported in the blood, disseminating the infection and leading to the formation of granulomatous lesions on the face and nose (figure 5.19). In severe cases, other major organs can become infected, leading to serious complications such as fatal meningitis.

Coccidioidomycosis can be diagnosed by culturing clinical samples. *C. immitis* readily grows on laboratory fungal media, such as Sabouraud's dextrose agar, at 35 °C (95 °F). Culturing the fungus, however, is rather dangerous. *C. immitis* is one of the most infectious fungal pathogens known and is capable of causing laboratory-acquired infections. Indeed, until 2012, this organism was considered a "select agent" of bioterrorism and classified as a BSL-3 microbe. Serological tests for antibody production are more often used for diagnosis. Although mild cases generally do not require intervention, disseminated infections can be treated with intravenous antifungal drugs like amphotericin B.

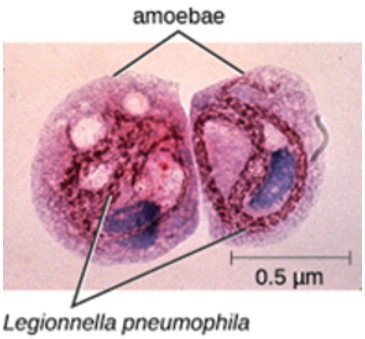


Figure 5.20: Legionella pneumophila (red intracellular rods) infecting amoebae from a contaminated water sample. Figure description available at the end of the chapter.

BLASTOMYCOSIS

Blastomycosis is a rare disease caused by another dimorphic fungus, *Blastomyces dermatitidis*. Like Histoplasma and Coccidioides, Blastomyces uses the soil as a reservoir, and fungal spores can be inhaled from disturbed soil. The pulmonary form of blastomycosis generally causes mild flu-like symptoms and is self-limiting. It can, however, become disseminated in immunocompromised people, leading to chronic cutaneous disease with subcutaneous lesions on the face and hands (figure 5.21). These skin lesions eventually become crusty and discolored and can result in deforming scars. Systemic blastomycosis is rare, but if left untreated, it is always fatal.

Preliminary diagnosis of pulmonary blastomycosis can be made by observing the characteristic budding yeast forms in sputum samples. Commercially available urine antigen tests are now also available. Additional confirmatory tests include serological assays such as immunodiffusion tests or EIA. Most cases of blastomycosis respond well to amphotericin B or ketoconazole treatments.

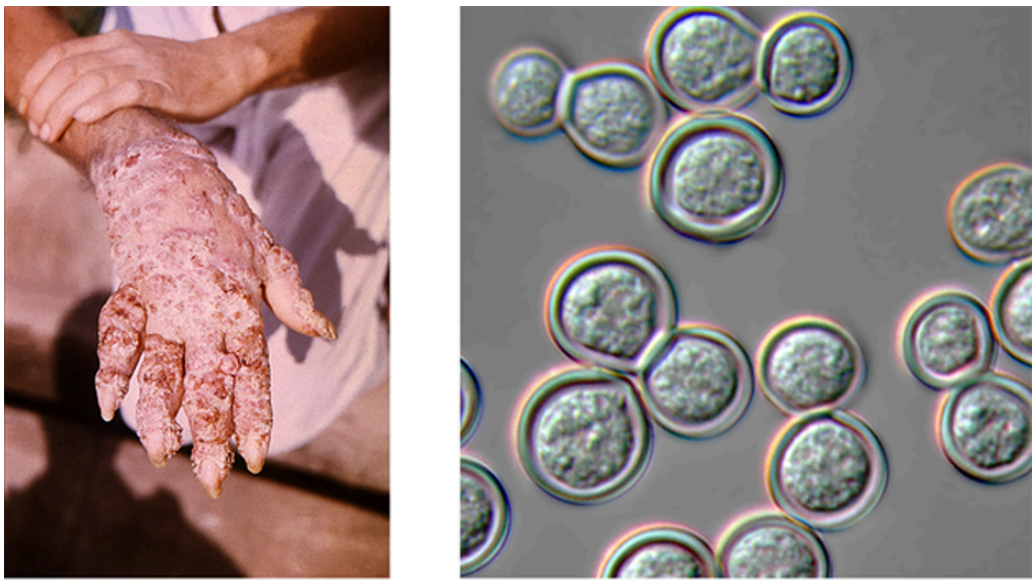


Figure 5.21: (a) These skin lesions are the result of disseminated cutaneous blastomycosis. (b) A differential interference contrast micrograph of B. dermatitidis yeast cultured on blood agar. Figure description available at the end of the chapter.

MUCORMYCOSIS

A variety of fungi in the order Mucorales cause mucormycosis, a rare fungal disease. These include bread molds, like Rhizopus and Mucor; the most commonly associated species is *Rhizopus arrhizus* (*oryzae*) (see figure 2.64). These fungi can colonize many different tissues in immunocompromised patients, but often infect the skin, sinuses, or the lungs.

Although most people are regularly exposed to the causative agents of mucormycosis, infections in healthy individuals are rare. Exposure to spores from the environment typically occurs through inhalation, but the spores can also infect the skin through a wound or the gastrointestinal tract if ingested. Respiratory mucormycosis primarily affects immunocompromised individuals, such as patients with cancer or those who have had a transplant.[26]

After the spores are inhaled, the fungi grow by extending hyphae into the host's tissues. Infections can occur in both the upper and lower respiratory tracts. Rhinocerebral mucormycosis is an infection of the sinuses and brain; symptoms include headache, fever, facial swelling, congestion, and tissue necrosis causing black lesions in the oral cavity. Pulmonary mucormycosis is an infection of the lungs; symptoms include fever, cough, chest pain, and shortness of breath. In severe cases, infections may become disseminated and involve the central nervous system, leading to coma and death.[27]

Diagnosing mucormycosis can be challenging. Currently, there are no serological or PCR-based tests available to identify these infections. Tissue biopsy specimens must be examined for the presence of the fungal pathogens. The causative agents, however, are often difficult to distinguish from other filamentous fungi. Infections are typically treated by the intravenous administration of amphotericin B, and superficial infections are removed by surgical debridement. Since the patients are often immunocompromised, viral and bacterial secondary infections commonly develop. Mortality rates vary depending on the site of the infection, the causative fungus, and other factors, but a recent study found an overall mortality rate of 54%.[28]

ASPERGILLOSIS

Aspergillus is a common filamentous fungus found in soils and organic debris. Nearly everyone has been exposed to this mold, yet very few people become sick. In immunocompromised patients, however, Aspergillus may become established and cause aspergillosis. Inhalation of spores can lead to asthma-like allergic reactions. The symptoms commonly include shortness of breath, wheezing, coughing, runny nose, and headaches. Fungal balls, or aspergilloma, can form when hyphal colonies collect in the lungs (figure 5.22). The fungal hyphae can invade the host tissues, leading to pulmonary hemorrhage and a bloody cough. In severe cases, the disease may progress to a disseminated form that is often fatal. Death most often results from pneumonia or brain hemorrhages.

Laboratory diagnosis typically requires chest radiographs and a microscopic examination of tissue and respiratory fluid samples. Serological tests are available to identify Aspergillus antigens. In addition, a skin test can be performed to determine if the patient has been exposed to the fungus. This test is similar to the Mantoux tuberculin skin test used for tuberculosis. Aspergillosis is treated with intravenous antifungal agents, including itraconazole and voriconazole. Allergic symptoms can be managed with corticosteroids because these drugs suppress the immune system and reduce inflammation. However, in disseminated infections, corticosteroids must be discontinued to allow a protective immune response to occur.

PNEUMOCYSTIS PNEUMONIA

A type of pneumonia called Pneumocystis pneumonia (PCP) is caused by *Pneumocystis jirovecii*. Once thought to be a protozoan, this organism was formerly named *P. carinii* but it has been reclassified as a fungus and renamed based on biochemical and genetic analyses. Pneumocystis is a leading cause of pneumonia in patients with acquired immunodeficiency syndrome (AIDS) and can be seen in other compromised patients and premature infants. Respiratory infection leads to fever, cough, and shortness of breath. Diagnosis of these infections can be difficult. The organism is typically identified by microscopic examination of tissue and fluid samples from the lungs (figure 5.23). A PCR-based test is available to detect *P. jirovecii* in asymptomatic patients with AIDS. The best treatment for these infections is the combination drug trimethoprim-sulfamethoxazole (TMP/SMZ). These sulfa drugs often have adverse effects, but the benefits outweigh these risks. Left untreated, PCP infections are often fatal.

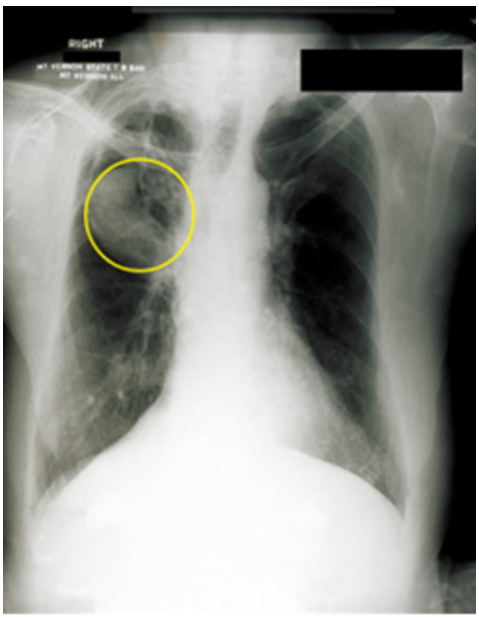


Figure 5.22: A fungal ball can be observed in the upper lobe of the right lung in this chest radiograph of a patient with aspergilloma. *Figure description available at the end of the chapter.*

CRYPTOCOCCOSIS

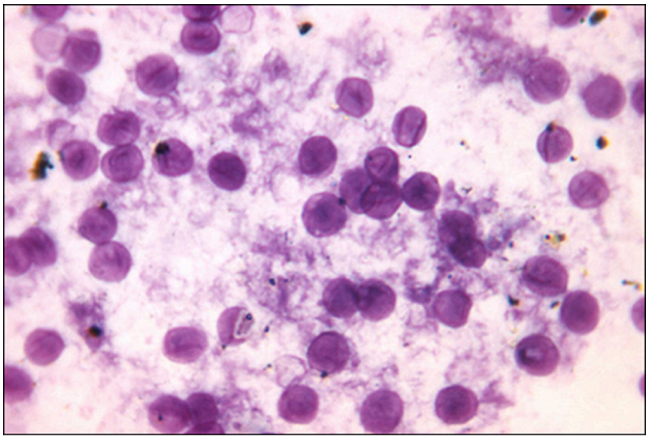


Figure 5.23: A light micrograph of a smear containing Pneumocystis jirovecii (dark purple cells) obtained from human lung tissue and stained with toluidine blue. *Figure description available at the end of the chapter.*

Infection by the encapsulated yeast *Cryptococcus neoformans* causes cryptococcosis. This fungus is ubiquitous in the soil and can be isolated from bird feces. Immunocompromised people are infected by inhaling basidiospores found in aerosols. The thick polysaccharide capsule surrounding these microbes enables them to avoid clearance by the alveolar macrophage. Initial symptoms of infection include fever, fatigue, and a dry cough. In immunocompromised patients, pulmonary infections often disseminate to the brain. The resulting meningitis produces headaches, sensitivity to light, and confusion. Left untreated, such infections are often fatal.

Cryptococcus infections are often diagnosed based on microscopic examination of lung tissues or cerebrospinal fluids. India ink preparations (figure 5.24) can be used to visualize the extensive capsules that surround the yeast cells. Serological tests are also available to confirm the diagnosis. Amphotericin B, in combination with flucytosine, is typically used for the initial treatment of pulmonary infections. Amphotericin B is a broad-spectrum antifungal drug that targets fungal cell membranes. It can also adversely impact host cells and produce side effects. For this reason, clinicians must carefully balance the risks and benefits of treatments in these patients. Because it is difficult to eradicate cryptococcal infections, patients usually need to take fluconazole for up to 6 months after treatment with amphotericin B and flucytosine to clear the fungus. Cryptococcal infections are more common in immunocompromised people, such as those with AIDS. These patients typically require lifelong suppressive therapy to control this fungal infection.

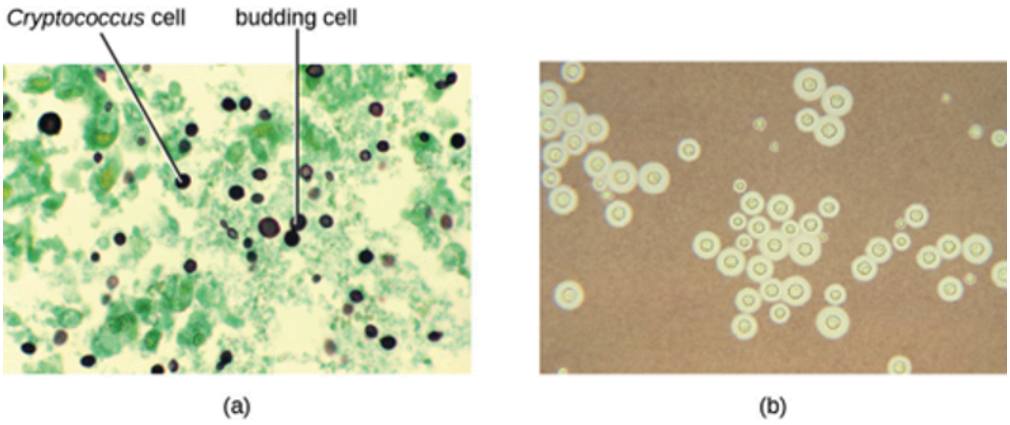


Figure 5.24: (a) The micrograph shows stained budding Cryptococcus yeast cells from the lungs of a patient with AIDS. (b) The large capsule of Cryptococcus neoformans is visible in this negative stain micrograph. *Figure description available at the end of the chapter.*

Disease	Pathogen	Signs and Symptoms	Diagnostic Tests	Antimicrobial Drugs
Aspergillosis	*Aspergillus fumigatus*	Shortness of breath, wheezing, coughing, runny nose, headaches; formation of aspergillomas causing severe pneumonia and pulmonary or brain hemorrhages; can be fatal	Chest radiograph, skin test, microscopic observation of sputum samples	Itraconazole, voriconazole
Blastomycosis	*Blastomyces dermatitidis*	Fever, chills, cough, headache, fatigue, chest pain, body aches; in disseminated infections, chronic, crusted lesions on face and hands with permanent scarring; can be fatal	Microscopic observation of sputum samples; urine antigen test; EIA	Amphotericin B, ketoconazole
Coccidioidomycosis (Valley fever)	*Coccidioides immitis*	Granulomatous lesions on face and nose; may spread to organs or brain, causing fatal meningitis	Culture (in BSL-3 lab only), serological antibody tests	Amphotericin B
Cryptococcosis	*Cryptococcus neoformans*	Fever, cough, shortness of breath; can cause fatal meningitis if disseminated to brain	Microscopic examination of lung tissue or cerebrospinal fluid	Amphotericin B, fluconazole, flucytosine
Histoplasmosis	*Histoplasma capsulatum*	Fever, headache, weakness, chest pain, lesions on lungs	Chest radiograph, culture, direct fluorescence antibody staining, complement fixation assay, histoplasmin sensitivity test	Amphotericin B, ketoconazole, itraconazole

Disease	Pathogen	Signs and Symptoms	Diagnostic Tests	Antimicrobial Drugs
Mucormycosis	*Rhizopus arrhizus*, other *Rhizopus* spp., *Mucor* spp.	Headache, fever, facial swelling, congestion, black lesions in oral cavity, cough, chest pain, shortness of breath; often fatal	Microscopic examination of tissue biopsy specimens	Amphotericin B
Pneumocystis pneumonia (PCP)	*Pneumocystis jirovecii*	Fever, cough, shortness of breath; can be fatal if untreated	Microscopic examination of lung tissue and fluid, PCR	Trimethoprim-sulfamethoxazole

Table 5.7: Fungal infections of the respiratory tract

SUMMARY

The following is a summary of the material covered throughout the chapter. It summarizes key aspects from each section and the pathogens included.

BACTERIAL INFECTIONS OF THE RESPIRATORY TRACT

- *Streptococcus pyogenes* causes **strep throat**, an infection of the pharynx that also causes high fever and can lead to **scarlet fever**, **acute rheumatic fever**, and **acute glomerulonephritis**.
- **Acute otitis media** is an infection of the middle ear that may be caused by several bacteria including, *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis*. The infection can block the eustachian tubes, leading to **otitis media with effusion**.
- **Diphtheria**, caused by *Corynebacterium diphtheriae*, is now a rare disease because of widespread vaccination. The bacteria produce exotoxins that kill cells in the pharynx, leading to the formation of a **pseudomembrane** and damage to other parts of the body.
- **Bacterial pneumonia** results from infections that cause inflammation and fluid accumulation in the alveoli. It is most commonly caused by *S. pneumoniae* or *H. influenzae*. The former is commonly multidrug-resistant.
- ***Mycoplasma* pneumonia** results from infection by *Mycoplasma pneumoniae*; it can spread quickly, but the disease is mild and self-limiting.
- **Chlamydial pneumonia** can be caused by three pathogens that are obligate intracellular parasites. *Chlamydophila pneumoniae* is typically transmitted from an infected person, whereas *C. psittaci* is typically transmitted from an infected bird. *Chlamydia trachomatis* may cause pneumonia in infants.
- Several other bacteria can cause pneumonia in immunocompromised individuals and those with cystic fibrosis.
- **Tuberculosis** is caused by *Mycobacterium tuberculosis*. Infection leads to the production of protective **tubercles** in the alveoli and calcified **Ghon complexes** that can harbor the bacteria for a long time. Antibiotic-resistant forms are common, and treatment is typically long term.
- **Pertussis** is caused by *Bordetella pertussis*. Mucus accumulation in the lungs leads to prolonged severe

coughing episodes (whooping cough) that facilitate transmission. Despite an available vaccine, outbreaks are still common.

- **Legionnaires disease** is caused by infection from environmental reservoirs of the *Legionella pneumophila* bacterium. The bacterium is endocytic within macrophages, and infection can lead to pneumonia, particularly among immunocompromised individuals.
- **Q fever** is caused by *Coxiella burnetii*, whose primary hosts are domesticated mammals (zoonotic disease). It causes pneumonia primarily in farm workers and can lead to serious complications, such as endocarditis.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs	Vaccine
Acute otitis media (AOM)	*Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis,* others	Earache, possible effusion; may cause fever, nausea, vomiting, diarrhea	Often a secondary infection; bacteria from respiratory tract become trapped in eustachian tube, cause infection	None	Cephalosporins, fluoroquinolones	None
Diphtheria	*Corynebacterium diphtheria*	Pseudomembrane on throat, possibly leading to suffocation and death	Inhalation of respiratory droplets or aerosols from infected person	Identification of bacteria in throat swabs; PCR to detect diphtheria toxin in vitro	Erythromycin, penicillin, antitoxin produced in horses	DtaP, Tdap, DT, Td, DTP
Legionnaires disease	*Legionella pneumophila*	Cough, fever, muscle aches, headaches, nausea, vomiting, confusion; sometimes fatal	Inhalation of aerosols from contaminated water reservoirs	Isolation, using Warthin-Starry procedure, of bacteria in sputum	Fluoroquinolones, macrolides	None
Pertussis (whooping cough)	*Bordetella pertussis*	Severe coughing with "whoop" sound; chronic cough lasting several months; can be fatal in infants	Inhalation of respiratory droplets from infected person	Direct culture of throat swab, PCR, ELISA	Macrolides	DTaP, Tdap

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs	Vaccine
Q fever	*Coxiella burnetii*	High fever, coughing, pneumonia, malaise; in chronic cases, potentially fatal endocarditis	Inhalation of aerosols of urine, feces, milk, or amniotic fluid of infected cattle, sheep, goats	PCR, ELISA	Doxycycline, hydroxychloroquine	None
Streptococcal pharyngitis, scarlet fever	*Streptococcus pyogenes*	Fever, sore throat, inflammation of pharynx and tonsils, petechiae, swollen lymph nodes; skin rash (scarlet fever), strawberry tongue	Direct contact, inhalation of respiratory droplets or aerosols from infected person	Direct culture of throat swab, rapid enzyme immunoassay	β-lactams	None
Tuberculosis	*Mycobacterium tuberculosis*	Formation of tubercles in lungs; rupture of tubercles, leading to chronic, bloody cough; healed tubercles (Ghon complexes) visible in radiographs; can be fatal	Inhalation of respiratory droplets or aerosols from infected person	Mantoux tuberculin skin test with chest radiograph to identify Ghon complexes	Isoniazid, rifampin, ethambutol, pyrazinamide	BCG

Table 5.8: Bacterial infections of the respiratory tract

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs	Vaccine
Chlamydial pneumonia	*Chlamydophila pneumoniae, C. psittaci, Chlamydia trachomatis*	Bronchitis; mild to severe respiratory distress	Inhalation of respiratory droplets or aerosols from infected person (*C. pneumoniae*); exposure to infected bird (*C. psittaci*); exposure in the birth canal (*Chlamydia trachomatis*)	Tissue culture, PCR	Tetracycline, macrolides	None
Haemophilus pneumonia	*Haemophilus influenza*	Cough, fever or low body temperature, chills, chest pain, headache, fatigue	Inhalation of respiratory droplets or aerosols from infected person or asymptomatic carrier	Culture on chocolate agar, serotyping of blood or cerebrospinal fluid samples	Cephalosporins, fluoroquinolones	Hib
Klebsiella pneumonia	*Klebsiella pneumoniae*, others	Lung necrosis, "currant jelly" sputum; often fatal	Health care associated; bacteria introduced via contaminated ventilators, intubation, or other medical equipment	Culture, PCR	Multidrug resistant; antibiotic susceptibility testing necessary	None
Mycoplasma pneumonia (walking pneumonia)	*Mycoplasma pneumoniae*	Low fever, persistent cough	Inhalation of respiratory droplets or aerosols from infected person	Culture with penicillin, thallium acetate	Macrolides	None
Pneumococcal pneumonia	*Streptococcus pneumoniae*	Productive cough, bloody sputum, fever, chills, chest pain, respiratory distress	Direct contact with respiratory secretions	Gram stain, blood agar culture with optichin and sodium deoxycholate, quellung reaction	β-lactams, macrolides, fluoroquinolones	Pneumococcal conjugate vaccine (PCV13), pneumococcal polysaccharide vaccine (PPSV23)

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs	Vaccine
Pseudomonas pneumonia	*Pseudomonas aeruginosa*	Viscous fluid and chronic inflammation of lungs; often fatal	Health care associated; bacteria introduced via contaminated ventilators; also frequently affects patients with cystic fibrosis	Culture from sputum or other body fluid	Multidrug resistant; antibiotic susceptibility testing necessary	None

Table 5.9: Bacterial causes of pneumonia

VIRAL INFECTIONS OF THE RESPIRATORY TRACT

- The **common cold** can be caused by more than 200 viruses, typically rhinoviruses, coronaviruses, and adenoviruses. They are transmitted by direct contact, aerosols, or via environmental surfaces.
- Due to its ability to rapidly mutate through **antigenic drift** and **antigenic shift**, **influenza** remains an important threat to human health. Two new influenza vaccines are developed annually.
- Several viral infections, including **respiratory syncytial virus** infections, which frequently occur in the very young, can begin with mild symptoms before progressing to viral pneumonia.
- **SARS** and **MERS** are acute respiratory infections caused by coronaviruses, and both appear to originate in animals. SARS has not been seen in the human population since 2004 but had a high mortality rate during its outbreak. MERS also has a high mortality rate and continues to appear in human populations.
- **Measles**, **rubella**, and **chickenpox** are highly contagious, systemic infections that gain entry through the respiratory system and cause rashes and fevers. Vaccines are available for all three. Measles is the most severe of the three and is responsible for significant mortality around the world. Chickenpox typically causes mild infections in children, but the virus can reactivate to cause painful cases of **shingles** later in life.

Disease	Pathogen	Signs and Symptoms	Transmission	Vaccine
Chickenpox (varicella)	Varicella-zoster virus	In children, fever, chills, pustular rash of lesions that burst and form crusty scabs; in adults, more severe symptoms and complications (e.g., pneumonia)	Highly contagious via contact with aerosols, particles, or droplets from infected individual's blisters or respiratory secretions	Chickenpox vaccine.

Disease	Pathogen	Signs and Symptoms	Transmission	Vaccine
Common cold	Rhinoviruses, adenoviruses, coronaviruses, others	Runny nose, congestion, sore throat, sneezing, headaches and muscle aches; may lead to otitis media, pharyngitis, laryngitis	Highly contagious via contact with respiratory secretions or inhalation of droplets or aerosols	None
Influenza	Influenza viruses A, B, C	Fever, chills, headaches, body aches, fatigue; may lead to pneumonia or complications such as Reye syndrome. Highly virulent strains may cause lethal complications	Highly contagious between humans via contact with respiratory secretions or inhalation of droplets or aerosols. Influenza A virus can be transmitted from animal reservoirs	Vaccines developed yearly against most prevalent strains
Measles	Measles virus (MeV)	High fever, conjunctivitis, sore throat, macular rash becoming confluent, Koplik's spots on oral mucosa; in severe cases, can lead to fatal pneumonia or encephalitis, especially in children	Highly contagious via contact with respiratory secretions, skin rash, or eye secretions of infected individual	MMR
MERS	Middle East respiratory syndrome coronavirus (MERS-CoV)	Fever, cough, shortness of breath; in some cases, complications such as pneumonia and kidney failure; can be fatal	Contact with respiratory secretions or inhalation of droplets or aerosols	None

Disease	Pathogen	Signs and Symptoms	Transmission	Vaccine
Rubella (German measles)	Rubella virus	Facial rash spreading to extremities, followed by low-grade fever, headache, conjunctivitis, cough, runny nose, swollen lymph nodes; congenital rubella may cause birth defects, miscarriage, or stillbirth	Contagious via inhalation of droplets or aerosols from infected person or asymptomatic carrier; ransplacental infection from a pregnant person to fetus	MMR
SARS	SARS-associated coronavrius (SARS-CoV)	High fever, headache, body aches, dry cough, pneumonia; can be fatal	Contact with respiratory secretions or inhalation of droplets or aerosols	None
Shingles	Varicella-zoster virus	Painful lesions on face or trunk lasting several weeks; may cause postherpetic neuralgia (chronic pain) or spread to organs in severe cases	Nontransmissible; occurs when dormant virus is reactivated, generally many years after initial chickenpox infection	Shingles vaccine
Viral pneumonia	Adenoviruses, influenza viruses, parainfluenza viruses, respiratory syncytial viruses, others	From mild cold-like symptoms to severe pneumonia; in infants, RSV infections may be life-threatening	Highly contagious via contact with respiratory secretions or inhalation of droplets or aerosols	None

Table 5.10: Viral infections of the respiratory tract

RESPIRATORY MYCOSES

- Antifungal drugs like amphotericin B can control most fungal respiratory infections.
- **Histoplasmosis** is caused by a mold that grows in soil rich in bird or bat droppings. Few exposed individuals become sick, but vulnerable individuals are susceptible. The yeast-like infectious cells grow inside phagocytes.
- **Coccidioidomycosis** is also acquired from soil and, in some individuals, will cause lesions on the face. Extreme cases may infect other organs, causing death.
- **Blastomycosis**, a rare disease caused by a soil fungus, typically producing a mild lung infection but can become disseminated in the immunocompromised. Systemic cases are fatal if untreated.

- **Mucormycosis** is a rare disease caused by fungi of the order *Mucorales*. It primarily affects immunocompromised people. Infection involves growth of the hyphae into infected tissues and can lead to death in some cases.
- **Aspergillosis** is caused by the common soil fungus *Aspergillus* and infects immunocompromised people. Hyphal balls may impede lung function and hyphal growth into tissues can cause damage. Disseminated forms can lead to death.
- ***Pneumocystis* pneumonia** is caused by the fungus *P. jirovecii*. The disease is found in patients with AIDS and other immunocompromised individuals. Sulfa drug treatments have side effects, but untreated cases may be fatal.
- **Cryptococcosis** is caused by *Cryptococcus neoformans*. Lung infections may move to the brain, causing meningitis, which can be fatal.

Disease	Pathogen	Signs and Symptoms	Diagnostic Tests	Antimicrobial Drugs
Aspergillosis	*Aspergillus fumigatus*	Shortness of breath, wheezing, coughing, runny nose, headaches; formation of aspergillomas causing severe pneumonia and pulmonary or brain hemorrhages; can be fatal	Chest radiograph, skin test, microscopic observation of sputum samples	Itraconazole, voriconazole
Blastomycosis	*Blastomyces dermatitidis*	Fever, chills, cough, headache, fatigue, chest pain, body aches; in disseminated infections, chronic, crusted lesions on face and hands with permanent scarring; can be fatal	Microscopic observation of sputum samples; urine antigen test; EIA	Amphotericin B, ketoconazole
Coccidioidomycosis (Valley fever)	*Coccidioides immitis*	Granulomatous lesions on face and nose; may spread to organs or brain, causing fatal meningitis	Culture (in BSL-3 lab only), serological antibody tests	Amphotericin B
Cryptococcosis	*Cryptococcus neoformans*	Fever, cough, shortness of breath; can cause fatal meningitis if disseminated to brain	Microscopic examination of lung tissue or cerebrospinal fluid	Amphotericin B, fluconazole, flucytosine

Disease	Pathogen	Signs and Symptoms	Diagnostic Tests	Antimicrobial Drugs
Histoplasmosis	*Histoplasma capsulatum*	Fever, headache, weakness, chest pain, lesions on lungs	Chest radiograph, culture, direct fluorescence antibody staining, complement fixation assay, histoplasmin sensitivity test	Amphotericin B, ketoconazole, itraconazole
Mucormycosis	*Rhizopus arrhizus*, other *Rhizopus* spp., *Mucor* spp.	Headache, fever, facial swelling, congestion, black lesions in oral cavity, cough, chest pain, shortness of breath; often fatal	Microscopic examination of tissue biopsy specimens	Amphotericin B
Pneumocystis pneumonia (PCP)	*Pneumocystis jirovecii*	Fever, cough, shortness of breath; can be fatal if untreated	Microscopic examination of lung tissue and fluid, PCR	Trimethoprim-sulfa methoxazole

Table 5.11: Fungal infections of the respiratory tract

Figure Descriptions

Figure 5.1: a) diagram of ear; a closeup shows the bones and membranes of the middle ear. The eardrum is a flat disk labeled tympanic membrane. Behind this is the tympanic cavity (middle ear) which contains the bones. A tube going downward from the middle ear is labeled Eustachian tube (auditory tube). b) A diagram of a cross section of the head. Above the nose is a space in the bone labeled frontal sinus. The space in the nose is the nasal cavity and a duct in the nose is the nasolacrimal duct. A space in the bone behind the nose is the sphenoid sinus. At the back of the nose is the opening of the Eustachian tube (auditory tube). Behind that is the pharyngeal tonsil. Below that is a tube labeled nasopharynx which becomes the pharynx which because the oropharynx (behind the mouth) which becomes the laryngopharynx, which becomes the esophagus. Vocal folds are found just beyond the laryngopharynx in the larynx a tube which becomes the trachea. The epiglottis is a flap the determines if material in the pharynx travels to the esophagus or the trachea because the mouth also leads to the pharynx. The mouth contains the tongue. Underneath the tongue is the lingual tonsil and at the back of the mouth is the palatine tonsil. At the very back of the mouth is the fauces. In front of the trachea is the thyroid gland.

Figure 5.2: A drawing of the lower respiratory system. The epiglottis is a flap that can allow material into the larynx. The larynx is a tube that leads to the trachea. The trachea branches to become the primary bronchi. These branch to become the secondary bronchi, these branch to become the tertiary bronchi. These branch to become the bronchioles. Terminal bronchioles end in clusters of balloon shapes called alveolar sacs. Each balloon shape is an alveolus. Thin, webbed capillaries cover the outside of the alveolus and are connected to pulmonary veins and pulmonary arteries. Oxygen from the alveolus travels into the capillary and carbon dioxide from the capillary travels into the alveolus of the lower respiratory system. The epiglottis is a flap that can allow material into the larynx. The larynx is a tube that leads to the trachea. The trachea branches to become the primary bronchi. These branch to become the secondary bronchi, these branch to become the tertiary bronchi. These branch to become the bronchioles. Terminal bronchioles end in clusters of balloon shapes called alveolar sacs. Each balloon shape is an alveolus. Thin, webbed capillaries cover the outside of the alveolus and are connected to pulmonary veins and pulmonary arteries. Oxygen from the alveolus travels into the capillary and carbon dioxide from the capillary travels into the alveolus.

Figure 5.3: A micrograph showing a space at the top labeled lumen of trachea. Underneath this are long cells with a brush border at the top. These cells are called pseudostratified columnar epithelia. The brush border is many cilia. Vase shaped cells in this layer are called goblet cells. Below this layer is tissue with small spheres labeled seromucous gland in submucosa.

Figure 5.4: Micrograph of chains of purple spheres.

Figure 5.5: a) Bright red inflammation at the back of the mouth. b) red inflamed spots covering a back.

Figure 5.6: a) A close-up of the eardrum (tympanic membrane), which looks like a translucent, thin covering. Labels point out the malleus, incus, and tympanum. B) Without the tympanic membrane, the region is red and swollen. The bones are deteriorating and yellow mucus builds up. Labels point to mucus, a torn membrane, and eroded inner ear bones.

Figure 5.7: A gray, leathery blob in the back of a person's mouth is shown and the label "pseudomembrane" points to it.

Figure 5.8: An X-ray that shows white bones on a black background. White regions within the lungs are labeled lesions.

Figure 5.9: Part a shows a micrograph of lancet (football) shaped cells, some of which have a clear ring around them. Part b shows two dumbbell-shaped blue cells on an orange background.

Figure 5.10: A micrograph of Haemophilus influenzae is shown. It looks like a brown disc with white streaks.

Figure 5.11: A micrograph showing a small oval cell binding to a much larger cell.

Figure 5.12: Diagram showing infectious cycle of tuberculosis. First droplet nuclei containing tubercle bacilli are inhaled, enter the lungs and travel to the alveoli. Next, the tubercle bacilli multiply in the alveoli. Next, the immune cells form a barrier shell around the tubercle bacilli, called a granuloma. Finally, the granuloma shell breaks down and the tubercle bacilli escape and rapidly multiply forming more tubercles.

Figure 5.13: a) a needle injects a small bubble into a person's skin. B) a ruler is used to measure a red area on a person's skin.

Figure 5.14: A sphere with strand of circles in the inside – this strand is labeled ribonucleoprotein. The outside of the sphere is made of 2 layers. The inner layer is the capsid. The outer layer is the lipid envelope. The lipid envelope has an M2 ion channel and two different surface components labeled hemagglutinin (H) and neuraminidase (N).

Figure 5.15: a and b) Red bumps on children's faces and mouths. c) Small red spots on a child's face. d) A micrograph of an oval structure containing a scale bar measuring 50 nanometers.

Figure 5.16: a) red bumps on a person's back. b) a micrograph of rubella.

Figure 5.17: (a) and (b) red bumps on skin. (c) a micrograph of human herpesvirus 3 is shown.

Figure 5.18: a) Large red spots on an adult's neck. b) Flaky inflamed patch on adult's face. c) Red bumps on skin.

Figure 5.19: a) Large, dark lesions on a face. B) A fluorescent micrograph of spheres in a larger sphere.

Figure 5.20: A micrograph of two circular cells next to each other. The label "amoebae" points to the exterior of both cells and the label "Legionella pneumophila" points to parts within. A scale bar indicates that the diameter of each cell is approximately half a micrometer.

Figure 5.21: a) Large crusty lesions on a deformed hand. B) A micrograph of round cells.

Figure 5.22: An X-ray showing white bones on a black background. White webbing in the upper lung is circled.

Figure 5.23: Micrograph of dark purple circles on a light purple background.

Figure 5.24: a) A micrograph with dark circles (some attached to form a figure 8) on a green background. The dark cells are labeled Cryptococcus. The figure 8 cells are labeled budding cells. b) a negative stain micrograph of cryptococcus neoformans is shown. It appears as green spots on a brown background.

Figure References

Figure 5.1: The ear is connected to the upper respiratory tract by the eustachian tube, which opens to the nasopharynx. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 5.2: The structures of the lower respiratory tract are identified in this illustration. Modification of work by National Cancer Institute. Public domain.

Figure 5.3: This micrograph shows the structure of the mucous membrane of the respiratory tract. (c) 2012. Regents of University of Michigan Medical School. Redistribution authorized with attribution.

Figure 5.4: This scanning electron micrograph of Streptococcus pyogenes shows the characteristic cellular phenotype resembling chains of cocci. Modification of work by U.S. Centers for Disease Control and Prevention – Medical Illustrator. Public domain.

Figure 5.5: Streptococcal infections of the respiratory tract may cause localized pharyngitis or systemic signs and symptoms. Left: Modification of work by Centers for Disease Control and Prevention. Public domain. Right: (c) modification of work by Alicia Williams. CC BY 4.0.

Figure 5.6: A healthy tympanic membrane; the middle ear bones can be seen behind the membrane. Left: Modification of work by (c) "DrER.tv"/YouTube. CC BY 4.0. Right: Figure 2 in Li MG, Hotez PJ, Vrabec JT, Donovan DT (2015) Is Chronic Suppurative Otitis Media a Neglected Tropical Disease? PLoS Negl Trop Dis 9(3): e0003485. https://doi.org/10.1371/journal.pntd.0003761.s002. CC BY 4.0.

Figure 5.7: The pseudomembrane in a patient with diphtheria presents as a leathery gray patch consisting of dead cells, pus, fibrin, red blood cells, and infectious microbes. (c) Unknown. Source cited as: Putnong N, Agustin G, Pasubillo M, Miyagi K, Dimaano EM.

Figure 5.8: A chest radiograph of a patient with pneumonia shows the consolidations (lesions) present as opaque patches. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 5.9: (a) This micrograph of Streptococcus pneumoniae grown from a blood culture shows the characteristic lancet-shaped diplococcal morphology. Left: modification of work by Centers for Disease Control and Prevention. Public domain. Right: modification of work by Janice Carr, Centers for Disease Control and Prevention. Public domain.

Figure 5.10: Culture of Haemophilus influenzae on a chocolate agar plate. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 5.11: The micrograph shows Mycoplasma pneumoniae using their specialized receptors to attach to epithelial cells in the trachea of an infected hamster. (c) American Society of Microbiology. Redistribution authorized with attribution.

Figure 5.12: In the infectious cycle of tuberculosis, the immune response of most infected individuals (approximately 90%) results in the formation of tubercles in which the infection is walled off. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 5.13: (a) The Mantoux skin test for tuberculosis involves injecting the subject with tuberculin protein derivative. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 5.14: The illustration shows the structure of an influenza virus. Modification of work by Dan Higgins, Centers for Disease Control and Prevention. Public domain.

Figure 5.15: Measles typically presents as a raised macular rash that begins on the face and spreads to the extremities. Top Left: Betty G. Partin /Centers for Disease Control and Prevention. Public Domain. Top Right, Bottom Left: modification of work by Centers for Disease Control and Prevention. Public Domain. Bottom Right: Centers for Disease Control and Prevention. Public Domain. https://commons.wikimedia.org/wiki/File:Measles_virus.JPG

Figure 5.16: (a) This photograph shows the appearance of the German measles (rubella) rash. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 5.17: The characteristic appearance of the pustular chickenpox rash is concentrated on the trunk region. Left: By Camiloaranzales. Public Domain. https://commons.wikimedia.org/wiki/File:Varicela_Aranzales.jpg. Middle: John Noble/Centers for Disease Control and Prevention. Public Domain. Right: Modification of work by Centers for Disease Control and Prevention. Public Domain.

Figure 5.18: An individual suffering from shingles. Left: modification of work by National Institute of Allergy and Infectious Diseases (NIAID). Public Domain. Middle: Robert E. Sumpter/Centers for Disease Control and Prevention. Public Domain. Right: modification of work by Centers for Disease Control and Prevention. Public Domain.

Figure 5.19: (a) This patient has extensive facial lesions due to a disseminated Coccidioides infection. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 5.20: Legionella pneumophila (red intracellular rods) infecting amoebae from a contaminated water sample. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 5.21: These skin lesions are the result of disseminated cutaneous blastomycosis. Left: modification of work by Centers for Disease Control and Prevention. Public Domain. Right: modification of work by Medmyco. Public Domain. https://commons.wikimedia.org/wiki/File:Blastomyces_dermatitidis_yeast_form.jpeg

Figure 5.22: A fungal ball can be observed in the upper lobe of the right lung in this chest radiograph of a patient with aspergilloma. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 5.23: A light micrograph of a smear containing Pneumocystis jirovecii (dark purple cells) obtained from human lung tissue and stained with toluidine blue. Centers for Disease Control and Prevention. Public domain.

Figure 5.24: (a) The micrograph shows stained budding Cryptococcus yeast cells from the lungs of a patient with AIDS. Modification of work by Centers for Disease Control and Prevention. Public domain.

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CHAPTER 6.

SYSTEMIC INFECTIONS OF THE CIRCULATORY AND LYMPHATIC SYSTEMS

6.1 INTRODUCTION TO THE CIRCULATORY AND LYMPHATIC SYSTEMS

INFECTIONS OF THE CIRCULATORY SYSTEM

Under normal circumstances, the circulatory system and the blood should be sterile; the **circulatory system has no normal microbiota**. Because the system is closed, there are no easy portals of entry into the circulatory system for microbes. Those that are able to breach the body's physical barriers and enter the bloodstream encounter a host of circulating immune defenses, such as antibodies, complement proteins, phagocytes, and other immune cells. Microbes often gain access to the circulatory system through a break in the skin (e.g., wounds, needles, intravenous catheters, insect bites) or spread to the circulatory system from infections in other body sites. For example, microorganisms causing pneumonia or renal infection may enter the local circulation of the lung or kidney and spread from there throughout the circulatory network (table 6.1).

If microbes in the bloodstream are not quickly eliminated, they can spread rapidly throughout the body, leading to serious, even life-threatening infections.

Various terms are used to describe conditions involving microbes in the circulatory system.

- The term **bacteremia** refers to bacteria in the blood.
- If bacteria are reproducing in the blood as they spread, this condition is called **septicemia**.
- The presence of viruses in the blood is called **viremia**.
- Microbial toxins can also be spread through the circulatory system, causing a condition termed **toxemia**.
- The presence of fungi in the blood is called **fungemia**, but is very rare, so not covered in this section

Microbes and microbial toxins in the blood can trigger an inflammatory response so severe that the inflammation damages host tissues and organs more than the infection itself. This counterproductive immune response is called **systemic inflammatory response syndrome** (SIRS), and it can lead to the life-threatening condition known as sepsis.

Sepsis is characterized by the production of excess cytokines that leads to classic signs of inflammation such as fever, vasodilation, and edema (see section 1.7). In a patient with sepsis, the inflammatory response becomes dysregulated and disproportionate to the threat of infection. Critical organs such as the heart, lungs, liver, and kidneys become dysfunctional, resulting in increased heart and respiratory rates, and disorientation. If not treated promptly and effectively, patients with sepsis can go into shock and die.

Certain infections can cause inflammation in the heart and blood vessels.

- Inflammation of the endocardium, the inner lining of the heart, is called **endocarditis** and can result in damage to the heart valves severe enough to require surgical replacement.
- Inflammation of the pericardium, the sac surrounding the heart, is called **pericarditis**.
- The term **myocarditis** refers to the inflammation of the heart's muscle tissue. Pericarditis and myocarditis can cause fluid to accumulate around the heart, that can compromise ventricle filling and thereby impact cardiac output.
- Inflammation of blood vessels is called **vasculitis**. Although somewhat rare, vasculitis can cause blood vessels to become damaged and rupture; as blood is released, small red or purple spots called petechiae appear on the skin. If the damage of tissues or blood vessels is severe, it can result in reduced blood flow to the surrounding tissues. This condition is called ischemia, and it can be very serious.

INFECTIONS OF THE LYMPHATIC SYSTEM

Like the circulatory system, the **lymphatic system does not have a normal microbiota**, and the large numbers of immune cells typically eliminate transient microbes before they can establish an infection. Only microbes with an array of virulence factors are able to overcome these defenses and establish infection in the lymphatic system. However, when a localized infection begins to spread, the lymphatic system is often the first place the invading microbes can be detected.

Infections in the lymphatic system also trigger an inflammatory response.

- Inflammation of lymphatic vessels, called **lymphangitis**, can produce visible red streaks under the skin.
- Inflammation in the lymph nodes can cause them to swell. A swollen lymph node is referred to as a **bubo**, and the condition is referred to as **lymphadenitis**.

6.2 BACTERIAL INFECTIONS OF THE CIRCULATORY AND LYMPHATIC SYSTEMS

Bacteria can enter the circulatory and lymphatic systems through acute infections or breaches of the skin barrier or mucosa. Breaches may occur through fairly common occurrences, such as insect bites or small wounds. Even the act of tooth brushing, which can cause small ruptures in the gums, may introduce bacteria into the circulatory system. In most cases, the bacteremia that results from such common exposures is transient and remains below the threshold of detection. In severe cases, bacteremia can lead to septicemia with dangerous complications such as toxemia, sepsis, and septic shock.

BACTERIAL SEPSIS, SEPTIC AND TOXIC SHOCK

At low concentrations, pro-inflammatory cytokines such as interleukin 1 (IL-1) and tumor necrosis factor-α (TNF-α) play important roles in the host's immune defenses. When they circulate systemically in larger amounts, however, the resulting immune response can be life threatening. IL-1 induces vasodilation (widening of blood vessels) and reduces the tight junctions between vascular endothelial cells, leading to widespread edema. As fluids move out of circulation into tissues, blood pressure begins to drop. If left unchecked, the blood pressure can fall below the level necessary to maintain proper kidney and respiratory functions, a condition known as septic shock. In addition, the excessive release of cytokines during the inflammatory response can lead to the formation of blood clots. The loss of blood pressure and occurrence of blood clots can result in multiple organ failure and death.

Bacteria are the most common pathogens associated with the development of sepsis, and septic shock.[1] The most common infection associated with sepsis is bacterial pneumonia (section 5.2), accounting for about half of all cases, followed by intra-abdominal infections (section 4.3) and urinary tract infections (section 7.2).[2] Infections associated with superficial wounds, animal bites, and indwelling catheters may also lead to sepsis and septic shock.

These initially minor, localized infections can be caused by a wide range of different bacteria, including *Staphylococcus, Streptococcus*, *Pseudomonas*, *Pasteurella, Acinetobacter,* and members of the Enterobacteriaceae. However, if left untreated, infections by these gram-positive and gram-negative pathogens can potentially progress to sepsis, shock, and death.

Toxic Shock Syndrome and Streptococcal Toxic Shock-Like Syndrome

Toxemia associated with infections caused by *Staphylococcus aureus* can cause staphylococcal toxic shock syndrome (TSS). Some strains of *S. aureus* produce a superantigen called toxic shock syndrome toxin-1 (TSST-1). TSS may occur as a complication of other localized or systemic infections such as pneumonia, osteomyelitis, sinusitis, and skin wounds (surgical, traumatic, or burns). Those at highest risk for staphylococcal TSS are women with preexisting *S. aureus* colonization of the vagina who leave tampons, contraceptive sponges, diaphragms, or other devices in the vagina for longer than the recommended time. The frequency of such cases, however, has declined.

Staphylococcal TSS is characterized by sudden onset of vomiting, diarrhea, myalgia, body temperature higher than 38.9 °C (102.0 °F), and rapid-onset hypotension with a systolic blood pressure less than 90 mm Hg for adults; a diffuse erythematous rash that leads to peeling and shedding skin (desquamation) 1 to 2 weeks after onset; and additional involvement of three or more organ systems.[3] The mortality rate associated with staphylococcal TSS is less than 3% of cases.

Diagnosis of staphylococcal TSS is based on clinical signs, symptoms, serologic tests to confirm bacterial species, and the detection of toxin production from staphylococcal isolates. Cultures of skin and blood are often negative; less than 5% are positive in cases of staphylococcal TSS. Treatment for staphylococcal TSS includes decontamination, debridement, vasopressors to elevate blood pressure, and (pending susceptibility results), antibiotic therapy with clindamycin plus vancomycin or daptomycin susceptibility to reduce toxin production

A syndrome with signs and symptoms similar to staphylococcal TSS can be caused by *Streptococcus pyogenes*. This condition, called streptococcal toxic shock-like syndrome (STSS), is characterized by more severe pathophysiology than staphylococcal TSS,[4] with about 50% of patients developing *S. pyogenes* bacteremia and necrotizing fasciitis. In contrast to staphylococcal TSS, STSS is more likely to cause acute respiratory distress syndrome (ARDS), a rapidly progressive disease characterized by fluid accumulation in the lungs that inhibits breathing and causes hypoxemia (low oxygen levels in the blood). STSS is associated with a higher mortality rate (20%–60%), even with aggressive therapy. STSS usually develops in patients with a streptococcal soft-tissue infection such as bacterial cellulitis, necrotizing fasciitis, pyomyositis (pus formation in muscle caused by infection), a recent influenza A infection, or chickenpox.

PUERPERAL SEPSIS

A type of sepsis called puerperal sepsis, also known as puerperal infection, puerperal fever, or childbed fever, is a nosocomial infection associated with the period of puerperium—the time following childbirth during which the mother's reproductive system returns to a nonpregnant state. Such infections may originate in the genital tract, breast, urinary tract, or a surgical wound. Initially the infection may be limited to the uterus or other local

site of infection, but it can quickly spread, resulting in peritonitis, septicemia, and death. Before the 19th century work of Ignaz Semmelweis (1818-1865) and the widespread acceptance of germ theory, puerperal sepsis was a major cause of mortality among new mothers in the first few days following childbirth.

Puerperal sepsis is often associated with *Streptococcus pyogenes*, but numerous other bacteria can also be responsible. Examples include gram-positive bacterial (e.g. *Streptococcus* spp., *Staphylococcus* spp., and *Enterococcus* spp.), gram-negative bacteria (e.g. *Chlamydia* spp., *Escherichia coli*, *Klebsiella* spp., and *Proteus* spp.), as well as anaerobes such as *Peptostreptococcus* spp., *Bacteroides* spp., and *Clostridium* spp. In cases caused by *S. pyogenes*, the bacteria attach to host tissues using M protein and produce a carbohydrate capsule to avoid phagocytosis. *S. pyogenes* also produces a variety of exotoxins, like streptococcal pyrogenic exotoxins A and B, that are associated with virulence and may function as superantigens.

Diagnosis of puerperal fever is based on the timing and extent of fever and isolation, and identification of the etiologic agent in blood, wound, or urine specimens. Because there are numerous possible causes, antimicrobial susceptibility testing must be used to determine the best antibiotic for treatment. Nosocomial incidence of puerperal fever can be greatly reduced through the use of antiseptics during delivery and strict adherence to hand washing protocols by doctors, midwives, and nurses.

INFECTIOUS ARTHRITIS

Also called septic arthritis, infectious arthritis can be either an acute or a chronic condition. Infectious arthritis is characterized by inflammation of joint tissues and is most often caused by bacterial pathogens. Most cases of acute infectious arthritis are secondary to bacteremia, with a rapid onset of moderate to severe joint pain and swelling that limits the motion of the affected joint. In adults and young children, the infectious pathogen is most often introduced directly through injury, such as a wound or a surgical site, and brought to the joint through the circulatory system. Acute infections may also occur after joint replacement surgery. Acute infectious arthritis often occurs in patients with an immune system impaired by other viral and bacterial infections. *S. aureus* is the most common cause of acute septic arthritis in the general population of adults and young children. *Neisseria gonorrhoeae* is an important cause of acute infectious arthritis in sexually active individuals.

Chronic infectious arthritis is responsible for 5% of all infectious arthritis cases and is more likely to occur in patients with other illnesses or conditions. Patients at risk include those living with HIV, a bacterial or fungal infection, prosthetic joints, rheumatoid arthritis (RA), or who are undergoing immunosuppressive chemotherapy. Onset is often in a single joint; there may be little or no pain, aching pain that may be mild, gradual swelling, mild warmth, and minimal or no redness of the joint area.

Diagnosis of infectious arthritis requires the aspiration of a small quantity of synovial fluid from the affected joint. Direct microscopic evaluation, culture, and polymerase chain reaction (PCR) analyses of the synovial fluid are used to identify the potential pathogen. Typical treatment includes administration of appropriate antimicrobial drugs based on antimicrobial susceptibility testing. For susceptible bacterial strains, β-lactams such as oxacillin and cefazolin are often prescribed for staphylococcal infections. Third-generation cephalosporins (e.g., ceftriaxone) are used for increasingly prevalent β-lactam-resistant *Neisseria* infections. Infections by *Mycobacterium* spp. or fungi are treated with appropriate long-term antimicrobial therapy. Even with treatment, the prognosis is often poor for those infected. About 40% of patients with nongonococcal infectious arthritis will suffer permanent joint damage and mortality rates range from 5% to 20%.[5] Mortality rates are higher among the elderly.[6]

OSTEOMYELITIS

Osteomyelitis is an inflammation of bone tissues most commonly caused by infection. These infections can either be acute or chronic and can involve a variety of different bacteria. The most common causative agent of osteomyelitis is *S. aureus*. However, *M. tuberculosis*, *Pseudomonas aeruginosa, Streptococcus pyogenes*, *S. agalactiae*, species in the Enterobacteriaceae, and other microorganisms can also cause osteomyelitis, depending on which bones are involved. In adults, bacteria usually gain direct access to the bone tissues through trauma or a surgical procedure involving prosthetic joints. In children, the bacteria are often introduced from the bloodstream, possibly spreading from focal infections. The long bones, such as the femur, are more commonly affected in children because of the more extensive vascularization of bones in the young.[7]

The signs and symptoms of osteomyelitis include fever, localized pain, swelling due to edema, and ulcers in soft tissues near the site of infection. The resulting inflammation can lead to tissue damage and bone loss. In addition, the infection may spread to joints, resulting in infectious arthritis, or disseminate into the blood, resulting in sepsis and thrombosis (formation of blood clots). Like septic arthritis, osteomyelitis is usually diagnosed using a combination of radiography, imaging, and identification of bacteria from blood cultures or from bone cultures if blood cultures are negative. Parenteral antibiotic therapy is typically used to treat osteomyelitis, but several studies support early transition to oral therapy. Because of the number of different possible etiologic agents, however, a variety of drugs might be used. Broad-spectrum antibacterial drugs such as nafcillin, oxacillin, or cephalosporin are typically prescribed for acute osteomyelitis, and ampicillin/sulbactam and piperacillin/tazobactam for chronic osteomyelitis. In cases of gram-positive organisms that are antibiotic resistant, vancomycin treatment is sometimes required to control infection. In serious cases, surgery to remove the site of infection may be required. Other forms of treatment include hyperbaric oxygen therapy and implantation of antibiotic beads or pumps.

RHEUMATIC FEVER

Infections with *S. pyogenes* have a variety of manifestations and complications generally called sequelae. As mentioned, the bacterium can cause suppurative infections like puerperal fever. However, this microbe can also cause nonsuppurative sequelae in the form of acute rheumatic fever (ARF), which can lead to rheumatic heart disease, thus impacting the circulatory system. Rheumatic fever occurs primarily in children a minimum of 2–3 weeks after an episode of untreated or inadequately treated pharyngitis (see section 5.2). At one time, rheumatic fever was a major killer of children in the US; today, however, it is rare in the US because of early diagnosis and treatment of streptococcal pharyngitis with antibiotics. In parts of the world where diagnosis and treatment are not readily available, acute rheumatic fever and rheumatic heart disease are still major causes of mortality in children.[8]

Rheumatic fever is characterized by a variety of diagnostic signs and symptoms caused by nonsuppurative, immune-mediated damage resulting from a cross-reaction between patient antibodies to bacterial surface proteins and similar proteins found on cardiac, neuronal, and synovial tissues. Damage to the nervous tissue or joints, which leads to joint pain and swelling, is reversible. However, damage to heart valves can be irreversible and is worsened by repeated episodes of acute rheumatic fever, particularly during the first 3–5 years after the first rheumatic fever attack. The inflammation of the heart valves caused by cross-reacting antibodies leads to scarring and stiffness of the valve leaflets. This, in turn, produces a characteristic heart murmur. Patients who have previously developed rheumatic fever and who subsequently develop recurrent pharyngitis due to *S. pyogenes* are at high risk for recurrent attacks of rheumatic fever.

The American Heart Association recommends[9] a treatment regimen consisting of benzathine benzylpenicillin every 3 or 4 weeks, depending on the patient's risk for reinfection. Additional prophylactic antibiotic treatment may be recommended depending on the age of the patient and risk for reinfection.

BACTERIAL ENDOCARDITIS AND PERICARDITIS

The endocardium is a tissue layer that lines the muscles and valves of the heart. This tissue can become infected by a variety of bacteria, including gram-positive cocci such as *Staphylococcus aureus*, viridans streptococci, *Enterococcus faecalis*, and the gram-negative so-called HACEK bacilli: *Haemophilus* spp., *Aggregatibacter actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingae*. The resulting inflammation is called endocarditis, which can be described as either acute or subacute. Causative agents typically enter the bloodstream during accidental or intentional breaches in the normal barrier defenses (e.g., dental procedures, body piercings, catheterization, wounds). Individuals with preexisting heart damage, prosthetic valves and other cardiac devices, and those with a history of rheumatic fever have a higher risk for endocarditis. This disease can rapidly destroy the heart valves and, if untreated, lead to death in just a few days.

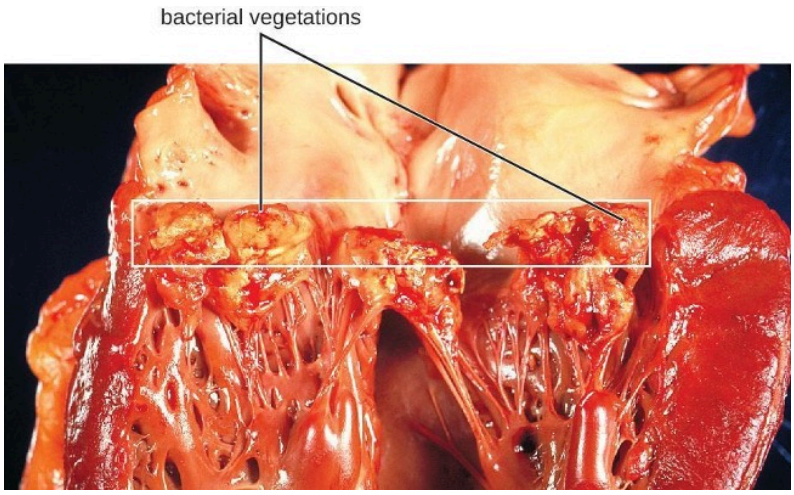

Figure 6.1: The heart of an individual who had subacute bacterial endocarditis of the mitral valve. Bacterial vegetations are visible on the valve tissues. Figure description available at the end of the chapter.

In subacute bacterial endocarditis, heart valve damage occurs slowly over a period of months. During this time, blood clots form in the heart, and these protect the bacteria from phagocytes. These patches of tissue-associated bacteria are called vegetations. The resulting damage to the heart, in part resulting from the immune response causing fibrosis of heart valves, can necessitate heart valve replacement (figure 6.1). Outward signs of subacute endocarditis may include a fever, splinter hemorrhages, Osler nodes or Janeway lesions (figure 6.2).

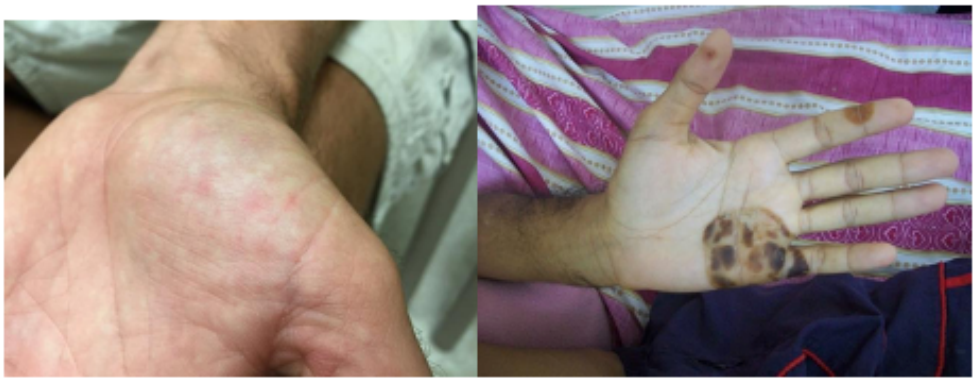

Figure 6.2: Janeway lesions (left) and Osler nodes (right). Figure description available at the end of the chapter.

Diagnosis of infective endocarditis is determined using the combination of blood cultures, echocardiogram, and clinical symptoms. In both acute and subacute endocarditis, treatment typically involves relatively high doses of intravenous antibiotics as determined by antimicrobial susceptibility testing. Acute endocarditis is often treated with a combination of ampicillin and nafcillin for synergistic coverage of *Staphylococcus* spp. and *Streptococcus* spp. Prosthetic-valve endocarditis is often treated with a combination of vancomycin, rifampin, and gentamicin. Rifampin is necessary to treat individuals with infection of prosthetic valves or other foreign bodies because rifampin can penetrate the biofilm of most of the pathogens that infect these devices.

Staphylcoccus spp. and *Streptococcus* spp. can also infect and cause inflammation in the tissues surrounding the heart, a condition called acute pericarditis. Pericarditis is marked by chest pain, difficulty breathing, and a dry cough. In most cases, pericarditis is self-limiting and clinical intervention is not necessary. Diagnosis is made with the aid of a chest radiograph, electrocardiogram, echocardiogram, aspiration of pericardial fluid, or biopsy of pericardium. Antibacterial medications may be prescribed for infections associated with pericarditis; however, pericarditis can also be caused other pathogens, including viruses (e.g., echovirus, influenza virus), fungi (e.g., *Histoplasma* spp., *Coccidioides* spp.), and eukaryotic parasites (e.g., *Toxoplasma* spp.).

GAS GANGRENE

Traumatic injuries or certain medical conditions, such as diabetes, can cause damage to blood vessels that interrupts blood flow to a region of the body. When blood flow is interrupted, tissues begin to die, creating an anaerobic environment in which anaerobic bacteria can thrive. This condition is called ischemia. Endospores of the anaerobic bacterium *Clostridium perfringens* (along with a number of other *Clostridium* spp. from the gut) can readily germinate in ischemic tissues and colonize the anaerobic tissues.

The resulting infection, called gas gangrene, is characterized by rapidly spreading myonecrosis (death of muscle tissue). The patient experiences a sudden onset of excruciating pain at the infection site and the rapid development of a foul-smelling wound containing gas bubbles and a thin, yellowish discharge tinged with a small amount of blood. As the infection progresses, edema and cutaneous blisters containing bluish-purple fluid form. The infected tissue becomes liquefied and begins sloughing off. The margin between necrotic and healthy tissue often advances several inches per hour even with antibiotic therapy. Septic shock and organ failure frequently accompany gas gangrene; when patients develop sepsis, the mortality rate is greater than 50%.

α-Toxin and theta (θ) toxin are the major virulence factors of *C. perfringens* implicated in gas gangrene. α-Toxin is a lipase responsible for breaking down cell membranes; it also causes the formation of thrombi (blood clots) in blood vessels, contributing to the spread of ischemia. θ-Toxin forms pores in the patient's cell membranes, causing cell lysis. The gas associated with gas gangrene is produced by *Clostridium*'s fermentation of butyric acid, which produces hydrogen and carbon dioxide that are released as the bacteria multiply, forming pockets of gas in tissues (figure 6.3).

Gas gangrene is initially diagnosed based on the presence of the clinical signs and symptoms described earlier in this section. Diagnosis can be confirmed through Gram stain and anaerobic cultivation of wound exudate (drainage) and tissue samples on blood agar, although onset of treatment should not wait for gram stain or culture diagnosis. Treatment typically involves surgical debridement of any necrotic tissue; advanced cases may require amputation. Surgeons may also use vacuum-assisted closure (VAC), a surgical technique in which vacuum-assisted drainage is used to remove blood or serous fluid from a wound or surgical site to speed recovery. The most common antibiotic treatments include penicillin G and clindamycin. Some cases are also treated with hyperbaric oxygen therapy because *Clostridium* spp. are incapable of surviving in oxygen-rich environments.

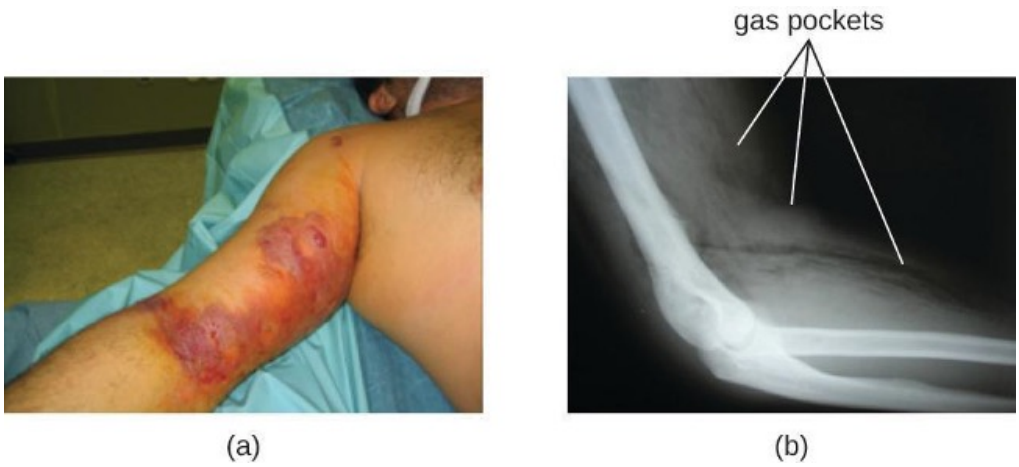

Figure 6.3: (a) In this image of a patient with gas gangrene, note the bluish-purple discoloration around the bicep and the irregular margin of the discolored tissue indicating the spread of infection. (b) A radiograph of the arm shows a darkening in the tissue, which indicates the presence of gas. *Figure description available at the end of the chapter.*

TULAREMIA

Infection with the gram-negative bacterium *Francisella tularensis* causes tularemia (or rabbit fever), a zoonotic infection in humans. *F. tularensis* is a facultative intracellular parasite that primarily causes illness in rabbits, although a wide variety of domesticated animals are also susceptible to infection. Humans can be infected through ingestion of contaminated meat or, more typically, handling of infected animal tissues (e.g., skinning an infected rabbit). Tularemia can also be transmitted by the bites of infected arthropods, including the dog tick (*Dermacentor variabilis*), the lone star tick (*Amblyomma americanum*), the wood tick (*Dermacentor andersoni*), and deer flies (*Chrysops* spp.). Although the disease is not directly communicable between humans, exposure to aerosols of *F. tularensis* can result in life-threatening infections. *F. tularensis* is highly contagious, with an infectious dose of as few as 10 bacterial cells. In addition, pulmonary infections have a 30%–60% fatality rate if untreated.[10] For these reasons, *F. tularensis* is currently classified and must be handled as a biosafety level-3 (BSL-3) organism and as a potential biological warfare agent.

Following introduction through a break in the skin, the bacteria initially move to the lymph nodes, where they are ingested by phagocytes. After escaping from the phagosome, the bacteria grow and multiply intracellularly in the cytoplasm of phagocytes. They can later become disseminated through the blood to other organs such as the liver, lungs, and spleen, where they produce masses of tissue called granulomas. After an incubation period of about 3 days, skin lesions develop at the site of infection (figure 6.4). Other signs and symptoms include fever, chills, headache, and swollen and painful lymph nodes.

A direct diagnosis of tularemia is challenging because it is so contagious. Once a presumptive diagnosis of tularemia is made, special handling is required to collect and process patients' specimens to prevent the infection of healthcare workers. Specimens suspected of containing *F. tularensis* can only be handled by BSL-3 laboratories registered with the Federal Select Agent Program, and individuals handling the specimen must wear protective equipment and use a class II biological safety cabinet.

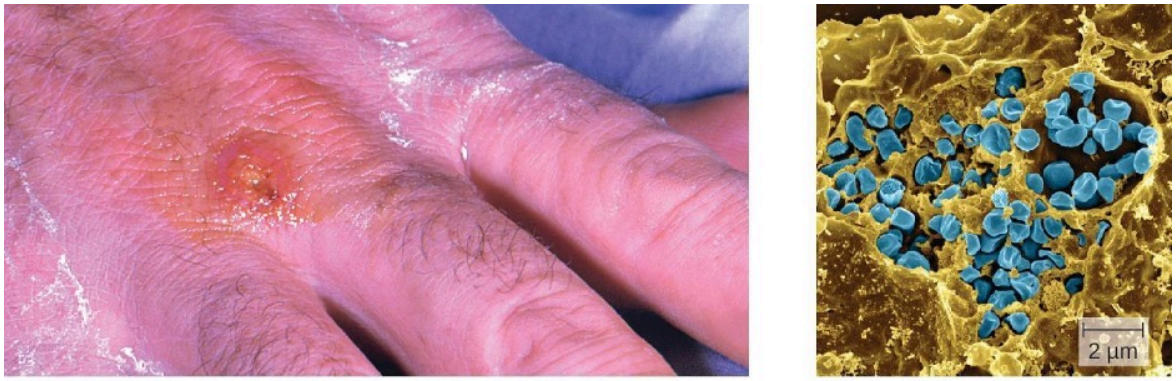

Figure 6.4: (a) A skin lesion appears at the site of infection on the hand of an individual infected with Francisella tularensis. (b) A scanning electron micrograph shows the coccobacilli cells (blue) of F. tularensis. *Figure description available at the end of the chapter.*

Tularemia is relatively rare in the US, and its signs and symptoms are similar to a variety of other infections that may need to be ruled out before a diagnosis can be made. Direct fluorescent-antibody (DFA) microscopic examination using antibodies specific for *F. tularensis* can rapidly confirm the presence of this pathogen. Culturing this microbe is difficult because of its requirement for the amino acid cysteine, which must be supplied as an extra nutrient in culturing media. Serological tests are available to detect an immune response against the bacterial pathogen. In patients with suspected infection, acute- and convalescent-phase serum samples are required to confirm an active infection. PCR-based tests can also be used for clinical identification of direct specimens from body fluids or tissues as well as cultured specimens. In most cases, diagnosis is based on clinical findings and likely incidents of exposure to the bacterium. The antibiotics streptomycin, gentamicin, doxycycline, and ciprofloxacin are effective in treating tularemia.

BRUCELLOSIS

Species in the genus *Brucella* are gram-negative facultative intracellular pathogens that appear as coccobacilli. Several species cause zoonotic infections in animals and humans, four of which have significant human pathogenicity: *B. abortus* from cattle and buffalo, *B. canis* from dogs, *B. suis* from swine, and *B. melitensis* from goats, sheep, and camels. Infections by these pathogens are called brucellosis, also known as undulant fever, "Mediterranean fever," or "Malta fever." Vaccination of animals has made brucellosis a rare disease in the US, but it is still common in the Mediterranean, south and central Asia, Central and South America, and the Caribbean. Human infections are primarily associated with the ingestion of meat or unpasteurized dairy products from infected animals. Infection can also occur through inhalation of bacteria in aerosols when handling animal products, or through direct contact with skin wounds. In the US, most cases of brucellosis are found in individuals with extensive exposure to potentially infected animals (e.g., slaughterhouse workers, veterinarians).

Two important virulence factors produced by *Brucella* spp. are urease, which allows ingested bacteria to avoid destruction by stomach acid, and lipopolysaccharide (LPS), which allows the bacteria to survive within phagocytes. After gaining entry to tissues, the bacteria are phagocytized by host neutrophils and macrophages. The bacteria then escape from the phagosome and grow within the cytoplasm of the cell. Bacteria phagocytized by macrophages are disseminated throughout the body. This results in the formation of granulomas within many body sites, including bones, the liver, spleen, lung, genitourinary tract, brain, heart, eyes, and skin. Acute infections can result in undulant (relapsing) fever, but untreated infections develop into chronic disease that usually manifests as acute febrile illness (fever of 40–41 °C [104–105.8 °F]) with recurring flu-like signs and symptoms.

Brucella is only reliably found in the blood during the acute fever stage; it is difficult to diagnose by cultivation. In addition, *Brucella* is considered a BSL-3 pathogen and is hazardous to handle in the clinical laboratory without protective clothing and at least a class II biological safety cabinet. Agglutination tests are most often used for serodiagnosis. In addition, enzyme-linked immunosorbent assays (ELISAs) are available to determine exposure to the organism. The antibiotics doxycycline or ciprofloxacin are typically prescribed in combination with rifampin; gentamicin, and streptomycin. Trimethoprim-sulfamethoxazole (TMP-SMZ) are also effective against *Brucella* infections and can be used if needed.

CAT-SCRATCH DISEASE

The zoonosis cat-scratch disease (CSD) (or cat-scratch fever) is a bacterial infection that can be introduced to the lymph nodes when a human is bitten or scratched by a cat. It is caused by the facultative intracellular gram-negative bacterium *Bartonella henselae*. Cats can become infected from flea feces containing *B. henselae* that they ingest while grooming. Humans become infected when flea feces or cat saliva (from claws or licking) containing *B. henselae* are introduced at the site of a bite or scratch. Once introduced into a wound, *B. henselae* infects red blood cells.

B. henselae invasion of red blood cells is facilitated by adhesins associated with outer membrane proteins and a secretion system that mediates the transport of virulence factors into the host cell. Evidence of infection is indicated if a small nodule with pus forms in the location of the scratch 1 to 3 weeks after the initial injury. The bacteria then migrate to the nearest lymph nodes, where they cause swelling and pain (lymphadenitis). Signs and symptoms may also include fever, chills, and fatigue. Most infections are mild and tend to be self-limiting. However, immunocompromised patients may develop bacillary angiomatosis (BA), characterized by the proliferation of blood vessels, resulting in the formation of tumor-like masses in the skin and internal organs; or bacillary peliosis (BP), characterized by multiple cyst-like, blood-filled cavities in the liver and spleen. Most cases of CSD can be prevented by keeping cats free of fleas and promptly cleaning a cat scratch with soap and warm water.

The diagnosis of CSD is difficult because the bacterium does not grow readily in the laboratory. When necessary, immunofluorescence, serological tests, PCR, and gene sequencing can be performed to identify the bacterial species. Given the limited nature of these infections, antibiotics are not normally prescribed. For immunocompromised patients, rifampin, azithromycin, ciprofloxacin, gentamicin (intramuscularly), or TMP-SMZ are generally the most effective options.

RAT-BITE FEVER

The zoonotic infection rat-bite fever can be caused by two different gram-negative bacteria: *Streptobacillus moniliformis*, which is more common in North America, and *Spirillum minor*, which is more common in Asia. Because of modern sanitation efforts, rat bites are rare in the US. However, contact with fomites, food, or water contaminated by rat feces or body fluids can also cause infections. Signs and symptoms of rat-bite fever include fever, vomiting, myalgia (muscle pain), arthralgia (joint pain), and a maculopapular rash on the hands and feet. An ulcer may also form at the site of a bite, along with some swelling of nearby lymph nodes. In most cases, the infection is self-limiting. Little is known about the virulence factors that contribute to these signs and symptoms of disease.

Cell culture, (matrix assisted laser desorption ionization-time of flight) MALDI-TOF mass spectrometry, PCR, or ELISA can be used in the identification of *Streptobacillus moniliformis*. The diagnosis of Spirillum *minus* may be confirmed by direct microscopic observation of the pathogens in blood using Giemsa or Wright stains, or dark-

field microscopy. Serological tests can be used to detect a host immune response to the pathogens after about 10 days. The most commonly used antibiotics to treat these infections are penicillin or doxycycline.

PLAGUE

The gram-negative bacillus *Yersinia pestis* causes the zoonotic infection plague. This bacterium causes acute febrile disease in animals, usually rodents or other small mammals, and humans. The disease is associated with a high mortality rate if left untreated. Historically, *Y. pestis* has been responsible for several devastating pandemics, resulting in millions of deaths. There are three forms of plague: bubonic plague (the most common form, accounting for about 80% of cases), pneumonic plague, and septicemic plague. These forms are differentiated by the mode of transmission and the initial site of infection. Figure 6.5 illustrates these various modes of transmission and infection between animals and humans.

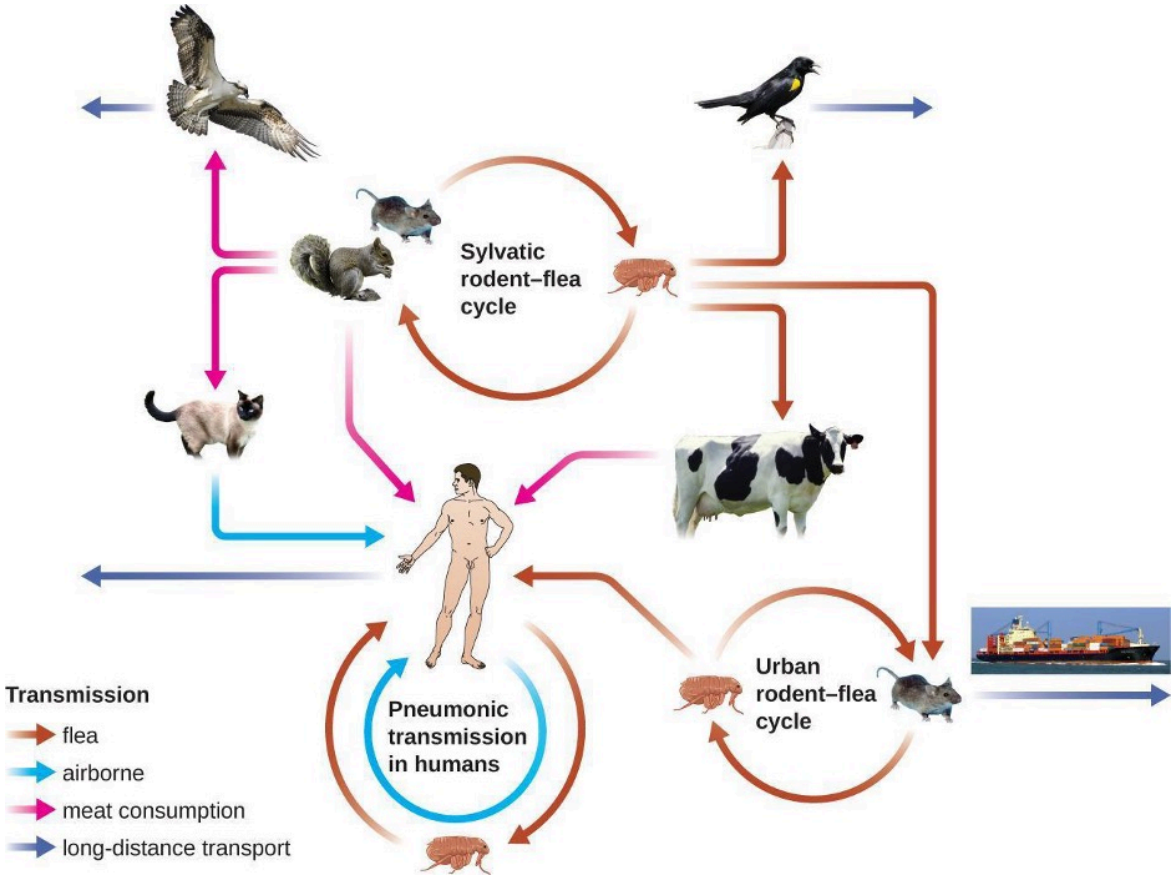

Figure 6.5: Yersinia pestis, the causative agent of plague, has numerous modes of transmission. The modes are divided into two ecological classes: urban and sylvatic (i.e., forest or rural). The urban cycle primarily involves transmission from infected urban mammals (rats) to humans by flea vectors (brown arrows). The disease may travel between urban centers (purple arrow) if infected rats find their way onto ships or trains. The sylvatic cycle involves mammals more common in nonurban environments. Sylvatic birds and mammals (including humans) may become infected after eating infected mammals (pink arrows) or by flea vectors. Pneumonic transmission occurs between humans or between humans and infected animals through the inhalation of Y. pestis in aerosols. *Figure description available at the end of the chapter.*

In bubonic plague, *Y. pestis* is transferred by the bite of infected fleas. Since most flea bites occur on the legs and ankles, *Y. pestis* is often introduced into the tissues and circulating blood in the lower extremities. After a 2- to 6-day incubation period, patients experience an abrupt onset fever (39.5–41 °C [103.1–105.8 °F]), headache,

hypotension, and chills. The pathogen localizes in lymph nodes, where it causes inflammation, swelling, and hemorrhaging that results in purple buboes (figure 6.6). Buboes often form in lymph nodes of the groin first because these are the nodes associated with the lower limbs; eventually, through circulation in the blood and lymph, lymph nodes throughout the body become infected and form buboes. The average mortality rate for bubonic plague is about 55% if untreated and about 10% with antibiotic treatment.

Septicemic plague occurs when *Y. pestis* is directly introduced into the bloodstream through a cut or wound and circulates through the body. The incubation period for septicemic plague is 1 to 3 days, after which patients develop fever, chills, extreme weakness, abdominal pain, and shock. Disseminated intravascular coagulation (DIC) can also occur, resulting in the formation of thrombi that obstruct blood vessels and promote ischemia and necrosis in surrounding tissues (figure 6.6). Necrosis occurs most commonly in extremities such as fingers and toes, which become blackened. Septicemic plague can quickly lead to death, with a mortality rate near 100% when it is untreated. Even with antibiotic treatment, the mortality rate is about 50%.

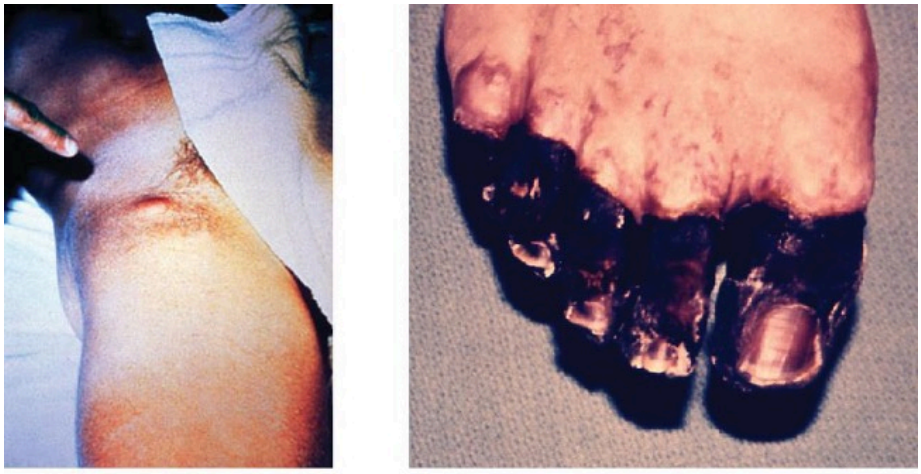

Figure 6.6: (a) Yersinia pestis infection can cause inflamed and swollen lymph nodes (buboes), like these in the groin of an infected patient. (b) Septicemic plague caused necrotic toes in this patient. Vascular damage at the extremities causes ischemia and tissue death. *Figure description available at the end of the chapter.*

Pneumonic plague occurs when *Y. pestis* causes an infection of the lungs. This can occur through inhalation of aerosolized droplets from an infected individual or, in patients with bubonic or septicemic plague, when the infection spreads to the lungs from elsewhere in the body. After an incubation period of 1 to 3 days, signs and symptoms include fever, headache, weakness, and a rapidly developing pneumonia with shortness of breath, chest pain, and cough producing bloody or watery mucus. The pneumonia may result in rapid respiratory failure and shock. Pneumonic plague is the only form of plague that can be spread from person to person by infectious aerosol droplets. If untreated, the mortality rate is near 100%; with antibiotic treatment, the mortality rate is about 50%, hence its potential as an agent of bioterrorism.

The high mortality rate for the plague is, in part, a consequence of it being unusually well equipped with virulence factors. To date, there are at least 15 different major virulence factors that have been identified from *Y. pestis* and, of these, eight are involved with adherence to host cells. In addition, the F1 component of the *Y. pestis* capsule is a virulence factor that allows the bacterium to avoid phagocytosis. F1 is produced in large quantities during mammalian infection and is the most immunogenic component.[11] Successful use of virulence factors allows the bacilli to disseminate from the area of the bite to regional lymph nodes and eventually the entire blood and lymphatic systems.

Culturing and direct microscopic examination of a sample of fluid from a bubo, blood, or sputum is the best way to identify *Y. pestis* and confirm a presumptive diagnosis of plague. Specimens may be stained using either a Gram, Giemsa, Wright, or Wayson's staining technique (figure 6.7). The bacteria show a characteristic bipolar staining pattern, resembling safety pins, that facilitates presumptive identification. Direct fluorescent antibody tests (rapid test of outer-membrane antigens) and serological tests like ELISA can be used to confirm the diagnosis. The confirmatory method for identifying *Y. pestis* isolates in the US is bacteriophage lysis.

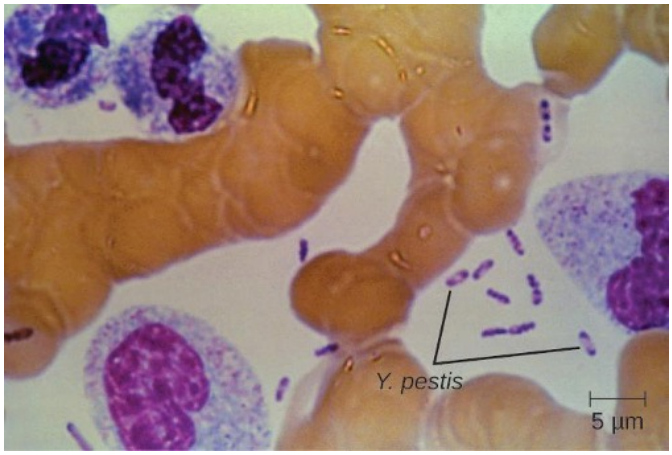

Figure 6.7: This Wright's stain of a blood sample from a patient with plague shows the characteristic "safety pin" appearance of Yersinia pestis. *Figure description available at the end of the chapter.*

Prompt antibiotic therapy can resolve most cases of bubonic plague, but septicemic and pneumonic plague are more difficult to treat because of their shorter incubation stages. Survival often depends on an early and accurate diagnosis and an appropriate choice of antibiotic therapy. In the US, the most common antibiotics used to treat patients with plague are gentamicin, streptomycin, fluoroquinolones (e.g., levofloxacin, ciprofloxacin) and doxycycline.

ZOONOTIC FEBRILE DISEASES

A wide variety of zoonotic febrile diseases (diseases that cause fever) are caused by pathogenic bacteria that require arthropod vectors. These pathogens are either obligate intracellular species of *Anaplasma, Bartonella*, *Ehrlichia, Orientia,* and *Rickettsia*, or spirochetes in the genus *Borrelia*. Isolation and identification of pathogens in this group are best performed in BSL2 or BSL-3 laboratories because of the low infective dose associated with the diseases.

Anaplasmosis

The zoonotic tick-borne disease human granulocytic anaplasmosis (HGA) is caused by the obligate intracellular pathogen *Anaplasma phagocytophilum*. HGA is endemic primarily in the central and northeastern US and in countries in Europe and Asia.

HGA is usually a mild febrile disease that causes flu-like symptoms in immunocompetent patients; however, symptoms are severe enough to require hospitalization in at least 50% of infections and, of those patients, less than 1% will die of HGA.[12] Small mammals such as white-footed mice, chipmunks, and voles have been identified as reservoirs of *A. phagocytophilum*, which is transmitted by the bite of an *Ixodes* tick. Five major virulence factors[13] have been reported in *Anaplasma*; three are adherence factors and two are factors that allow the pathogen to avoid the human immune response. Diagnostic approaches include locating intracellular microcolonies (morulae) of *Anaplasma* through microscopic examination of neutrophils or eosinophils stained with Giemsa or Wright stain, PCR for detection of *A. phagocytophilum*, and serological tests to detect antibody titers against the pathogens. The primary antibiotic used for treatment is doxycycline.

Ehrlichiosis

Human monocytotropic ehrlichiosis (HME) is a zoonotic, tick-borne disease caused by the BSL-2, obligate intracellular pathogen *Ehrlichia chaffeensis*. Currently, the primary geographic distribution of HME is in the eastern half of the US. A few cases are reported in the West, which corresponds with the known geographic distribution of the primary vector, the lone star tick (*Amblyomma americanum*). Symptoms of HME are similar to the flu-like symptoms observed in anaplasmosis, but a rash is more common, with 60% of children and less than 30% of adults developing petechial, macula, and maculopapular rashes.[14] Virulence factors allow *E. chaffeensis* to adhere to and infect monocytes, forming intracellular microcolonies in monocytes that are diagnostic for HME. Diagnosis of HME can be confirmed with PCR and serologic tests. The first-line treatment for adults and children of all ages with HME is doxycycline.

Epidemic Typhus

The disease epidemic typhus is caused by *Rickettsia prowazekii* and is transmitted by the body lice, *Pediculus humanus*. Flying squirrels are animal reservoirs of *R. prowazekii* in North America and can also be sources of lice capable of transmitting the pathogen. Epidemic typhus is characterized by a high fever and body aches that last for about 2 weeks. A rash develops on the abdomen and chest and radiates to the extremities. Severe cases can result in death from shock or damage to heart and brain tissues. Infected humans are an important reservoir for this bacterium because *R. prowazekii* is the only *Rickettsia* that can establish a chronic carrier state in humans.

Epidemic typhus has played an important role in human history, causing large outbreaks with high mortality rates during times of war or adversity. During World War I, epidemic typhus killed more than 3 million people on the Eastern front.[15] With the advent of effective insecticides and improved personal hygiene, epidemic typhus is now quite rare in the US. In the developing world, however, epidemics can lead to mortality rates of up to 40% in the absence of treatment.[16] In recent years, most outbreaks have taken place in Burundi, Ethiopia, and Rwanda. For example, an outbreak in Burundi refugee camps in 1997 resulted in 45,000 illnesses in a population of about 760,000 people.[17]

A rapid diagnosis is difficult because of the similarity of the primary symptoms with those of many other diseases. Molecular and immunohistochemical diagnostic tests are the most useful methods for establishing a diagnosis during the acute stage of illness when therapeutic decisions are critical. PCR to detect distinctive genes from *R. prowazekii* can be used to confirm the diagnosis of epidemic typhus, along with immunofluorescent staining of tissue biopsy specimens. Serology is usually used to identify rickettsial infections. However, adequate antibody titers take up to 10 days to develop. Antibiotic therapy is typically begun before the diagnosis is complete, which may be only achieved retrospectively with convalescent titers. The most common drugs used to treat patients with epidemic typhus are doxycycline or chloramphenicol.

Murine (Endemic) Typhus

Murine typhus (also known as endemic typhus) is caused by *Rickettsia typhi* and is transmitted by the bite of the rat flea, *Xenopsylla cheopis.* Infected rats are the main reservoir. Clinical signs and symptoms of murine typhus include a rash and chills accompanied by headache and fever that last about 12 days. Some patients also exhibit a cough and pneumonia-like symptoms. Severe illness can develop in immunocompromised patients, with seizures, coma, and renal and respiratory failure.

Clinical diagnosis of murine typhus can be confirmed from a biopsy specimen from the rash. Diagnostic tests include indirect immunofluorescent antibody (IFA) staining, PCR for *R. typhi*, and acute and convalescent sero-

logic testing. Primary treatment is doxycycline, with fluoroquinolones as the second choice and chloramphenicol in the case of a patient who is pregnant.

Rocky Mountain Spotted Fever

The disease Rocky Mountain spotted fever (RMSF) is caused by *Rickettsia rickettsii* and is transmitted by the bite of a hard-bodied tick such as the American dog tick (*Dermacentor variabilis*), Rocky Mountain wood tick (*D. andersoni*), or brown dog tick (*Rhipicephalus sanguineus*).

This disease is endemic in North and South America and its incidence is coincident with the arthropod vector range. Despite its name, most cases in the US do not occur in the Rocky Mountain region but in the Southeast and South Central regions with North Carolina, Tennessee, Missouri, Arkansas, and Oklahoma accounting for greater than 60% of all cases.[18] The map in figure 6.8 shows the distribution of prevalence in the US in 2021.

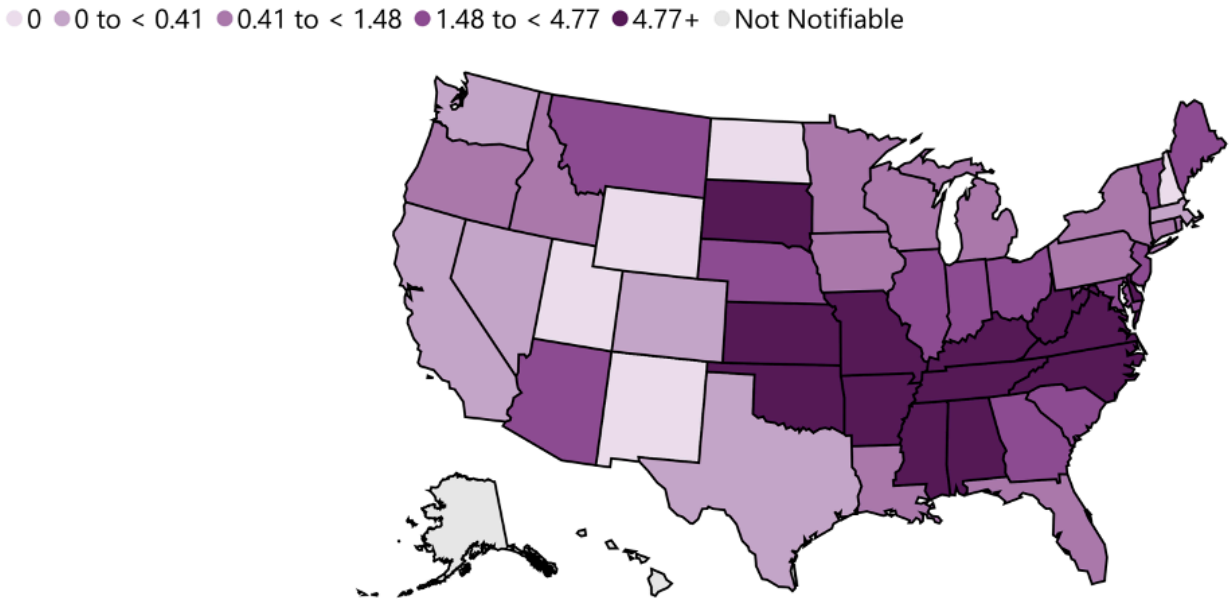

Figure 6.8: Annual incidence (per million of population) of reported spotted fever rickettsiosis in the United States in 2021. In the US, Rocky Mountain spotted fever is most prevalent in the southeastern states. *Figure description available at the end of the chapter.*

Signs and symptoms of RMSF include a high fever, headache, body aches, nausea, and vomiting. A petechial rash (similar in appearance to measles) begins on the hands and wrists, and spreads to the trunk, face, and extremities (figure 6.9). If untreated, RMSF is a serious illness that can be fatal in the first 8 days even in otherwise healthy patients. Ideally, treatment should begin before petechiae develop, because this is a sign of progression to severe disease; however, the rash usually does not appear until day 6 or later after onset of symptoms and only occurs in 35%–60% of patients with the infection. Increased vascular permeability associated with petechiae formation can result in fatality rates of 3% or greater, even in the presence of clinical support. Most deaths are due to hypotension and cardiac arrest or from ischemia following blood coagulation.

Diagnosis can be challenging because the disease mimics several other diseases that are more prevalent. The diagnosis of RMSF is made based on symptoms, fluorescent antibody staining of a biopsy specimen from the rash, PCR for *Rickettsia rickettsii*, and acute and convalescent serologic testing. Primary treatment is doxycycline, with chloramphenicol as the second choice.

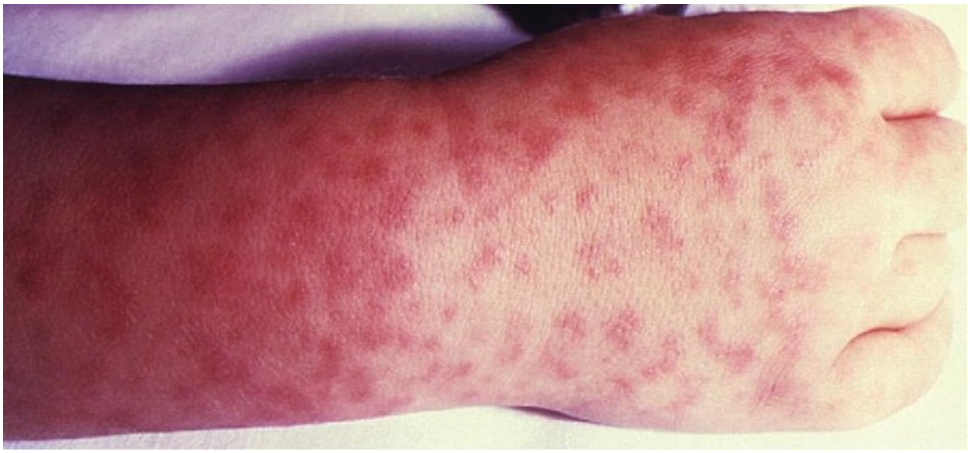

Figure 6.9: Rocky Mountain spotted fever causes a petechial rash. Unlike epidemic or murine typhus, the rash begins at the hands and wrists and then spreads to the trunk. *Figure description available at the end of the chapter.*

Lyme Disease

Lyme disease is caused by the spirochete *Borrelia burgdorferi* that is transmitted by the bite of a hard-bodied, black-legged *Ixodes* tick. *I. scapularis* is the biological vector transmitting *B. burgdorferi* in the eastern and north-central US and *I. pacificus* transmits *B. burgdorferi* in the western US. Different species of *Ixodes* ticks are responsible for *B. burgdorferi* transmission in Asia and Europe. In the US, Lyme disease is the most commonly reported vector-borne illness. In 2014, it was the fifth most common Nationally Notifiable disease.[19]

Ixodes ticks have complex life cycles and deer, mice, and even birds can act as reservoirs. Over 2 years, the ticks pass through four developmental stages and require a blood meal from a host at each stage. In the spring, tick eggs hatch into six-legged larvae. These larvae do not carry *B. burgdorferi* initially. They may acquire the spirochete when they take their first blood meal (typically from a mouse). The larvae then overwinter and molt into eight-legged nymphs in the following spring. Nymphs take blood meals primarily from small rodents, but may also feed on humans, burrowing into the skin. The feeding period can last several days to a week, and it typically takes 24 hours for an infected nymph to transmit enough *B. burgdorferi* to cause infection in a human host. Risk of infection can be significantly reduced with immediate removal of the tick (figure 6.10). Nymphs ultimately mature into male and female adult ticks, which tend to feed on larger animals like deer or, occasionally, humans. The adults then mate and produce eggs to continue the cycle (figure 6.11).

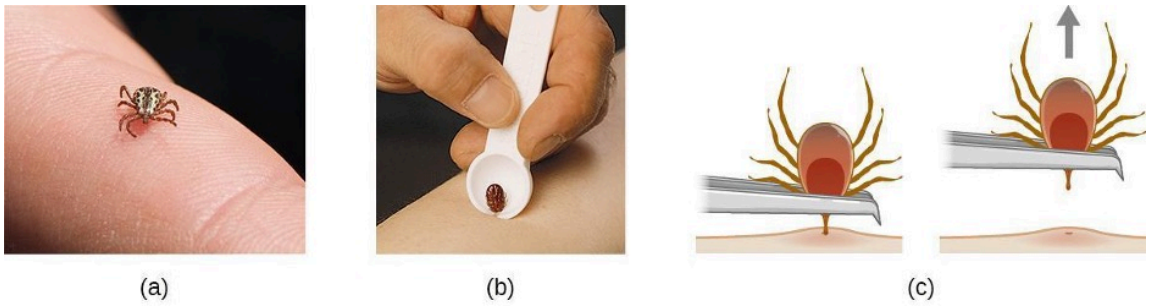

Figure 6.10: (a) This black-legged tick, also known as the deer tick, has not yet attached to the skin. (b) A notched tick extractor can be used for removal. (c) To remove an attached tick with fine-tipped tweezers, pull gently on the mouth parts until the tick releases its hold on the skin. Avoid squeezing the tick's body, because this could release pathogens and thus increase the risk of contracting Lyme disease. *Figure description available at the end of the chapter.*

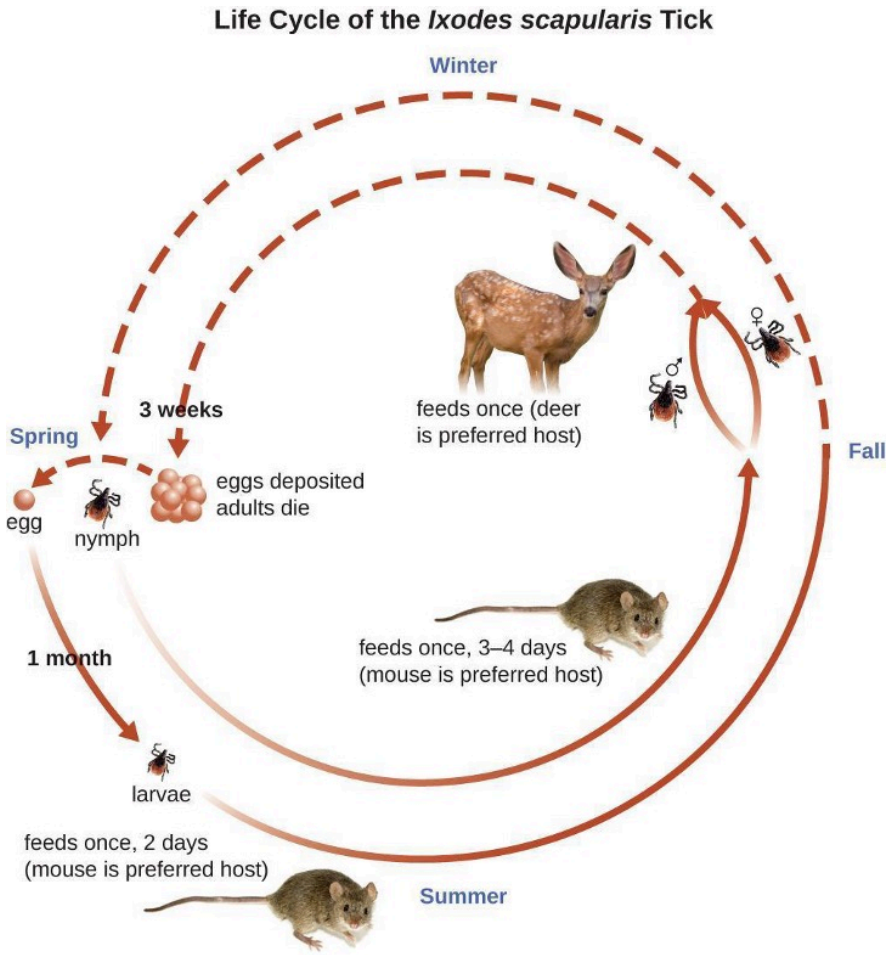

Figure 6.11: This image shows the 2-year life cycle of the black-legged tick, the biological vector of Lyme disease. *Figure description available at the end of the chapter.*

The symptoms of Lyme disease follow three stages: early localized, early disseminated, and late stage. During the early-localized stage, approximately 70%–80%[20] of cases may be characterized by a bull's-eye rash, called erythema migrans, at the site of the initial tick bite. The rash forms 3 to 30 days after the tick bite (7 days is the average) and may also be warm to the touch (figure 6.12).[21] This diagnostic sign is often overlooked if the tick bite occurs on the scalp or another less visible location. Other early symptoms include flu-like symptoms such as malaise, headache, fever, and muscle stiffness. If the patient goes untreated, the second early-disseminated stage of the disease occurs days to weeks later. The symptoms at this stage may include severe headache, neck stiffness, facial paralysis, arthritis, and carditis. The late-stage manifestations of the disease may occur years after exposure. Chronic inflammation causes damage that can eventually cause severe arthritis, meningitis, encephalitis, and altered mental states. The disease may be fatal if untreated.

A presumptive diagnosis of Lyme disease can be made based solely on the presence of a bull's-eye rash at the site of infection, if it is present, in addition to other associated symptoms (figure 6.12). In addition, indirect immunofluorescent antibody (IFA) labeling can be used to visualize bacteria from blood or skin biopsy specimens. Serological tests like ELISA can also be used to detect serum antibodies produced in response to infection. During the early stage of infection (about 30 days), antibacterial drugs such as amoxicillin and doxycycline are effective. In the later stages, penicillin G or ceftriaxone can be given intravenously.

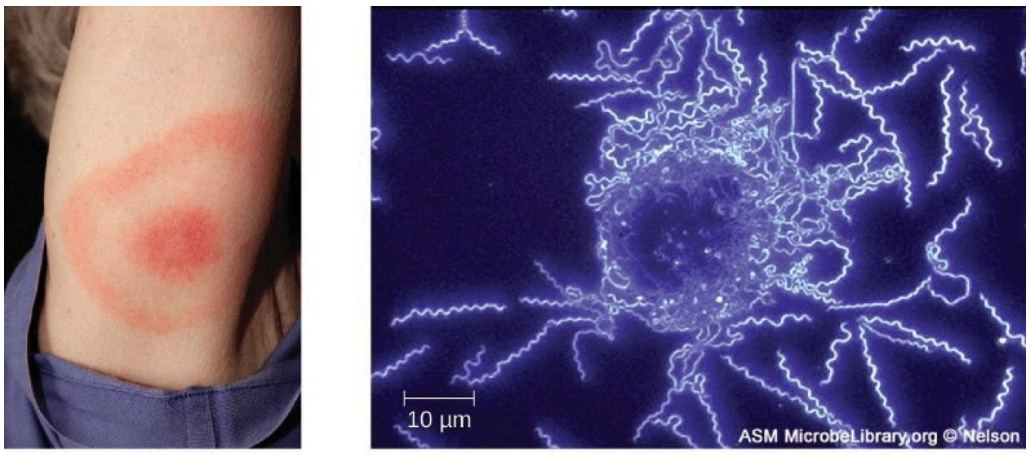

Figure 6.12: (a) A characteristic bull's eye rash of Lyme disease forms at the site of a tick bite. (b) A darkfield micrograph shows Borrelia burgdorferi, the causative agent of Lyme disease. *Figure description available at the end of the chapter.*

Relapsing Fever

Borrelia spp. also can cause relapsing fever. Two of the most common species are *B. recurrentis*, which causes epidemics of louse-borne relapsing fever, and *B. hermsii*, which causes tick-borne relapsing fevers. These *Borrelia* species are transmitted by the body louse *Pediculus humanus* and the soft-bodied tick *Ornithodoros hermsi*, respectively. Lice acquire the spirochetes from human reservoirs, whereas ticks acquire them from rodent reservoirs. Spirochetes infect humans when *Borrelia* in the vector's saliva or excreta enter the skin rapidly as the vector bites.

In both louse- and tick-borne relapsing fevers, bacteremia usually occurs after the initial exposure, leading to a sudden high fever (39–43 °C [102.2–109.4 °F) typically accompanied by headache and muscle aches. After about 3 days, these symptoms typically subside, only to return again after about a week. After another 3 days, the symptoms subside again but return a week later, and this cycle may repeat several times unless it is disrupted by antibiotic treatment. Immune evasion through bacterial antigenic variation is responsible for the cyclical nature of the symptoms in these diseases.

The diagnosis of relapsing fever can be made by observation of spirochetes in blood, using darkfield microscopy (figure 6.13). For louse-borne relapsing fever, doxycycline or erythromycin are the first-line antibiotics. For tick-borne relapsing fever, doxycycline or erythromycin are the first-line antibiotics.

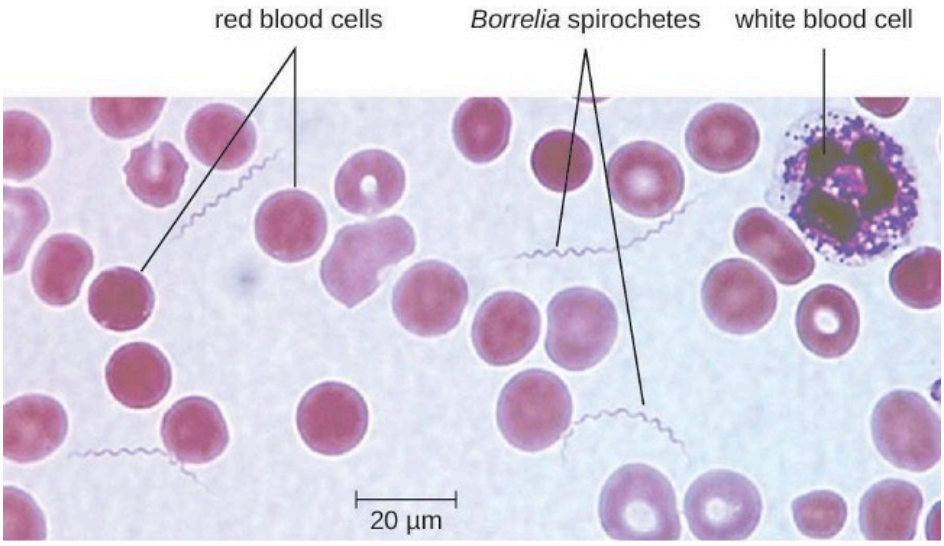

Figure 6.13: A peripheral blood smear from a patient with tick-borne relapsing fever. Borrelia appears as thin spirochetes among the larger red blood cells. *Figure description available at the end of the chapter.*

Trench Fever

The louse-borne disease trench fever was first characterized as a specific disease during World War I, when approximately 1 million soldiers were infected. Today, it is primarily limited to areas of the developing world where conditions lead to infestations of lice (e.g., overpopulated urban areas and refugee camps). Trench fever is caused by the gram-negative bacterium *Bartonella quintana*, which is transmitted when feces from infected body lice, *Pediculus humanus* var *corporis*, are rubbed into the louse bite, abraded skin, or the conjunctiva. The symptoms typically follow a 5-day course marked by a high fever, body aches, conjunctivitis, ocular pain, severe headaches, and severe bone pain in the shins, neck, and back. Diagnosis can be made using blood cultures; serological tests like ELISA can be used to detect antibody titers to the pathogen and PCR can also be used. The first-line antibiotics are doxycycline, macrolide antibiotics, and ceftriaxone.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Anaplasmosis (HGA)	*Anaplasma phagocytophilum*	Fever, flu-like symptoms	From small-mammal reservoirs via tick vector	Blood smear, PCR	Doxycycline
Brucellosis	*Brucella melitensis, B. abortus, B. canis, B. suis*	Granuloma, undulating fever, chronic flu-like symptoms	Direct contact with infected livestock or animals	Agglutination tests, ELISA	Doxycycline, rifampin
Cat-scratch disease	*Bartonella henselae*	Lymph-node swelling and pain, fever, chills, fatigue	Bite or scratch from domestic cats	Immunofluorescence, serological tests, PCR	None for immunocompetent patients
Ehrlichiosis (HME)	*Ehrlichia chaffeensis*	Flu-like symptoms, rash	Lone star tick vector	Serologic tests, PCR	Doxycycline

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Endocarditis/ pericarditis	*Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., *HACEK* bacilli	Chest pain, difficulty breathing, dry cough, fever; potentially fatal damage to heart valves	Pathogens introduced to bloodstream via contaminated catheters, dental procedures, piercings, or wounds	Echocardiogram, blood culture	Ampicillin, nafcillin, gentamicin, others; based on susceptibility testing
Epidemic typhus	*Rickettsia prowazekii*	High fever, body aches, rash; potentially fatal damage to heart and brain	From rodent reservoir via body louse vector	PCR, immunofluorescenc e	Doxycycline, chloramphenicol
Gas gangrene	*Clostridium perfringens,* other *Clostridium* spp.	Rapidly spreading myonecrosis, edema, yellowish and then purple discharge from wound, pockets of gas in tissues, septic shock and death	Germination of endospores in ischemic tissues, typically due to injury or chronic disease (e.g., diabetes)	Wound culture	Penicillin G, clindamycin, metronidazole
Infectious arthritis (septic arthritis)	*Staphylococcus aureus, Neisseria gonorrhoeae*	Joint pain and swelling, limited range of motion	Infection spreads to joint via circulatory system from wound or surgical site	Synovial fluid culture	Oxacillin, cefazolin, cephtriaxone
Lyme disease	*Borrelia burgdorferi*	Early localized: bull's eye rash, malaise, headache, fever, muscle stiffness; early disseminated: stiff neck, facial paralysis, arthritis, carditis; late-stage: arthritis, meningitis, possibly fatal	From deer, rodent, bird reservoirs via tick vector	IFA, serology, and ELISA	Amoxicillin, doxycycline, penicillin G, chloramphenicol, ceftriaxone
Murine (endemic) typhus	*Rickettsia typhi*	Low-grade fever, rash, headache, cough	From rodents or between humans via rat flea vector	Biopsy, IFA, PCR	Doxycycline, chloramphenicol
Osteomyelitis	*Staphylococcus aureus, Streptococcus pyogenes,* others	Inflammation of bone tissue, leading to fever, localized pain, edema, ulcers, bone loss	Pathogens introduced through trauma, prosthetic joint replacement, or from other infected body site via bloodstream	Radiograph of affected bone, culture of bone biopsy specimen	Cephalosporin, penicillins, others

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Plague	*Yersinia pestis*	Bubonic: buboes, fever, internal hemorrhaging; septicemic: fever, abdominal pain, shock, DIC, necrosis in extremities; pneumonic: acute pneumonia, respiratory failure, shock. All forms have high mortality rates.	Transmitted from mammal reservoirs via flea vectors or consumption of infected animal; transmission of pneumonic plague between humans via respiratory aerosols	Culture of bacteria from lymph, blood, or sputum samples; DFA, ELISA	Gentamycin, fluoroquinolones, others
Puerperal sepsis	*Streptococcus pyogenes*, many others	Rapid-onset fever, shock, and death	Pathogens introduced during or immediately following childbirth	Wound, urine, or blood culture	As determined by susceptibility testing
Rat-bite fever	*Streptobacillus moniliformis, Spirillum minor*	Fever, muscle and joint pain, rash, ulcer	Bite from infected rat or exposure to rat feces or body fluids in contaminated food or water	Observation of the organism from samples and antibody tests	Penicillin
Relapsing fever	*Borrelia recurrentis, B. hermsii,* other *Borrelia* spp.	Recurring fever, headache, muscle aches	From rodent or human reservoir via body louse or tick vector	Darkfield microscopy	Doxycycline, tetracycline, erythromycin
Rheumatic fever	*Streptococcus pyogenes*	Joint pain and swelling, inflammation and scarring of heart valves, heart murmur	Sequela of streptococcal pharyngitis	Serology, electrocardiogram, echocardiogram	Benzathine benzylpenicillin
Rocky Mountain spotted fever	*Rickettsia rickettsia*	High fever, headache, body aches, nausea and vomiting, petechial rash; potentially fatal hypotension and ischemia due to blood coagulation	From rodent reservoir via tick vectors	Biopsy, serology, PCR	Doxycycline, chloramphenicol
Toxic shock syndrome (TSS)	*Staphylococcus aureus*	Sudden high fever, vomiting, diarrhea, hypotension, death	Pathogens from localized infection spread to bloodstream; pathogens introduced on tampons or other intravaginal products	Serology, toxin identification from isolates	Clindamycin, vancomycin

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Toxic shock-like syndrome (STSS)	*Streptococcus pyogenes*	Sudden high fever, vomiting, diarrhea, acute respiratory distress syndrome (ARDS), hypoxemia, necrotizing fasciitis, death	Sequela of streptococcal skin or soft-tissue infection	Serology, blood culture, urinalysis	Penicillin, cephalosporin
Trench fever	*Bartonella Quintana*	High fever, conjunctivitis, ocular pain, headaches, severe pain in bones of shins, neck, and back	Between humans via body louse vector	Blood culture, ELISA, PCR	Doxycycline, macrolide antibiotics, ceftriaxone
Tularemia (rabbit fever)	*Francisella tularensis*	Skin lesions, fever, chills, headache, buboes	Eating or handling infected rabbit; transmission from infected animal via tick or fly vector; aerosol transmission (in laboratory or as bioweapon)	DFA	Streptomycin, gentamycin, others

Table 6.1: Bacterial infections of the circulatory and lymphatic systems

6.3 VIRAL INFECTIONS OF THE CIRCULATORY AND LYMPHATIC SYSTEMS

Viral pathogens of the circulatory system vary tremendously both in their virulence and distribution worldwide. Some of these pathogens are practically global in their distribution. Fortunately, the most ubiquitous viruses tend to produce the mildest forms of disease (table 6.2). In the majority of cases, those infected remain asymptomatic. On the other hand, other viruses are associated with life-threatening diseases that have impacted human history.

INFECTIOUS MONONUCLEOSIS AND BURKITT LYMPHOMA

Human herpesvirus 4, also known as Epstein-Barr virus (EBV), has been associated with a variety of human diseases, such as mononucleosis and Burkitt lymphoma. Exposure to the human herpesvirus 4 (HHV-4) is widespread and nearly all people have been exposed at some time in their childhood, as evidenced by serological tests on populations. The virus primarily resides within B lymphocytes and, like all herpes viruses, can remain dormant in a latent state for a long time.

When uninfected young adults are exposed to EBV, they may experience infectious mononucleosis. The virus is mainly spread through contact with body fluids (e.g., saliva, blood, and semen). The main symptoms include pharyngitis, fever, fatigue, and lymph node swelling. Abdominal pain may also occur as a result of spleen and liver enlargement in the second or third week of infection. The disease typically is self-limiting after about a month. The main symptom, extreme fatigue, can continue for several months, however. Complications in

immunocompetent patients are rare but can include jaundice, anemia, and possible rupture of an enlarged spleen.

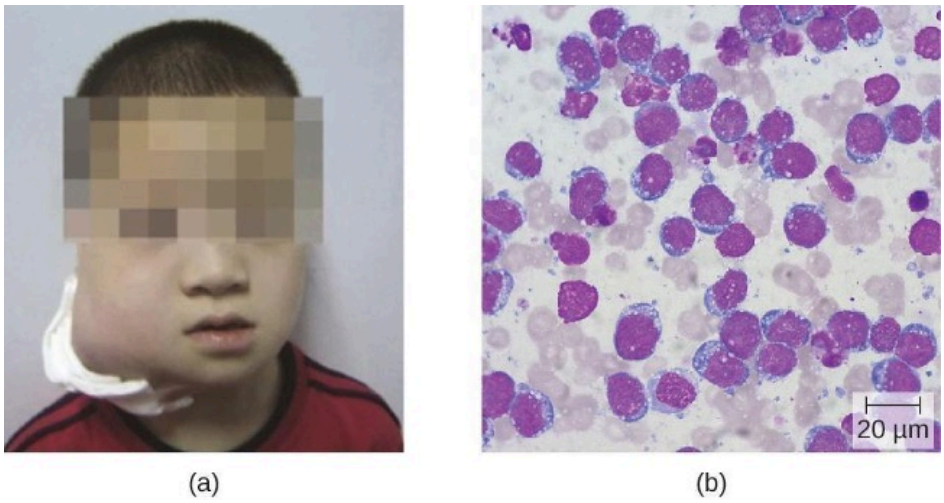

Figure 6.14: (a) Burkitt lymphoma can cause large tumors. (b) Characteristic irregularly shaped abnormal lymphocytes (large purple cells) with vacuoles (white spots) from a fine-needle aspirate of a tumor from a patient with Burkitt lymphoma. *Figure description available at the end of the chapter.*

In patients with malaria or HIV, Epstein-Barr virus can lead to a fast-growing malignant cancer known as Burkitt lymphoma (figure 6.14). This condition is a form of non-Hodgkin lymphoma that produces solid tumors chiefly consisting of aberrant B cells. Burkitt lymphoma is more common in Africa, where the prevalence of HIV and malaria is high, and Burkitt lymphoma more frequently afflicts children. Repeated episodes of viremia caused by reactivation of the virus are common in immunocompromised individuals. In some patients with AIDS, EBV may induce the formation of malignant B-cell lymphomas or oral hairy leukoplakia. Immunodeficiency-associated Burkitt lymphoma primarily occurs in patients with HIV. HIV infection, similar to malaria, leads to polyclonal B-cell activation and permits poorly controlled proliferation of EBV+ B cells, leading to the formation of lymphomas.

Infectious mononucleosis is typically diagnosed based on the initial clinical symptoms and a test for antibodies to EBV-associated antigens. Because the disease is self-limiting, antiviral treatments are rare for mononucleosis. Cases of Burkitt lymphoma are diagnosed from a biopsy specimen from a lymph node or tissue from a suspected tumor. Staging of the cancer includes computed tomography (CT) scans of the chest, abdomen, pelvis, and cytologic and histologic evaluation of biopsy specimens. Because the tumors grow so rapidly, staging studies must be expedited and treatment must be initiated promptly. An intensive alternating regimen of cyclophosphamide, vincristine, doxorubicin, methotrexate, ifosfamide, etoposide, and cytarabine (CODOX-M/IVAC) plus rituximab results in a cure rate greater than 90% for children and adults.

CYTOMEGALOVIRUS INFECTIONS

Also known as cytomegalovirus (CMV), human herpesvirus 5 (HHV-5) is a virus with high infection rates in the human population. It is currently estimated that 50% of people in the US have been infected by the time they reach adulthood.[22] CMV is the major cause of non-Epstein-Barr infectious mononucleosis in the general human population. It is also an important pathogen in immunocompromised hosts, including patients with AIDS, neonates, and transplant recipients. However, the vast majority of CMV infections are asymptomatic. In adults, if symptoms do occur, they typically include fever, fatigue, swollen glands, and pharyngitis.

CMV can be transmitted between individuals through contact with body fluids such as saliva or urine. Common modes of transmission include sexual contact, nursing, blood transfusions, and organ transplants. In addition, pregnant women with active infections frequently pass this virus to their fetus, resulting in congenital CMV

infections, which occur in approximately one in every 200 infants in the US.[23] Infants can also be infected during passage through the birth canal or through breast milk and saliva from the mother.

Perinatal infections tend to be milder but can occasionally cause lung, spleen, or liver damage. Serious symptoms in newborns include growth retardation, jaundice, deafness, blindness, and mental retardation if the virus crosses the placenta during the embryonic state when the body systems are developing in utero. However, a majority (approximately 80%) of infected infants will never have symptoms or experience long-term problems.[24] Diagnosis of CMV infection during pregnancy is usually achieved by serology; CMV is the "C" in prenatal TORCH screening.

Many patients receiving blood transfusions or transplants ultimately become infected with CMV. Approximately 60% of transplant recipients will have CMV infection and more than 20% will develop symptomatic disease.[25] These infections may result from CMV-contaminated tissues but also may be a consequence of immunosuppression required for transplantation causing reactivation of prior CMV infections. The resulting viremia can lead to fever and leukopenia, a decrease in the number of white blood cells in the bloodstream. Serious consequences may include liver damage, transplant rejection, and death. For similar reasons, many patients with AIDS develop active CMV infections that can manifest as encephalitis or progressive retinitis leading to blindness.[26]

Diagnosis of a localized CMV infection can be achieved through direct microscopic evaluation of tissue specimens stained with routine stains (e.g., Wright-Giemsa, hematoxylin and eosin, Papanicolaou) and immunohistochemical stains. Cells infected by CMV produce characteristic inclusions with an "owl's eye" appearance; this sign is less sensitive than molecular methods like PCR but more predictive of localized disease (figure 6.15). For more severe CMV infection, tests such as enzyme immunoassay (EIA), indirect immunofluorescence antibody (IFA) tests, and PCR, which are based on detection of CMV antigen or DNA, have a higher sensitivity and can determine viral load. Cultivation of the virus from saliva or urine is still the method for detecting CMV in newborn babies up to 3 weeks old. Ganciclovir and valganciclovir are the first-line antiviral drugs for serious CMV infections.

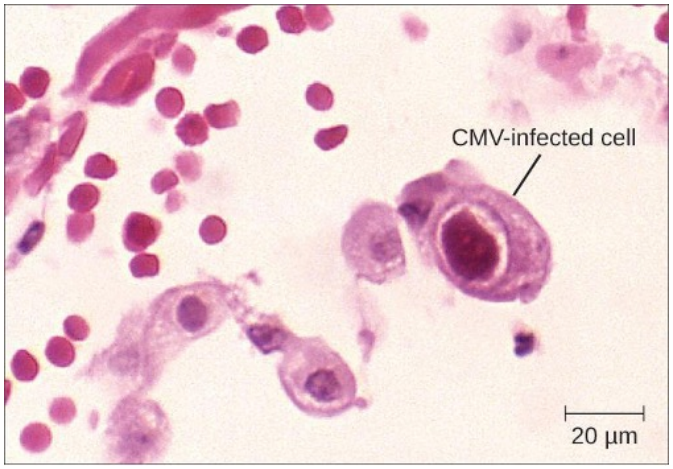

Figure 6.15: Cells infected with CMV become enlarged and have a characteristic "owl's eye" nucleus. This micrograph shows kidney cells from a patient with CMV. *Figure description available at the end of the chapter.*

ARTHROPOD-BORNE VIRAL DISEASES

There are a number of arthropod-borne viruses, or arboviruses, that can cause human disease. Among these are several important hemorrhagic fevers transmitted by mosquitoes. We will discuss three that pose serious threats: yellow fever, chikungunya fever, and dengue fever.

Yellow Fever

Yellow fever was once common in the US and caused several serious outbreaks between 1700 and 1900.[27] Through vector control efforts, however, this disease has been eliminated in the US. Currently, yellow fever occurs primarily in tropical and subtropical areas in South America and Africa. It is caused by the yellow

fever virus of the genus *Flavivirus* (named for the Latin word *flavus* meaning *yellow*), which is transmitted to humans by mosquito vectors. Sylvatic yellow fever occurs in tropical jungle regions of Africa and Central and South America, where the virus can be transmitted from infected monkeys to humans by the mosquitoes *Aedes africanus* or *Haemagogus* spp. In urban areas, the *Aedes aegypti* mosquito is mostly responsible for transmitting the virus between humans.

Most individuals infected with yellow fever virus have no illness or only mild disease. Onset of milder symptoms is sudden, with dizziness, fever of 39–40 °C (102–104 °F), chills, headache, and myalgias. As symptoms worsen, the face becomes flushed, and nausea, vomiting, constipation, severe fatigue, restlessness, and irritability are common. Mild disease may resolve after 1 to 3 days. However, approximately 15% of cases progress to develop moderate to severe yellow fever disease.[28]

In moderate or severe disease, the fever falls suddenly 2 to 5 days after onset, but recurs several hours or days later. Symptoms of jaundice, petechial rash, mucosal hemorrhages, oliguria (scant urine), epigastric tenderness with bloody vomit (hematemesis), confusion, and apathy also often occur for approximately 7 days of moderate to severe disease. After more than a week, patients may have a rapid recovery and no sequelae.

In its most severe form, called malignant yellow fever, symptoms include delirium, bleeding, seizures, shock, coma, and multiple organ failure; in some cases, death occurs. Patients with malignant yellow fever also become severely immunocompromised, and even those in recovery may become susceptible to bacterial superinfections and pneumonia.

Diagnosis of yellow fever is often based on clinical signs and symptoms and, if applicable, the patient's travel history, but infection can be confirmed by culture, serologic tests, PCR or immunohistochemistry. There are no effective treatments for patients with yellow fever. Whenever possible, patients with yellow fever should be hospitalized for close observation and given supportive care. Prevention is the best method of controlling yellow fever. Use of mosquito netting, window screens, insect repellents, and insecticides are all effective methods of reducing exposure to mosquito vectors. An effective vaccine is also available, but in the US, it is only administered to those traveling to areas with endemic yellow fever. In West Africa, the World Health Organization (WHO) launched a Yellow Fever Initiative in 2006 and, since that time, significant progress has been made in combating yellow fever.

Dengue Fever

The disease dengue fever, also known as breakbone fever, is caused by four serotypes of dengue virus called dengue 1–4. These are *Flavivirus* species that are transmitted to humans by *A. aegypti* or *A. albopictus* mosquitoes. The disease is distributed worldwide but is predominantly located in tropical regions. The WHO estimates that 50 million to 100 million infections occur yearly, including 500,000 dengue hemorrhagic fever (DHF) cases and 22,000 deaths, most among children.[29] Dengue fever is primarily a self-limiting disease characterized by abrupt onset of high fever up to 40 °C (104 °F), intense headaches, rash, slight nose or gum bleeding, and extreme muscle, joint, and bone pain, causing patients to feel as if their bones are breaking, hence the alternative name breakbone fever. As the body temperature returns to normal, in some patients, signs of dengue hemorrhagic fever may develop that include drowsiness, irritability, severe abdominal pain, severe nose or gum bleeding, persistent vomiting, vomiting blood, and black, tarry stools, as the disease progresses to DHF or dengue shock syndrome (DSS). Patients who develop DHF experience circulatory system failure caused by increased blood vessel permeability. Patients with dengue fever can also develop DSS from vascular collapse because of the severe drop in blood pressure. Patients who develop DHF or DSS are at greater risk for death without prompt and appropriate supportive treatment. About 30% of patients with severe hemorrhagic disease with poor supportive treatment die, but mortality can be less than 1% with experienced support.[30]

Diagnostic tests for dengue fever include serologic testing, ELISA, and reverse transcriptase-polymerase chain reaction (RT-PCR) of blood. There are no specific antiviral treatments for dengue fever. Instead, supportive clinical care is provided to treat the symptoms of the disease. The best way to limit the impact of this viral pathogen is vector control.

Chikungunya Fever

The arboviral disease chikungunya fever is caused by chikungunya virus (CHIKV), which is transmitted to humans by *A. aegypti* and *A. albopictus* mosquitoes. Until 2013, the disease had not been reported outside of Africa, Asia, and a few European countries; however, CHIKV has now spread to mosquito populations in North and South America. Chikungunya fever is characterized by high fever, joint pain, rash, and blisters, with joint pain persisting for several months. These infections are typically self-limiting and rarely fatal.

The diagnostic approach for chikungunya fever is similar to that for dengue fever. Viruses can be cultured directly from patient serum during early infections. IFA, EIA, ELISA, PCR, and RT-PCR are available to detect CHIKV antigens and patient antibody response to the infection. There are no specific treatments for this disease except to manage symptoms with fluids, analgesics, and bed rest. As with most arboviruses, the best strategy for combating the disease is vector control.

EBOLA VIRUS DISEASE

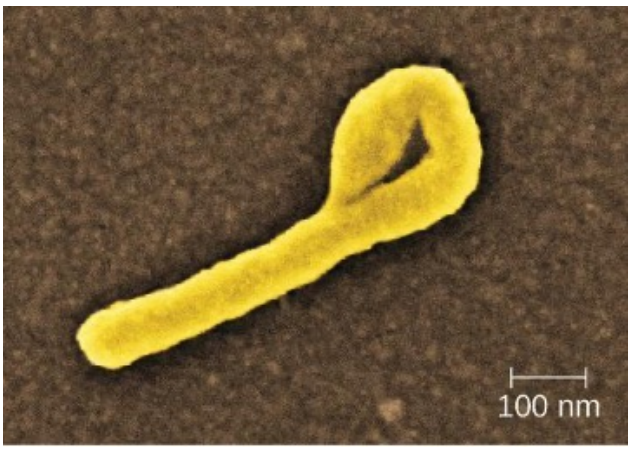

Figure 6.16: An Ebola virus particle viewed with electron microscopy. These filamentous viruses often exhibit looped or hooked ends. *Figure description available at the end of the chapter.*

The Ebola virus disease (EVD) is a highly contagious disease caused by species of *Ebolavirus*, a BSL-4 filovirus (figure 6.16). Transmission to humans occurs through direct contact with body fluids (e.g., blood, saliva, sweat, urine, feces, or vomit), and indirect contact by contaminated fomites. Infected patients can easily transmit Ebola virus to others if appropriate containment and use of personal protective equipment is not available or used. Handling and working with patients with EVD is extremely hazardous to the general population and healthcare workers. In almost every EVD outbreak there have been Ebola infections among healthcare workers. This ease of Ebola virus transmission was recently demonstrated in the Ebola epidemic in Guinea, Liberia, and Sierra Leone in 2014, in which more than 28,000 people in 10 countries were infected and more than 11,000 died.[31]

After infection, the initial symptoms of Ebola are unremarkable: fever, severe headache, myalgia, cough, chest pain, and pharyngitis. As the disease progresses, patients experience abdominal pain, diarrhea, and vomiting. Hemorrhaging begins after about 3 days, with bleeding occurring in the gastrointestinal tract, skin, and many other sites. This often leads to delirium, stupor, and coma, accompanied by shock, multiple organ failure, and death. The mortality rates of EVD often range from 50% to 90%.

The initial diagnosis of Ebola is difficult because the early symptoms are so similar to those of many other illnesses. It is possible to directly detect the virus from patient samples within a few days after symptoms begin, using antigen-capture ELISA, immunoglobulin M (IgM) ELISA, PCR, and virus isolation. There are currently

no effective, approved treatments for Ebola other than supportive care and proper isolation techniques to contain its spread.

HANTAVIRUS

The genus *Hantavirus* consists of at least four serogroups with nine viruses causing two major clinical (sometimes overlapping) syndromes: hantavirus pulmonary syndrome (HPS) in North America and hemorrhagic fever with renal syndrome (HFRS) in other continents. Hantaviruses are found throughout the world in wild rodents that shed the virus in their urine and feces. Transmission occurs between rodents and to humans through inhalation of aerosols of the rodent urine and feces. Hantaviruses associated with outbreaks in the US and Canada are transmitted by the deer mouse, white-footed mouse, or cotton rat.

HPS begins as a nonspecific flu-like illness with headache, fever, myalgia, nausea, vomiting, diarrhea, and abdominal pain. Patients rapidly develop pulmonary edema and hypotension resulting in pneumonia, shock, and death, with a mortality rate of up to 50%.[32] This virus can also cause HFRS, which has not been reported in the US. The initial symptoms of this condition include high fever, headache, chills, nausea, inflammation or redness of the eyes, or a rash. Later symptoms are hemorrhaging, hypotension, kidney failure, shock, and death. The mortality rate of HFRS can be as high as 15%.[33]

ELISA, Western blot, rapid immunoblot strip assay (RIBA), and RT-PCR detect host antibodies or viral proteins produced during infection. Immunohistological staining may also be used to detect the presence of viral antigens. There are no clinical treatments other than general supportive care available for HPS infections. Patients with HFRS can be treated with ribavirin.[34]

HUMAN IMMUNODEFICIENCY VIRUS

Human T-lymphotropic viruses (HTLV), also called human immunodeficiency viruses (HIV) are retroviruses that are the causative agent of acquired immune deficiency syndrome (AIDS). There are two main variants of human immunodeficiency virus (HIV). HIV-1 (figure 6.17) occurs in human populations worldwide, whereas HIV-2 is concentrated in West Africa. Currently, the most affected region in the world is sub-Saharan Africa, with an estimated 25.6 million people living with HIV in 2022.[35] Sub-Saharan Africa also accounts for two-thirds of the global total of new HIV infections.[36]

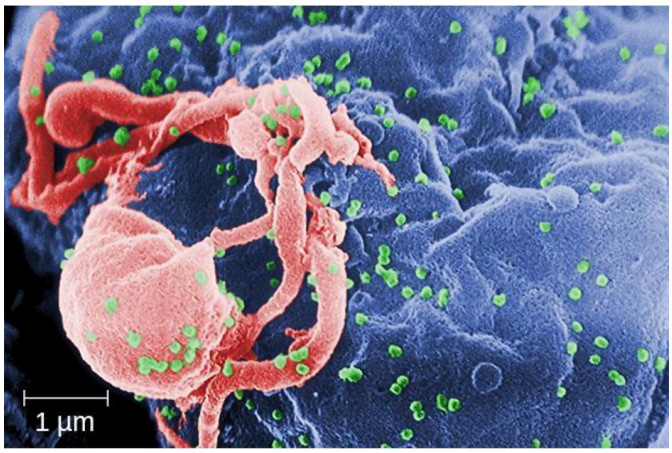

Figure 6.17: This micrograph shows HIV particles (green) budding from a lymphocyte (lower left). *Figure description available at the end of the chapter.*

HIV is spread through direct contact with certain body fluids. Casual contact and insect vectors are not sufficient for disease transmission; common modes of transmission include sexual contact and sharing of needles by intravenous (IV) drug users. It generally takes many years before the effects of an HIV infection are detected. HIV infections are not dormant during this period: virions are continually produced, and the immune system continually attempts to clear the viral infection, while the virus persistently infects additional CD4 T cells. Over time, the CD4 T-cell population is devastated, ultimately leading to AIDS.

When people are infected with HIV, their disease progresses through three stages based on CD4 T-cell counts and the presence of clinical symptoms (figure 6.18).

- **Stage 1: Acute HIV infection.** Two to 4 weeks after infection with HIV, patients may experience a flu-like illness, which can last for a few weeks. Patients with acute HIV infection have lower-than-normal CD4 T-cells and a large amount of virus in their blood. Patients are very contagious during this stage. To confirm acute infection, either a fourth-generation antibody-antigen test or a nucleic acid test (NAT) must be performed.
- **Stage 2: Clinical latency.** During this period, HIV enters a period of dormancy. HIV is still active but reproduces at low levels, and patients may not experience any symptoms of illness. For patients who are not taking medicine to treat HIV, this period can last a decade or longer. For patients receiving antiretroviral therapy, the stage may last for several decades, and those with low levels of the virus in their blood are much less likely to transmit HIV than those who are not virally suppressed. Near the end of the latent stage, the patient's viral load starts to increase and the CD4 T-cell count begins to decrease, leading to the development of symptoms and increased susceptibility to opportunistic infections.
- **Stage 3: Acquired immunodeficiency syndrome (AIDS).** Patients are diagnosed with AIDS when their CD4 T-cell count drops below 200 cells/μL or when they develop certain opportunistic illnesses. During this stage, the immune system is severely damaged by HIV. Common symptoms of AIDS include chills, fever, sweats, swollen lymph glands, weakness, and weight loss; in addition, patients often develop rare cancers such as Kaposi's sarcoma and opportunistic infections such as *Pneumocystis* pneumonia, tuberculosis, cryptosporidiosis, and toxoplasmosis. This is a fatal progression that, in the terminal stages, includes wasting syndrome and dementia (HAND–HIV-Associated Neurocognitive Disorder). Patients with AIDS have a high viral load and are highly infectious.

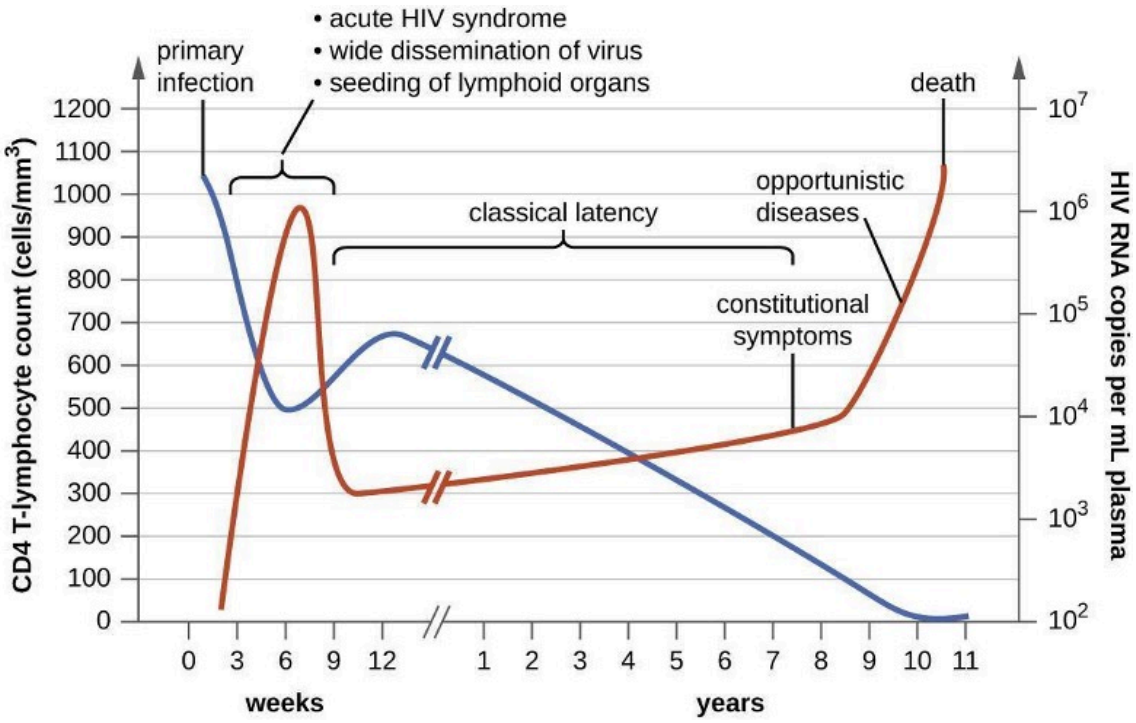

Figure 6.18: This graph shows the clinical progression of CD4 T cells (blue line), clinical symptoms, and viral RNA (red line) during an HIV infection. *Figure description available at the end of the chapter.*

The initial diagnosis of HIV is performed using a serological test for antibody production against the pathogen. Positive test results are confirmed by PCR tests. It can take weeks or months for the body to produce antibodies in response to an infection. There are fourth-generation tests that detect HIV antibodies and also HIV antigens that are present before those antibodies are formed. Nucleic acid tests (NATs) are a third type of test that is relatively expensive and uncommon; NAT can detect HIV in blood and determine the viral load.

As a consequence of integration and the formation of provirus, it is currently not possible to eliminate HIV from an infected patient's body. Elimination by specific antibodies is ineffective because the virus mutates rapidly—a result of the error-prone reverse transcriptase and the inability to correct errors. Antiviral treatments, however, can greatly extend life expectancy. To combat the problem of drug resistance, combinations of antiretroviral drugs called antiretroviral therapy (ART), sometimes called highly active ART or combined ART, are used. There are several different targets for antiviral drug action, and a growing list of drugs for each of these targets. One class of drugs inhibits HIV entry; other classes inhibit reverse transcriptase by blocking viral RNA-dependent and DNA-dependent DNA polymerase activity; and still others inhibit one of the three HIV enzymes needed to replicate inside human cells.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
AIDS/HIV infection	Human immunodeficiency virus (HIV)	Flu-like symptoms during acute stage, followed by long period of clinical latency; final stage (AIDS) includes fever, weight loss, wasting syndrome, dementia, and opportunistic secondary infections leading to death	Contact with body fluids (e.g., sexual contact, use of contaminated needles)	Serological tests for antibodies and/or HIV antigens; nucleic acid test (NAT) for presence of virus	Antiretroviral therapy (ART) using various combinations of drugs
Burkitt lymphoma	Epstein-Barr virus (human herpesvirus-4 [HHV-4])	Rapid formation of malignant B-cell tumors, oral hairy leukoplakia; fatal if not promptly treated	Contact with body fluids (e.g., saliva, blood, semen); primarily affects patients immunocompromised by HIV or malaria	CT scans, tumor biopsy	Intensive alternating chemotherapy regimen
Chikungunya fever	Chikungunya virus	Fever, rash, joint pain	Transmitted between humans by *Aedes aegypti* and *A. albopictus* vectors	Viral culture, IFA, EIA, ELISA, PCR, RT-PCR	None

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Cytomegalovirus infection	Cytomegalovirus (HHV-5)	Usually asymptomatic but may cause non-Epstein-Barr mononucleosis in adults; may cause developmental issues in developing fetus; in transplant recipients, may cause fever, transplant rejection, death	Contact with body fluids, blood transfusions, organ transplants; infected people can transmit virus to fetus transplacentally or to newborn in breastmilk, saliva	Histology, culture, EIA, IFA, PCR	Ganciclovir, valganciclovir, foscarnet, cidofovir
Dengue fever (breakbone fever)	Dengue fever viruses 1–4	Fever, headache, extreme bone and joint pain, abdominal pain, vomiting, hemorrhaging; can be fatal	Transmitted between humans by *A. aegypti* and *A. albopictus* vectors	Serologic testing, ELISA, and RT-PCR	None
Ebola virus disease (EVD)	Ebola virus	Fever, headache, joint pain, diarrhea, vomiting, hemorrhaging in gastrointestinal tract, organ failure; often fatal	Contact with body fluids (e.g., blood, saliva, sweat, urine, feces, vomit); highly contagious	ELISA, IgM ELISA, PCR, and virus isolation	None
Hantavirus pulmonary syndrome (HPS)	Hantavirus	Initial flu-like symptoms followed by pulmonary edema and hypotension leading to pneumonia and shock; can be fatal	Inhalation of dried feces, urine from infected mouse or rat	ELISA, Western blot, RIBA, RT-PCR	None
Hemorrhagic fever with renal syndrome	Hantavirus	Fever, headache, nausea, rash, or eye inflammation, followed by hemorrhaging and kidney failure; can be fatal	Inhalation of dried feces, urine from infected mouse or rat	ELISA, Western blot, RIBA, RT-PCR	None
Infectious mononucleosis	Epstein-Barr virus (HHV-4), cytomegalovirus (HHV-5)	Pharyngitis, fever, extreme fatigue; swelling of lymph nodes, spleen, and liver	Contact with body fluids (e.g., saliva, blood, semen)	Tests for antibodies to various EBV-associated antigens	None

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Yellow fever	Yellow fever virus	Dizziness, fever, chills, headache, myalgia, nausea, vomiting, constipation, fatigue; moderate to severe cases may include jaundice, rash, mucosal, hemorrhaging, seizures, shock, and death	From monkeys to humans or between humans via *Aedes* or *Haemagogus* mosquito vectors	Culture, serology, PCR	None for treatment; preventive vaccine available

Table 6.2: Viral diseases of the circulatory and lymphatic system

6.4 PARASITIC INFECTIONS OF THE CIRCULATORY AND LYMPHATIC SYSTEMS

Some protozoa and parasitic flukes are also capable of causing infections of the human circulatory system (summarized in table 6.3). Although these infections are rare in the US, they continue to cause widespread suffering in the developing world today. Fungal infections of the circulatory system are very rare. Therefore, they are not discussed in this section.

MALARIA

Despite more than a century of intense research and clinical advancements, malaria remains one of the most pronounced infectious diseases in the world today. Its widespread distribution places more than half of the world's population in jeopardy. In 2015, the WHO estimated there were about 214 million cases of malaria worldwide, resulting in about 438,000 deaths; about 88% of cases and 91% of deaths occurred in Africa.[37] Although malaria is not currently a major threat in the US, the possibility of its reintroduction is a concern. Malaria is caused by several protozoan parasites in the genus *Plasmodium*: *P. falciparum, P. knowlesi, P. malariae, P. ovale,* and *P. vivax. Plasmodium* primarily infect red blood cells and are transmitted through the bite of *Anopheles* mosquitoes.

Currently, *P. falciparum* is the most common and most lethal cause of malaria, often called falciparum malaria. Falciparum malaria is widespread in highly populated regions of Africa and Asia, putting many people at risk for the most severe form of the disease.

The classic signs and symptoms of malaria are cycles of extreme fever and chills. The sudden, violent symptoms of malaria start with malaise, abrupt chills, and fever (39–41° C [102.2–105.8 °F]), rapid and faint pulse, polyuria, headache, myalgia, nausea, and vomiting. After 2 to 6 hours of these symptoms, the fever falls, and profuse sweating occurs for 2 to 3 hours, followed by extreme fatigue. These symptoms are a result of *Plasmodium* emerging from red blood cells synchronously, leading to simultaneous rupture of a large number of red blood cells, resulting in damage to the spleen, liver, lymph nodes, and bone marrow. The organ damage resulting from hemolysis causes patients to develop sludge blood (i.e., blood in which the red blood cells agglutinate into clumps) that can lead to lack of oxygen, necrosis of blood vessels, organ failure, and death.

In established infections, malarial cycles of fever and chills typically occur every 2 days in the disease described as tertian malaria, which is caused by *P. vivax* and *P. ovale*. The cycles occur every 3 days in the disease described as quartan malaria, which is caused by *P. malariae*. These intervals may vary among cases.

Plasmodium has a complex life cycle that includes several developmental stages alternately produced in mosquitoes and humans (figure 6.19). When an infected mosquito takes a blood meal, sporozoites in the mosquito's salivary gland are injected into the host's blood. These parasites circulate to the liver, where they develop into schizonts. The schizonts then undergo schizogony, resulting in the release of many merozoites at once. The merozoites move to the bloodstream and infect red blood cells. Inside red blood cells, merozoites develop into trophozoites that produce more merozoites. The synchronous release of merozoites from red blood cells in the evening leads to the symptoms of malaria.

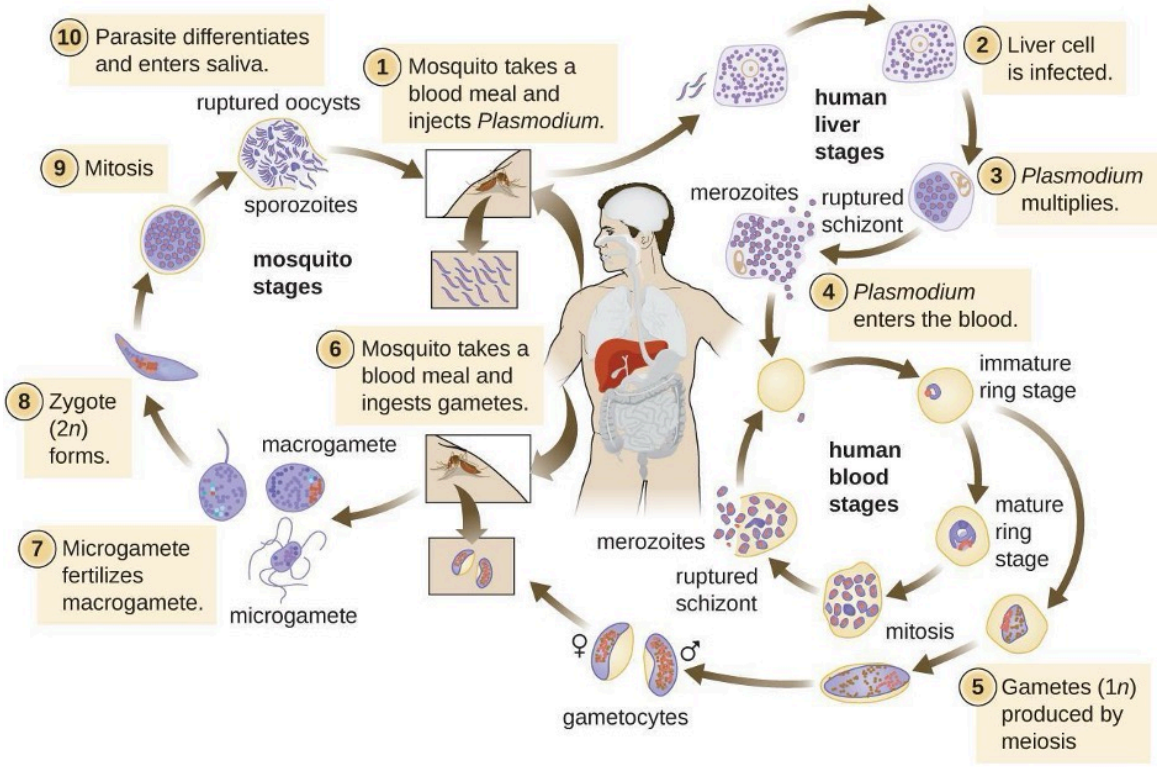

Figure 6.19: The life cycle of Plasmodium. *Figure description available at the end of the chapter.*

In addition, some trophozoites alternatively develop into male and female gametocytes. The gametocytes are taken up when the mosquito takes a blood meal from an infected individual. Sexual sporogony occurs in the gut of the mosquito. The gametocytes fuse to form zygotes in the insect gut. The zygotes become motile and elongate into an ookinete. This form penetrates the midgut wall and develops into an oocyst. Finally, the oocyst releases new sporozoites that migrate to the mosquito salivary glands to complete the life cycle.

Diagnosis of malaria is by microscopic observation of developmental forms of *Plasmodium* in blood smears and rapid EIA assays that detect *Plasmodium* antigens or enzymes (figure 6.20). Drugs such as chloroquine, atovaquone, artemether, and lumefantrine may be prescribed for both acute and prophylactic therapy, although some *Plasmodium* spp. have shown resistance to antimalarial drugs. Use of insecticides and insecticide-treated bed nets can limit the spread of malaria. Despite efforts to develop a vaccine for malaria, none is currently available. However, there are prophylactic antimalarials that can be taken in addition to the environmental preventative measures mentioned.

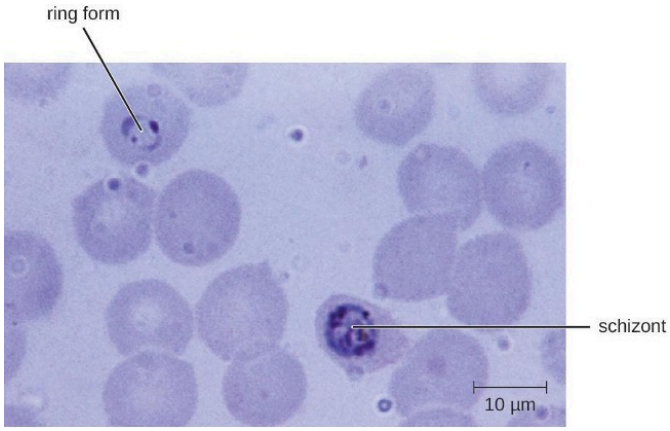

Figure 6.20: A blood smear (human blood stage) shows an early trophozoite in a delicate ring form (upper left) and an early stage schizont form (center) of Plasmodium falciparum from a patient with malaria. *Figure description available at the end of the chapter.*

TOXOPLASMOSIS

The disease toxoplasmosis is caused by the protozoan *Toxoplasma gondii*. *T. gondii* is found in a wide variety of birds and mammals,[38] and human infections are common. The Centers for Disease Control and Prevention (CDC) estimates that 11% of the population 6 years and older has been infected with *T. gondii*; but immunocompetent individuals are typically asymptomatic.[39] Domestic cats are the only known definitive hosts for the sexual stages of *T. gondii* and, thus, are the main reservoirs of infection. Infected cats shed *T. gondii* oocysts in their feces, and these oocysts typically spread to humans through contact with fecal matter on cats' bodies, in litter boxes, or in garden beds where outdoor cats defecate.

T. gondii has a complex life cycle that involves multiple hosts. The *T. gondii* life cycle begins when unsporulated oocysts are shed in the cat's feces. These oocysts take 1–5 days to sporulate in the environment and become infective. Intermediate hosts in nature include birds and rodents, which become infected after ingesting soil, water, or plant material contaminated with the infective oocysts. Once ingested, the oocysts transform into tachyzoites that localize in the bird or rodent neural and muscle tissue, where they develop into tissue cysts. Cats may become infected after consuming birds and rodents harboring tissue cysts. Cats and other animals may also become infected directly by ingestion of sporulated oocysts in the environment. Interestingly, *Toxoplasma* infection appears to be able to modify the host's behavior. Mice infected by *Toxoplasma* lose their fear of cat pheromones. As a result, they become easier prey for cats, facilitating the transmission of the parasite to the cat definitive host[40] (figure 6.21).

Toxoplasma infections in humans are extremely common, but most infected people are asymptomatic or have subclinical symptoms. Some studies suggest that the parasite may be able to influence the personality and psychomotor performance of infected humans, similar to the way it modifies behavior in other mammals.[41] When symptoms do occur, they tend to be mild and similar to those of mononucleosis. However, asymptomatic toxoplasmosis can become problematic in certain situations. Cysts can lodge in a variety of human tissues and lie dormant for years. Reactivation of these quiescent infections can occur in immunocompromised patients following transplantation, cancer therapy, or the development of an immune disorder such as AIDS. In patients with AIDS who have toxoplasmosis, the immune system cannot combat the growth of *T. gondii* in body tissues; as a result, these cysts can cause encephalitis, retinitis, pneumonitis, cognitive disorders, and seizures that can eventually be fatal.

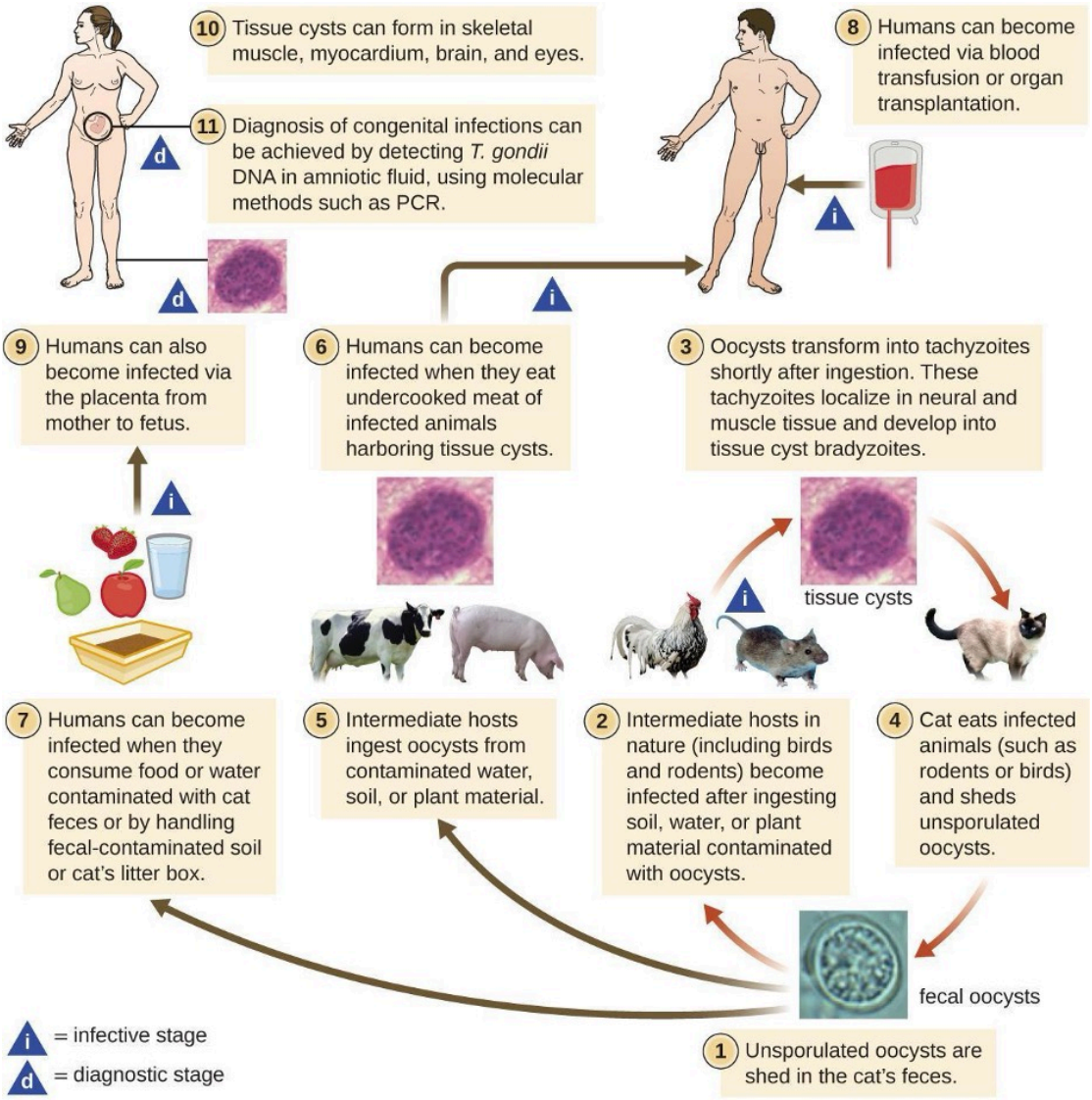

Figure 6.21: The infectious cycle of Toxoplasma gondii. Figure description available at the end of the chapter.

Toxoplasmosis can also pose a risk during pregnancy because tachyzoites can cross the placenta and cause serious infections in the developing fetus. The extent of fetal damage resulting from toxoplasmosis depends on the severity of maternal disease, the damage to the placenta, the gestational age of the fetus when infected, and the virulence of the organism. Congenital toxoplasmosis often leads to fetal demise or premature birth and can result in damage to the central nervous system, manifesting as mental retardation, deafness, or blindness. Consequently, pregnant women are advised by the CDC to take particular care in preparing meat, gardening, and caring for pet cats.[42] Diagnosis of toxoplasmosis infection during pregnancy is usually achieved by serology including TORCH testing (the "T" in TORCH stands for toxoplasmosis). Diagnosis of congenital infections can also be achieved by detecting *T. gondii* DNA in amniotic fluid, using molecular methods such as PCR.

In adults, diagnosis of toxoplasmosis can include observation of tissue cysts in tissue specimens. Tissue cysts may be observed in Giemsa- or Wright-stained biopsy specimens, and CT and magnetic resonance imaging. Lumbar puncture can also be used to confirm infection (figure 6.22).

Preventing infection is the best first-line defense against toxoplasmosis. Preventive measures include washing hands thoroughly after handling raw meat, soil, or cat litter, and avoiding consumption of vegetables possibly contaminated with cat feces. All meat should be cooked to an internal temperature of 73.9–76.7 °C (165–170 °F).

Most immunocompetent patients do not require clinical intervention for *Toxoplasma* infections. However, neonates, pregnant women, and immunocompromised patients can be treated with pyrimethamine and sulfadiazine—except during the first trimester of pregnancy, because these drugs can cause birth defects. Spiramycin has been used safely to reduce transmission in pregnant women with primary infection during the first trimester because it does not cross the placenta.

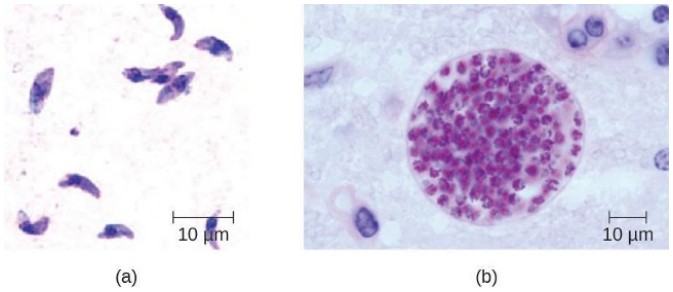

Figure 6.22: (a) Giemsa-stained Toxoplasma gondii tachyzoites from a smear of peritoneal fluid obtained from a mouse inoculated with T. gondii. Tachyzoites are typically crescent shaped with a prominent, centrally placed nucleus. (b) Microscopic cyst containing T. gondii from mouse brain tissue. Thousands of resting parasites (stained red) are contained in a thin parasite cyst wall. Figure description available at the end of the chapter.

BABESIOSIS

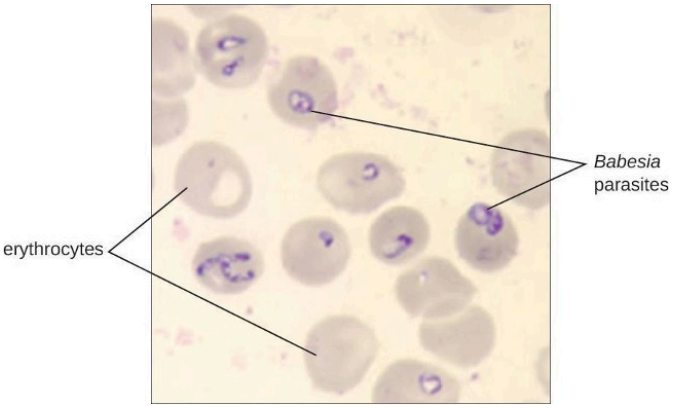

Figure 6.23: In this blood smear from a patient with babesiosis, Babesia parasites can be observed in the red blood cells. Figure description available at the end of the chapter.

Babesiosis is a rare zoonotic infectious disease caused by *Babesia* spp. These parasitic protozoans infect various wild and domestic animals and can be transmitted to humans by black-legged *Ixodes* ticks. In humans, *Babesia* infect red blood cells and replicate inside the cell until it ruptures. The *Babesia* released from the ruptured red blood cell continues the growth cycle by invading other red blood cells. Patients may be asymptomatic, but those who do have symptoms often initially experience malaise, fatigue, chills, fever, headache, myalgia, and arthralgia. In rare cases, particularly in asplenic (absence of the spleen) patients, the elderly, and patients with AIDS, babesiosis may resemble falciparum malaria, with high fever, hemolytic anemia, hemoglobinuria (hemoglobin or blood in urine), jaundice, and renal failure, and the infection can be fatal. Previously acquired asymptomatic *Babesia* infections may become symptomatic if a splenectomy is performed.

Diagnosis is based mainly on the microscopic observation of parasites in blood smears (figure 6.23). Serologic and antibody detection by IFA can also be performed, and PCR-based tests are available. Many people do not require clinical intervention for *Babesia* infections, however, symptomatic infections can be cleared with a combination of atovaquone and azithromycin or a combination of clindamycin and quinine.

CHAGAS DISEASE

Also called American trypanosomiasis, Chagas disease is a zoonosis classified as a neglected tropical disease (NTD). It is caused by the flagellated protozoan *Trypanosoma cruzi* (figure 6.24) and is most commonly transmitted to animals and people through the feces of triatomine bugs. The triatomine bug (figure 6.24) is nicknamed the kissing bug because it frequently bites humans on the face or around the eyes. The bite itself is painless and, initially, many people show no signs of the disease. Alternative modes of transmission include contaminated blood transfusions, organ transplants from infected donors, and congenital transmission from mother to fetus.

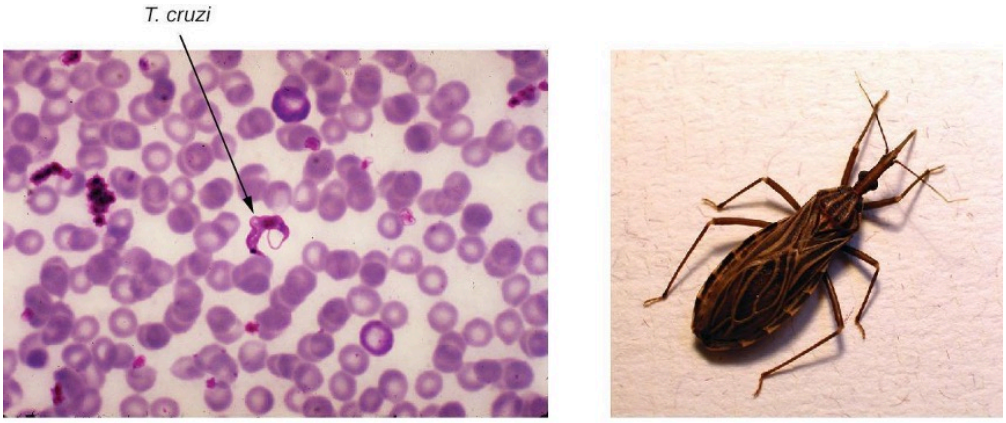

Figure 6.24: (a) Trypanosoma cruzi protozoan in a blood smear from a patient with Chagas disease. (b) The triatomine bug (also known as the kissing bug or assassin bug) is the vector of Chagas disease. *Figure description available at the end of the chapter.*

Chagas disease is endemic throughout much of Mexico, Central America, and South America, where, according to WHO, an estimated 6 million to 7 million people are infected.[43] Currently, Chagas disease is not endemic in the US, even though triatomine bugs are found in the southern half of the country.

Triatomine bugs typically are active at night, when they take blood meals by biting the faces and lips of people or animals as they sleep and often defecate near the site of the bite. Infection occurs when the host rubs the feces into their eyes, mouth, the bite wound, or another break in the skin. The protozoan then enters the blood and invades tissues of the heart and central nervous system, as well as macrophages and monocytes. Nonhuman reservoirs of *T. cruzi* parasites include wild animals and domesticated animals such as dogs and cats, which also act as reservoirs of the pathogen.[44]

There are three phases of Chagas disease: acute, intermediate, and chronic. These phases can be either asymptomatic or life-threatening depending on the immunocompetence status of the patient.

In acute phase disease, symptoms include fever, headache, myalgia, rash, vomiting, diarrhea, and enlarged spleen, liver, and lymph nodes. In addition, a localized nodule called a chagoma may form at the portal of entry, and swelling of the eyelids or the side of the face, called Romaña's sign, may occur near the bite wound. Symptoms of the acute phase may resolve spontaneously, but if untreated, the infection can persist in tissues, causing irreversible damage to the heart or brain. In rare cases, young children may die of myocarditis or meningoencephalitis during the acute phase of Chagas disease.

Following the acute phase is a prolonged intermediate phase during which few or no parasites are found in the blood and most people are asymptomatic. Many patients will remain asymptomatic for life; however, decades after exposure, an estimated 20%–30% of infected people will develop chronic disease that can be debilitating

and sometimes life threatening. In the chronic phase, patients may develop painful swelling of the colon, leading to severe twisting, constipation, and bowel obstruction; painful swelling of the esophagus, leading to dysphagia and malnutrition; and flaccid cardiomegaly (enlargement of the heart), which can lead to heart failure and sudden death.

Diagnosis can be confirmed through several different tests, including direct microscopic observation of trypanosomes in the blood, IFA, EIAs, PCR, and culturing in artificial media. In endemic regions, xenodiagnosis may be used; this method involves allowing uninfected kissing bugs to feed on the patient and then examining their feces for the presence of *T. cruzi*.

The medications nifurtimox and benznidazole are effective treatments during the acute phase of Chagas disease. The efficacy of these drugs is much lower when the disease is in the chronic phase. Avoiding exposure to the pathogen through vector control is the most effective method of limiting this disease.

LEISHMANIASIS

Although it is classified as an NTD, leishmaniasis is relatively widespread in tropical and subtropical regions, affecting people in more than 90 countries. It is caused by approximately 20 different species of *Leishmania*, protozoan parasites that are transmitted by sand fly vectors such as *Phlebotomus* spp. and *Lutzomyia* spp. Dogs, cats, sheep, horses, cattle rodents, and humans can all serve as reservoirs.

The *Leishmania* protozoan is phagocytosed by macrophages but uses virulence factors to avoid destruction within the phagolysosome. The virulence factors inhibit the phagolysosome enzymes that would otherwise destroy the parasite. The parasite reproduces within the macrophage, lyses it, and the progeny infect new macrophages.

The three major clinical forms of leishmaniasis are cutaneous (sores or boils), and mucosal (espundia). The most common form of disease is cutaneous leishmaniasis, which is characterized by the formation of sores at the site of the insect bite that may start out as papules or nodules before becoming large ulcers (figure 6.25).

It may take visceral leishmaniasis months and sometimes years to develop, leading to enlargement of the lymph nodes, liver, spleen, and bone marrow. The damage to these body sites triggers fever, weight loss, and swelling of the spleen and liver. It also causes a decrease in the number of red blood cells (anemia), white blood cells (leukopenia), and platelets (thrombocytopenia), causing the patient to become immunocompromised and more susceptible to fatal infections of the lungs and gastrointestinal tract.

The mucosal form of leishmaniasis is one of the less common forms of the disease. It causes a lesion similar to the cutaneous form but mucosal leishmaniasis is associated with mucous membranes of the mouth, nares, or pharynx, and can be destructive and disfiguring. Mucosal leishmaniasis occurs less frequently when the original cutaneous (skin) infection is promptly treated.

Definitive diagnosis of leishmaniasis is made by visualizing organisms in Giemsa-stained smears, by isolating *Leishmania* protozoans in cultures, or by PCR-based assays of aspirates from infected tissues. Specific DNA probes or analysis of cultured parasites can help to distinguish *Leishmania* species that are causing simple cutaneous leishmaniasis from those capable of causing mucosal leishmaniasis.

Cutaneous leishmaniasis is often not treated, depending on the severity of the lesions. The lesions will resolve after weeks (or several months), but may result in scarring. Recurrence rates are low for this disease. More serious infections can be treated with stibogluconate (antimony gluconate), amphotericin B, and miltefosine.

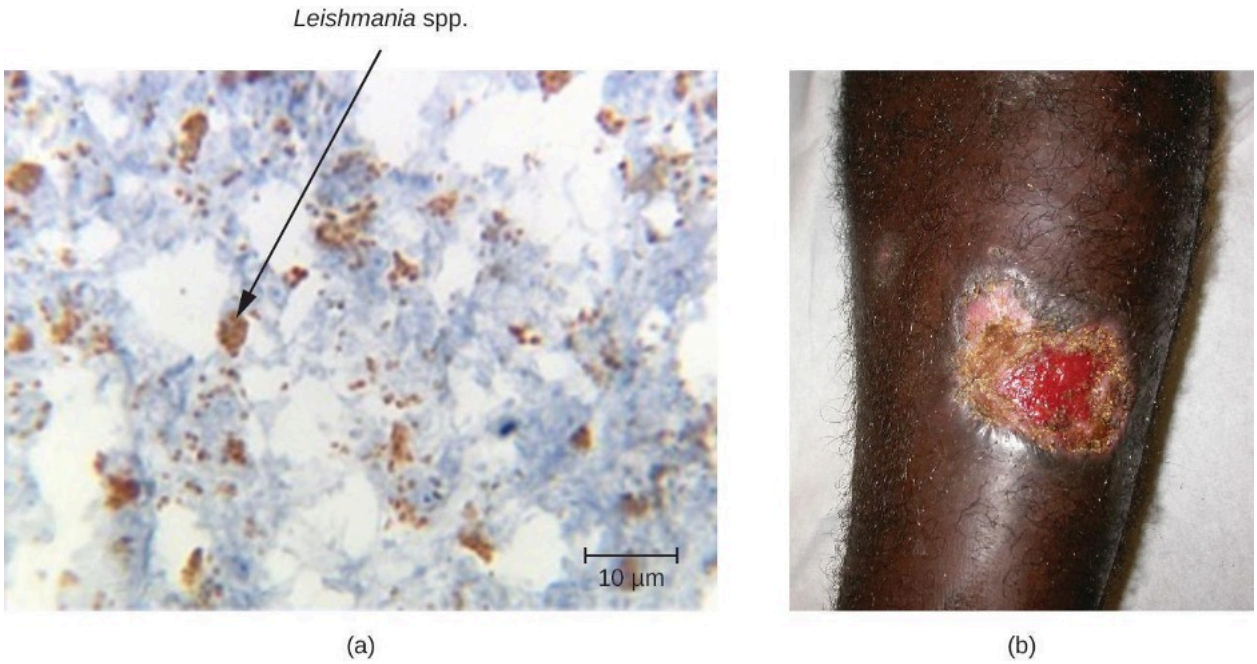

Figure 6.25: (a) A micrograph of a tissue sample from a patient with localized cutaneous leishmaniasis. Parasitic Leishmania mexicana (black arrow) are visible in and around the host cells. (b) Large skin ulcers are associated with cutaneous leishmaniasis. *Figure description available at the end of the chapter.*

SCHISTOSOMIASIS

Schistosomiasis (bilharzia) is an NTD caused by blood flukes in the genus *Schistosoma* that are native to the Caribbean, South America, Middle East, Asia, and Africa. Most human schistosomiasis cases are caused by *Schistosoma mansoni, S. haematobium*, or *S. japonicum*. *Schistosoma* are the only trematodes that invade through the skin; all other trematodes infect by ingestion. WHO estimates that at least 258 million people required preventive treatment for schistosomiasis in 2014.[45]

Infected human hosts shed *Schistosoma* eggs in urine and feces, which can contaminate freshwater habitats of snails that serve as intermediate hosts. The eggs hatch in the water, releasing miracidia, an intermediate growth stage of the *Schistosoma* that infects the snails. The miracidia mature and multiply inside the snails, transforming into cercariae that leave the snail and enter the water where they can penetrate the skin of swimmers and bathers. The cercariae migrate through human tissue and enter the bloodstream, where they mature into adult male and female worms that mate and release fertilized eggs. The eggs travel through the bloodstream and penetrate various body sites, including the bladder or intestine, from which they are excreted in urine or stool to start the life cycle over again.

A few days after infection, patients may develop a rash or itchy skin associated with the site of cercariae penetration. Within 1–2 months of infection, symptoms may develop, including fever, chills, cough, and myalgia, as eggs that are not excreted circulate through the body. After years of infection, the eggs become lodged in tissues and trigger inflammation and scarring that can damage the liver, central nervous system, intestine, spleen, lungs, and bladder. This may cause abdominal pain, enlargement of the liver, blood in the urine or stool, and problems passing urine. Increased risk for bladder cancer is also associated with chronic *Schistosoma* infection. In addition, children who are repeatedly infected can develop malnutrition, anemia, and learning difficulties.

Diagnosis of schistosomiasis is made by the microscopic observation of eggs in feces or urine, intestine or bladder tissue specimens, or serologic tests. The drug praziquantel is effective for the treatment of all schistosome infections. Improving wastewater management and educating at-risk populations to limit exposure to contaminated water can help control the spread of the disease.

Cercarial Dermatitis

The cercaria of some species of *Schistosoma* can only transform into adult worms and complete their life cycle in animal hosts such as migratory birds and mammals. The cercaria of these worms are still capable of penetrating human skin, but they are unable to establish a productive infection in human tissue. Still, the presence of the cercaria in small blood vessels triggers an immune response, resulting in itchy raised bumps called cercarial dermatitis (also known as swimmer's itch or clam digger's itch). Although it is uncomfortable, cercarial dermatitis is typically self-limiting and rarely serious. Antihistamines and antipruritics can be used to limit inflammation and itching, respectively.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Protozoa					
Babesiosis	*Babesia* spp.	Malaise, chills, fever, headache, myalgia, arthralgia	From animals to humans via *Ixodes* tick vectors	Blood smear, serology, IFA, and PCR	Atovaquone and azithromycin or clindamycin and quinine
Chagas disease	*Trypanosoma cruzi*	Fever, headache, body aches, swollen lymph nodes; potentially fatal	Between humans or from animal reservoirs via triatomine (kissing bug) vector	Blood smear, IFA, EIA, PCR, xenodiagnoses	Nifurtimox, benznidazole
Leishmaniasis	*Leishmania* spp.	Ulcer; enlargement of the lymph nodes, liver, spleen, and other organs	Between humans or from animal reservoirs via sand fly (*Phlebotomus* spp., *Lutzomyia* spp.) vectors	Blood smear, culture, PCR, DNA probe, biopsy	Stibogluconate, amphotericin B, miltefosine
Malaria	*Plasmodium vivax, P. malariae, P. falciparum, P. ovale, P. knowlesi*	Extreme fever, chills, myalgia, nausea, and vomiting, possibly leading to organ failure and death	Between humans via Anopheles mosquito vectors	Blood smear, EIA	Chloroquine, atovaquone, artemether, and lumefantrine
Toxoplasmosis	*Toxoplasma gondii*	Tissue cysts; in pregnant people, birth defects or miscarriage	Contact with feces of infected cat; eating contaminated vegetables or undercooked meat of infected animal	Serological tests, direct detection of pathogen in tissue sections	Sulfadiazine, pyrimethamine, spiramycin
Helminths					

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Schistosomiasis	*Schistosoma* spp.	Rash, fever, chills, myalgia; chronic inflammation and scarring of liver, spleen, and other organs where cysts develop	Snail hosts release cercaria into freshwater; cercaria burrow into skin of swimmers and bathers	Eggs in stool or urine, tissue biopsy, serological testing	Praziquantel

Table 6.3: Parasitic diseases of the circulatory and lymphatic systems

SUMMARY

The following is a summary of the material covered throughout the chapter. It summarizes key aspects from each section and the pathogens included.

BACTERIAL INFECTIONS OF THE CIRCULATORY AND LYMPHATIC SYSTEMS

- Bacterial pathogens usually require a breach in the immune defenses to colonize the circulatory system. Most often, this involves a wound or the bite of an arthropod vector, but it can also occur in hospital settings,resulting in nosocomial infections.
- **Sepsis** from both gram-negative and gram-positive bacteria**, puerperal fever, rheumatic fever, endocarditis, gas gangrene, osteomyelitis,** and **toxic shock syndrome** are typically a result of injury or introduction of bacteria by medical or surgical intervention.
- **Tularemia, brucellosis, cat-scratch fever, rat-bite fever,** and **bubonic plague** are zoonotic diseases transmitted by biological vectors.
- **Ehrlichiosis, anaplasmosis, endemic** and **murine typhus, Rocky Mountain spotted fever, Lyme disease, relapsing fever,** and **trench fever** are transmitted by arthropod vectors.
- Because their symptoms are so similar to those of other diseases, many bacterial infections of the circulatory system are difficult to diagnose.
- Standard antibiotic therapies are effective for the treatment of most bacterial infections of the circulatory system, unless the bacterium is resistant. In these cases, synergistic treatment may be required.
- The systemic immune response to a bacteremia, which involves the release of excessive amounts of cytokines, can sometimes be more damaging to the host than the infection itself.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Anaplasmosis (HGA)	*Anaplasma phagocytophilum*	Fever, flu-like symptoms	From small-mammal reservoirs via tick vector	Blood smear, PCR	Doxycycline

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Brucellosis	*Brucella melitensis, B. abortus, B. canis, B. suis*	Granuloma, undulating fever, chronic flu-like symptoms	Direct contact with infected livestock or animals	Agglutination tests, ELISA	Doxycycline, rifampin
Cat-scratch disease	*Bartonella henselae*	Lymph-node swelling and pain, fever, chills, fatigue	Bite or scratch from domestic cats	Immunofluorescence, serological tests, PCR	None for immunocompetent patients
Ehrlichiosis (HME)	*Ehrlichia chaffeensis*	Flu-like symptoms, rash	Lone star tick vector	Serologic tests, PCR	Doxycycline
Endocarditis/ pericarditis	*Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., *HACEK* bacilli	Chest pain, difficulty breathing, dry cough, fever; potentially fatal damage to heart valves	Pathogens introduced to bloodstream via contaminated catheters, dental procedures, piercings, or wounds	Echocardiogram, blood culture	Ampicillin, nafcillin, gentamicin, others; based on susceptibility testing
Epidemic typhus	*Rickettsia prowazekii*	High fever, body aches, rash; potentially fatal damage to heart and brain	From rodent reservoir via body louse vector	PCR, immunofluorescence	Doxycycline, chloramphenicol
Gas gangrene	*Clostridium perfringens,* other *Clostridium* spp.	Rapidly spreading myonecrosis, edema, yellowish and then purple discharge from wound, pockets of gas in tissues, septic shock and death	Germination of endospores in ischemic tissues, typically due to injury or chronic disease (e.g., diabetes)	Wound culture	Penicillin G, clindamycin, metronidazole
Infectious arthritis (septic arthritis)	*Staphylococcus aureus, Neisseria gonorrhoeae*	Joint pain and swelling, limited range of motion	Infection spreads to joint via circulatory system from wound or surgical site	Synovial fluid culture	Oxacillin, cefazolin, cephtriaxone

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Lyme disease	*Borrelia burgdorferi*	Early localized: bull's eye rash, malaise, headache, fever, muscle stiffness; early disseminated: stiff neck, facial paralysis, arthritis, carditis; late-stage: arthritis, meningitis, possibly fatal	From deer, rodent, bird reservoirs via tick vector	IFA, serology, and ELISA	Amoxicillin, doxycycline, penicillin G, chloramphenicol, ceftriaxone
Murine (endemic) typhus	*Rickettsia typhi*	Low-grade fever, rash, headache, cough	From rodents or between humans via rat flea vector	Biopsy, IFA, PCR	Doxycycline, chloramphenicol
Osteomyelitis	*Staphylococcus aureus, Streptococcus pyogenes,* others	Inflammation of bone tissue, leading to fever, localized pain, edema, ulcers, bone loss	Pathogens introduced through trauma, prosthetic joint replacement, or from other infected body site via bloodstream	Radiograph of affected bone, culture of bone biopsy specimen	Cephalosporin, penicillins, others
Plague	*Yersinia pestis*	Bubonic: buboes, fever, internal hemorrhaging; septicemic: fever, abdominal pain, shock, DIC, necrosis in extremities; pneumonic: acute pneumonia, respiratory failure, shock. All forms have high mortality rates.	Transmitted from mammal reservoirs via flea vectors or consumption of infected animal; transmission of pneumonic plague between humans via respiratory aerosols	Culture of bacteria from lymph, blood, or sputum samples; DFA, ELISA	Gentamycin, fluoroquinolones , others
Puerperal sepsis	*Streptococcus pyogenes*, many others	Rapid-onset fever, shock, and death	Pathogens introduced during or immediately following childbirth	Wound, urine, or blood culture	As determined by susceptibility testing

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Rat-bite fever	*Streptobacillus moniliformis, Spirillum minor*	Fever, muscle and joint pain, rash, ulcer	Bite from infected rat or exposure to rat feces or body fluids in contaminated food or water	Observation of the organism from samples and antibody tests	Penicillin
Relapsing fever	*Borrelia recurrentis, B. hermsii,* other *Borrelia* spp.	Recurring fever, headache, muscle aches	From rodent or human reservoir via body louse or tick vector	Darkfield microscopy	Doxycycline, tetracycline, erythromycin
Rheumatic fever	*Streptococcus pyogenes*	Joint pain and swelling, inflammation and scarring of heart valves, heart murmur	Sequela of streptococcal pharyngitis	Serology, electrocardiogram, echocardiogram	Benzathine benzylpenicillin
Rocky Mountain spotted fever	*Rickettsia rickettsia*	High fever, headache, body aches, nausea and vomiting, petechial rash; potentially fatal hypotension and ischemia due to blood coagulation	From rodent reservoir via tick vectors	Biopsy, serology, PCR	Doxycycline, chloramphenicol
Toxic shock syndrome (TSS)	*Staphylococcus aureus*	Sudden high fever, vomiting, diarrhea, hypotension, death	Pathogens from localized infection spread to bloodstream; pathogens introduced on tampons or other intravaginal products	Serology, toxin identification from isolates	Clindamycin, vancomycin
Toxic shock-like syndrome (STSS)	*Streptococcus pyogenes*	Sudden high fever, vomiting, diarrhea, acute respiratory distress syndrome (ARDS), hypoxemia, necrotizing fasciitis, death	Sequela of streptococcal skin or soft-tissue infection	Serology, blood culture, urinalysis	Penicillin, cephalosporin

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Trench fever	*Bartonella Quintana*	High fever, conjunctivitis, ocular pain, headaches, severe pain in bones of shins, neck, and back	Between humans via body louse vector	Blood culture, ELISA, PCR	Doxycycline, macrolide antibiotics, ceftriaxone
Tularemia (rabbit fever)	*Francisella tularensis*	Skin lesions, fever, chills, headache, buboes	Eating or handling infected rabbit; transmission from infected animal via tick or fly vector; aerosol transmission (in laboratory or as bioweapon)	DFA	Streptomycin, gentamycin, others

Table 6.4: Bacterial infections of the circulatory and lymphatic systems

VIRAL INFECTIONS OF THE CIRCULATORY AND LYMPHATIC SYSTEMS

- Human herpesviruses, such as **Epstein-Barr virus** (HHV-4) and **cytomegalovirus** (HHV-5), are widely distributed. The former is associated with infectious mononucleosis and Burkitt lymphoma. The latter can cause serious congenital infections as well as serious disease in immunocompromised adults.
- Arboviral diseases, such as y**ellow fever, dengue fever,** and **chikungunya fever,** are characterized by high fevers and vascular damage that can often be fatal. **Ebola virus disease** is a highly contagious and often fatal infection spread through contact with bodily fluids.
- Although there is a vaccine available for yellow fever, treatments for patients with yellow fever, dengue, chikungunya fever, and Ebola virus diseases are limited to supportive therapies.
- Patients infected with **human immunodeficiency virus (HIV)** progress through three stages of disease, culminating in **AIDS**. **Antiretroviral therapy (ART)** uses various combinations of drugs to suppress viral replication, extending the period of latency and reducing the likelihood of transmission.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
AIDS/HIV infection	Human immunodeficiency virus (HIV)	Flu-like symptoms during acute stage, followed by long period of clinical latency; final stage (AIDS) includes fever, weight loss, wasting syndrome, dementia, and opportunistic secondary infections leading to death	Contact with body fluids (e.g., sexual contact, use of contaminated needles)	Serological tests for antibodies and/or HIV antigens; nucleic acid test (NAT) for presence of virus	Antiretroviral therapy (ART) using various combinations of drugs
Burkitt lymphoma	Epstein-Barr virus (human herpesvirus-4 [HHV-4])	Rapid formation of malignant B-cell tumors, oral hairy leukoplakia; fatal if not promptly treated	Contact with body fluids (e.g., saliva, blood, semen); primarily affects patients immunocompromised by HIV or malaria	CT scans, tumor biopsy	Intensive alternating chemotherapy regimen
Chikungunya fever	Chikungunya virus	Fever, rash, joint pain	Transmitted between humans by *Aedes aegypti* and *A. albopictus* vectors	Viral culture, IFA, EIA, ELISA, PCR, RT-PCR	None
Cytomegalovirus infection	Cytomegalovirus (HHV-5)	Usually asymptomatic but may cause non-Epstein-Barr mononucleosis in adults; may cause developmental issues in developing fetus; in transplant recipients, may cause fever, transplant rejection, death	Contact with body fluids, blood transfusions, organ transplants; infected people can transmit virus to fetus transplacentally or to newborn in breastmilk, saliva	Histology, culture, EIA, IFA, PCR	Ganciclovir, valganciclovir, foscarnet, cidofovir

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Dengue fever (breakbone fever)	Dengue fever viruses 1–4	Fever, headache, extreme bone and joint pain, abdominal pain, vomiting, hemorrhaging; can be fatal	Transmitted between humans by *A. aegypti* and *A. albopictus* vectors	Serologic testing, ELISA, and RT-PCR	None
Ebola virus disease (EVD)	Ebola virus	Fever, headache, joint pain, diarrhea, vomiting, hemorrhaging in gastrointestinal tract, organ failure; often fatal	Contact with body fluids (e.g., blood, saliva, sweat, urine, feces, vomit); highly contagious	ELISA, IgM ELISA, PCR, and virus isolation	None
Hantavirus pulmonary syndrome (HPS)	Hantavirus	Initial flu-like symptoms followed by pulmonary edema and hypotension leading to pneumonia and shock; can be fatal	Inhalation of dried feces, urine from infected mouse or rat	ELISA, Western blot, RIBA, RT-PCR	None
Hemorrhagic fever with renal syndrome	Hantavirus	Fever, headache, nausea, rash, or eye inflammation, followed by hemorrhaging and kidney failure; can be fatal	Inhalation of dried feces, urine from infected mouse or rat	ELISA, Western blot, RIBA, RT-PCR	None
Infectious mononucleosis	Epstein-Barr virus (HHV-4), cytomegalovirus (HHV-5)	Pharyngitis, fever, extreme fatigue; swelling of lymph nodes, spleen, and liver	Contact with body fluids (e.g., saliva, blood, semen)	Tests for antibodies to various EBV-associated antigens	None

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Yellow fever	Yellow fever virus	Dizziness, fever, chills, headache, myalgia, nausea, vomiting, constipation, fatigue; moderate to severe cases may include jaundice, rash, mucosal, hemorrhaging, seizures, shock, and death	From monkeys to humans or between humans via *Aedes* or *Haemagogus* mosquito vectors	Culture, serology, PCR	None for treatment; preventive vaccine available

Table 6.5: Viral diseases of the circulatory and lymphatic system

PARASITIC INFECTIONS OF THE CIRCULATORY AND LYMPHATIC SYSTEMS

- **Malaria** is a protozoan parasite that remains an important cause of death, primarily in the tropics. Several species in the genus *Plasmodium* are responsible for malaria. All species are transmitted by *Anopheles* mosquitoes. *Plasmodium* infects and destroys human red blood cells, leading to organ damage, anemia, blood vessel necrosis, and death. Malaria can be treated with various antimalarial drugs and prevented through vector control.
- **Toxoplasmosis** is a widespread protozoal infection that can cause serious infections in the immunocompromised and in developing fetuses. Domestic cats are the definitive host.
- **Babesiosis** is a generally an asymptomatic infection of red blood cells that can cause malaria-like symptoms in elderly, immunocompromised, or asplenic patients.
- **Chagas disease** is a tropical disease transmitted by triatomine bugs. The trypanosome infects heart tissues, neural tissues, monocytes, and phagocytes, often remaining latent for many years before causing serious and sometimes fatal damage to the digestive system and heart.
- **Leishmaniasis** is caused by the protozoan *Leishmania* and is transmitted by sand flies. Symptoms are generally mild, but serious cases may cause organ damage, anemia, and loss of immune competence.
- **Schistosomiasis** is caused by a fluke transmitted by snails. The fluke moves throughout the body in the bloodstream and chronically infects various tissues, leading to organ damage.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Protozoa					
Babesiosis	*Babesia* spp.	Malaise, chills, fever, headache, myalgia, arthralgia	From animals to humans via *Ixodes* tick vectors	Blood smear, serology, IFA, and PCR	Atovaquone and azithromycin or clindamycin and quinine

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Chagas disease	*Trypanosoma cruzi*	Fever, headache, body aches, swollen lymph nodes; potentially fatal	Between humans or from animal reservoirs via triatomine (kissing bug) vector	Blood smear, IFA, EIA, PCR, xenodiagnoses	Nifurtimox, benznidazole
Leishmaniasis	*Leishmania* spp.	Ulcer; enlargement of the lymph nodes, liver, spleen, and other organs	Between humans or from animal reservoirs via sand fly (*Phlebotomus* spp., *Lutzomyia* spp.) vectors	Blood smear, culture, PCR, DNA probe, biopsy	Stibogluconate, amphotericin B, miltefosine
Malaria	*Plasmodium vivax, P. malariae, P. falciparum, P. ovale, P. knowlesi*	Extreme fever, chills, myalgia, nausea, and vomiting, possibly leading to organ failure and death	Between humans via Anopheles mosquito vectors	Blood smear, EIA	Chloroquine, atovaquone, artemether, and lumefantrine
Toxoplasmosis	*Toxoplasma gondii*	Tissue cysts; in pregnant people, birth defects or miscarriage	Contact with feces of infected cat; eating contaminated vegetables or undercooked meat of infected animal	Serological tests, direct detection of pathogen in tissue sections	Sulfadiazine, pyrimethamine, spiramycin
Helminths					
Schistosomiasis	*Schistosoma* spp.	Rash, fever, chills, myalgia; chronic inflammation and scarring of liver, spleen, and other organs where cysts develop	Snail hosts release cercaria into freshwater; cercaria burrow into skin of swimmers and bathers	Eggs in stool or urine, tissue biopsy, serological testing	Praziquantel

Table 6.6: Parasitic diseases of the circulatory and lymphatic systems

Figure Descriptions

Figure 6.1: a heart with subacute bacterial endocarditis. There are thick, swollen strands in the heart. There are also large lumpy structures at the ends of the chordae tendineae.

Figure 6.2: The image on the left is of lesions, small red or hemorrhagic (blood-filled) spots on the palm of a hand. The image on the right shows a hand that has small, tender, red or purple bumps on the palm.

Figure 6.3: a) Photo of an arm with large purple and red regions. B) X-ray of an arm showing white muscles and cloudy skin. A dark black band cuts through the skin.

Figure 6.4: a) photo of a red blister on a hand. B) micrograph of circular cells.

Figure 6.5: Diagram of plague transmission. The sylvatic rodent-flea cycle is when rodents (such as squirrels and chipmunks) and fleas transmit the pathogen to each other. Fleas and rodents can also transmit the pathogen to birds, which can carry the pathogen long distances. Fleas can also transmit to cows, which can then transmit to humans. Fleas can also transmit to rodents, which are involved in long-distance transport if they travel on a boat. The urban rodent-flea cycle is when urban rodents (such as mice) and fleas transmit the pathogen to each other. Fleas can infect humans. Pneumonic transmission in humans is when one human transmits to another via the airborne route. Humans can carry the pathogen long distances when they travel. The squirrels and chipmunks in the sylvatic cycle can also transmit to humans; or they can transmit to cats which can then transmit to humans.

Figure 6.6: Part a shows the upper leg of a person with a large red bump near the groin. Part b is a photo of blackened toes.

Figure 6.7: A micrograph showing small rod-shaped purple cells in between larger human cells. The purple bacterial cells have a small clear circle in the center.

Figure 6.8: Map of geographic distribution of RMS incidence in 2010; cases per millions. Not notifiable in Alaska and Hawaii. 0 in: NV, SD, NE, WV, VT, MA. 0.2 – 1.5 in WA, OR, CA, UT, CO, NM, TX, ND, MN, WI, MI, OH, PA, LI, FL, LA, KY. 1.9 – 19 in ID, MT, WY, NE, IA, IL, IN, AZ, MS, AL, GA, SC, VA, DC. 19 – 63 in OK, MO, AR, TN, NC

Figure 6.9: Photo of many red spots on a person's hand.

Figure 6.10: a) a tick on a finger. B) a spoon-shaped tool with a notch is used to pull the tick. C) tweezers can be used to pull the tick straight out.

Figure 6.11: The life cycle of the Ixodes scapularis In whether it feeds one (deer is the preferred host). Eggs are deposited and adults die within 3 weeks. In the spring the egg becomes a larvae and feeds once, 2 days (mouse is preferred host). The larva becomes a nymph.

Figure 6.12: a) a rash with a red ring that contains a red spot in the center. B) A micrograph of spiral-shaped cells.

Figure 6.13: A micrograph showing red circles labeled red blood cells and larger white blood cells. Small spirals (approximately the length of 2 red blood cells; 20 μm) are labeled Borrelia spirochetes.

Figure 6.14: a) photo of a child with a very large swelling on the side of the neck. B) micrograph of a blood smear with a lot of white blood cells that are oddly shaped with white spots.

Figure 6.15: Micrograph of cells. A large one with a large, dark nucleus is labeled CMV-infected cell.

Figure 6.16: Micrograph of a straight cell with a straight cell that forms a loop on one end.

Figure 6.17: A micrograph of tiny green particles on the surface of a cell.

Figure 6.18: A graph with time on the X axis and two Y axes – CD4+ T lymphocyte count (cells/mm cubed) and HIV RNA copies per ml plasma. The primary infection is set at time 0 when there is a high CD4 count (over 1000) and a low RNA count (near 0). During the first weeks – macrophage infection, increase in virus production and HIV-1 reservoirs. At about 6 weeks – acute HIV syndrome, wide dissemination of virus, seeding of lymphoid organs. During this time the RNA count increases to about 10 to the 6 and the CD4 count decreases to about 500. From 9 weeks to about 12 weeks the CD4 count increases and the RNA count decreases. From 9 weeks to about 7 years is classic latency – T-cell depletion/immune dysfunction and neurocognitive impairment. During this time CD4 count steadily decreases to near 0 and RNA count steadily increases to over 10 to the 6. Constitutional symptoms occur at about 8 years. After this, opportunistic diseases occur; HIV-D and HIVAN. Then death.

Figure 6.19: "Life cycle of Plasmodium. [Human Liver Stages] 1 – Mosquito take a blood meal and injects Plasmodium into a human. 2 – Plasmodium infects liver cell. 3 – Plasmodium multiplies in liver cell. [Human Blood Stages] 4 – Plasmodium enters blood. An immature ring stage looks like a signet ring in a red blood cell. This becomes a mature ring stage and undergoes mitosis to produce schizonts which are released by rupturing the red blood cells. 5 – Gametes (1n) produced by meiosis. [Mosquito Stages] 6 – Mosquito takes a blood meal and ingests gametes. 7 – Microgametes fertilizes macrogamete. 8 – Zygote (2n) forms. 9 – Zygote undergoes mitosis. 10 – Parasite differentiates and enters the saliva of the mosquito.

Figure 6.20: A micrograph showing red blood cells. A dark ring in the center of one cell is labeled ring form. A larger dark region in another cell is labeled schizont.

Figure 6.21: Life cycle of Toxoplasma gondii. 1 – Unsporulated oocysts are shed in the cat's feces. 2 – Intermediate hosts in nature (including birds and rodents) become infected after ingesting soil, water or plant material contaminated with oocysts. 3 – Oocysts transform into tachyzoites shortly after ingestion. These tachyzoites localize in neural and muscle tissue and develop into tissue cyst bradyzoites. 4 – Cats eat infected animals (such as rodents or birds) and shed unsporulated oocysts. 5 – Intermediate hosts (such as pigs and cows) ingest oocysts from contaminated water, soil, or plant material. 6 – Humans can become infected when they eat undercooked meat of infected animals harboring tissue cysts. 7 – Humans can also become infected when they consume food or water contaminated with cat feces or by handling fecal-contaminated soil or cat's litter box. 8 – Humans can also become infected via the placenta from fetus to mother. 10 – Tissue cysts can form in skeletal muscle, myocardium, brain, and eyes. 11 – Diagnosis of congenital infection can be achieved by detecting T. gondii DNA in amniotic fluid using molecular methods such as PCR.

Figure 6.22: A) A micrograph of curved cells with a nucleus. B) micrograph of a sphere with many smaller spheres inside.

Figure 6.23: Micrograph of red blood cells with dark circles inside.

Figure 6.24: a) Micrograph of red blood cells with an arrow labeled T. Cruzi pointing towards a crescent-shaped protozoan. B) photo of a triatomine bug.

Figure 6.25: a) Micrograph of a tissue sample. A black arrow points to Leishmania mexicana. B) a large, open wound on skin.

Figure References

Figure 6.1: The heart of an individual who had subacute bacterial endocarditis of the mitral valve. Bacterial vegetations are visible on the valve tissues. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 6.2: Janeway lesions (left) and Osler nodes (right). Left (c) Galindo, Roberto J. Osler Nodules Hand/ CC BY 4.0. https://commons.wikimedia.org/wiki/File:Osler_Nodules_Hand.jpg. Right (c) Warfieldian. Janeway Lesion. CC BY 4.0. https://commons.wikimedia.org/wiki/File:Janeway_lesion.JPG.

Figure 6.3: Gas gangrene. Figure 1 from Aggelidakis, J., Lasithiotakis, K., Topalidou, A. et al. Limb salvage after gas gangrene: a case report and review of the literature. World J Emerg Surg 6, 28 (2011). https://doi.org/10.1186/1749-7922-6-28. CC BY 2.0.

Figure 6.4: A skin lesion appears at the site of infection on the hand of an individual infected with Francisella tularensis. Left: modification of work by Centers for Disease Control and Prevention. Public Domain. Right: modification of work by NIAID. Public Domain.

Figure 6.5: Yersinia pestis, the causative agent of plague, has numerous modes of transmission. Illustration: Modification of Figure 2 from Stenseth NC, Atshabar BB, Begon M, Belmain SR, Bertherat E, Carniel E, et al. (2008) Plague: Past, Present, and Future. PLoS Med 5(1): e3. https://doi.org/10.1371/journal.pmed.0050003. CC0/Public Domain. Cat Image: modification of work (c) KaCey97078. CC BY 2.0. https://flic.kr/p/EWJE2

Figure 6.6: Yersinia pestis infection. Left: (c) American Society of Microbiology. Redistribution authorized with attribution. Right: Modification of work by Centers for Disease Control and Prevention. Public Domain. https://phil.cdc.gov/Details.aspx?pid=4139

Figure 6.7: This Wright's stain of a blood sample from a patient with plague shows the characteristic "safety pin" appearance of Yersinia pestis. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 6.8: Annual incidence (per million of population) of reported spotted fever rickettsiosis in the United States in 2021. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 6.9: Rocky Mountain spotted fever causes a petechial rash. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 6.10: This black-legged tick, also known as the deer tick, has not yet attached to the skin. Left: modification of work (c) Jerry Kirkhart. CC BY 4.0. https://commons.wikimedia.org/wiki/File:American_Dog_Tick_(Dermacentor_variabilis).jpg. Middle: modification of Marjadeteek. Public Domain. https://commons.wikimedia.org/wiki/File:Tekenlepel_Teek_verwijderen.jpg. Right: modification of work by Centers for Disease Control and Prevention. Public Domain.

Figure 6.11: This image shows the 2-year life cycle of the black-legged tick, the biological vector of Lyme disease. Illustration: (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology. Mouse Image: Modification of work (c) George Shuklin. CC BY 2.0. https://flic.kr/p/bP2MWP

Figure 6.12: A characteristic bull's eye rash of Lyme disease forms at the site of a tick bite. Left: Modification of work by Centers for Disease Control and Prevention. Public Domain. https://commons.wikimedia.org/wiki/File:Erythema_migrans_-_erythematous_rash_in_Lyme_disease_-_PHIL_9875.jpg. Right: (c) American Society of Microbiology. Redistribution authorized with attribution.

Figure 6.13: A peripheral blood smear from a patient with tick-borne relapsing fever. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 6.14: Burkitt lymphoma can cause large tumors. Left: Figure 1C in Bi, CF., Tang, Y., Zhang, WY. et al. Sporadic Burkitt lymphomas of children and adolescents in Chinese: a clinicopathological study of 43 cases. Diagn Pathol 7, 72 (2012). https://doi.org/10.1186/1746-1596-7-72. CC BY 2.0. Right: Modification of work (c) Ed Uthman. CC BY 2.0. https://flic.kr/p/88y3tw

Figure 6.15: Cells infected with CMV become enlarged and have a characteristic "owl's eye" nucleus. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 6.16: An Ebola virus particle viewed with electron microscopy. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 6.17: This micrograph shows HIV particles (green) budding from a lymphocyte (lower left). Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 6.18: This graph shows the clinical progression of CD4 T cells (blue line), clinical symptoms, and viral RNA (red line) during an HIV infection. Modification of Figure 3 from Kogan, M., Rappaport, J. HIV-1 Accessory Protein Vpr: Relevance in the pathogenesis of HIV and potential for therapeutic intervention. Retrovirology 8, 25 (2011). https://doi.org/10.1186/1742-4690-8-25. CC BY 2.0.

Figure 6.19: The life cycle of Plasmodium. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 6.20: A blood smear (human blood stage) shows an early trophozoite in a delicate ring form (upper left) and an early stage schizont form (center) of Plasmodium falciparum from a patient with malaria. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 6.21: The infectious cycle of Toxoplasma gondii. Illustration: modification of work by Centers for Disease Control and Prevention. Public Domain. Cat Image: modification of work (c) KaCey97078. CC BY 2.0. https://flic.kr/p/EWJE2

Figure 6.22: (a) Giemsa-stained Toxoplasma gondii tachyzoites from a smear of peritoneal fluid obtained from a mouse inoculated with T. gondii. Left: modification of work by Centers for Disease Control and Prevention. Public domain. Right: modification of work by USDA. Public domain.

Figure 6.23: In this blood smear from a patient with babesiosis, Babesia parasites can be observed in the red blood cells. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 6.24: Trypanosoma cruzi protozoan in a blood smear from a patient with Chagas disease. Left: Centers for Disease Control and Prevention. Public Domain. Right: modification of work by Erwin Huebner. Public Domain. https://commons.wikimedia.org/wiki/File:Rhodnius_prolixus70-300.jpg

Figure 6.25: A micrograph of a tissue sample from a patient with localized cutaneous leishmaniasis. Left: Figure 3F in Edith A. Fernández-Figueroa,Claudia Rangel-Escareño,Valeria Espinosa-Mateos,Karol Carrillo-Sánchez,Norma Salaiza-Suazo,Georgina Carrada-Figueroa,Santiago March-Mifsut,Ingeborg Becker (2012) "Disease Severity in Patients Infected with Leishmania mexicana Relates to IL-1β" PLOS Neglected Tropical Diseases. https://doi.org/10.1371/journal.pntd.0001533. CC BY 2.0. Right:

Work by (c) Jean Fortunet. CC BY-SA https://commons.wikimedia.org/wiki/File:Leishmaniose_cutan%C3%A9e_-_Guyane_fr.JPG

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CHAPTER 7.

SYSTEMIC INFECTIONS OF THE URINARY SYSTEM

7.1 INTRODUCTION TO THE ANATOMY AND NORMAL MICROBIOTA OF THE UROGENITAL TRACT

The normal microbiota of different body sites provides an important nonspecific defense against infectious diseases (see section 1.2), and the urogenital tract is no exception. In both men and women, however, the kidneys are sterile. Although urine does contain some antibacterial components, bacteria will grow in urine left out at room temperature. Therefore, it is primarily the flushing action that keeps the ureters and bladder free of microbes.

Below the bladder, the normal microbiota of the male urogenital system is found primarily within the distal urethra and includes bacterial species that are commonly associated with the skin microbiota. In women, the normal microbiota is found within the distal one third of the urethra and the vagina. The normal microbiota of the vagina becomes established shortly after birth and is a complex and dynamic population of bacteria that fluctuates in response to environmental changes.

Members of the vaginal microbiota play an important role in the nonspecific defense against vaginal infections and sexually transmitted infections by occupying cellular binding sites and competing for nutrients. In addition, the production of lactic acid by members of the microbiota provides an acidic environment within the vagina that also serves as a defense against infections. For the majority of women, the lactic-acid–producing bacteria in the vagina are dominated by a variety of species of *Lactobacillus*. For women who lack sufficient lactobacilli in their vagina, lactic acid production comes primarily from other species of bacteria such as *Leptotrichia* spp., *Megasphaera* spp., and *Atopobium vaginae*. *Lactobacillus* spp. use glycogen from vaginal epithelial cells for metabolism and production of lactic acid. This process is tightly regulated by the hormone estrogen. Increased levels of estrogen correlate with increased levels of vaginal glycogen, increased production of lactic acid, and a lower vaginal pH. Therefore, decreases in estrogen during the menstrual cycle and with menopause are associated with decreased levels of vaginal glycogen and lactic acid, and a higher pH. In addition to producing lactic acid, *Lactobacillus* spp. also contribute to the defenses against infectious disease through their production of hydrogen peroxide and bacteriocins (antibacterial peptides).

GENERAL SIGNS AND SYMPTOMS OF UROGENITAL INFECTIONS

Infections of the urinary tract most commonly cause inflammation of the **bladder (cystitis)** or of the **urethra (urethritis)**. Urethritis can be associated with cystitis, but can also be caused by sexually transmitted infections. Symptoms of urethritis in men include burning sensation while urinating, discharge from the penis, and blood in the semen or the urine. In women, urethritis is associated with painful and frequent urination, vaginal discharge, fever, chills, and abdominal pain. The symptoms of cystitis are similar to those of urethritis. When urethritis is caused by a sexually transmitted pathogen, additional symptoms involving the genitalia can occur. These can include painful vesicles (blisters), warts, and ulcers. Ureteritis, a rare infection of the ureter, can also occur with cystitis. These infections can be acute or chronic.

Pyelonephritis and **glomerulonephritis** are infections of the kidney that are potentially serious. **Pyelonephritis** is an infection of one or both of the kidneys and may develop from a lower urinary tract infection; the upper urinary tract, including the ureters, is often affected. Signs and symptoms of pyelonephritis include fever, chills, nausea, vomiting, lower back pain, and frequent painful urination. Pyelonephritis usually only becomes chronic in individuals who have malformations in or damage to the kidneys.

Glomerulonephritis is an inflammation of the glomeruli of the nephrons. Symptoms include excessive protein and blood in urine, increased blood pressure, and fluid retention leading to edema of face, hands, and feet. Glomerulonephritis may be an acute infection or it can become chronic.

Infections occurring within the reproductive structures of males include **epididymitis**, **orchitis**, and **prostatitis**. Bacterial infections may cause inflammation of the epididymis, called epididymitis. This inflammation causes pain in the scrotum, testicles, and groin; swelling, redness, and warm skin in these areas may also be observed. Inflammation of the testicle, called **orchitis**, is usually caused by a bacterial infection spreading from the epididymis, but it can also be a complication of the viral disease mumps. The symptoms are similar to those of epididymitis, and it is not uncommon for them both to occur together, in which case the condition is called epididymo-orchitis. Inflammation of the prostate gland, called prostatitis, can result from a bacterial infection. The signs and symptoms of prostatitis include fever, chills, and pain in the bladder, testicles, and penis. Patients may also experience burning during urination, difficulty emptying the bladder, and painful ejaculation.

Because of its proximity to the exterior, the vagina is a common site for infections in women. The general term for any inflammation of the vagina is **vaginitis.** Vaginitis often develops as a result of an overgrowth of bacteria or fungi that normally reside in the vaginal microbiota, although it can also result from infections by transient pathogens. Bacterial infections of the vagina are called bacterial vaginosis, whereas fungal infections (typically involving *Candida*spp.) are called yeast infections. Dynamic changes affecting the normal microbiota, acid production, and pH variations can be involved in the initiation of the microbial overgrowth and the development of vaginitis. Although some individuals may have no symptoms, vaginosis and vaginitis can be associated with discharge, odor, itching, and burning.

Pelvic inflammatory disease (PID) is an infection of the female reproductive organs including the uterus, cervix, fallopian tubes, and ovaries. The two most common pathogens are the sexually transmitted bacterial pathogens *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Inflammation of the fallopian tubes, called **salpingitis**, is the most serious form of PID. Symptoms of PID can vary between women and include pain in the lower abdomen, vaginal discharge, fever, chills, nausea, diarrhea, vomiting, and painful urination.

GENERAL CAUSES AND MODES OF TRANSMISSION OF UROGENITAL INFECTIONS

Hormonal changes, particularly shifts in estrogen in women due to pregnancy or menopause, can increase susceptibility to urogenital infections. As discussed earlier, estrogen plays an important role in regulating the availability of glycogen and subsequent production of lactic acid by *Lactobacillus* species. Low levels of estrogen are associated with an increased vaginal pH and an increased risk of bacterial vaginosis and yeast infections. Estrogen also plays a role in maintaining the elasticity, strength, and thickness of the vaginal wall, and keeps the vaginal wall lubricated, reducing dryness. Low levels of estrogen are associated with thinning of the vaginal wall. This thinning increases the risk of tears and abrasions, which compromise the protective barrier and increase susceptibility to pathogens.

Another common cause of urogenital infections in females is fecal contamination that occurs because of the close proximity of the anus and the urethra. *Escherichia coli*, an important member of the digestive tract micro-

biota, is the most common cause of urinary tract infections(urethritis and cystitis) in women; it generally causes infection when it is introduced to the urethra in fecal matter. Good hygiene can reduce the risk of urinary tract infections by this route. In men, urinary tract infections are more commonly associated with other conditions, such as an enlarged prostate, kidney stones, or placement of a urinary catheter. All of these conditions impair the normal emptying of the bladder, which serves to flush out microbes capable of causing infection.

Infections that are transmitted between individuals through sexual contact are called sexually transmitted infections (STIs) or sexually transmitted diseases (STDs). (The CDC prefers the term STD, but WHO prefers STI,[1] which encompasses infections that result in disease as well as those that are subclinical or asymptomatic.) STIs often affect the external genitalia and skin, where microbes are easily transferred through physical contact. Lymph nodes in the genital region may also become swollen as a result of infection. However, many STIs have systemic effects as well, causing symptoms that range from mild (e.g., general malaise) to severe (e.g., liver damage or serious immunosuppression).

7.2 BACTERIAL INFECTIONS OF THE URINARY SYSTEM

Urinary tract infections (UTIs) include infections of the urethra, bladder, and kidneys, and are common causes of urethritis, cystitis, pyelonephritis, and glomerulonephritis. Bacteria are the most common causes of UTIs, especially in the urethra and bladder. Tables 7.1 and 7.2 provide a summary of common pathogens.

CYSTITIS

In women, bladder infections are more common because the urethra is short and located in close proximity to the anus, which can result in infections of the urinary tract by fecal bacteria. Bladder infections are also more common in the elderly because the bladder may not empty fully, causing urine to pool; the elderly may also have weaker immune systems that make them more vulnerable to infection. Conditions such as prostatitis in men or kidney stones in both men and women can impact proper drainage of urine and increase risk of bladder infections. Catheterization can also increase the risk of bladder infection.

Figure 7.1: A urine dipstick is compared against a color key to determine levels of various chemicals, proteins, or cells in the urine. Abnormal levels may indicate an infection. *Figure description available at the end of the chapter.*

Gram-negative bacteria such as *Escherichia coli* (most commonly), *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* cause most bladder infections. Gram-positive pathogens associated with cystitis include the coagulase-negative *Staphylococcus saprophyticus*, *Enterococcus faecalis*, and *Streptococcus agalactiae*. Routine manual urinalysis using a urine dipstick or test strip can be used for rapid screening of infection. These test strips (figure 7.1) are either held in a urine stream or dipped in a sample of urine to test for the presence of nitrites, leukocyte esterase, protein, or blood that can indicate an active bacterial infection. The presence of nitrite may indicate the presence of *E. coli* or *K. pneumonia;* these bacteria produce nitrate reductase, which converts nitrate to nitrite. The leukocyte esterase (LE) test detects the presence of neutrophils as a potential indication of active infection.

Low specificity, sensitivity, or both, associated with these rapid screening tests require that care be taken in interpretation of results and in their use in diagnosis of urinary tract infections. Therefore, positive LE or nitrite results are followed by a urine culture to confirm a bladder infection. Urine culture is generally accomplished using blood agar and MacConkey agar, and it is important to culture a clean catch of urine to minimize contamination with normal microbiota of the penis and vagina. A clean catch of urine is accomplished by first washing the labia and urethral opening of female patients or the penis of male patients. The patient then releases a small amount of urine into the toilet bowl before stopping the flow of urine. Finally, the patient resumes urination, this time filling the container used to collect the specimen.

Bacterial cystitis is commonly treated with fluoroquinolones, nitrofurantoin, cephalosporins, or a combination of trimethoprim and sulfamethoxazole. Pain medications may provide relief for patients with dysuria. Treatment is more difficult in elderly patients, who experience a higher rate of complications such as sepsis and kidney infections.

KIDNEY INFECTIONS (PYELONEPHRITIS AND GLOMERULONEPHRITIS)

Pyelonephritis, an inflammation of the kidney, can be caused by bacteria that have spread from other parts of the urinary tract (such as the bladder). In addition, pyelonephritis can develop from bacteria that travel through the bloodstream to the kidney. When the infection spreads from the lower urinary tract, the causative agents are typically fecal bacteria such as *E. coli*. Common signs and symptoms include back pain (due to the location of the kidneys), fever, and nausea or vomiting. Gross hematuria (visible blood in the urine) occurs in 30–40% of women but is rare in men.[2] The infection can become serious, potentially leading to bacteremia and systemic effects that can become life-threatening. Scarring of the kidney can occur and persist after the infection has cleared, which may lead to dysfunction.

Diagnosis of pyelonephritis is made using microscopic examination of urine, culture of urine, testing for leukocyte esterase and nitrite levels, and examination of the urine for blood or protein. It is also important to use blood cultures to evaluate the spread of the pathogen into the bloodstream. Imaging of the kidneys may be performed in high-risk patients with diabetes or immunosuppression, the elderly, patients with previous renal damage, or to rule out an obstruction in the kidney. Pyelonephritis can be treated with either oral or intravenous antibiotics, including penicillins, cephalosporins, vancomycin, fluoroquinolones, carbapenems, and aminoglycosides.

Glomerulonephritis occurs when the glomeruli of the nephrons are damaged from inflammation. Whereas pyelonephritis is usually acute, glomerulonephritis may be acute or chronic. The most well-characterized mechanism of glomerulonephritis is the post-streptococcal sequelae associated with *Streptococcus pyogenes* throat and skin infections. Although *S. pyogenes* does not directly infect the glomeruli of the kidney, immune complexes that form in blood between *S. pyogenes* antigens and antibodies lodge in the capillary endothelial cell junctions of the glomeruli and trigger a damaging inflammatory response. Glomerulonephritis can also occur in patients with bacterial endocarditis (infection and inflammation of heart tissue); however, it is currently unknown whether glomerulonephritis associated with endocarditis is also immune-mediated.

Leptospirosis

Leptospira are generally harmless spirochetes that are commonly found in the soil. However, some pathogenic species can cause an infection called leptospirosis in the kidneys and other organs (figure 7.2). Leptospirosis can produce fever, headache, chills, vomiting, diarrhea, and rash with severe muscular pain. If the disease continues to progress, infection of the kidney, meninges, or liver may occur and may lead to organ failure or meningitis.

When the kidney and liver become seriously infected, it is called Weil's disease. Pulmonary hemorrhagic syndrome can also develop in the lungs, and jaundice may occur.

Leptospira spp. are found widely in animals such as dogs, horses, cattle, pigs, and rodents, and are excreted in their urine. Humans generally become infected by coming in contact with contaminated soil or water, often while swimming or during flooding; infection can also occur through contact with body fluids containing the bacteria. The bacteria may enter the body through mucous membranes, skin injuries, or by ingestion. The mechanism of pathogenicity is not well understood.

Leptospirosis is extremely rare in the United States, although it is endemic in Hawaii. In fact, 50% of all cases in the United States come from Hawaii.[3] It is more common in tropical climates than in temperate climates, and individuals who work with animals or animal products are most at risk. The bacteria can also be cultivated in specialized media, with growth observed in broth in a few days to four weeks; however, diagnosis of leptospirosis is generally made using faster methods, such as detection of antibodies to *Leptospira* spp. in patient samples using serologic testing. Polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), slide agglutination, and indirect immunofluorescence tests may all be used for diagnosis. Treatment for leptospirosis involves broad-spectrum antibiotics such as penicillin and doxycycline. For more serious cases of leptospirosis, antibiotics may be given intravenously.

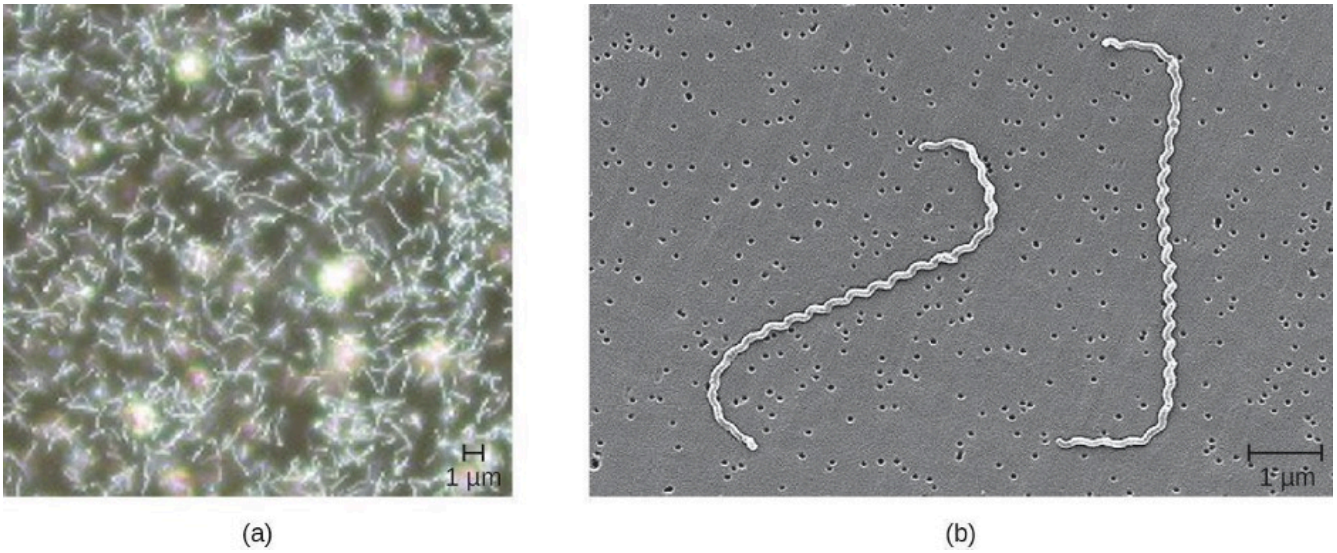

Figure 7.2: (a) Dark field view of Leptospira spp. (b) A scanning electron micrograph of Leptospira interrogans, a pathogenic species, shows the distinctive spirochete morphology of this genus. *Figure description available at the end of the chapter.*

NONGONOCOCCAL URETHRITIS (NGU)

There are two main categories of bacterial urethritis: gonorrheal and nongonococcal. Gonorrheal urethritis is caused by *Neisseria gonorrhoeae* and is associated with gonorrhea, a common STI. This cause of urethritis will be discussed in section 7.3. The term nongonococcal urethritis (NGU) refers to inflammation of the urethra that is unrelated to *N. gonorrhoeae*. In women, NGU is often asymptomatic. In men, NGU is typically a mild disease, but can lead to purulent discharge and dysuria. Because the symptoms are often mild or nonexistent, most infected individuals do not know that they are infected, yet they are carriers of the disease. Asymptomatic patients also have no reason to seek treatment, and although not common, untreated NGU can spread to the reproductive organs, causing pelvic inflammatory disease and salpingitis in women and epididymitis and prostatitis in men.

Important bacterial pathogens that cause nongonococcal urethritis include *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, and *Mycoplasma hominis*.

C. trachomatis is a difficult-to-stain, gram-negative bacterium with an ovoid shape. An intracellular pathogen, *C. trachomatis* causes the most frequently reported STI in the United States, chlamydia. Although most persons infected with *C. trachomatis* are asymptomatic, some patients can present with NGU. *C. trachomatis* can also cause non-urogenital infections such as the ocular disease trachoma (see section 3.2). The life cycle of *C. trachomatis* is illustrated in figure 2.31.

C. trachomatis has multiple possible virulence factors that are currently being studied to evaluate their roles in causing disease. These include polymorphic outer-membrane autotransporter proteins, stress response proteins, and type III secretion effectors. The type III secretion effectors have been identified in gram-negative pathogens, including *C. trachomatis*. This virulence factor is an assembly of more than 20 proteins that form what is called an injectisome for the transfer of other effector proteins that target the infected host cells. The outer-membrane autotransporter proteins are also an effective mechanism of delivering virulence factors involved in colonization, disease progression, and immune system evasion.

Other species associated with NGU include *Mycoplasma genitalium, Ureaplasma urealyticum*, and *Mycoplasma hominis*. These bacteria are commonly found in the normal microbiota of healthy individuals, who may acquire them during birth or through sexual contact, but they can sometimes cause infections leading to urethritis (in males and females) or vaginitis and cervicitis (in females).

M. genitalium is a more common cause of urethritis in most settings than *N. gonorrhoeae*, although it is less common than *C. trachomatis*. It is responsible for approximately 30% of recurrent or persistent infections, 20–25% of nonchlamydial NGU cases, and 15%–20% of NGU cases. *M. genitalium* attaches to epithelial cells and has substantial antigenic variation that helps it evade host immune responses. It has lipid-associated membrane proteins that are involved in causing inflammation.

Several possible virulence factors have been implicated in the pathogenesis of *U. urealyticum* (figure 7.3). These include the ureaplasma proteins phospholipase A, phospholipase C, multiple banded antigen (MBA), urease, and immunoglobulin α protease. The phospholipases are virulence factors that damage the cytoplasmic membrane of target cells. The immunoglobulin α protease is an important defense against antibodies. It can generate hydrogen peroxide, which may adversely affect host cell membranes through the production of reactive oxygen species.

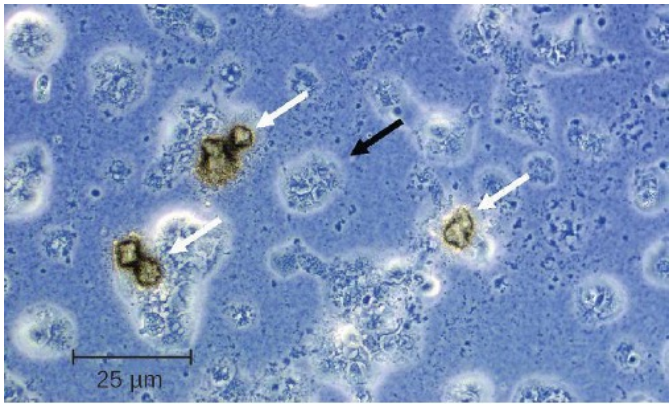

Figure 7.3: Ureaplasma urealyticum microcolonies (white arrows) on agar surface after anaerobic incubation, visualized using phase contrast microscopy (800×). The black arrow indicates cellular debris. *Figure description available at the end of the chapter.*

Treatments differ for gonorrheal and nongonococcal urethritis. However, *N. gonorrhoeae* and *C. trachomatis* are often simultaneously present, which is an important consideration for treatment. NGU is most commonly treated using tetracyclines (such as doxycycline) and azithromycin; erythromycin is an alternative option. Tetracyclines and fluoroquinolones are most commonly used to treat *U. urealyticum*, but resistance to tetracyclines is becoming an increasing problem.[4] While tetracyclines have been the treatment of choice for *M. hominis*, increasing resistance means that other options must be used. Clindamycin and fluoroquinolones are alternatives. *M. genitalium* is generally susceptible

to doxycycline, azithromycin, and moxifloxacin. Like other mycoplasma, *M. genitalium* does not have a cell wall and therefore β-lactams (including penicillins and cephalosporins) are not effective treatments.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Cystitis	*Escherichia coli, Enterococcus faecalis, Streptococcus agalactiae, Klebsiella pneumoniae, Staphylococcus saprophyticus,* others	Dysuria, pyuria, hematuria, and bladder pain; most common in females due to the shorter urethra and abundant normal vaginal microbiota	Nontransmissible; opportunistic infections occur when fecal bacteria are introduced to urinary tract or when normal urination or immune function is impaired	Urine dipstick, urine culture for confirmation	Fluoroquinolones, nitrofurantoin, cephalosporins, trimethoprim, sulfamethoxazole
Leptospirosis	*Leptospira* spp.	Fever, headache, chills, vomiting, diarrhea, rash, muscular pain; in disseminated infections, may cause jaundice, pulmonary hemorrhaging, meningitis	From animals to humans via contact with urine or body fluids	PCR, ELISA, slide agglutination, indirect immunofluorescence	Doxycycline, amoxicillin, ampicillin, erythromycin, penicillin
Nongonococcal urethritis (NGU)	*Chlamydia trachomatis, Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma urealyticum*	Mild or asymptomatic; may cause purulent discharge and dysuria	Transmitted sexually or from a pregnant person to neonate during birth	Urethral swabs and urine culture, PCR, NAAT	Azithromycin, doxycycline, erythromycin, fluoroquinolones
Pyelonephritis, glomerulonephritis	*E. coli, Proteus spp., Klebsiella spp., Streptococcus pyogenes,* others	Back pain, fever, nausea, vomiting, blood in urine; possible scarring of the kidneys and impaired kidney function; severe infections may lead to sepsis and death	Nontransmissible; infection spreads to kidneys from urinary tract or through bloodstream	Urinalysis, urine culture, radioimaging of kidneys	Penicillins, cephalosporins, fluoroquinolones, aminoglycosides, others

Table 7.1: Bacterial infections of the urinary tract

7.3 BACTERIAL INFECTIONS OF THE REPRODUCTIVE SYSTEM

In addition to infections of the urinary tract, bacteria commonly infect the reproductive tract. As with the urinary tract, parts of the reproductive system closest to the external environment are the most likely sites of infection. Often, the same microbes are capable of causing urinary tract and reproductive tract infections.

BACTERIAL VAGINITIS AND VAGINOSIS

Inflammation of the vagina is called vaginitis, often caused by a bacterial infection. It is also possible to have an imbalance in the normal vaginal microbiota without inflammation called bacterial vaginosis (BV). Vaginosis may be asymptomatic or may cause mild symptoms such as a thin, white-to-yellow, homogeneous vaginal discharge, burning, odor, and itching. The major causative agent is *Gardnerella vaginalis*, a gram-variable to gram-negative pleomorphic bacterium. Other causative agents include anaerobic species such as members of the genera *Bacteroides* and *Fusobacterium*. Additionally, ureaplasma and mycoplasma may be involved. The disease is usually self-limiting, although antibiotic treatment is recommended if symptoms develop.

G. vaginalis appears to be more virulent than other vaginal bacterial species potentially associated with BV. Like *Lactobacillus* spp., *G. vaginalis* is part of the normal vaginal microbiota, but when the population of *Lactobacillus* spp. decreases and the vaginal pH increases, *G. vaginalis* flourishes, causing vaginosis by attaching to vaginal epithelial cells and forming a thick protective biofilm. *G. vaginalis* also produces a cytotoxin called vaginolysin that lyses vaginal epithelial cells and red blood cells.

Since *G. vaginalis* can also be isolated from healthy women, the "gold standard" for the diagnosis of BV is direct examination of vaginal secretions and not the culture of *G. vaginalis*. Diagnosis of bacterial vaginosis from vaginal secretions can be accurately made in three ways. The first is to use a DNA probe. The second method is to assay for sialidase activity (sialidase is an enzyme produced by *G. vaginalis* and other bacteria associated with vaginosis, including *Bacteroides* spp., *Prevotella* spp., and *Mobiluncus* spp.). The third method is to assess gram-stained vaginal smears for microscopic morphology and relative numbers and types of bacteria, squamous epithelial cells, and leukocytes. By examining slides prepared from vaginal swabs, it is possible to distinguish lactobacilli (long, gram-positive rods) from other gram-negative species responsible for BV. A shift in predominance from gram-positive bacilli to gram-negative coccobacilli can indicate BV. Additionally, the slide may contain so-called clue cells, which are epithelial cells that appear to have a granular or stippled appearance due to bacterial cells attached to their surface (figure 7.4). Presumptive diagnosis of bacterial vaginosis can involve an assessment of clinical symptoms and evaluation of vaginal fluids using Amsel's diagnostic criteria which include 3 out of 4 of the following characteristics:

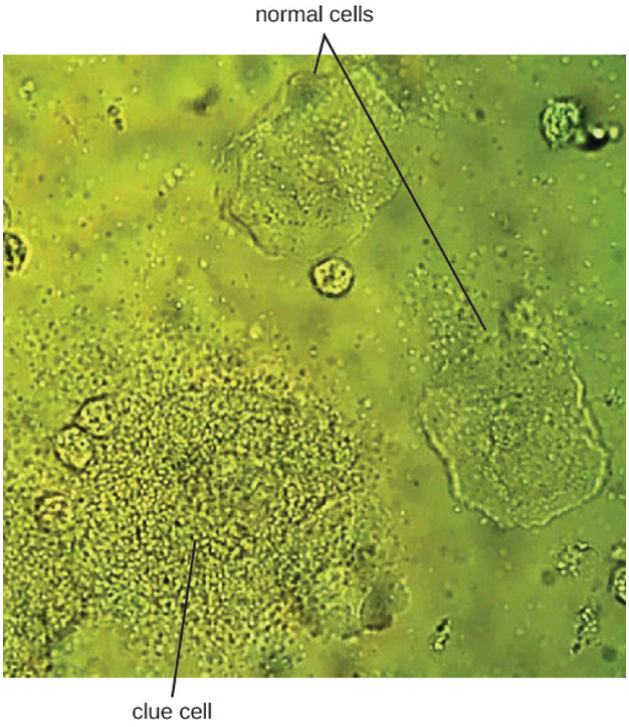

Figure 7.4: In this vaginal smear, the cell at the lower left is a clue cell with a unique appearance caused by the presence of bacteria on the cell that obscures the normally visible borders of the cell. The cell on the right with easily discernible borders is a normal cell. *Figure description available at the end of the chapter.*

1. white to yellow discharge;
2. a fishy odor, most noticeable when 10% KOH is added;
3. pH greater than 4.5;
4. the presence of clue cells.

Treatment is often unnecessary because the infection often clears on its own. However, in some cases, antibiotics such as topical or oral clindamycin or metronidazole may be prescribed. Alternative treatments include oral tinidazole or clindamycin ovules (vaginal suppositories).

GONORRHEA

Also known as the clap, gonorrhea is a common sexually transmitted disease of the reproductive system that is especially prevalent in individuals between the ages of 15 and 24. It is caused by *Neisseria gonorrhoeae*, often called gonococcus or GC, which have fimbriae that allow the cells to attach to epithelial cells. It also has a type of lipopolysaccharide endotoxin called lipooligosaccharide as part of the outer membrane structure that enhances its pathogenicity. In addition to causing urethritis, *N. gonorrhoeae* can infect other body tissues such as the skin, meninges, pharynx, and conjunctiva.

Many infected individuals (both men and women) are asymptomatic carriers of gonorrhea. When symptoms do occur, they manifest differently in males and females. Males may develop pain and burning during urination and discharge from the penis that may be yellow, green, or white (figure 7.5). Less commonly, the testicles may become swollen or tender. Over time, these symptoms can increase and spread. In some cases, chronic infection develops. The disease can also develop in the rectum, causing symptoms such as discharge, soreness, bleeding, itching, and pain (especially in association with bowel movements).

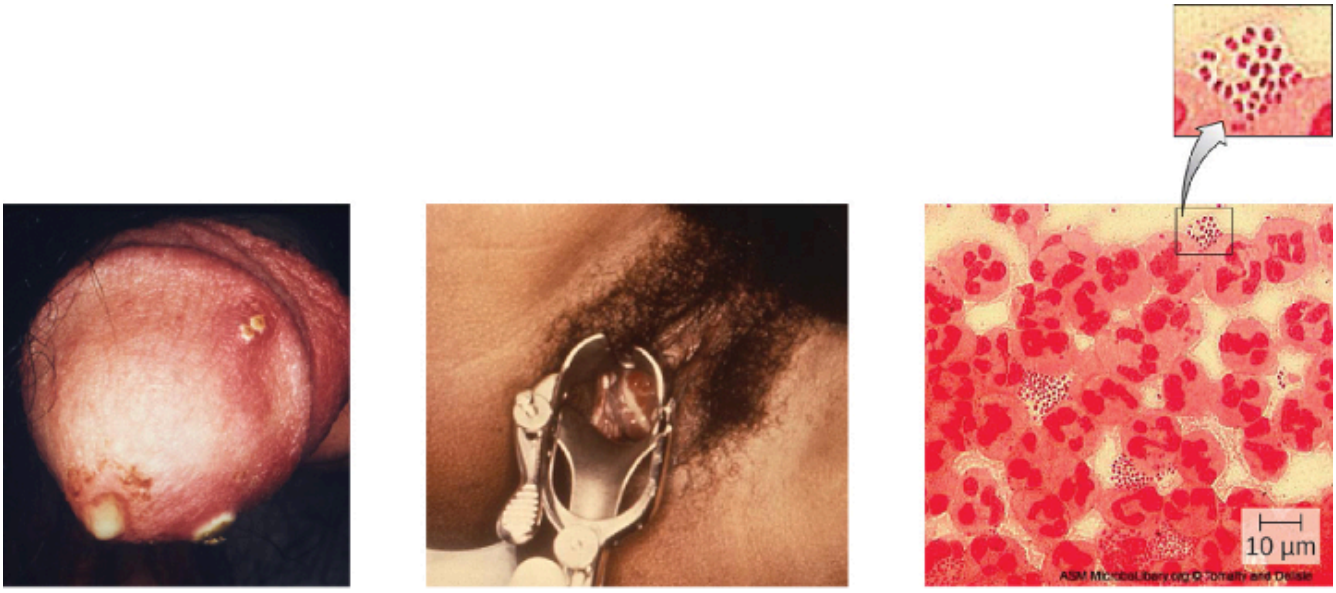

Figure 7.5: (a) Clinical photograph of gonococcal discharge from penis. The lesions on the skin could indicate co-infection with another STI. (b) Purulent discharge originating from the cervix and accumulating in the vagina of a patient with gonorrhea. (c) A micrograph of urethral discharge shows gram-negative diplococci (paired cells) both inside and outside the leukocytes (large cells with lobed nuclei). These results could be used to diagnose gonorrhea in a male patient, but female vaginal samples may contain other Neisseria spp. even if the patient is not infected with N. gonorrhoeae. *Figure description available at the end of the chapter.*

Women may develop pelvic pain, discharge from the vagina, intermenstrual bleeding (i.e., bleeding not associated with normal menstruation), and pain or irritation associated with urination. As with men, the infection can become chronic. In women, however, chronic infection can cause increases in menstrual flow. Rectal infection can also occur, with the symptoms previously described for men. Infections that spread to the endometrium and fallopian tubes can cause pelvic inflammatory disease (PID), characterized by pain in the lower abdominal

region, dysuria, vaginal discharge, and fever. PID can also lead to infertility through scarring and blockage of the fallopian tubes (salpingitis); it may also increase the risk of a life-threatening ectopic pregnancy, which occurs when a fertilized egg begins developing somewhere other than the uterus (e.g., in the fallopian tube or ovary).

When a gonorrhea infection disseminates throughout the body, serious complications can develop. The infection may spread through the blood (bacteremia) and affect organs throughout the body, including the heart (gonorrheal endocarditis), joints (gonorrheal arthritis), and meninges encasing the brain (meningitis).

Urethritis caused by *N. gonorrhoeae* can be difficult to treat due to antibiotic resistance. Some strains have developed resistance to the fluoroquinolones, so cephalosporins are often a first choice for treatment. Because co-infection with *C. trachomatis* is common, the CDC recommends treating with a combination regimen of ceftriaxone and azithromycin. Treatment of sexual partners is also recommended to avoid reinfection and spread of infection to others.[5]

CHLAMYDIA

Chlamydia trachomatis is the causative agent of the STI chlamydia (figure 7.6). While many *Chlamydia* infections are asymptomatic, chlamydia is a major cause of nongonococcal urethritis (NGU) and may also cause epididymitis and orchitis in men. In women, chlamydia infections can cause urethritis, salpingitis, and PID. In addition, chlamydial infections may be associated with an increased risk of cervical cancer.

Because chlamydia is widespread, often asymptomatic, and has the potential to cause substantial complications, routine screening is recommended for sexually active women who are under age 25, at high risk (i.e., not in a monogamous relationship), or beginning prenatal care.

Certain serovars of *C. trachomatis* can cause an infection of the lymphatic system in the groin known as lymphogranuloma venereum. This condition is commonly found in tropical regions and can also co-occur in conjunction with human immunodeficiency virus (HIV) infection. After the microbes invade the lymphatic system, buboes (large lymph nodes, see figure 7.6) form and can burst, releasing pus through the skin. The male genitals can become greatly enlarged and in women the rectum may become narrow.

Urogenital infections caused by *C. trachomatis* can be treated using azithromycin or doxycycline (the recommended regimen from the CDC). Erythromycin, levofloxacin, and ofloxacin are alternatives.

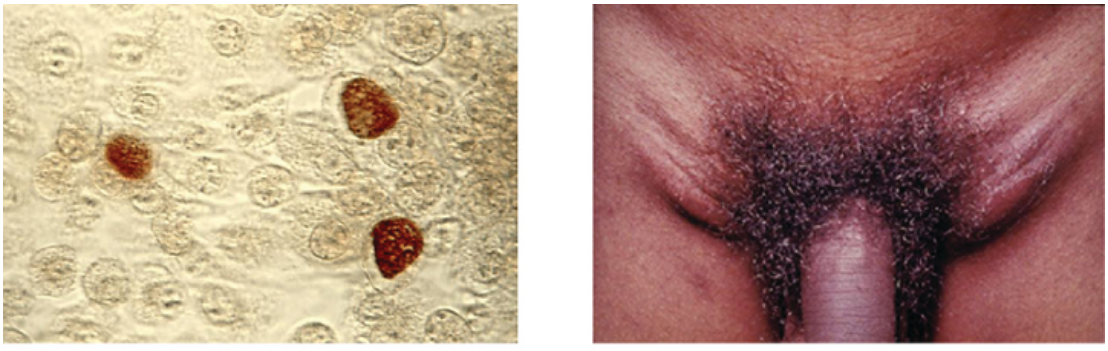

Figure 7.6: (a) Chlamydia trachomatis inclusion bodies within McCoy cell monolayers. Inclusion bodies are distinguished by their brown color. (b) Lymphogranuloma venereum infection can cause swollen lymph nodes in the groin called buboes. *Figure description available at the end of the chapter.*

SYPHILIS

Syphilis is spread through direct physical (generally sexual) contact, and is caused by the gram-negative spirochete *Treponema pallidum*. *T. pallidum* has a relatively simple genome and lacks lipopolysaccharide endotoxin characteristic of gram-negative bacteria. However, it does contain lipoproteins that trigger an immune response in the host, causing tissue damage that may enhance the pathogen's ability to disseminate while evading the host immune system.

After entering the body, *T. pallidum* moves rapidly into the bloodstream and other tissues. If not treated effectively, syphilis progresses through three distinct stages: primary, secondary, and tertiary. Primary syphilis appears as a single lesion on the cervix, penis, or anus within 10 to 90 days of transmission. Such lesions contain many *T. pallidum* cells and are highly infectious. The lesion, called a hard chancre, is initially hard and painless, but it soon develops into an ulcerated sore (figure 7.7). Localized lymph node swelling may occur as well. In some cases, these symptoms may be relatively mild, and the lesion may heal on its own within two to six weeks. Because the lesions are painless and often occur in hidden locations (e.g., the cervix or anus), infected individuals sometimes do not notice them.

The secondary stage generally develops once the primary chancre has healed or begun to heal. Secondary syphilis is characterized by a rash that affects the skin and mucous membranes of the mouth, vagina, or anus. The rash often begins on the palms or the soles of the feet and spreads to the trunk and the limbs (figure 7.7). The rash may take many forms, such as macular or papular. On mucous membranes, it may manifest as mucus patches or white, wart-like lesions called condylomata lata. The rash may be accompanied by malaise, fever, and swelling of lymph nodes. Individuals are highly contagious in the secondary stage, which lasts two to six weeks and is recurrent in about 25% of cases.

After the secondary phase, syphilis can enter a latent phase, in which there are no symptoms but microbial levels remain high. Blood tests can still detect the disease during latency. The latent phase can persist for years.

Tertiary syphilis, which may occur 10 to 20 years after infection, produces the most severe symptoms, and can be fatal. Granulomatous lesions called gummas may develop in a variety of locations, including mucous membranes, bones, and internal organs (figure 7.7). Gummas can be large and destructive, potentially causing massive tissue damage. The most deadly lesions are those of the cardiovascular system (cardiovascular syphilis) and the central nervous system (neurosyphilis). Cardiovascular syphilis can result in a fatal aortic aneurysm (rupture of the aorta) or coronary stenosis (a blockage of the coronary artery). Damage to the central nervous system can cause dementia, personality changes, seizures, general paralysis, speech impairment, loss of vision and hearing, and loss of bowel and bladder control.

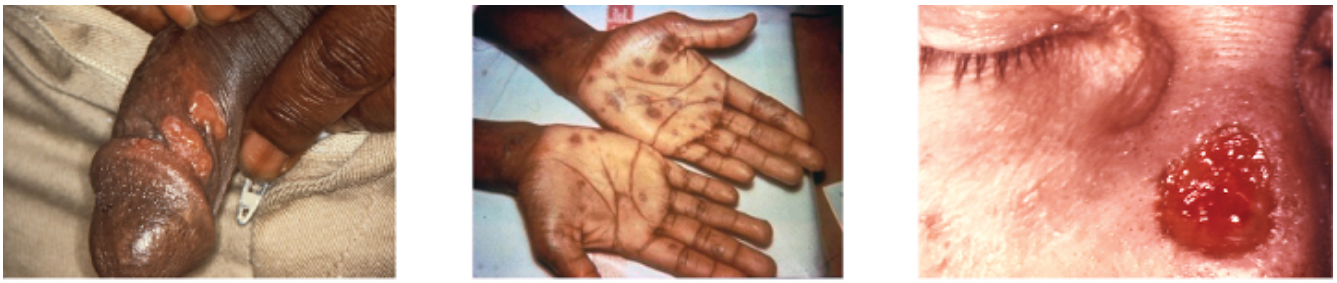

Figure 7.7: (a) This ulcerated sore is a hard chancre caused by syphilis. (b) This individual has a secondary syphilis rash on the hands. (c) Tertiary syphilis produces lesions called gummas, such as this one located on the nose. *Figure description available at the end of the chapter.*

The recommended methods for diagnosing early syphilis are darkfield or brightfield (silver stain) microscopy of tissue or exudate from lesions to detect *T. pallidum* (figure 7.8). If these methods are not available, two types of serologic tests (treponemal and nontreponemal) can be used for a presumptive diagnosis once the spirochete has spread in the body. Nontreponemal serologic tests include the Venereal Disease Research Laboratory (VDRL) and rapid plasma reagin (RPR) tests. These are similar screening tests that detect nonspecific antibodies (those for lipid antigens produced during infection) rather than those produced against the spirochete. Treponemal serologic tests measure antibodies directed against *T. pallidum* antigens using particle agglutination (*T. pallidum* passive particle agglutination or TP-PA), immunofluorescence (the fluorescent *T. pallidum* antibody absorption or FTA-ABS), various enzyme reactions (enzyme immunoassays or EIAs) and chemiluminescence immunoassays (CIA). Confirmatory testing, rather than screening, must be done using treponemal rather than nontreponemal tests because only the former tests for antibodies to spirochete antigens. Both treponemal and nontreponemal tests should be used (as opposed to just one) since both tests have limitations that can result in false positives or false negatives.

Neurosyphilis cannot be diagnosed using a single test. With or without clinical signs, it is generally necessary to assess a variety of factors, including reactive serologic test results, cerebrospinal fluid cell count abnormalities, cerebrospinal fluid protein abnormalities, or reactive VDRL-CSF (the VDRL test of cerebrospinal fluid). The VDRL-CSF is highly specific, but not sufficiently sensitive for conclusive diagnosis.

The recommended treatment for syphilis is parenteral penicillin G (especially long-acting benzathine penicillin, although the exact choice depends on the stage of disease). Other options include tetracycline and doxycycline.

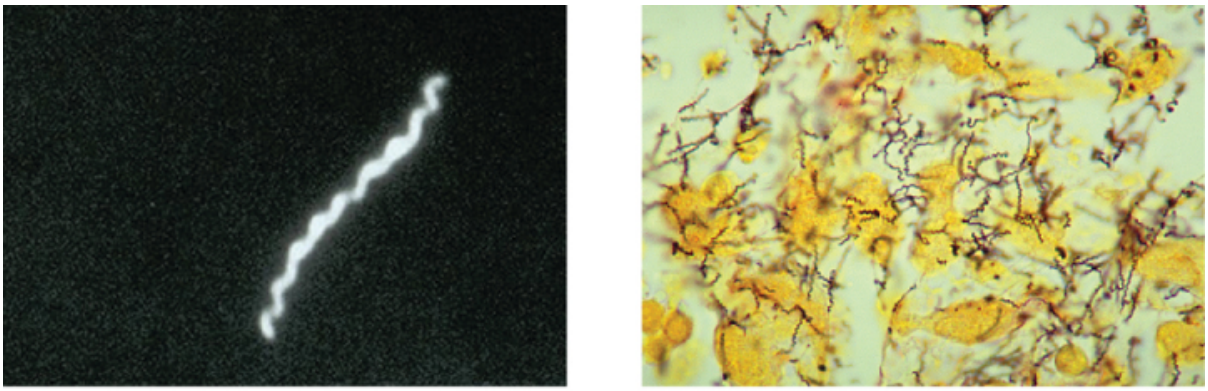

Figure 7.8: (a) Darkfield micrograph of Treponema pallidum. (b) Silver stain micrograph of the same species. *Figure description available at the end of the chapter.*

Congenital Syphilis

Congenital syphilis is passed by mother to fetus when untreated primary or secondary syphilis is present. In many cases, infection may lead to miscarriage or stillbirth. Children born with congenital syphilis show symptoms of secondary syphilis and may develop mucus patches that deform the nose. In infants, gummas can cause significant tissue damage to organs and teeth. Many other complications may develop, such as osteochondritis, anemia, blindness, bone deformations, neurosyphilis, and cardiovascular lesions. Because congenital syphilis poses such a risk to the fetus, expectant mothers are screened for syphilis infection during the first trimester of pregnancy as part of the TORCH panel of prenatal tests.

CHANCROID

The sexually transmitted infection chancroid is caused by the gram-negative rod *Haemophilus ducreyi*. It is characterized by soft chancres (figure 7.9) on the genitals or other areas associated with sexual contact, such as the mouth and anus. Unlike the hard chancres associated with syphilis, soft chancres develop into painful, open sores that may bleed or produce fluid that is highly contagious. In addition to causing chancres, the bacteria can invade the lymph nodes, potentially leading to pus discharge through the skin from lymph nodes in the groin. Like other genital lesions, soft chancres are of particular concern because they compromise the protective barriers of the skin or mucous membranes, making individuals more susceptible to HIV and other sexually transmitted diseases.

Several virulence factors have been associated with *H. ducreyi*, including lipooligosaccharides, protective outer membrane proteins, antiphagocytic proteins, secretory proteins, and collagen-specific adhesin NcaA. The collagen-specific adhesion NcaA plays an important role in initial cellular attachment and colonization. Outer membrane proteins DsrA and DltA have been shown to provide protection from serum-mediated killing by antibodies and complement.

H. ducreyi is difficult to culture; thus, diagnosis is generally based on clinical observation of genital ulcers and tests that rule out other diseases with similar ulcers, such as syphilis and genital herpes. PCR tests for *H. ducreyi* have been developed in some laboratories, but as of 2015 none had been cleared by the US Food and Drug Administration (FDA).[6] Recommended treatments for chancroid include antibiotics such as azithromycin, ciprofloxacin, erythromycin and ceftriaxone. It should be noted that resistance to ciprofloxacin and erythromycin has been reported.[7]

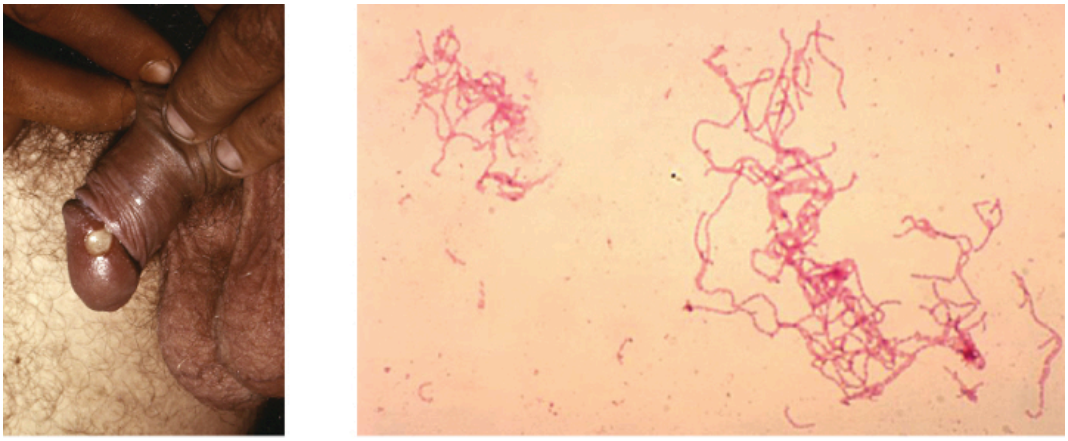

Figure 7.9: (a) A soft chancre on the penis of a man with chancroid. (b) Chancroid is caused by the gram-negative bacterium Haemophilus ducreyi, seen here in a gram-stained culture of rabbit blood. *Figure description available at the end of the chapter.*

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Bacterial vaginosis (BV)	*Gardnerella vaginalis, Bacteroides* spp., *Fusobacterium* spp., others	Often asymptomatic; vaginal discharge, burning, odor, or itching	Opportunistic infection caused by imbalance of normal vaginal microbiota	Vaginal smear	Clindamycin, metronidazole, tinidazole
Chancroid	*Haemophilus ducreyi*	Soft, painful chancres on genitals, mouth, or anus; swollen lymph nodes; pus discharge	Sexual contact or contact with open lesions or discharge	Observation of clinical symptoms and negative tests for syphilis and herpes	Azithromycin, ceftriaxone, erythromycin, ciprofloxacin
Chlamydia	*Chlamydia trachomatis*	Often asymptomatic; in men, urethritis, epididymitis, orchitis; in females, urethritis, vaginal discharge or bleeding, pelvic inflammatory disease, salpingitis, increased risk of cervical cancer	Sexual contact or from a pregnant person to neonate during birth	NAAT, urine sample, vaginal swab, culture	Azithromycin, doxycycline, erythromycin, ofloxacin, or levofloxacin.
Gonorrhea	*Neisseria gonorrhoeae*	Urethritis, dysuria, penile or vaginal discharge, rectal pain and bleeding; in females, pelvic pain, intermenstrual bleeding, pelvic inflammatory disease, salpingitis, increased risk of infertility or ectopic pregnancy; in disseminated infections, arthritis, endocarditis, meningitis	Sexual contact	Urine sample or culture, NAAT, PCR, ELISA	Ceftriaxone, azithromycin
Syphilis	*Treponema pallidum*	Primary: hard chancre; Secondary: rash, cutaneous lesions, condylomata, malaise, fever, swollen lymph nodes; Tertiary: gummas, cardiovascular syphilis, neurosyphilis, possibly fatal	Sexual contact or from a pregnant person to neonate during birth	Darkfield or brightfield silver stain examination of lesion tissue or exudate, treponemal and non-treponemal serological testing, VDRL-CSF for neurosyphilis, prenatal TORCH panel	Penicillin G, tetracycline, doxycycline

Table 7.2: Bacterial infections of the reproductive tract

7.4 VIRAL INFECTIONS OF THE REPRODUCTIVE SYSTEM

Several viruses can cause serious problems for the human reproductive system. Most of these viral infections are incurable, increasing the risk of persistent sexual transmission (table 7.3). In addition, such viral infections are very common in the United States. For example, human papillomavirus (HPV) is the most common STI in the country, with an estimated prevalence of 79.1 million infections in 2008; herpes simplex virus 2 (HSV-2) is the next most prevalent STI at 24.1 million infections.[8] In this section, we will examine these and other major viral infections of the reproductive system.

GENITAL HERPES

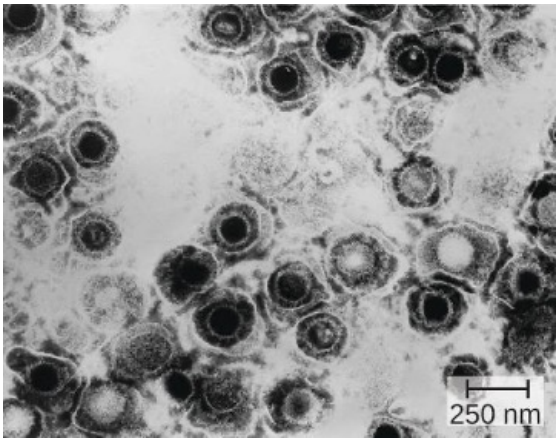

Figure 7.10: Virions of the herpes simplex virus are shown here in this transmission electron micrograph. Figure description available at the end of the chapter.

Genital herpes is a common condition caused by the herpes simplex virus (figure 7.10), an enveloped, double-stranded DNA virus that is classified into two distinct types. Herpes simplex virus has several virulence factors, including infected cell protein (ICP) 34.5, which helps in replication and inhibits the maturation of dendritic cells as a mechanism of avoiding elimination by the immune system. In addition, surface glycoproteins on the viral envelope promote the coating of herpes simplex virus with antibodies and complement factors, allowing the virus to appear as "self" and prevent immune system activation and elimination.

There are two herpes simplex virus types. While herpes simplex virus type 1 (HSV-1) is generally associated with oral lesions like cold sores or fever blisters (see section 3.2), herpes simplex virus type 2 (HSV-2) is usually associated with genital herpes. However, both viruses can infect either location as well as other parts of the body. Oral-genital contact can spread either virus from the mouth to the genital region or vice versa.

Many infected individuals do not develop symptoms, and thus do not realize that they carry the virus. However, in some infected individuals, fever, chills, malaise, swollen lymph nodes, and pain precede the development of fluid-filled vesicles that may be irritating and uncomfortable. When these vesicles burst, they release infectious fluid and allow transmission of HSV. In addition, open herpes lesions can increase the risk of spreading or acquiring HIV.

In men, the herpes lesions typically develop on the penis and may be accompanied by a watery discharge. In women, the vesicles develop most commonly on the vulva, but may also develop on the vagina or cervix (figure 7.11). The symptoms are typically mild, although the lesions may be irritating or accompanied by urinary discomfort. Use of condoms may not always be an effective means of preventing transmission of genital herpes since the lesions can occur on areas other than the genitals.

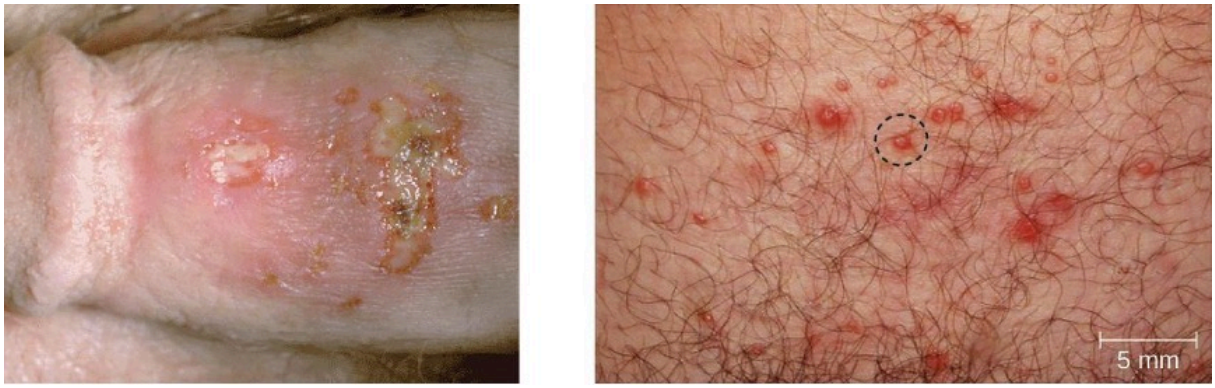

Figure 7.11: Genital herpes is typically characterized by lesions on the genitals (left), but lesions can also appear elsewhere on the skin or mucous membranes (right). The lesions can be large and painful or small and easily overlooked. Figure description available at the end of the chapter.

Herpes simplex viruses can cause recurrent infections because the virus can become latent and then be reactivated. This occurs more commonly with HSV-2 than with HSV-1.[9] The virus moves down peripheral nerves, typically sensory neurons, to ganglia in the spine (either the trigeminal ganglion or the lumbar-sacral ganglia) and becomes latent. Reactivation can later occur, causing the formation of new vesicles. HSV-2 most effectively reactivates from the lumbar-sacral ganglia. Not everyone infected with HSV-2 experiences reactivations, which are typically associated with stressful conditions, and the frequency of reactivation varies throughout life and among individuals. Between outbreaks or when there are no obvious vesicles, the virus can still be transmitted.

Virologic and serologic techniques are used for diagnosis. The virus may be cultured from lesions. The immunostaining methods that are used to detect viruses from cultures generally require less expertise than methods based on cytopathic effect (CPE), as well as being a less expensive option. However, PCR or other DNA amplification methods may be preferred because they provide the most rapid results without waiting for culture amplification. PCR is also best for detecting systemic infections. Serologic techniques are also useful in some circumstances, such as when symptoms persist but PCR testing is negative.

While there is no cure or vaccine for HSV-2 infections, antiviral medications are available that manage the infection by keeping the virus in its dormant or latent phase, reducing signs and symptoms. If the medication is discontinued, then the condition returns to its original severity. The recommended medications, which may be taken at the start of an outbreak or daily as a method of prophylaxis, are acyclovir, famciclovir, and valacyclovir.

Neonatal Herpes

Herpes infections in newborns, referred to as neonatal herpes, are generally transmitted from the mother to the neonate during childbirth, when the child is exposed to pathogens in the birth canal. Infections can occur regardless of whether lesions are present in the birth canal. In most cases, the infection of the newborn is limited to skin, mucous membranes, and eyes, and outcomes are good. However, sometimes the virus becomes disseminated and spreads to the central nervous system, resulting in motor function deficits or death.

In some cases, infections can occur before birth when the virus crosses the placenta. This can cause serious complications in fetal development and may result in spontaneous abortion or severe disabilities if the fetus survives. The condition is most serious when the mother is infected with HSV for the first time during pregnancy. Thus, expectant mothers are screened for HSV infection during the first trimester of pregnancy as part of the TORCH panel of prenatal tests (see section 2.13). Systemic acyclovir treatment is recommended to treat newborns with neonatal herpes.

HUMAN PAPILLOMAS

Warts of all types are caused by a variety of strains of human papillomavirus (HPV) (see section 3.2). *Condylomata acuminata*, more commonly called genital warts or venereal warts (figure 7.12), are an extremely prevalent STI caused by certain strains of HPV. Condylomata are irregular, soft, pink growths that are found on external genitalia or the anus.

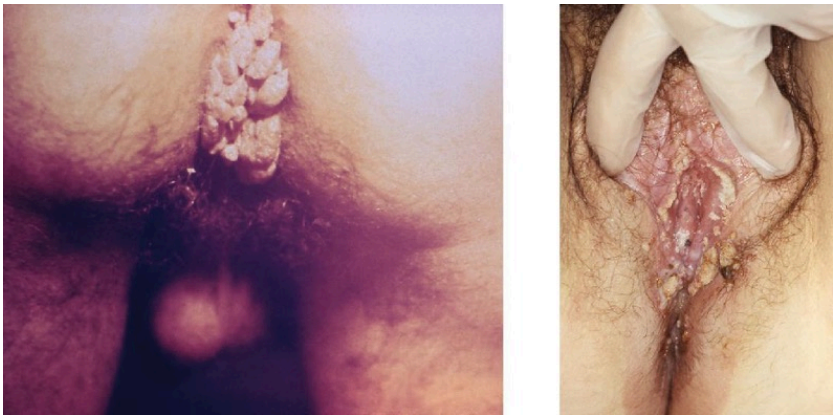

Figure 7.12: Genital warts may occur around the anus (left) or genitalia (right). Figure description available at the end of the chapter. *Figure description available at the end of the chapter.*

HPV is a small, non-enveloped virus with a circular double-stranded DNA genome. Researchers have identified over 200 different strains (called types) of HPV, with approximately 40 causing STIs. While some types of HPV cause genital warts, HPV infection is often asymptomatic and self-limiting. However, genital HPV infection often co-occurs with other STIs like syphilis or gonorrhea. Additionally, some forms of HPV (not the same ones associated with genital warts) are associated with cervical cancers. At least 14 oncogenic (cancer-causing) HPV types are known to have a causal association with cervical cancers. Examples of oncogenic HPV are types 16 and 18, which are associated with 70% of cervical cancers.[10] Oncogenic HPV types can also cause oropharyngeal cancer, anal cancer, vaginal cancer, vulvar cancer, and penile cancer. Most of these cancers are caused by HPV type 16. HPV virulence factors include proteins (E6 and E7) that are capable of inactivating tumor suppressor proteins, leading to uncontrolled cell division and the development of cancer.

HPV cannot be cultured, so molecular tests are the primary method used to detect HPV. While routine HPV screening is not recommended for men, it is included in guidelines for women. An initial screening for HPV at age 30, conducted at the same time as a Pap test, is recommended. If the tests are negative, then further HPV testing is recommended every five years. More frequent testing may be needed in some cases. The protocols used to collect, transport, and store samples vary based on both the type of HPV testing and the purpose of the testing. This should be determined in individual cases in consultation with the laboratory that will perform the testing.

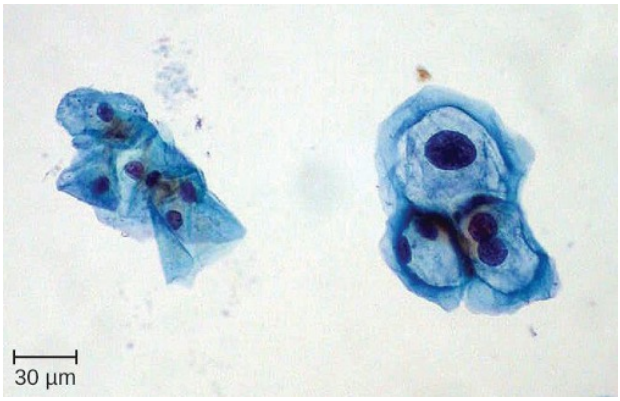

Figure 7.13: In this image, the cervical cells on the left are normal and those on the right show enlarged and sometimes multiple nuclei with hyperchromasia (darkly stained nuclei) typical of HPV-infected koilocytes. *Figure description available at the end of the chapter.*

Because HPV testing is often conducted concurrently with Pap testing, the most common approach uses a single sample collection within one vial for both. This approach uses liquid-based cytology (LBC). The samples are then used for Pap smear cytology as well as HPV testing and genotyping. HPV can be recognized in Pap smears by the presence of cells called koilocytes (called koilocytosis or koilocytotic atypia). Koilocytes have a hyperchromatic atypical nucleus that stains darkly and a high ratio of nuclear material to cytoplasm. There is a distinct clear appearance around the nucleus called a perinuclear halo (figure 7.13).

Most HPV infections resolve spontaneously; however, various therapies are used to treat and remove warts. Topical medications such as imiquimod (which stimulates the production of interferon), podofilox, or sinecatechins, may be effective. Warts can also be removed using cryotherapy or surgery, but these approaches are less effective for genital warts than for other types of warts. Electrocauterization and carbon dioxide laser therapy are also used for wart removal.

Regular Pap testing can detect abnormal cells that might progress to cervical cancer, followed by biopsy and appropriate treatment. Vaccines for some of the high risk HPV types are now available. Gardasil vaccine includes types 6, 11, 16 and 18 (types 6 and 11 are associated with 90% of genital wart infections and types 16 and 18 are associated with 70% of cervical cancers). Gardasil 9 vaccinates against the previous four types and an additional five high-risk types (31, 33, 45, 52, and 58). Cervarix vaccine includes just HPV types 16 and 18. Vaccination is the most effective way to prevent infection with oncogenic HPV, but it is important to note that not all oncogenic HPV types are covered by the available vaccines. It is recommended for everyone prior to sexual activity (usually between the ages of nine and fifteen).

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs/Vaccines
Cervical cancer	HPV types 16, 18, and others	Development of cancer in cervix (or elsewhere)	Direct contact, including sexual	Pap smear	Gardasil vaccine, Cervarix vaccine
Genital herpes	Herpes simplex virus (HSV-1 or HSV-2)	Recurring outbreaks of skin vesicles on genitalia and elsewhere; asymptomatic in many individuals	Sexual contact or contact with open lesions	Viral culture, PCR, ELISA	Acyclovir, famciclovir, valacyclovir
Human papillomas	Human papillomavirus (HPV) (various strains)	Genital warts or warts in other areas	Direct contact, including sexual	Pap smear	Imiquimod, podofilox, sinecatechins.
Neonatal herpes	Herpes simplex virus (HSV-1 or HSV-2)	Vesicles on the skin, mucous membranes, eyes; in disseminated infections, motor impairment and possible death of fetus or newborn	Exposure to pathogens in the birth canal; transplacental infection in some cases	Viral culture or PCR	Acyclovir

Table 7.3: Viral infections of the reproductive tract

7.5 FUNGAL INFECTIONS OF THE REPRODUCTIVE SYSTEM

Only one major fungal pathogen affects the urogenital system. *Candida* is a genus of fungi capable of existing in a yeast form or as a multicellular fungus. *Candida* spp. are commonly found in the normal, healthy microbiota of the skin, gastrointestinal tract, respiratory system, and female urogenital tract (table 7.4 and figure 7.14). They can be pathogenic due to their ability to adhere to and invade host cells, form biofilms, secrete hydrolases (e.g., proteases, phospholipases, and lipases) that assist in their spread through tissues, and change their phenotypes to protect themselves from the immune system. However, they typically only cause disease in the female reproductive tract under conditions that compromise the host's defenses. While there are at least 20 *Candida* species of clinical importance, *C. albicans* is the species most commonly responsible for fungal vaginitis.

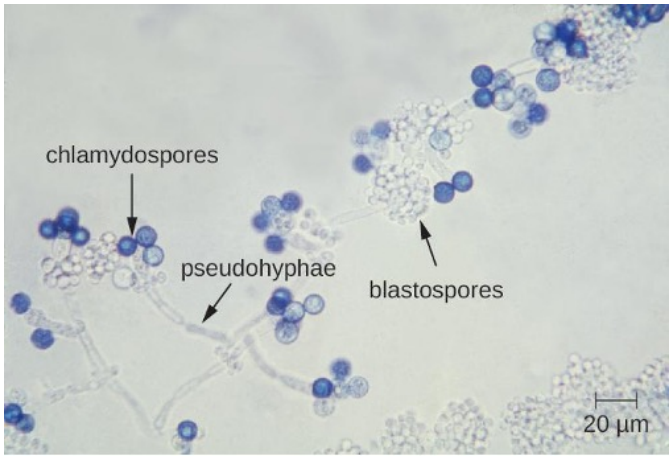

Figure 7.14: Candida blastospores (asexual spores that result from budding) and chlamydospores (resting spores produced through asexual reproduction) are visible in this micrograph. Figure description available at the end of the chapter.

As discussed earlier, lactobacilli in the vagina inhibit the growth of other organisms, including bacteria and *Candida*, but disruptions can allow *Candida* to increase in numbers. Typical disruptions include antibiotic therapy, illness (especially diabetes), pregnancy, and the presence of transient microbes. Immunosuppression can also play a role, and the severe immunosuppression associated with HIV infection often allows *Candida* to thrive. This can cause genital or vaginal candidiasis, a condition characterized by vaginitis and commonly known as a yeast infection. When a yeast infection develops, inflammation occurs along with symptoms of pruritus (itching), a thick white or yellow discharge, and odor.

Other forms of candidiasis include cutaneous candidiasis (see section 3.3) and oral thrush (see section 4.2). Although *Candida* spp. are found in the normal microbiota, *Candida* spp. may also be transmitted between individuals. Sexual contact is a common mode of transmission, although candidiasis is not considered an STI.

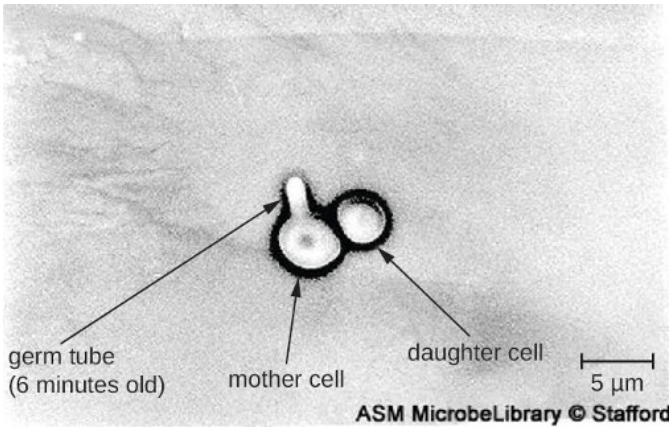

Figure 7.15: Candida can produce germ tubes, like the one in this micrograph, that develop into hyphae. Figure description available at the end of the chapter.

Diagnosis of vaginal candidiasis can be made using microscopic evaluation of vaginal secretions to determine whether there is an excess of *Candida* (figure 7.16). Culturing approaches are less useful because *Candida* is part of the normal microbiota and will regularly appear. It is also easy to contaminate samples with *Candida* because it is so common, so care must be taken to handle clinical material appropriately. Samples can be refrigerated if there is a delay in handling. *Candida* is a dimorphic fungus, so it does not only exist in a yeast form; cultivation can be used to identify chlamydospores and pseudohyphae, which develop from germ tubes (figure 7.15). The presence of the germ tube can be used in a diagnostic test in which cultured yeast cells are combined with rabbit

serum and observed after a few hours for the presence of germ tubes. Molecular tests are also available if needed. The Affirm VPII Microbial Identification Test, for instance, tests simultaneously for the vaginal microbes *C. albicans*, *G. vaginalis* (see section 7.2), and *Trichomonas vaginalis* (see section 7.6).

Topical antifungal medications for vaginal candidiasis include butoconazole, miconazole, clotrimazole, tioconazole, and nystatin. Oral treatment with fluconazole can be used. There are often no clear precipitating factors for infection, so prevention is difficult.

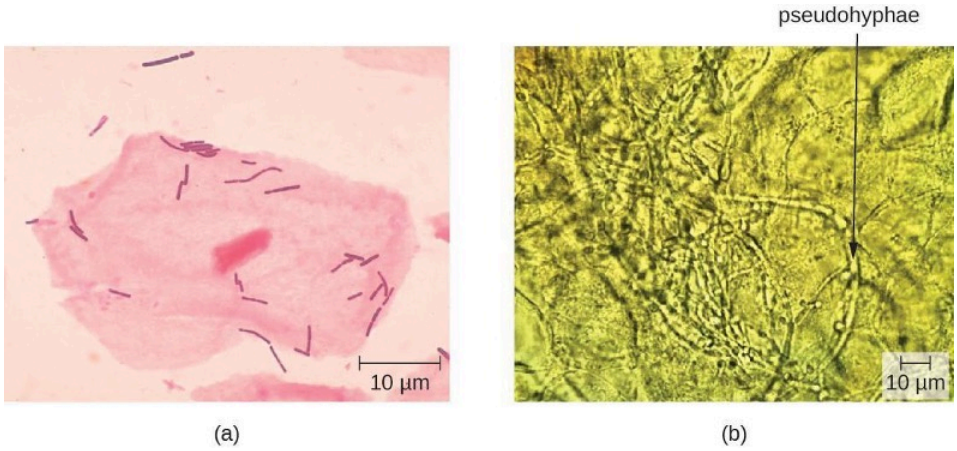

Figure 7.16: (a) Lactobacilli are visible as gram-positive rods on and around this squamous epithelial cell. (b) This wet mount prepared with KOH shows Candida albicans pseudohyphae and squamous epithelial cells in a vaginal sample from a patient with candidiasis. Figure description available at the end of the chapter.

7.6 PROTOZOAN INFECTIONS OF THE UROGENITAL SYSTEM

Only one major protozoan species causes infections in the urogenital system. Trichomoniasis, or "trich," is the most common non-viral STI and is caused by a flagellated protozoan *Trichomonas vaginalis*. *T. vaginalis* has an undulating membrane and, generally, an amoeboid shape when attached to cells in the vagina. In culture, it has an oval shape.

T. vaginalis is commonly found in the normal microbiota of the vagina (table 7.4). As with other vaginal pathogens, it can cause vaginitis when there is disruption to the normal microbiota. It is found only as a trophozoite and does not form cysts. *T. vaginalis* can adhere to cells using adhesins such as lipoglycans; it also has other cell-surface virulence factors, including tetraspanins that are involved in cell adhesion, motility, and tissue invasion. In addition, *T. vaginalis* is capable of phagocytosing other microbes of the normal microbiota, contributing to the development of an imbalance that is favorable to infection.

Both men and women can develop trichomoniasis. Men are generally asymptomatic, and although women are more likely to develop symptoms, they are often asymptomatic as well. When symptoms do occur, they are characteristic of urethritis. Men experience itching, irritation, discharge from the penis, and burning after urination or ejaculation. Women experience dysuria (itching, burning, redness, and soreness of the genitalia) and vaginal discharge. The infection may also spread to the cervix. Infection increases the risk of transmitting or acquiring HIV and is associated with pregnancy complications such as preterm birth.

Microscopic evaluation of wet mounts is an inexpensive and convenient method of diagnosis, but the sensitivity of this method is low (figure 7.17). Nucleic acid amplification testing (NAAT) is preferred due to its high sensitivity. Using wet mounts and then NAAT for those who initially test negative is one option to improve sensitivity. Samples may be obtained for NAAT using urine, vaginal, or endocervical specimens for women and with urine and urethral swabs for men. It is also possible to use other methods such as the OSOM *Trichomonas* Rapid Test (an immunochromatographic test that detects antigen) and a DNA probe test for multiple species associated with vaginitis (the Affirm VPII Microbial Identification Test discussed in section 7.5).[11] *T. vaginalis* is sometimes detected on a Pap test, but this is not considered diagnostic due to high rates of false positives and negatives. The recommended treatment for trichomoniasis is oral metronidazole or tinidazole. Sexual partners should be treated as well.

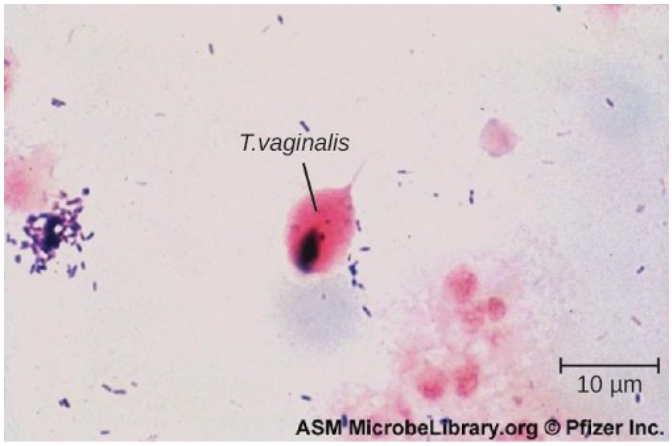

Figure 7.17: Trichomonas vaginalis is visible in this Gram stained specimen. Figure description available at the end of the chapter.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Trichomoniasis	*Trichomonas vaginalis*	Urethritis, vaginal or penile discharge; redness or soreness of female genitalia	Sexual contact	Wet mounts, NAAT of urine or vaginal samples; OSOM Trichomonas Rapid Test, Affirm VPII Microbial Identification Test	Metronidazole, tinidazole
Vaginal candidiasis (yeast infection)	*Candida* spp., especially *C. albicans*	Dysuria; vaginal burning, itching, discharge	Transmissible by sexual contact, but typically only causes opportunistic infections after immunosuppression or disruption of vaginal microbiota	Culture, Affirm VPII Microbial Identification Test	Fluconazole, miconazole, clotrimazole, tioconazole, nystatin

Table 7.4: Fungal and protozoan infections of the reproductive tract

SUMMARY

The following is a summary of the material covered throughout the chapter. It summarizes key aspects from each section and the pathogens included.

BACTERIAL INFECTIONS OF THE URINARY SYSTEM

- Bacterial **cystitis** is commonly caused by fecal bacteria such as *E. coli.*
- **Pyelonephritis** is a serious kidney infection that is often caused by bacteria that travel from infections elsewhere in the urinary tract. This disease may cause systemic complications.
- **Leptospirosis** is a bacterial infection of the kidney that can be transmitted by exposure to infected animal urine, especially in contaminated water. It is more common in tropical climates rather than in temperate climates.
- **Nongonococcal urethritis (NGU)** is commonly caused by *C. trachomatis, M. genitalium, Ureaplasma urealyticum,* and *M. hominis.*
- Diagnosis and treatment for bacterial urinary tract infections varies. Urinalysis (e.g., for leukocyte esterase levels, nitrite levels, microscopic evaluation, and culture of urine) is an important component in most cases. Broad-spectrum antibiotics are typically used.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Cystitis	*Escherichia coli, Enterococcus faecalis, Streptococcus agalactiae, Klebsiella pneumoniae, Staphylococcus saprophyticus,* others	Dysuria, pyuria, hematuria, and bladder pain; most common in females due to the shorter urethra and abundant normal vaginal microbiota	Nontransmissible; opportunistic infections occur when fecal bacteria are introduced to urinary tract or when normal urination or immune function is impaired	Urine dipstick, urine culture for confirmation	Fluoroquinolones, nitrofurantoin, cephalosporins, trimethoprim, sulfamethoxazole
Leptospirosis	*Leptospira* spp.	Fever, headache, chills, vomiting, diarrhea, rash, muscular pain; in disseminated infections, may cause jaundice, pulmonary hemorrhaging, meningitis	From animals to humans via contact with urine or body fluids	PCR, ELISA, slide agglutination, indirect immunofluorescence	Doxycycline, amoxicillin, ampicillin, erythromycin, penicillin

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Nongonococcal urethritis (NGU)	*Chlamydia trachomatis, Mycoplasma genitalium, Mycoplasma hominis, Ureaplasma urealyticum*	Mild or asymptomatic; may cause purulent discharge and dysuria	Transmitted sexually or from a pregnant person to neonate during birth	Urethral swabs and urine culture, PCR, NAAT	Azithromycin, doxycycline, erythromycin, fluoroquinolones
Pyelonephritis, glomerulonephritis	*E. coli, Proteus spp., Klebsiella spp., Streptococcus pyogenes,* others	Back pain, fever, nausea, vomiting, blood in urine; possible scarring of the kidneys and impaired kidney function; severe infections may lead to sepsis and death	Nontransmissible; infection spreads to kidneys from urinary tract or through bloodstream	Urinalysis, urine culture, radioimaging of kidneys	Penicillins, cephalosporins, fluoroquinolones, aminoglycosides, others

Table 7.5: Bacterial infections of the urinary tract

BACTERIAL INFECTIONS OF THE REPRODUCTIVE SYSTEM

- **Bacterial vaginosis** is caused by an imbalance in the vaginal microbiota, with a decrease in lactobacilli and an increase in vaginal pH. *G. vaginalis* is the most common cause of bacterial vaginosis, which is associated with vaginal discharge, odor, burning, and itching.
- **Gonorrhea** is caused by *N. gonorrhoeae*, which can cause infection of the reproductive and urinary tracts and is associated with symptoms of urethritis. If left untreated, it can progress to epididymitis, salpingitis, and pelvic inflammatory disease and may enter the bloodstream, leading to infections in other sites of the body.
- **Chlamydia** is the most commonly reported STI and is caused by *C. trachomatis*. Most infections are asymptomatic, and infections that are not treated can spread to involve the epididymis of men and cause salpingitis and pelvic inflammatory disease in women.
- **Syphilis** is caused by *T. pallidum* and has three stages, primary, secondary, and tertiary. Primary syphilis is associated with a painless hard chancre lesion on genitalia. Secondary syphilis is associated with skin and mucous membrane lesions. Tertiary syphilis is the most serious and life-threatening and can involve serious nervous system damage.
- **Chancroid** is an infection of the reproductive tract caused by *H. ducreyi* that results in the development of characteristic **soft chancres**.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Bacterial vaginosis (BV)	*Gardnerella vaginalis, Bacteroides* spp., *Fusobacterium* spp., others	Often asymptomatic; vaginal discharge, burning, odor, or itching	Opportunistic infection caused by imbalance of normal vaginal microbiota	Vaginal smear	Clindamycin, metronidazole, tinidazole
Chancroid	*Haemophilus ducreyi*	Soft, painful chancres on genitals, mouth, or anus; swollen lymph nodes; pus discharge	Sexual contact or contact with open lesions or discharge	Observation of clinical symptoms and negative tests for syphilis and herpes	Azithromycin, ceftriaxone, erythromycin, ciprofloxacin
Chlamydia	*Chlamydia trachomatis*	Often asymptomatic; in men, urethritis, epididymitis, orchitis; in females, urethritis, vaginal discharge or bleeding, pelvic inflammatory disease, salpingitis, increased risk of cervical cancer	Sexual contact or from a pregnant person to neonate during birth	NAAT, urine sample, vaginal swab, culture	Azithromycin, doxycycline, erythromycin, ofloxacin, or levofloxacin.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Gonorrhea	*Neisseria gonorrhoeae*	Urethritis, dysuria, penile or vaginal discharge, rectal pain and bleeding; in females, pelvic pain, intermenstrual bleeding, pelvic inflammatory disease, salpingitis, increased risk of infertility or ectopic pregnancy; in disseminated infections, arthritis, endocarditis, meningitis	Sexual contact	Urine sample or culture, NAAT, PCR, ELISA	Ceftriaxone, azithromycin
Syphilis	*Treponema pallidum*	Primary: hard chancre; Secondary: rash, cutaneous lesions, condylomata, malaise, fever, swollen lymph nodes; Tertiary: gummas, cardiovascular syphilis, neurosyphilis, possibly fatal	Sexual contact or from a pregnant person to neonate during birth	Darkfield or brightfield silver stain examination of lesion tissue or exudate, treponemal and non-treponemal serological testing, VDRL-CSF for neurosyphilis, prenatal TORCH panel	Penicillin G, tetracycline, doxycycline

Table 7.6: Bacterial infections of the reproductive tract

VIRAL INFECTIONS OF THE REPRODUCTIVE SYSTEM

- **Genital herpes** is usually caused by **HSV-2** (although HSV-1 can also be responsible) and may cause the development of infectious, potentially recurrent vesicles
- **Neonatal herpes** can occur in babies born to infected mothers and can cause symptoms that range from relatively mild (more common) to severe.
- **Human papillomaviruses** are the most common sexually transmitted viruses and include strains that

cause **genital warts** as well as strains that cause **cervical cancer**.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs/Vaccines
Cervical cancer	HPV types 16, 18, and others	Development of cancer in cervix (or elsewhere)	Direct contact, including sexual	Pap smear	Gardasil vaccine, Cervarix vaccine
Genital herpes	Herpes simplex virus (HSV-1 or HSV-2)	Recurring outbreaks of skin vesicles on genitalia and elsewhere; asymptomatic in many individuals	Sexual contact or contact with open lesions	Viral culture, PCR, ELISA	Acyclovir, famciclovir, valacyclovir
Human papillomas	Human papillomavirus (HPV) (various strains)	Genital warts or warts in other areas	Direct contact, including sexual	Pap smear	Imiquimod, podofilox, sinecatechins.
Neonatal herpes	Herpes simplex virus (HSV-1 or HSV-2)	Vesicles on the skin, mucous membranes, eyes; in disseminated infections, motor impairment and possible death of fetus or newborn	Exposure to pathogens in the birth canal; transplacental infection in some cases	Viral culture or PCR	Acyclovir

Table 7.7: Viral infections of the reproductive tract

FUNGAL INFECTIONS OF THE REPRODUCTIVE SYSTEM

- *Candida* spp. are typically present in the normal microbiota in the body, including the skin, respiratory tract, gastrointestinal tract, and female urogenital system.
- Disruptions in the normal vaginal microbiota can lead to an overgrowth of *Candida*, causing vaginal **candidiasis**.
- Vaginal candidiasis can be treated with topical or oral fungicides. Prevention is difficult.

PROTOZOAN INFECTIONS OF THE UROGENITAL SYSTEM

- **Trichomoniasis** is a common STI caused by *Trichomonas vaginalis*.
- *T. vaginalis* is common at low levels in the normal microbiota.
- Trichomoniasis is often asymptomatic. When symptoms develop, trichomoniasis causes urinary discomfort, irritation, itching, burning, discharge from the penis (in men), and vaginal discharge (in women).

- Trichomoniasis is treated with the anti-flagellate drugs tinidazole and metronidazole.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Trichomoniasis	*Trichomonas vaginalis*	Urethritis, vaginal or penile discharge; redness or soreness of female genitalia	Sexual contact	Wet mounts, NAAT of urine or vaginal samples; OSOM Trichomonas Rapid Test, Affirm VPII Microbial Identification Test	Metronidazole, tinidazole
Vaginal candidiasis (yeast infection)	*Candida* spp., especially *C. albicans*	Dysuria; vaginal burning, itching, discharge	Transmissible by sexual contact, but typically only causes opportunistic infections after immunosuppression or disruption of vaginal microbiota	Culture, Affirm VPII Microbial Identification Test	Fluconazole, miconazole, clotrimazole, tioconazole, nystatin

Table 7.8: Fungal and protozoan infections of the reproductive tract

Figure Descriptions

Figure 7.1: A thin strip with 4 colored regions. Each region matches a set of colors on a container. Each different color indicates a different measurement for a particular test.

Figure 7.2: (a) Micrograph of many spiral-shaped cells. (b) Higher magnification shows the spiral shape more clearly.

Figure 7.3: Micrograph showing oddly (roughly round) shaped structures, additionally there are three darker spherical shaped objects with white arrows pointing to them.

Figure 7.4: Micrograph of larger human cells and smaller bacterial cells.

Figure 7.5: Part A shows a penis with white discharge. Part B shows a vagina with a metal tool. Part C is a micrograph of urethral discharge showing red spots on a yellow background.

Figure 7.6: a) Micrograph showing brown coloration inside cells. B) photo of a swollen region on either side of the penis.

Figure 7.7: a) Photo of red, open sores on a penis. B) Photo of brown spots on the palm of the hands. C) Photo of a red, open sore on the nose.

Figure 7.8: a) micrograph of a spiral cell. b) micrograph of many spiral cells.

Figure 7.9: a) Photo of a white swelling on a penis. B) micrograph of rod-shaped pink cells.

Figure 7.10: Micrograph of round structures.

Figure 7.11: Photo of penis with white sores. B) Photo of skin with red raised bumps.

Figure 7.12: Photos of lumpy protrusions in the anus and vaginal regions.

Figure 7.13: Micrograph of cells. On the left are thin flaky cells with nuclei. On the right are cells with much larger nuclei.

Figure 7.14: a) The micrograph shows long strands with dark blue spheres labeled chlamydospores on the tips of the strands, which are labeled pseudohyphae. Smaller clear spheres in clusters on the strand are labeled blastospores.

Figure 7.15: Micrograph of two circular cells attached to each other; one is labeled daughter cell and the other is labeled mother cell. The mother cell has a small protrusion labeled germ tube (6 minutes old).

Figure 7.16: a) micrograph of a large pink cell with a nucleus and smaller pink rod-shaped cells. B) Micrograph of long tubes labeled pseudohyphae.

Figure 7.17: Micrograph of small purple cells and larger oval cells labeled T. vaginalis.

Figure References

Figure 7.1: A urine dipstick is compared against a color key to determine levels of various chemicals, proteins, or cells in the urine. (c) modification of work by Suzanne Wakim. CC BY 4.0.

Figure 7.2: Dark field view of Leptospira spp. Left: Modification of work by Bluuurgh at Dutch Royal Tropical Institute. Public Domain. https://commons.wikimedia.org/wiki/File:Leptospirosis_darkfield.jpg. Right: Modification of work by Janice Carr, Centers for Disease Control and Prevention. Public Domain.

Figure 7.3: Ureaplasma urealyticum microcolonies (white arrows) on agar surface after anaerobic incubation, visualized using phase contrast microscopy (800×). (c) American Society of Microbiology. Redistribution authorized with attribution.

Figure 7.4: In this vaginal smear, the cell at the lower left is a clue cell with a unique appearance caused by the presence of bacteria on the cell that obscures the normally visible borders of the cell. By Mikael Haggstrom. CC0/Public Domain. https://commons.wikimedia.org/wiki/File:Vaginal_wet_mount_with_a_clue_cell.jpg

Figure 7.5: Clinical photograph of gonococcal discharge from penis. Left, Middle: Centers for Disease Control and Prevention. Public Domain. Right: (c) American Society of Microbiology. Redistribution authorized with attribution.

Figure 7.6: Chlamydia trachomatis inclusion bodies within McCoy cell monolayers. Inclusion bodies are distinguished by their brown color. Left: Centers for Disease Control and Prevention. Public Domain. Right: Modification of work (c) Herbert L. Fred and Hendrik A. van Dijk. CC BY 4.0.

Figure 7.7: (a) This ulcerated sore is a hard chancre caused by syphilis. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 7.8: (a) Darkfield micrograph of Treponema pallidum. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 7.9: (a) A soft chancre on the penis of a man with chancroid. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 7.10: Virions of the herpes simplex virus are shown here in this transmission electron micrograph. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 7.11: Genital herpes. Left: Figure 2 CC0/Public Domain. https://commons.wikimedia.org/w/index.php?curid=12666289. in McIntosh, L.S. (2019). Herpes Infections: Cutaneous Manifestations. In: Russell, J., Ryan Jr., E. (eds) Common Dermatologic Conditions in Primary Care. Current Clinical Practice. Humana, Cham. https://doi.org/10.1007/978-3-030-18065-2_8. Right: Figure 2 in Schiffer JT, Swan D, Al Sallaq R, Magaret A, Johnston C, Mark KE, Selke S, Ocbamichael N, Kuntz S, Zhu J, Robinson B, Huang ML, Jerome KR, Wald A, Corey L. Rapid localized spread and immunologic containment define Herpes simplex virus-2 reactivation in the human genital tract. Elife. 2013 Apr 16;2:e00288. doi: 10.7554/eLife.00288. CC BY 3.0.

Figure 7.12: Genital warts may occur around the anus (left) or genitalia (right). Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 7.13: In this image, the cervical cells on the left are normal and those on the right show enlarged and sometimes multiple nuclei with hyperchromasia (darkly stained nuclei) typical of HPV-infected koilocytes. Modification of work by (c) Ed Uthman. CC BY SA 2.0. https://commons.wikimedia.org/wiki/File:ThinPrep_Pap_smear_HPV.jpeg

Figure 7.14: Candida blastospores (asexual spores that result from budding) and chlamydospores (resting spores produced through asexual reproduction) are visible in this micrograph. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 7.15: Candida can produce germ tubes, like the one in this micrograph, that develop into hyphae. (c) American Society of Microbiology. Redistribution authorized with attribution.

Figure 7.16: Lactobacilli are visible as gram-positive rods on and around this squamous epithelial cell. Left: Modification of work by Centers for Disease Control and Prevention. Public Domain. Right: Modification of work (c) Häggström, Mikael (2014). "Medical gallery of Mikael Häggström 2014". WikiJournal of Medicine 1 (2). DOI:10.15347/wjm/2014.008. CC0/Public Domain. https://commons.wikimedia.org/wiki/File:Vaginal_wet_mount_of_candidal_vulvovaginitis.jpg

Figure 7.17: Trichomonas vaginalis is visible in this Gram stained specimen. (c) American Society of Microbiology. Redistribution authorized with attribution.

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CHAPTER 8.

SYSTEMIC INFECTIONS OF THE NERVOUS SYSTEM

8.1 INTRODUCTION TO BACTERIAL DISEASES OF THE NERVOUS SYSTEM

Bacterial infections that affect the nervous system are serious and can be life-threatening. Fortunately, there are only a few bacterial species commonly associated with neurological infections. Common pathogens are summarized in table 8.1.

BACTERIAL MENINGITIS

Bacterial meningitis is one of the most serious forms of meningitis. Bacteria that cause meningitis often gain access to the CNS through the bloodstream after trauma or as a result of the action of bacterial toxins. Bacteria may also spread from structures in the upper respiratory tract, such as the oropharynx, nasopharynx, sinuses, and middle ear. Patients with head wounds or cochlear implants (an electronic device placed in the inner ear) are also at risk for developing meningitis.

Many of the bacteria that can cause meningitis are commonly found in healthy people. The most common causes of non-neonatal bacterial meningitis are *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*. All three of these bacterial pathogens are spread from person to person by respiratory secretions. Each can colonize and cross through the mucous membranes of the oropharynx and nasopharynx, and enter the blood. Once in the blood, these pathogens can disseminate throughout the body and are capable of both establishing an infection and triggering inflammation in any body site, including the meninges (figure 8.1). Without appropriate systemic antibacterial therapy, the case-fatality rate can be as high as 70%, and 20% of survivors may be left with irreversible nerve damage or tissue destruction, resulting in hearing loss, neurologic disability, or loss of a limb. Mortality rates are much lower (as low as 15%) in populations where appropriate therapeutic drugs and preventive vaccines are available.[1]

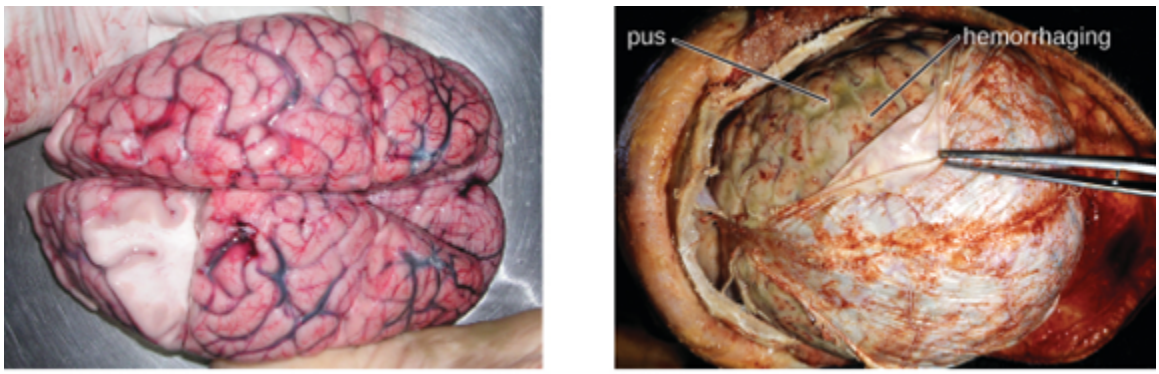

Figure 8.1: (a) A normal human brain removed during an autopsy. (b) The brain of a patient who died from bacterial meningitis. Note the pus under the dura mater (being retracted by the forceps) and the red hemorrhagic foci on the meninges. *Figure description available at the end of the chapter.*

A variety of other bacteria, including *Listeria monocytogenes* and *Escherichia coli*, are also capable of causing meningitis. These bacteria cause infections of the arachnoid mater and CSF after spreading through the circulation in blood or by spreading from an infection of the sinuses or nasopharynx. *Streptococcus agalactiae*, commonly found in the microbiota of the vagina and gastrointestinal tract, can also cause bacterial meningitis in newborns after transmission from the mother either before or during birth.

The profound inflammation caused by these microbes can result in early symptoms that include severe headache, fever, confusion, nausea, vomiting, photophobia, and stiff neck. Systemic inflammatory responses associated with some types of bacterial meningitis can lead to hemorrhaging and purpuric lesions on skin, followed by even more severe conditions that include shock, convulsions, coma, and death—in some cases, in the span of just a few hours.

Diagnosis of bacterial meningitis is best confirmed by analysis of CSF obtained by a lumbar puncture. Abnormal levels of polymorphonuclear neutrophils (PMNs) (> 10 PMNs/mm3), glucose (< 45 mg/dL), and protein (> 45 mg/dL) in the CSF are suggestive of bacterial meningitis.[2] Characteristics of specific forms of bacterial meningitis are detailed in the subsections that follow.

Meningococcal Meningitis

Meningococcal meningitis is a serious infection caused by the gram-negative coccus *N. meningitidis*. In some cases, death can occur within a few hours of the onset of symptoms. Nonfatal cases can result in irreversible nerve damage, resulting in hearing loss and brain damage, or amputation of extremities because of tissue necrosis.

Meningococcal meningitis can infect people of any age, but its prevalence is highest among infants, adolescents, and young adults.[3] Meningococcal meningitis was once the most common cause of meningitis epidemics in human populations. This is still the case in a swath of sub-Saharan Africa known as the meningitis belt, but meningococcal meningitis epidemics have become rare in most other regions, thanks to meningococcal vaccines. However, outbreaks can still occur in communities, schools, colleges, prisons, and other populations where people are in close direct contact.

N. meningitidis has a high affinity for mucosal membranes in the oropharynx and nasopharynx. Contact with respiratory secretions containing *N. meningitidis* is an effective mode of transmission. The pathogenicity of *N. meningitidis* is enhanced by virulence factors that contribute to the rapid progression of the disease. These include lipooligosaccharide (LOS) endotoxin, type IV pili for attachment to host tissues, and polysaccharide capsules that help the cells avoid phagocytosis and complement-mediated killing. Additional virulence factors include IgA protease (which breaks down IgA antibodies), the invasion factors Opa, Opc, and porin (which facilitate transcellular entry through the blood-brain barrier), iron-uptake factors (which strip heme units from hemoglobin in host cells and use them for growth), and stress proteins that protect bacteria from reactive oxygen molecules.

A unique sign of meningococcal meningitis is the formation of a petechial rash on the skin or mucous membranes, characterized by tiny, red, flat, hemorrhagic lesions. This rash, which appears soon after disease onset, is a response to LOS endotoxin and adherence virulence factors that disrupt the endothelial cells of capillaries and small veins in the skin. The blood vessel disruption triggers the formation of tiny blood clots, causing blood to leak into the surrounding tissue. As the infection progresses, the levels of virulence factors increase, and the hemorrhagic lesions can increase in size as blood continues to leak into tissues. Lesions larger than 1.0 cm usually occur in patients developing shock, as virulence factors cause increased hemorrhage and clot formation.

Sepsis, as a result of systemic damage from meningococcal virulence factors, can lead to rapid multiple organ failure, shock, disseminated intravascular coagulation, and death.

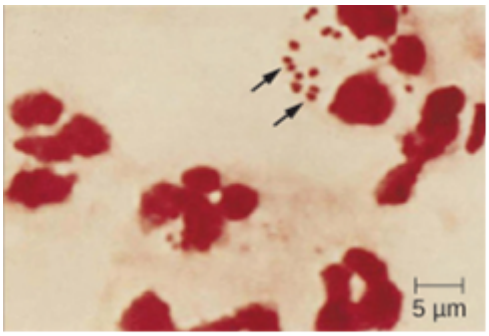

Figure 8.2: N. meningitidis (arrows) associated with neutrophils (the larger stained cells) in a gram-stained CSF sample. *Figure description available at the end of the chapter.*

Because meningococcal meningitis progresses so rapidly, a greater variety of clinical specimens are required for the timely detection of *N. meningitidis*. Required specimens can include blood, CSF, naso- and oropharyngeal swabs, urethral and endocervical swabs, petechial aspirates, and biopsies. Safety protocols for handling and transport of specimens suspected of containing *N. meningitidis* should always be followed, since cases of fatal meningococcal disease have occurred in healthcare workers exposed to droplets or aerosols from patient specimens. Prompt presumptive diagnosis of meningococcal meningitis can occur when CSF is directly evaluated by Gram stain, revealing extra- and intracellular, gram-negative diplococci with a distinctive coffee-bean microscopic morphology associated with PMNs (figure 8.2). Identification can also be made directly from CSF using latex agglutination and immunochromatographic rapid diagnostic tests specific for *N. meningitidis*. Species identification can also be performed using DNA sequence-based typing schemes for hypervariable outer membrane proteins of *N. meningitidis*, which has replaced sero(sub)typing.

Meningococcal infections can be treated with antibiotic therapy, and third-generation cephalosporins are most often employed. However, because outcomes can be negative even with treatment, preventive vaccination is the best form of treatment. In 2010, countries in Africa's meningitis belt began using a new serogroup A meningococcal conjugate vaccine. This program has dramatically reduced the number of cases of meningococcal meningitis by conferring individual and herd immunity.

Twelve different capsular serotypes of *N. meningitidis* are known to exist. Serotypes A, B, C, W, X, and Y are the most prevalent worldwide. The CDC recommends that children between 11–12 years of age be vaccinated with a single dose of a quadrivalent vaccine that protects against serotypes A, C, W, and Y, with a booster at age 16.[4] An additional booster or injections of serogroup B meningococcal vaccine may be given to individuals in high-risk settings (such as epidemic outbreaks on college campuses).

Pneumococcal Meningitis

Pneumococcal meningitis is caused by the encapsulated, gram-positive bacterium *S. pneumoniae* (pneumococcus, also called strep pneumo). This organism is commonly found in the microbiota of the pharynx of 30–70% of young children, depending on the sampling method, while *S. pneumoniae* can be found in fewer than 5% of healthy adults. Although it is often present without disease symptoms, this microbe can cross the blood-brain barrier in susceptible individuals. In some cases, it may also result in septicemia. Since the introduction of the Hib vaccine, *S. pneumoniae* has become the leading cause of meningitis in humans aged 2 months through adulthood.

S. pneumoniae can be identified in CSF samples using gram-stained specimens, latex agglutination, and immunochromatographic RDT specific for *S. pneumoniae*. In gram-stained samples, *S. pneumoniae* appears as gram-positive, lancet-shaped diplococci (figure 8.3). Identification of *S. pneumoniae* can also be achieved using cultures of CSF and blood, and at least 93 distinct serotypes can be identified based on the quellung reaction to unique capsular polysaccharides. PCR and RT-PCR assays are also available to confirm identification.

Major virulence factors produced by *S. pneumoniae* include PI-1 pilin for adherence to host cells (pneumococcal adherence) and virulence factor B (PavB) for attachment to cells of the respiratory tract; choline-binding proteins (cbpA) that bind to epithelial cells and interfere with immune factors IgA and C3; and the cytoplasmic bacterial toxin pneumolysin that triggers an inflammatory response.

With the emergence of drug-resistant strains of *S. pneumoniae*, pneumococcal meningitis is typically treated with broad-spectrum antibiotics, such as levofloxacin, cefotaxime, penicillin, or other β-lactam antibiotics. The two available pneumococcal vaccines are described in section 5.2.

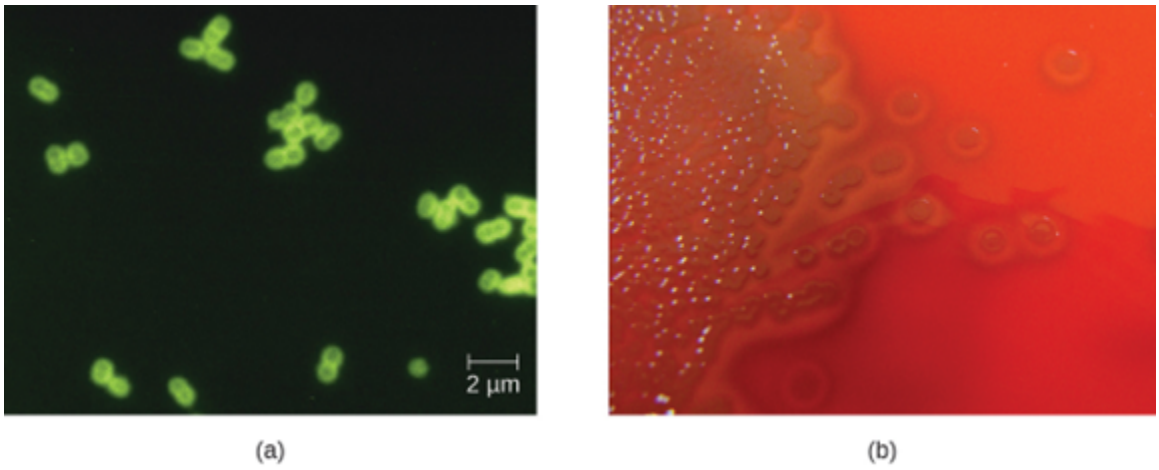

Figure 8.3: (a) Digitally colorized fluorescent antibody stained micrograph of Streptococcus pneumoniae in CSF. (b) S. pneumoniae growing on blood agar. Figure description available at the end of the chapter.

Haemophilus influenzae Type b

Meningitis due to *H. influenzae* serotype b (Hib), an encapsulated pleomorphic gram-negative coccobacilli, is now uncommon in most countries because of the use of the effective Hib vaccine. Without the use of the Hib vaccine, *H. influenzae* can be the primary cause of meningitis in children 2 months through 5 years of age. *H. influenzae* can be found in the throats of healthy individuals, including infants and young children. By five years of age, most children have developed immunity to this microbe. Infants older than 2 months of age, however, do not produce a sufficient protective antibody response and are susceptible to serious disease. The intracranial pressure caused by this infection leads to a 5% mortality rate and 20% incidence of deafness or brain damage in survivors.[5]

H. influenzae produces at least 16 different virulence factors, including LOS, which triggers inflammation, and Haemophilus adhesion and penetration factor (Hap), which aids in attachment and invasion into respiratory epithelial cells. The bacterium also has a polysaccharide capsule that helps it avoid phagocytosis, as well as factors such as IgA1 protease and P2 protein that allow it to evade antibodies secreted from mucous membranes. In addition, factors such as hemoglobin-binding protein (Hgp) and transferrin-binding protein (Tbp) acquire iron from hemoglobin and transferrin, respectively, for bacterial growth.

Preliminary diagnosis of *H. influenzae* infections can be made by direct PCR and a smear of CSF. Stained smears will reveal intracellular and extracellular PMNs with small, pleomorphic, gram-negative coccobacilli or filamentous forms that are characteristic of *H. influenzae*. Initial confirmation of this genus can be based on its fastidious growth on chocolate agar. Identification is confirmed with requirements for exogenous biochemical growth cofactors NAD and heme (by MALDI-TOF), latex agglutination, and RT-PCR.

Meningitis caused by *H. influenzae* is usually treated with doxycycline, fluoroquinolones, second- and third-generation cephalosporins, and carbapenems. The best means of preventing *H. influenzae* infection is with the use of the Hib polysaccharide conjugate vaccine. It is recommended that all children receive this vaccine at 2, 4, and 6 months of age, with a final booster dose at 12 to 15 months of age.[6]

Neonatal Meningitis

S. agalactiae, Group B streptococcus (GBS), is an encapsulated gram-positive bacterium that is the most common cause of neonatal meningitis, a term that refers to meningitis occurring in babies up to 3 months of age.[7] *S. agalactiae* can also cause meningitis in people of all ages and can be found in the urogenital and gastrointestinal microbiota of about 10–30% of humans.

Neonatal infection occurs as either early onset or late-onset disease. Early onset disease is defined as occurring in infants up to 7 days old. The infant initially becomes infected by *S. agalactiae* during childbirth, when the bacteria may be transferred from the mother's vagina. Incidence of early onset neonatal meningitis can be greatly reduced by giving intravenous antibiotics to the mother during labor.

Late-onset neonatal meningitis occurs in infants between 1 week and 3 months of age. Infants born to mothers with *S. agalactiae* in the urogenital tract have a higher risk of late-onset meningitis, but late-onset infections can be transmitted from sources other than the mother; often, the source of infection is unknown. Infants who are born prematurely (before 37 weeks of pregnancy) or to mothers who develop a fever also have a greater risk of contracting late-onset neonatal meningitis.

Signs and symptoms of early onset disease include temperature instability, apnea (cessation of breathing), bradycardia (slow heart rate), hypotension, difficulty feeding, irritability, and limpness. When asleep, the baby may be difficult to wake up. Symptoms of late-onset disease are more likely to include seizures, bulging fontanel (soft spot), stiff neck, hemiparesis (weakness on one side of the body), and opisthotonos (rigid body with arched back and head thrown backward).

S. agalactiae produces at least 12 virulence factors that include FbsA that attaches to host cell surface proteins, PI-1 pili that promotes the invasion of human endothelial cells, a polysaccharide capsule that prevents the activation of the alternative complement pathway and inhibits phagocytosis, and the toxin CAMP factor, which forms pores in host cell membranes and binds to IgG and IgM antibodies.

Diagnosis of neonatal meningitis is often, but not uniformly, confirmed by positive results from cultures of CSF or blood. Tests include routine culture, antigen detection by enzyme immunoassay, serotyping of different capsule types, PCR, and RT-PCR. It is typically treated with β-lactam antibiotics such as intravenous penicillin or ampicillin plus gentamicin. Even with treatment, roughly 10% mortality is seen in infected neonates.[8]

CLOSTRIDIUM-ASSOCIATED DISEASES

Species in the genus *Clostridium* are gram-positive, endospore-forming rods that are obligate anaerobes. Endospores of *Clostridium* spp. are widespread in nature, commonly found in soil, water, feces, sewage, and marine sediments. *Clostridium* spp. produce more types of protein exotoxins than any other bacterial genus, including two exotoxins with protease activity that are the most potent known biological toxins: botulinum neurotoxin (BoNT) and tetanus neurotoxin (TeNT). These two toxins have lethal doses of 0.2–10 ng per kg body weight.

BoNT can be produced by unique strains of *C. butyricum*, and *C. baratii*; however, it is primarily associated with *C. botulinum* and the condition of botulism. TeNT, which causes tetanus, is only produced by *C. tetani*. These powerful neural exotoxins are the primary virulence factors for these pathogens. The mode of action for these toxins was described in section 2.14 and illustrated in figure 2.96.

Diagnosis of tetanus or botulism typically involves bioassays that detect the presence of BoNT and TeNT in fecal specimens, blood (serum), or suspect foods. In addition, both *C. botulinum* and *C. tetani* can be isolated and cultured using commercially available media for anaerobes. ELISA and RT-PCR tests are also available.

Tetanus

Tetanus is a noncommunicable disease characterized by uncontrollable muscle spasms (contractions) caused by the action of TeNT. It generally occurs when *C. tetani* infects a wound and produces TeNT, which rapidly binds to neural tissue, resulting in an intoxication (poisoning) of neurons. Depending on the site and extent of infection, cases of tetanus can be described as localized, cephalic, or generalized. Generalized tetanus that occurs in a newborn is called neonatal tetanus.

Localized tetanus occurs when TeNT only affects the muscle groups close to the injury site. There is no CNS involvement, and the symptoms are usually mild, with localized muscle spasms caused by a dysfunction in the surrounding neurons. Individuals with partial immunity—especially previously vaccinated individuals who neglect to get the recommended booster shots—are most likely to develop localized tetanus as a result of *C. tetani* infecting a puncture wound.

Cephalic tetanus is a rare, localized form of tetanus generally associated with wounds on the head or face. In rare cases, it has occurred in cases of otitis media (middle ear infection). Cephalic tetanus often results in patients seeing double images, because of the spasms affecting the muscles that control eye movement.

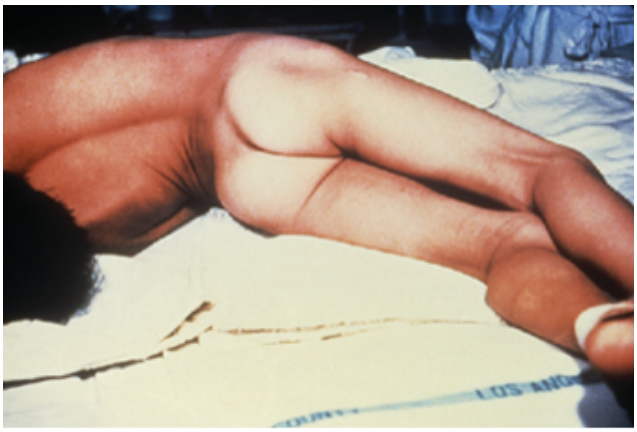

Figure 8.4: A tetanus patient exhibiting the rigid body posture known as opisthotonos. *Figure description available at the end of the chapter.*

Both localized and cephalic tetanus may progress to generalized tetanus—a much more serious condition—if TeNT is able to spread further into body tissues. In generalized tetanus, TeNT enters neurons of the PNS. From there, TeNT travels from the site of the wound, usually on an extremity of the body, retrograde (back up) to inhibitory neurons in the CNS. It then prevents the release of gamma aminobutyric acid (GABA), the neurotransmitter responsible for muscle relaxation. The resulting muscle spasms often first occur in the jaw muscles, leading to the characteristic symptom of lockjaw (inability to open the mouth). As the toxin progressively continues to block neurotransmitter release, other muscles become involved, resulting in uncontrollable, sudden muscle spasms that are powerful enough to cause tendons to rupture and bones to fracture. Spasms in the muscles in the neck, back, and legs may cause the body to form a rigid, stiff arch, a posture called opisthotonos (figure 8.4). Spasms in the larynx, diaphragm, and muscles of the chest restrict the patient's ability to swallow and breathe, eventually leading to death by asphyxiation (insufficient supply of oxygen).

Neonatal tetanus typically occurs when the stump of the umbilical cord is contaminated with spores of *C. tetani* after delivery. Although this condition is rare in the United States, neonatal tetanus is a major cause of infant

mortality in countries that lack maternal immunization for tetanus and where birth often occurs in unsanitary conditions. At the end of the first week of life, infected infants become irritable, feed poorly, and develop rigidity with spasms. Neonatal tetanus has a very poor prognosis with a mortality rate of 70%–100%.[9]

Treatment for patients with tetanus includes assisted breathing through the use of a ventilator, wound debridement, fluid balance, and antibiotic therapy with metronidazole or penicillin to halt the growth of *C. tetani*. In addition, patients are treated with TeNT antitoxin, preferably in the form of human immunoglobulin to neutralize non-fixed toxins and benzodiazepines to enhance the effect of GABA for muscle relaxation and anxiety.

A tetanus toxoid (TT) vaccine is available for protection and prevention of tetanus. It is the "T" component of vaccines such as DTaP, Tdap, and Td. The CDC recommends children receive doses of the DTaP vaccine at 2, 4, 6, and 15–18 months of age and another at 4–6 years of age. One dose of Td is recommended for adolescents and adults as a TT booster every 10 years.[10]

Botulism

Botulism is a rare but frequently fatal illness caused by intoxication by BoNT. It can occur either as the result of an infection by *C. botulinum*, in which case the bacteria produce BoNT in vivo, or as the result of a direct introduction of BoNT into tissues.

Infection and production of BoNT in vivo can result in wound botulism, infant botulism, and adult intestinal toxemia. Wound botulism typically occurs when *C. botulinum* is introduced directly into a wound after a traumatic injury, deep puncture wound, or via an injection site. Infant botulism, which occurs in infants younger than 1 year of age, and adult intestinal toxemia, which occurs in immunocompromised adults, results from ingesting *C. botulinum* endospores in food. The endospores germinate in the body, resulting in the production of BoNT in the intestinal tract.

Intoxications occur when BoNT is produced outside the body and then introduced directly into the body through food (foodborne botulism), air (inhalation botulism), or a clinical procedure (iatrogenic botulism). Foodborne botulism, the most common of these forms, occurs when BoNT is produced in contaminated food and then ingested along with the food. Inhalation botulism is rare because BoNT is unstable as an aerosol and does not occur in nature; however, it can be produced in the laboratory and was used (unsuccessfully) as a bioweapon by terrorists in Japan in the 1990s. A few cases of accidental inhalation botulism have also occurred. Iatrogenic botulism is also rare; it is associated with injections of BoNT used for cosmetic purposes.

When BoNT enters the bloodstream in the gastrointestinal tract, wound, or lungs, it is transferred to the neuromuscular junctions of motor neurons where it binds irreversibly to presynaptic membranes and prevents the release of acetylcholine from the presynaptic terminal of motor neurons into the neuromuscular junction. The consequence of preventing acetylcholine release is the loss of muscle activity, leading to muscle relaxation and eventually paralysis.

If BoNT is absorbed through the gastrointestinal tract, early symptoms of botulism include blurred vision, drooping eyelids, difficulty swallowing, abdominal cramps, nausea, vomiting, constipation, or possibly diarrhea. This is followed by progressive flaccid paralysis, a gradual weakening and loss of control over the muscles. A patient's experience can be particularly terrifying, because hearing remains normal, consciousness is not lost, and he or she is fully aware of the progression of his or her condition. In infants, notable signs of botulism include weak cry, decreased ability to suckle, and hypotonia (limpness of head or body). Eventually, botulism ends in death from respiratory failure caused by the progressive paralysis of the muscles of the upper airway, diaphragm, and chest.

Botulism is treated with an antitoxin specific for BoNT. If administered in time, the antitoxin stops the progression of paralysis but does not reverse it. Once the antitoxin has been administered, the patient will slowly regain neurological function, but this may take several weeks or months, depending on the severity of the case. During recovery, patients generally must remain hospitalized and receive breathing assistance through a ventilator.

Listeriosis

The foodborne pathogen that causes listeriosis, Listeria monocytogenes, is a nonencapsulated, non-sporulating, gram-positive rod. At-risk groups include pregnant women, neonates, the elderly, and the immunocompromised. Listeriosis leads to meningitis in about 20% of cases, particularly neonates and patients over the age of 60. The CDC identifies listeriosis as the third leading cause of death due to foodborne illness, with overall mortality rates reaching 16%.[11] In pregnant women, listeriosis can also cause spontaneous abortion in pregnant women because of the pathogen's unique ability to cross the placenta.

L. monocytogenes is generally introduced into food items by contamination with soil or animal manure used as fertilizer. Foods commonly associated with listeriosis include fresh fruits and vegetables, frozen vegetables, processed meats, soft cheeses, and raw milk.[12] Unlike most other foodborne pathogens, *L. monocytogenes* is able to grow at temperatures between 0 °C and 50 °C, and can therefore continue to grow, even in refrigerated foods.

Ingestion of contaminated food leads initially to infection of the gastrointestinal tract. However, *L. monocytogenes* produces several unique virulence factors that allow it to cross the intestinal barrier and spread to other body systems. Surface proteins called internalins (InlA and InlB) help *L. monocytogenes* invade nonphagocytic cells and tissues, penetrating the intestinal wall and becoming disseminating through the circulatory and lymphatic systems. Internalins also enable *L. monocytogenes* to breach other important barriers, including the blood-brain barrier and the placenta. Within tissues, *L. monocytogenes* uses other proteins called listeriolysin O and ActA to facilitate intercellular movement, allowing the infection to spread from cell to cell (figure 8.5).

L. monocytogenes is usually identified by cultivation of samples from a normally sterile site (e.g., blood or CSF). Recovery of viable organisms can be enhanced using cold enrichment by incubating samples in a broth at 4 °C for a week or more. Distinguishing types and subtypes of *L. monocytogenes*—an important step for diagnosis and epidemiology—is typically done using pulsed-field gel electrophoresis. Identification can also be achieved using chemiluminescence DNA probe assays and MALDI-TOF.

Treatment for listeriosis involves antibiotic therapy, most commonly with ampicillin and gentamicin. There is no vaccine available.

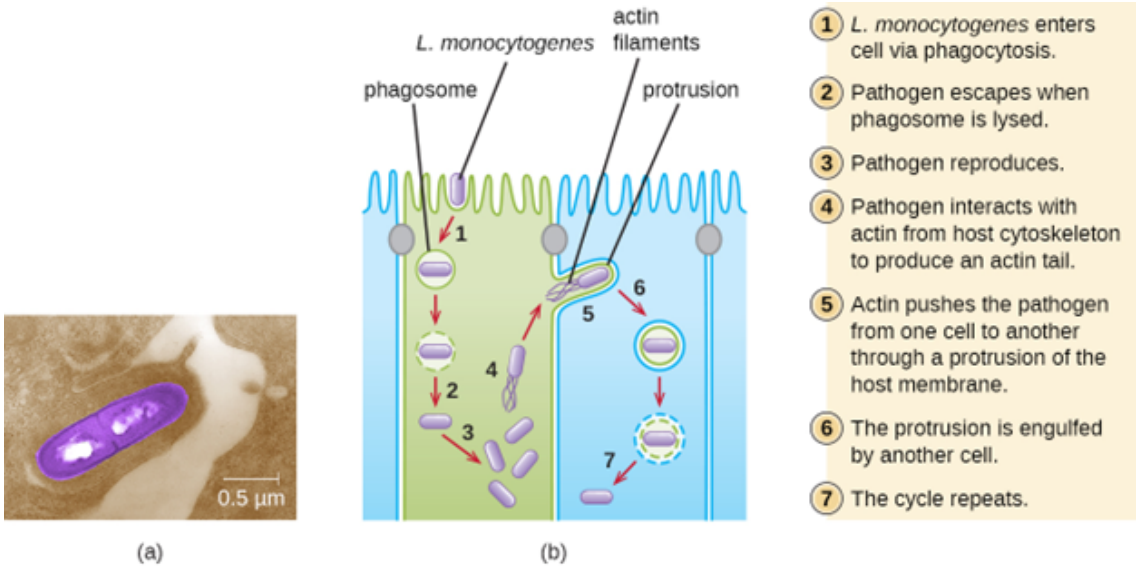

Figure 8.5: (a) An electron micrograph of Listeria monocytogenes infecting a host cell. (b) Listeria is able to use host cell components to cause infection. For example, phagocytosis allows it to enter host cells, and the host's cytoskeleton provides the materials to help the pathogen move to other cells. *Figure description available at the end of the chapter.*

HANSEN'S DISEASE (LEPROSY)

Hansen's disease (also known as leprosy) is caused by a long, thin, filamentous rod-shaped bacterium *Mycobacterium leprae*, an obligate intracellular pathogen. *M. leprae* is classified as gram-positive bacteria, but it is best visualized microscopically with an acid-fast stain and is generally referred to as an acid-fast bacterium. Hansen's disease affects the PNS, leading to permanent damage and loss of appendages or other body parts.

Hansen's disease is communicable but not highly contagious; approximately 95% of the human population cannot be easily infected because they have a natural immunity to *M. leprae*. Person-to-person transmission occurs by inhalation into nasal mucosa or prolonged and repeated contact with infected skin. Armadillos, one of only five mammals susceptible to Hansen's disease, have also been implicated in transmission of some cases.[13]

In the human body, *M. leprae* grows best at the cooler temperatures found in peripheral tissues like the nose, toes, fingers, and ears. Some of the virulence factors that contribute to *M. leprae's* pathogenicity are located on the capsule and cell wall of the bacterium. These virulence factors enable it to bind to and invade Schwann cells, resulting in progressive demyelination that gradually destroys neurons of the PNS. The loss of neuronal function leads to hypoesthesia (numbness) in infected lesions. *M. leprae* is readily phagocytized by macrophages but is able to survive within macrophages in part by neutralizing reactive oxygen species produced in the oxidative burst of the phagolysosome. Like *L. monocytogenes*, *M. leprae* also can move directly between macrophages to avoid clearance by immune factors.

The extent of the disease is related to the immune response of the patient. Initial symptoms may not appear for as long as 2 to 5 years after infection. These often begin with small, blanched, numb areas of the skin. In most individuals, these will resolve spontaneously, but some cases may progress to a more serious form of the disease. Tuberculoid (paucibacillary) Hansen's disease is marked by the presence of relatively few (three or less) flat, blanched skin lesions with small nodules at the edges and few bacteria present in the lesion. Although these lesions can persist for years or decades, the bacteria are held in check by an effective immune response including cell-mediated cytotoxicity. Individuals who are unable to contain the infection may later develop leproma-

tous (multibacillary) Hansen's disease. This is a progressive form of the disease characterized by nodules filled with acid-fast bacilli and macrophages. Impaired function of infected Schwann cells leads to peripheral nerve damage, resulting in sensory loss that leads to ulcers, deformities, and fractures. Damage to the ulnar nerve (in the wrist) by *M. leprae* is one of the most common causes of crippling of the hand. In some cases, chronic tissue damage can ultimately lead to loss of fingers or toes. When mucosal tissues are also involved, disfiguring lesions of the nose and face can also occur (figure 8.6).

Hansen's disease is diagnosed on the basis of clinical signs and symptoms of the disease, and confirmed by the presence of acid-fast bacilli on skin smears or in skin biopsy specimens (figure 8.6). *M. leprae* does not grow in vitro on any known laboratory media, but it can be identified by culturing in vivo in the footpads of laboratory mice or armadillos. Where needed, PCR and genotyping of *M. leprae* DNA in infected human tissue may be performed for diagnosis and epidemiology.

Hansen's disease responds well to treatment and, if diagnosed and treated early, does not cause disability. In the United States, most patients with Hansen's disease are treated in ambulatory care clinics in major cities by the National Hansen's Disease program, the only institution in the United States exclusively devoted to Hansen's disease. Since 1995, WHO has made multidrug therapy for Hansen's disease available free of charge to all patients worldwide. As a result, global prevalence of Hansen's disease has declined from about 5.2 million cases in 1985 to roughly 176,000 in 2014.[14] Multidrug therapy consists of dapsone and rifampicin for all patients and a third drug, clofazimine, for patients with multibacillary disease.

Currently, there is no universally accepted vaccine for Hansen's disease. India and Brazil use a tuberculosis vaccine against Hansen's disease because both diseases are caused by species of Mycobacterium. The effectiveness of this method is questionable, however, since it appears that the vaccine works in some populations but not in others.

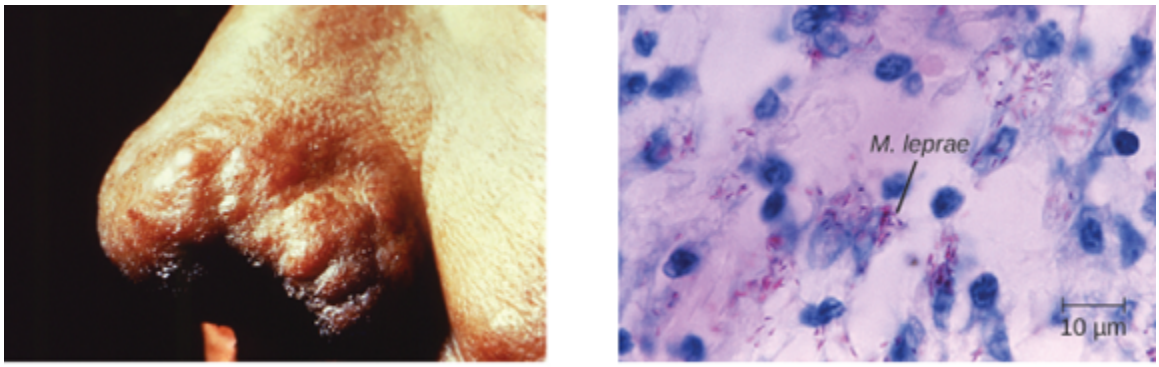

Figure 8.6: (a) The nose of a patient with Hansen's disease. Note the lepromatous/multibacillary lesions around the nostril. (b) Hansen's disease is caused by Mycobacterium leprae, a gram-positive bacillus. *Figure description available at the end of the chapter.*

Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs	Vaccine
Botulism	*Clostridium botulinum*	Blurred vision, drooping eyelids, difficulty swallowing and breathing, nausea, vomiting, often fatal	Ingestion of preformed toxin in food, ingestion of endospores in food by infants or immunocompromised adults, bacterium introduced via wound or injection	Antitoxin; penicillin (for wound botulism)	None
Hansen's disease (leprosy)	*Mycobacterium leprae*	Hypopigmented skin, skin lesions, and nodules, loss of peripheral nerve function, loss of fingers, toes, and extremities	Inhalation, possible transmissible from armadillos to humans	Dapsone, rifampin, clofazimin	None
Haemophilus influenzae type b meningitis	*Haemophilus influenza*	Nausea, vomiting, photophobia, stiff neck, confusion	Direct contact, inhalation of aerosols	Doxycycline, fluoroquinolones, second- and third-generation cephalosporins, and carbapenems	Hib vaccine
Listeriosis	*Listeria monocytogenes*	Initial flu-like symptoms, sepsis and potentially fatal meningitis in susceptible individuals, miscarriage in pregnant people	Bacterium ingested with contaminated food or water	Ampicillin, gentamicin	None
Meningococcal meningitis	*Neisseria meningitidis*	Nausea, vomiting, photophobia, stiff neck, confusion; often fatal	Direct contact	Cephalosporins or penicillins	Meningococcal conjugate
Neonatal meningitis	*Streptococcus agalactiae*	Temperature instability, apnea, bradycardia, hypotension, feeding difficulty, irritability, limpness, seizures, bulging fontanel, stiff neck, opisthotonos, hemiparesis, often fatal	Direct contact in birth canal	Ampicillin plus gentamicin, cefotaxime, or both	None
Pneumococcal meningitis	*Streptococcus pneumoniae*	Nausea, vomiting, photophobia, stiff neck, confusion, often fatal	Direct contact, aerosols	Cephalosporins, penicillin	Pneumococcal vaccines
Tetanus	*Clostridium tetani*	Progressive spasmatic paralysis starting with the jaw, often fatal	Bacterium introduced in puncture wound	Penicillin, antitoxin	DTaP, Tdap

Table 8.1: Bacterial infections of the nervous system

8.2 ACELLULAR DISEASES OF THE NERVOUS SYSTEM

A number of different viruses and subviral particles can cause diseases that affect the nervous system. Viral diseases tend to be more common than bacterial infections of the nervous system today. Fortunately, viral infections are generally milder than their bacterial counterparts and often spontaneously resolve. Some of the more important acellular pathogens of the nervous system are described in this section (and summarized in table 8.2).

VIRAL MENINGITIS

Although it is much more common than bacterial meningitis, viral meningitis is typically less severe. Many different viruses can lead to meningitis as a sequela of the primary infection, including those that cause herpes, influenza, measles, and mumps. Most cases of viral meningitis spontaneously resolve, but severe cases do occur.

Arboviral Encephalitis

Several types of insect-borne viruses can cause encephalitis. Collectively, these viruses are referred to as arboviruses (because they are arthropod-borne), and the diseases they cause are described as arboviral encephalitis. Most arboviruses are endemic to specific geographical regions. Arboviral encephalitis diseases found in the United States include eastern equine encephalitis (EEE), western equine encephalitis (WEE), St. Louis encephalitis, and West Nile encephalitis (WNE). Expansion of arboviruses beyond their endemic regions sometimes occurs, generally as a result of environmental changes that are favorable to the virus or its vector. Increased travel of infected humans, animals, or vectors has also allowed arboviruses to spread into new regions.

In most cases, arboviral infections are asymptomatic or lead to a mild disease. However, when symptoms do occur, they include high fever, chills, headaches, vomiting, diarrhea, and restlessness. In elderly patients, severe arboviral encephalitis can rapidly lead to convulsions, coma, and death.

Mosquitoes are the most common biological vectors for arboviruses, which tend to be enveloped ssRNA viruses. Thus, prevention of arboviral infections is best achieved by avoiding mosquitoes—using insect repellent, wearing long pants and sleeves, sleeping in well-screened rooms, using bed nets, etc.

Diagnosis of arboviral encephalitis is based on clinical symptoms and serologic testing of serum or CSF. There are no antiviral drugs to treat any of these arboviral diseases, so treatment consists of supportive care and management of symptoms.

Eastern equine encephalitis (EEE) is caused by eastern equine encephalitis virus (EEEV), which can cause severe disease in horses and humans. Birds are reservoirs for EEEV with accidental transmission to horses and humans by *Aedes*, *Coquillettidia*, and *Culex* species of mosquitoes. Neither horses nor humans serve as reservoirs. EEE is most common in US Gulf Coast and Atlantic states. EEE is one of the more severe mosquito-transmitted diseases in the United States, but fortunately, it is a very rare disease in the United States (figure 8.7).[1516]

Western equine encephalitis (WEE) is caused by western equine encephalitis virus (WEEV). WEEV is usually transmitted to horses and humans by the *Culex tarsalis* mosquitoes and, in the past decade, has caused very few cases of encephalitis in humans in the United States. In humans, WEE symptoms are less severe than EEE and include fever, chills, and vomiting, with a mortality rate of 3–4%. Like EEEV, birds are the natural reservoir for WEEV. Periodically, for indeterminate reasons, epidemics in human cases have occurred in North America in the past. The largest on record was in 1941, with more than 3400 cases.[17]

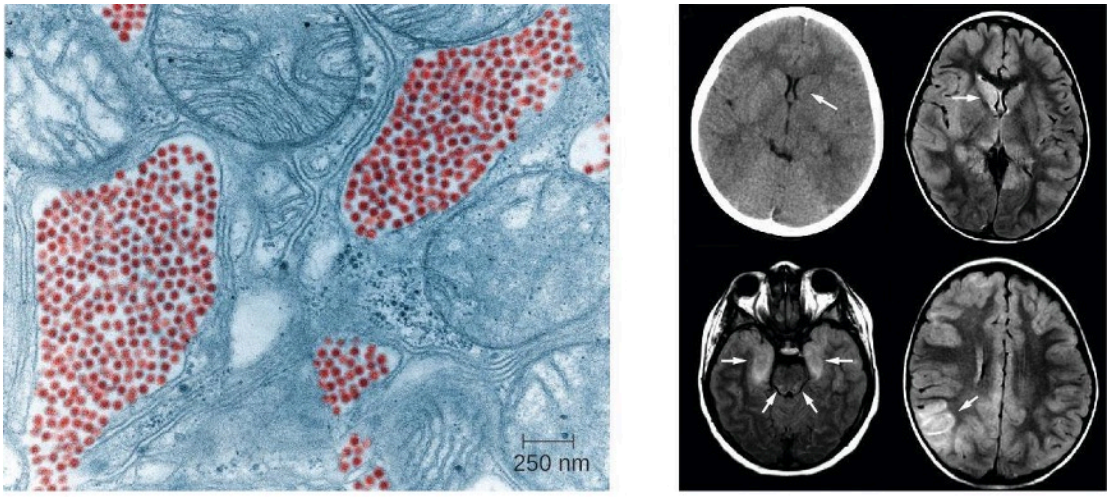

Figure 8.7: (a) A false color TEM of a mosquito salivary gland cell shows an infection of the eastern equine encephalitis virus (red). (b) CT (left) and MRI (right) scans of the brains of children with eastern equine encephalitis infections, showing abnormalities (arrows) resulting from the infection. *Figure description available at the end of the chapter.*

St. Louis encephalitis (SLE), caused by St. Louis encephalitis virus (SLEV), is a rare form of encephalitis with symptoms occurring in fewer than 1% of infected patients. The natural reservoirs for SLEV are birds. SLEV is most often found in the Ohio-Mississippi River basin of the central United States and was named after a severe outbreak in Missouri in 1934. The worst outbreak of St. Louis encephalitis occurred in 1975, with over 2000 cases reported.[18] Humans become infected when bitten by *C. tarsalis*, *C. quinquefasciatus*, or *C. pipiens* mosquitoes carrying SLEV. Most patients are asymptomatic, but in a small number of individuals, symptoms range from mild flu-like syndromes to fatal encephalitis. The overall mortality rate for symptomatic patients is 5–15%.[19]

Japanese encephalitis, caused by Japanese encephalitis virus (JEV), is the leading cause of vaccine-preventable encephalitis in humans and is endemic to some of the most populous countries in the world, including China, India, Japan, and all of Southeast Asia. JEV is transmitted to humans by *Culex* mosquitoes, usually the species *C. tritaeniorhynchus*. The biological reservoirs for JEV include pigs and wading birds. Most patients with JEV infections are asymptomatic, with symptoms occurring in fewer than 1% of infected individuals. However, about 25% of those who do develop encephalitis die, and among those who recover, 30–50% have psychiatric, neurologic, or cognitive impairment.[20] Fortunately, there is an effective vaccine that can prevent infection with JEV. The CDC recommends this vaccine for travelers who expect to spend more than one month in endemic areas.

As the name suggests, West Nile virus (WNV) and its associated disease, West Nile encephalitis (WNE), did not originate in North America. Until 1999, it was endemic in the Middle East, Africa, and Asia; however, the first US cases were identified in New York in 1999, and by 2004, the virus had spread across the entire continental United States. Over 35,000 cases, including 1400 deaths, were confirmed in the five-year period between 1999 and 2004. WNV infection remains reportable to the CDC.

WNV is transmitted to humans by *Culex* mosquitoes from its natural reservoir, infected birds, with 70–80% of infected patients experiencing no symptoms. Most symptomatic cases involve only mild, flu-like symptoms, but fewer than 1% of infected people develop severe and sometimes fatal encephalitis or meningitis. The mortality rate in WNV patients who develop neurological disease is about 10%.

ZIKA VIRUS INFECTION

Zika virus infection is an emerging arboviral disease associated with human illness in Africa, Southeast Asia, and South and Central America; however, its range is expanding as a result of the widespread range of its mosquito vector. The first cases originating in the United States were reported in 2016. The Zika virus was initially described in 1947 from monkeys in the Zika Forest of Uganda through a network that monitored yellow fever. It was not considered a serious human pathogen until the first large-scale outbreaks occurred in Micronesia in 2007;[21] however, the virus has gained notoriety over the past decade, as it has emerged as a cause of symptoms similar to other arboviral infections that include fever, skin rashes, conjunctivitis, muscle and joint pain, malaise, and headache. Mosquitoes of the *Aedes* genus are the primary vectors, although the virus can also be transmitted sexually, from mother to baby during pregnancy, or through a blood transfusion.

Most Zika virus infections result in mild symptoms such as fever, a slight rash, or conjunctivitis. However, infections in pregnant women can adversely affect the developing fetus. Reports in 2015 indicate fetal infections can result in brain damage, including a serious birth defect called microcephaly, in which the infant is born with an abnormally small head (figure 8.8).[22]

Diagnosis of Zika is primarily based on clinical symptoms. However, the FDA recently authorized the use of a Zika virus RNA assay, Trioplex RT-PCR, and Zika MAC-ELISA to test patient blood and urine to confirm Zika virus disease. There are currently no antiviral treatments or vaccines for Zika virus, and treatment is limited to supportive care.

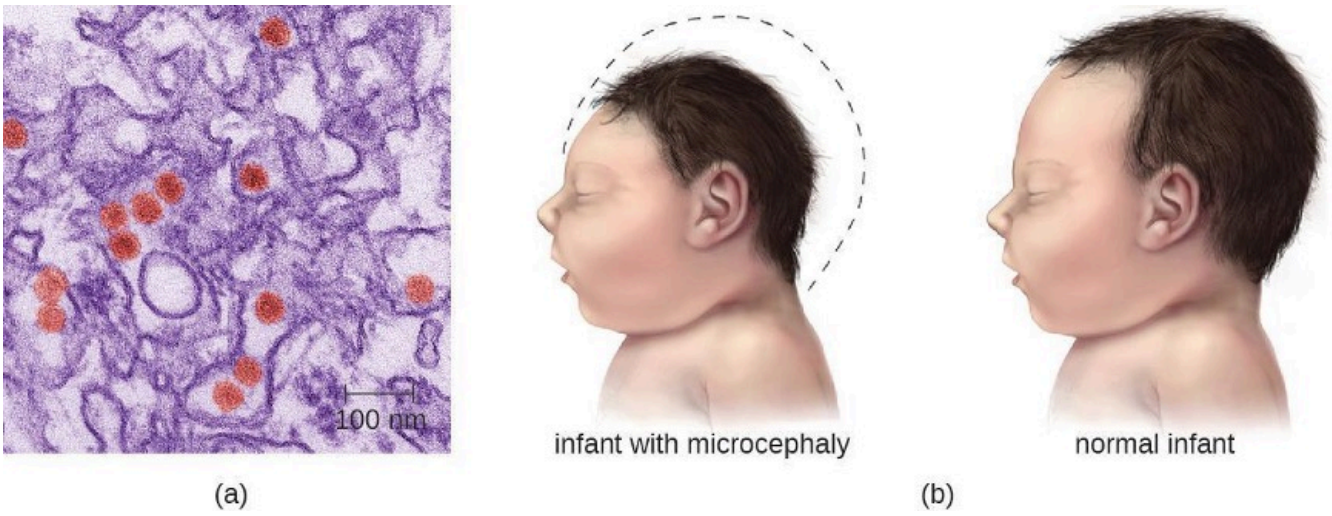

Figure 8.8: (a) This colorized electron micrograph shows Zika virus particles (red). (b) Women infected by the Zika virus during pregnancy may give birth to children with microcephaly, a deformity characterized by an abnormally small head and brain. *Figure description available at the end of the chapter.*

RABIES

Rabies is a deadly zoonotic disease that has been known since antiquity. The disease is caused by rabies virus (RV), a member of the family Rhabdoviridae, and is primarily transmitted through the bite of an infected mammal. Rhabdoviridae are enveloped RNA viruses that have a distinctive bullet shape (figure 8.9); they were first studied by Louis Pasteur (1822-1895), who obtained rabies virus from rabid dogs and cultivated the virus in rabbits. He successfully prepared a rabies vaccine using dried nerve tissues from infected animals. This vaccine was used to first treat an infected human in 1885.

The most common reservoirs in the United States are wild animals such as raccoons (30.2% of all animal cases during 2014), bats (29.1%), skunks (26.3%), and foxes (4.1%); collectively, these animals were responsible for a total of 92.6% of animal rabies cases in the United States in 2014. The remaining 7.4% of cases that year were in domesticated animals such as dogs, cats, horses, mules, sheep, goats, and llamas.[23] While there are typically only one or two human cases per year in the United States, rabies still causes tens of thousands of human deaths per year worldwide, primarily in Asia and Africa.

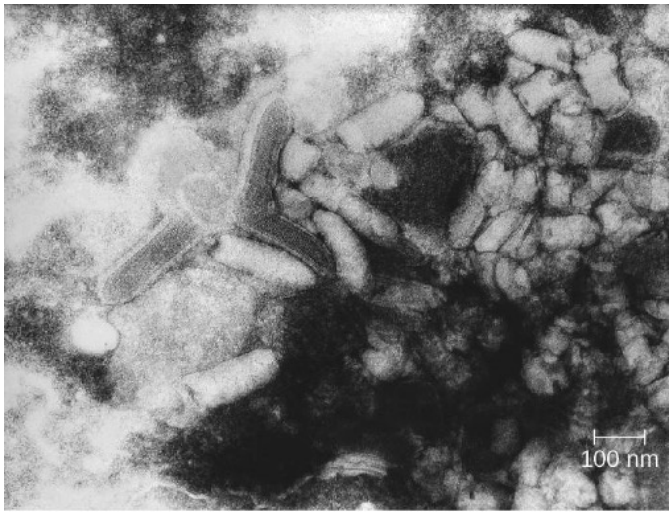

Figure 8.9: Virions of the rabies virus have a characteristic bullet-like shape. *Figure description available at the end of the chapter.*

The low incidence of rabies in the United States is primarily a result of the widespread vaccination of dogs and cats. An oral vaccine is also used to protect wild animals, such as raccoons and foxes, from infection. Oral vaccine programs tend to focus on geographic areas where rabies is endemic.[24] The oral vaccine is usually delivered in a package of bait that is dropped by airplane, although baiting in urban areas is done by hand to maximize safety.[25] Many countries require a quarantine or proof of rabies vaccination for domestic pets being brought into the country. These procedures are especially strict in island nations where rabies infections are rare, such as Australia.

The incubation period for rabies can be lengthy, ranging from several weeks or months to over a year. As the virus replicates, it moves from the site of the bite into motor and sensory axons of peripheral nerves and spreads from nerve to nerve using a process called retrograde transport, eventually making its way to the CNS through the spinal ganglia. Once the rabies virus reaches the brain, the infection leads to encephalitis caused by the disruption of normal neurotransmitter function, resulting in the symptoms associated with rabies. The virions act in the synaptic spaces as competitors with a variety of neurotransmitters for acetylcholine, GABA, and glycine receptors. Thus, the action of rabies virus is neurotoxic rather than cytotoxic. After the rabies virus infects the brain, it can continue to spread through other neuronal pathways, traveling out of the CNS to tissues such as the salivary glands, where the virus can be released. As a result, as the disease progresses the virus can be found in many other tissues, including the salivary glands, taste buds, nasal cavity, and tears.

The early symptoms of rabies include discomfort at the site of the bite, fever, and headache. Once the virus reaches the brain and later symptoms appear, the disease is always fatal. Terminal rabies cases can end in one of two ways: either furious or paralytic rabies. Individuals with furious rabies become very agitated and hyperactive. Hydrophobia (a fear of water) is common in patients with furious rabies, which is caused by muscular spasms in the throat when swallowing or thinking about water. Excess salivation and a desire to bite can lead to foaming of the mouth. These behaviors serve to enhance the likelihood of viral transmission, although contact with infected secretions like saliva or tears alone is sufficient for infection. The disease culminates after just a few days with terror and confusion, followed by cardiovascular and respiratory arrest. In contrast, individuals with paralytic rabies generally follow a longer course of disease. The muscles at the site of infection become paralyzed. Over a period of time, the paralysis slowly spreads throughout the body. This paralytic form of disease culminates in coma and death.

There are no tests that can detect rabies virus in humans at the time of the bite or shortly thereafter. Once the virus has begun to replicate (but before clinical symptoms occur), the virus can be detected using an immuno-

fluorescence test on cutaneous nerves found at the base of hair follicles. Saliva can also be tested for viral genetic material by reverse transcription followed by polymerase chain reaction (RT-PCR). Even when these tests are performed, most suspected infections are treated as positive in the absence of contravening evidence. It is better that patients undergo unnecessary therapy because of a false-positive result, rather than die as the result of a false-negative result.

Human rabies infections are treated by immunization with multiple doses of an attenuated vaccine to develop active immunity in the patient . Vaccination of an already-infected individual has the potential to work because of the slow progress of the disease, which allows time for the patient's immune system to develop antibodies against the virus. Patients may also be treated with human rabies immune globulin (antibodies to the rabies virus) to encourage passive immunity. These antibodies will neutralize any free viral particles. Although the rabies infection progresses slowly in peripheral tissues, patients are not normally able to mount a protective immune response on their own.

POLIOMYELITIS

Poliomyelitis (polio), caused by poliovirus, is a primarily intestinal disease that, in a small percentage of cases, proceeds to the nervous system, causing paralysis and, potentially, death. Poliovirus is highly contagious, with transmission occurring by the fecal-oral route or by aerosol or droplet transmission. Approximately 72% of all poliovirus infections are asymptomatic; another 25% result only in mild intestinal disease, producing nausea, fever, and headache.[26] However, even in the absence of symptoms, patients infected with the virus can shed it in feces and oral secretions, potentially transmitting the virus to others. In about one case in every 200, the poliovirus affects cells in the CNS.[27]

After it enters through the mouth, initial replication of poliovirus occurs at the site of implantation in the pharynx and gastrointestinal tract. As the infection progresses, poliovirus is usually present in the throat and in the stool before the onset of symptoms. One week after the onset of symptoms, there is less poliovirus in the throat, but for several weeks, poliovirus continues to be excreted in the stool. Poliovirus invades local lymphoid tissue, enters the bloodstream, and then may infect cells of the CNS. Replication of poliovirus in motor neurons of the anterior horn cells in the spinal cord, brain stem, or motor cortex results in cell destruction and leads to flaccid paralysis. In severe cases, this can involve the respiratory system, leading to death. Patients with impaired respiratory function are treated using positive-pressure ventilation systems. In the past, patients were sometimes confined to Emerson respirators, also known as iron lungs (figure 8.10).

Direct detection of the poliovirus from the throat or feces can be achieved using reverse transcriptase PCR (RT-PCR) or genomic sequencing to identify the genotype of the poliovirus infecting the patient. Serological tests can be used to determine whether the patient has been previously vaccinated. There are no therapeutic measures for polio; treatment is limited to various supportive measures. These include pain relievers, rest, heat therapy to ease muscle spasms, physical therapy and corrective braces if necessary to help with walking, and mechanical ventilation to assist with breathing if necessary.

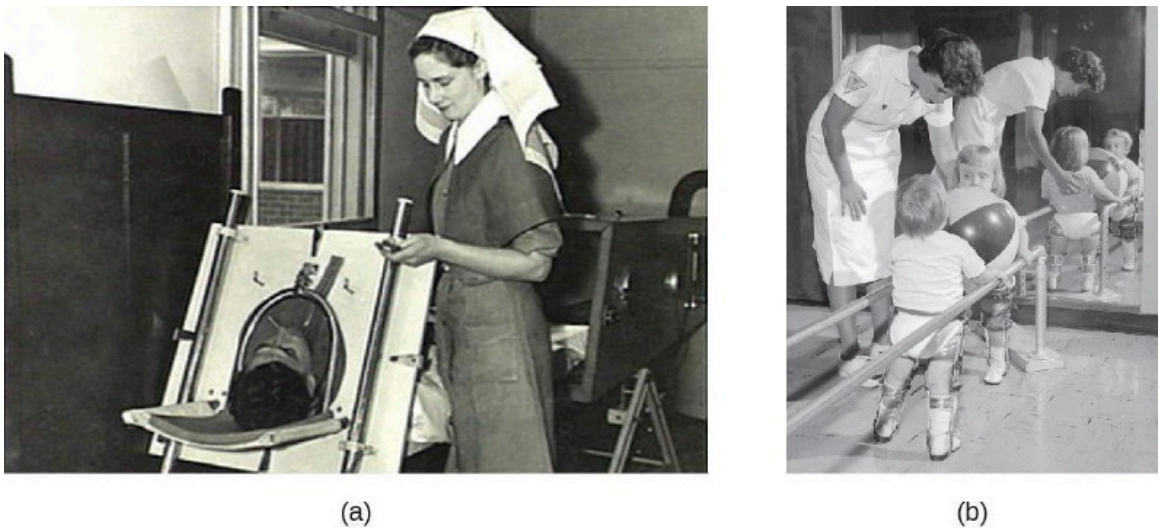

Figure 8.10: (a) An Emerson respiratory (or iron lung) that was used to help some polio victims to breathe. (b) Polio can also result in impaired motor function. Figure description available at the end of the chapter.

Two different vaccines were introduced in the 1950s that have led to the dramatic decrease in polio worldwide (figure 8.11). The Salk vaccine is an inactivated polio virus that was first introduced in 1955. This vaccine is delivered by intramuscular injection. The Sabin vaccine is an oral polio vaccine that contains an attenuated virus; it was licensed for use in 1962. There are three serotypes of poliovirus that cause disease in humans; both the Salk and the Sabin vaccines are effective against all three.

Attenuated viruses from the Sabin vaccine are shed in the feces of immunized individuals and thus have the potential to infect non-immunized individuals. By the late 1990s, the few polio cases originating in the United States could be traced back to the Sabin vaccine. In these cases, mutations of the attenuated virus following vaccination likely allowed the microbe to revert to a virulent form. For this reason, the United States switched exclusively to the Salk vaccine in 2000. Because the Salk vaccine contains an inactivated virus, there is no risk of transmission to others (see section 1.12). Currently four doses of the vaccine are recommended for children: at 2, 4, and 6–18 months of age, and at 4–6 years of age.

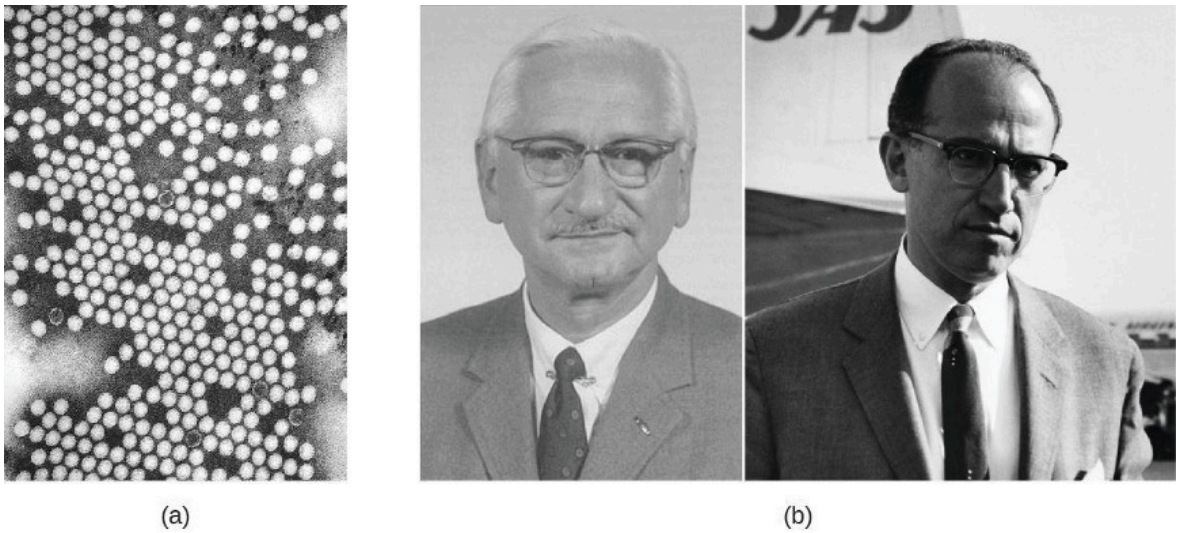

Figure 8.11: (a) Polio is caused by the poliovirus. (b) Two American virologists developed the first polio vaccines: Albert Sabin (1906-1993) (left) and Jonas Salk (1914-1995) (right). Figure description available at the end of the chapter.

TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

Acellular infectious agents called prions are responsible for a group of related diseases known as transmissible spongiform encephalopathies (TSEs) that occur in humans and other animals (see section 2.12). All TSEs are degenerative, fatal neurological diseases that occur when brain tissue becomes infected by prions. These diseases have a slow onset; symptoms may not become apparent until after an incubation period of years and perhaps decades, but death usually occurs within months to a few years after the first symptoms appear.

TSEs in animals include scrapie, a disease in sheep that has been known since the 1700s, and chronic wasting disease, a disease of deer and elk in the United States and Canada. Mad cow disease is seen in cattle and can be transmitted to humans through the consumption of infected nerve tissues. Human prion diseases include Creutzfeldt-Jakob disease and kuru, a rare disease endemic to Papua New Guinea.

Prions are infectious proteinaceous particles that are not viruses and do not contain nucleic acid. They are typically transmitted by exposure to and ingestion of infected nervous system tissues, tissue transplants, blood transfusions, or contaminated fomites. Prion proteins are normally found in healthy brain tissue in a form called PrPC. However, if this protein is misfolded into a denatured form (PrPSc), it can cause disease. Although the exact function of PrPC is not currently understood, the protein folds into mostly alpha helices and binds copper. The rogue protein, on the other hand, folds predominantly into beta-pleated sheets and is resistant to proteolysis. In addition, PrPSc can induce PrPC to become misfolded and produce more rogue protein (figure 8.12).

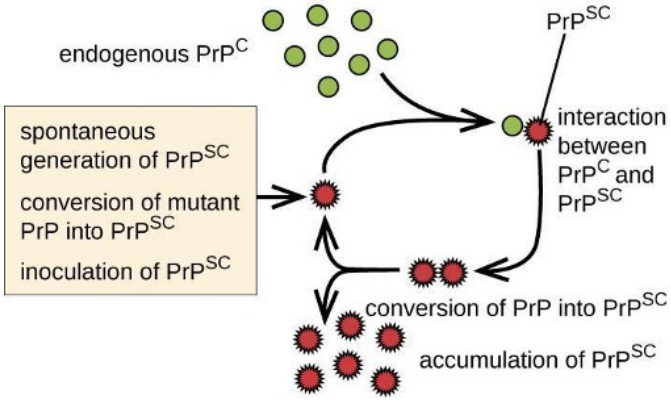

Figure 8.12: The replicative cycle of misfolded prion proteins.
Figure description available at the end of the chapter.

As PrPSc accumulates, it aggregates and forms fibrils within nerve cells. These protein complexes ultimately cause the cells to die. As a consequence, brain tissues of infected individuals form masses of neurofibrillary tangles and amyloid plaques that give the brain a spongy appearance, which is why these diseases are called spongiform encephalopathy (see figure 2.84). Damage to brain tissue results in a variety of neurological symptoms. Most commonly, affected individuals suffer from memory loss, personality changes, blurred vision, uncoordinated movements, and insomnia. These symptoms gradually worsen over time and culminate in coma and death.

The gold standard for diagnosing TSE is the histological examination of brain biopsies for the presence of characteristic amyloid plaques, vacuoles, and prion proteins. Great care must be taken by clinicians when handling suspected prion-infected materials to avoid becoming infected themselves. Other tissue assays search for the presence of the 14-3-3 protein, a marker for prion diseases like Creutzfeldt-Jakob disease. New assays, like RT-QuIC (real-time quaking-induced conversion), offer new hope to effectively detect the abnormal prion proteins in tissues earlier in the course of infection. Prion diseases cannot be cured. However, some medications may help slow their progress. Medical support is focused on keeping patients as comfortable as possible despite progressive and debilitating symptoms.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs	Vaccine
Arboviral encephalitis (eastern equine, western equine, St. Louis, West Nile, Japanese)	EEEV, WEEV, SLEV, WNV, JEV	In mild cases, fever, chills, headaches, and restlessness; in serious cases, encephalitis leading to convulsions, coma, and death	From bird reservoirs to humans (and horses) by mosquito vectors of various species	Serologic testing of serum or CSF	None	Human vaccine available for JEV only; no vaccines available for other arboviruses
Creutzfeldt-Jacob Disease and other TSEs	Prions	Memory loss, confusion, blurred vision, uncoordinated movement, insomnia, coma, death	Exposure to infected nerve tissue via consumption or transplant, inherited;	Tissue biopsy	None	None
Poliomyelitis	Poliovirus	Asymptomatic or mild nausea, fever, headache in most cases; in neurological infections, flaccid paralysis and potentially fatal respiratory paralysis	Fecal-oral route or contact with droplets or aerosols	Culture of poliovirus, PCR	None	Attenuated vaccine (Sabin), killed vaccine (Salk)
Rabies	Rabies virus (RV)	Fever, headaches, hyperactivity, hydrophobia, excessive salivation, terrors, confusion, spreading paralysis, coma, always fatal if not promptly treated	From bite of infected mammal	Viral antigen in tissue, antibodies to virus	Attenuated vaccine, rabies immunoglobulin	Attenuated vaccine
Viral meningitis	HSV-1, HSV-2, varicella zoster virus, mumps virus, influenza virus, measles virus	Nausea, vomiting, photophobia, stiff neck, confusion, symptoms generally resolve within 7–10 days	Sequela of primary viral infection	Testing of oral, fecal, blood, or CSF samples	Varies depending on cause	Varies depending on cause
Zika virus infection	Zika virus	Fever, rash, conjunctivitis; in pregnant people, can cause fetal brain damage and microcephaly	Between humans by *Aedes* spp. mosquito vectors, also may be transmitted sexually or via blood transfusion	Zika virus RNA assay, Trioplex RT-PCR, Zika MAC-ELISA test	None	None

Table 8.2: Acellular infections of the nervous system

8.3 FUNGAL AND PARASITIC DISEASES OF THE NERVOUS SYSTEM

Fungal infections of the nervous system, called neuromycoses, are rare in healthy individuals. However, neuromycoses can be devastating in immunocompromised or elderly patients. Several eukaryotic parasites are also capable of infecting the nervous system of human hosts. Although relatively uncommon, these infections can also be life-threatening in immunocompromised individuals. In this section, we will first discuss neuromycoses (table 8.3), followed by parasitic infections of the nervous system (table 8.4).

CRYPTOCOCCAL MENINGITIS

Cryptococcus neoformans is a fungal pathogen that can cause meningitis. This yeast is commonly found in soils and is particularly associated with pigeon droppings. It has a thick capsule that serves as an important virulence factor, inhibiting clearance by phagocytosis. Most *C. neoformans* cases result in subclinical respiratory infections that, in healthy individuals, generally resolve spontaneously with no long-term consequences (see section 5.4). In immunocompromised patients or those with other underlying illnesses, the infection can progress to cause meningitis and granuloma formation in brain tissues. *Cryptococcus* antigens can also serve to inhibit cell-mediated immunity and delayed-type hypersensitivity.

Cryptococcus can be easily cultured in the laboratory and identified based on its extensive capsule (figure 8.13). *C. neoformans* is frequently cultured from urine samples of patients with disseminated infections.

Prolonged treatment with antifungal drugs is required to treat cryptococcal infections. Combined therapy is required with amphotericin B plus flucytosine for at least 10 weeks. Many antifungal drugs have difficulty crossing the blood-brain barrier and have strong side effects that necessitate low doses; these factors contribute to the lengthy time of treatment. Patients with AIDS are particularly susceptible to *Cryptococcus* infections because of their compromised immune state. AIDS patients with cryptococcosis can also be treated with antifungal drugs, but they often have relapses; lifelong doses of fluconazole may be necessary to prevent reinfection.

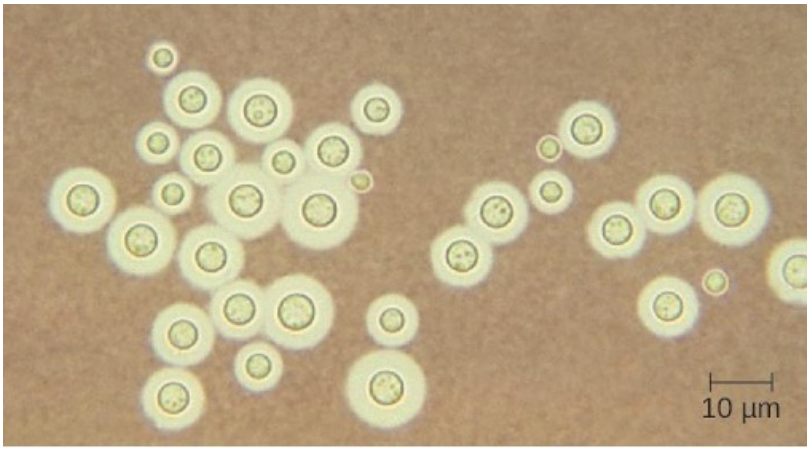

Figure 8.13: An India ink-negative stain of C. neoformans showing the thick capsules around the spherical yeast cells. *Figure description available at the end of the chapter.*

Disease	Pathogen	Signs and Symptomes	Transmission	Diagnostic Tests	Antimicrobial Drugs
Aspergillosis	*Aspergillus fumigatus*	Meningitis, brain abscesses	Dissemination from respiratory infection	CSF, routine culture	Amphotericin B, voriconazole
Candidiasis	*Candida albicans*	Meningitis	Oropharynx or urogenital	CSF, routine culture	Amphotericin B, flucytosine
Coccidioidomycosis (Valley fever)	*Coccidioides immitis*	Meningitis (in about 1% of infections)	Dissemination from respiratory infection	CSF, routine culture	Amphotericin B, azoles
Cryptococcosis	*Cryptococcus neoformans*	Meningitis, granuloma formation in brain	Inhalation	Negative stain of CSF, routine culture	Amphotericin B, flucytosine
Histoplasmosis	*Histoplasma capsulatum*	Meningitis, granulomas in the brain	Dissemination from respiratory infection	CSF, routine culture	Amphotericin B, itraconazole
Mucormycosis	*Rhizopus arrhizus*	Brain abscess	Nasopharynx	CSF, routine culture	Amphotericin B, azoles

Table 8.3: Neuromycoses

AMOEBIC MENINGITIS

Primary amoebic meningoencephalitis (PAM) is caused by *Naegleria fowleri*. This amoeboflagellate is commonly found free-living in soils and water. It can exist in one of three forms—the infective amoebic trophozoite form, a motile flagellated form, and a resting cyst form. PAM is a rare disease that has been associated with young and otherwise healthy individuals. Individuals are typically infected by the amoeba while swimming in warm bodies of freshwater such as rivers, lakes, and hot springs. The pathogenic trophozoite infects the brain by initially entering through nasal passages to the sinuses; it then moves down olfactory nerve fibers to penetrate the submucosal nervous plexus, invades the cribriform plate, and reaches the subarachnoid space. The subarachnoid space is highly vascularized and is a route of dissemination of trophozoites to other areas of the CNS, including the brain (figure 8.14). Inflammation and destruction of gray matter leads to severe headaches and fever. Within days, confusion and convulsions occur and quickly progress to seizures, coma, and death. The progression can be very rapid, and the disease is often not diagnosed until autopsy.

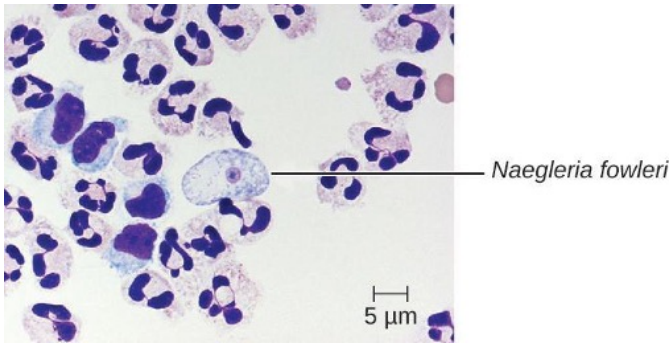

Figure 8.14: Free-living amoeba in human brain tissue from a patient suffering from PAM. *Figure description available at the end of the chapter.*

N. fowleri infections can be confirmed by direct observation of CSF; the amoebae can often be seen moving while viewing a fresh CSF wet mount through a microscope. Flagellated forms can occasionally also be found in CSF. The amoebae can be stained with several stains for identification, including Giemsa-Wright or a modified trichrome stain. Detection of antigens with indirect immunofluorescence, or genetic analysis with PCR, can be used to confirm an initial diagnosis. *N. fowleri* infections are nearly always fatal; only 3 of 138 patients with

PAM in the United States have survived.[28] A new experimental drug called miltefosine shows some promise for treating these infections. This drug is a phosphatidylcholine derivative that is thought to inhibit membrane function in *N. fowleri*, triggering apoptosis and disturbance of lipid-dependent cell signaling pathways.[29] When administered early in infection and coupled with therapeutic hypothermia (lowering the body's core temperature to reduce the cerebral edema associated with infection), this drug has been successfully used to treat primary amoebic encephalitis.

GRANULOMATOUS AMOEBIC ENCEPHALITIS

Acanthamoeba and *Balamuthia* species are free-living amoebae found in many bodies of fresh water. Human infections by these amoebae are rare. However, they can cause amoebic keratitis in contact lens wearers, disseminated infections in immunocompromised patients, and granulomatous amoebic encephalitis (GAE) in severe cases. Compared to PAM, GAE tends to be a subacute infection. The microbe is thought to enter through either the nasal sinuses or breaks in the skin. It is disseminated hematogenously and can invade the CNS. There, the infections lead to inflammation, formation of lesions, and development of typical neurological symptoms of encephalitis (figure 8.15). GAE is nearly always fatal.

GAE is often not diagnosed until late in the infection. Lesions caused by the infection can be detected using CT or MRI. The live amoebae can be directly detected in CSF or tissue biopsies. Serological tests are available but generally are not necessary to make a correct diagnosis, since the presence of the organism in CSF is definitive. Some antifungal drugs, like fluconazole, have been used to treat acanthamoeba infections. In addition, a combination of miltefosine and voriconazole (an inhibitor of ergosterol biosynthesis) has recently been used to successfully treat GAE. Even with treatment, however, the mortality rate for patients with these infections is high.

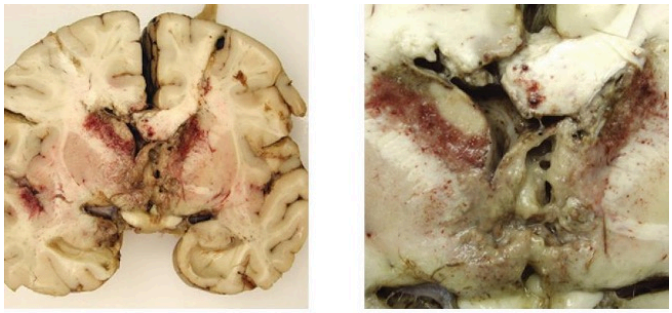

Figure 8.15: (a) Brain tissue from a patient who died of granulomatous amebic encephalitis (GAE) caused by Balamuthia mandrillaris. (b) A close-up of the necrosis in the center of the brain section. *Figure description available at the end of the chapter.*

HUMAN AFRICAN TRYPANOSOMIASIS

Human African trypanosomiasis (also known as African sleeping sickness) is a serious disease endemic to two distinct regions in sub-Saharan Africa. It is caused by the insect-borne hemoflagellate *Trypanosoma brucei*. The subspecies *Trypanosoma brucei rhodesiense* causes East African trypanosomiasis (EAT), and another subspecies, *Trypanosoma brucei gambiense* causes West African trypanosomiasis (WAT). A few hundred cases of EAT are currently reported each year.[30] WAT is more commonly reported and tends to be a more chronic disease. Around 7,000 to 10,000 new cases of WAT are identified each year.[31]

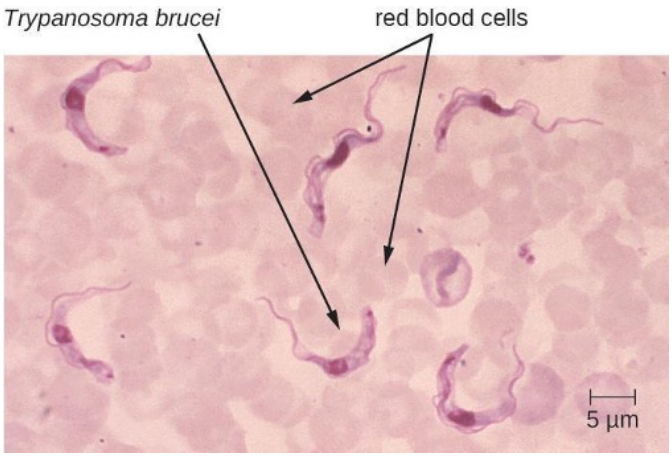

Figure 8.16: Trypanosoma brucei, the causative agent of African sleeping sickness, in a human blood smear. *Figure description available at the end of the chapter.*

T. brucei is primarily transmitted to humans by the bite of the tsetse fly (*Glossina* spp.). Soon after the bite of a tsetse fly, a chancre forms at the site of infection. The flagellates then spread, moving into the circulatory system (figure 8.16). These systemic infections result in an undulating fever, during which symptoms persist for two or three days with remissions of about a week between bouts. As the disease enters its final phase, the pathogens move from the lymphatics into the CNS. Neurological symptoms include daytime sleepiness, insomnia, and mental deterioration. In EAT, the disease runs its course over a span of weeks to months. In contrast, WAT often occurs over a span of months to years.

Clinical symptoms can be used to recognize the early signs of African trypanosomiasis. These include the formation of a chancre at the site of infection and Winterbottom's sign. Winterbottom's sign refers to the enlargement of lymph nodes on the back of the neck—often indicative of cerebral infections. *Trypanosoma* can be directly observed in stained samples including blood, lymph, CSF, and skin biopsies of chancres from patients. Antibodies against the parasite are found in most patients with acute or chronic disease. Serologic testing is generally not used for diagnosis, however, since the microscopic detection of the parasite is sufficient. Early diagnosis is important for treatment. Before the nervous system is involved, drugs like pentamidine (an inhibitor of nuclear metabolism) and suramin (mechanism unclear) can be used. These drugs have fewer side effects than the drugs needed to treat the second stage of the disease. Once the sleeping sickness phase has begun, harsher drugs including melarsoprol (an arsenic derivative) and eflornithine can be effective. Following successful treatment, patients still need to have follow-up examinations of their CSF for two years to detect possible relapses of the disease. The most effective means of preventing these diseases is to control the insect vector populations.

NEUROTOXOPLASMOSIS

Toxoplasma gondii is an ubiquitous intracellular parasite that can cause neonatal infections. Cats are the definitive host, and humans can become infected after eating infected meat or, more commonly, by ingesting oocysts shed in the feces of cats (see section 6.4). *T. gondii* enters the circulatory system by passing between the endothelial cells of blood vessels.[32] Most cases of toxoplasmosis are asymptomatic. However, in immunocompromised patients, neurotoxoplasmosis caused by *T. gondii* infections are one of the most common causes of brain abscesses.[33] The organism is able to cross the blood-brain barrier by infecting the endothelial cells of capillaries in the brain. The parasite reproduces within these cells, a step that appears to be necessary for entry to the brain, and then causes the endothelial cell to lyse, releasing the progeny into brain tissues. This mechanism is quite different than the method it uses to enter the bloodstream in the first place.[34]

The brain lesions associated with neurotoxoplasmosis can be detected radiographically using MRI or CAT scans (figure 8.17). Diagnosis can be confirmed by direct observation of the organism in CSF. RT-PCR assays can also be used to detect *T. gondii* through genetic markers.

Treatment of neurotoxoplasmosis caused by *T. gondii* infections requires six weeks of multi-drug therapy with pyrimethamine, sulfadiazine, and folinic acid. Long-term maintenance doses are often required to prevent recurrence.

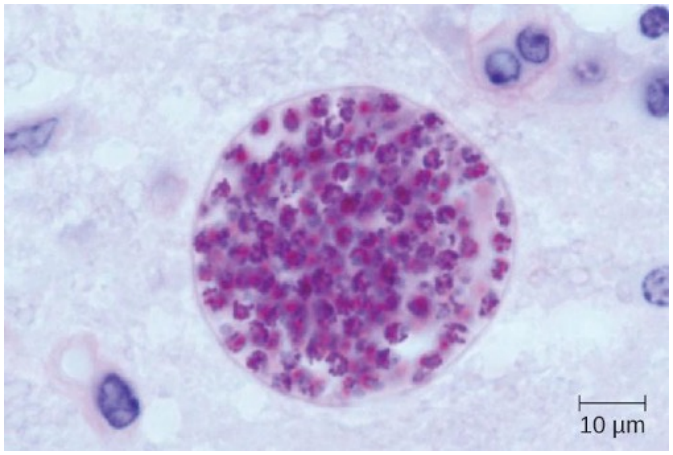

Figure 8.17: This Toxoplasma gondii cyst, observed in mouse brain tissue, contains thousands of inactive parasites. *Figure description available at the end of the chapter.*

NEUROCYSTICERCOSIS

Cysticercosis is a parasitic infection caused by the larval form of the pork tapeworm, *Taenia solium*. When the larvae invade the brain and spinal cord, the condition is referred to as **neurocysticercosis**. This condition affects millions of people worldwide and is the leading cause of adult onset epilepsy in the developing world. Cysticercosis is endemic in Central and South America, Africa, and Asia.[35]

The life cycle of *T. solium* is discussed in section 4.6. Following ingestion, the eggs hatch in the intestine to form larvae called **cysticerci**. Adult tapeworms form in the small intestine and produce eggs that are shed in the feces. These eggs can infect other individuals through fecal contamination of food or other surfaces. Eggs can also hatch within the intestine of the original patient and lead to an ongoing autoinfection. The cysticerci can migrate to the blood and invade many tissues in the body, including the CNS.

Neurocysticercosis is usually diagnosed through noninvasive techniques and epidemiological information can be used as an initial screen. Radiological imaging (MRI and CT scans) is the primary method used to diagnose neurocysticercosis; imaging can be used to detect the one- to two-centimeter cysts that form around the parasites (figure 8.18). Elevated levels of eosinophils in the blood can also indicate a parasitic infection. EIA and ELISA are also used to detect antigens associated with the pathogen.

The treatment for neurocysticercosis depends on the location, number, size, and stage of cysticerci present. Anthelmintic chemotherapy includes albendazole and praziquantel. Because these drugs kill viable cysts, they may acutely increase symptoms by provoking an inflammatory response caused by the release of Taenia cysticerci antigens, as the cysts are destroyed by the drugs. To alleviate this response, corticosteroids that cross the blood-brain barrier (e.g., dexamethasone) can be used to mitigate these effects. Surgical intervention may be required to remove intraventricular cysts.

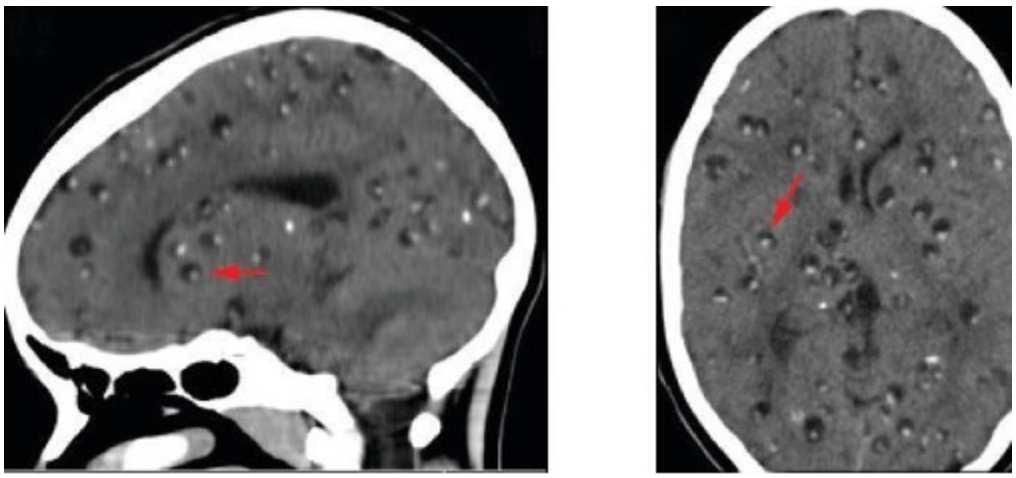

Figure 8.18: Brain CT scans of sagittal (left) and axial (right) sections of a brain with neurocysticercosis. Numerous cysts are visible in both images, as indicated by the arrows. *Figure description available at the end of the chapter.*

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Granulomatous amoebic encephalitis (GAE)	*Acanthamoeba* spp., *Balamuthia mandrillaris*	Inflammation, lesions in CNS, almost always fatal	Freshwater ameobae invade CNS via breaks in skin or sinuses	CT scan, MRI, CSF	Fluconazole, miltefosine, voriconazole
Human African trypanosomiasis	*Trypanosoma brucei gambiense, T. brucei rhodesiense*	Chancre, Winterbottom's sign, undulating fever, lethargy, insomnia, usually fatal if untreated	Protozoan transmitted via bite of tsetse fly	Blood smear	Pentamidine and suramine (initial phase); melarsoprol and eflornithine (final phase)
Neurocysticercosis	*Taenia solium*	Brain cysts, epilepsy	Ingestion of tapeworm eggs in fecally contaminated food or surfaces	CT scan, MRI	Albendazole, praziquantel, dexamethasone
Neurotoxoplasmosis	*Toxoplasma gondii*	Brain abscesses, chronic encephalitis	Protozoan transmitted via contact with oocytes in cat feces	CT scan, MRI, CSF	Pyrimethamine, sulfadiazine, folinic acid
Primary amoebic meningoencephalitis (PAM)	*Naegleria fowleri*	Headache, seizures, coma, almost always fatal	Freshwater ameobae invade brain via nasal passages	CSF, IFA, PCR	Miltefosine (experimental)

Table: 8.4: Parasitic diseases of the nervous system

SUMMARY

The following is a summary of the material covered throughout the chapter. It summarizes key aspects from each section and the pathogens included.

BACTERIAL DISEASES OF THE NERVOUS SYSTEM

- **Bacterial meningitis** can be caused by several species of encapsulated bacteria, including *Haemophilus influenzae*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Streptococcus agalactiae* (group B streptococci). *H. influenzae* affects primarily young children and neonates. *N. meningitidis* is the only communicable pathogen and mostly affects children and young adults. *S. pneumoniae* affects mostly young children, and *S. agalactiae* affects newborns during or shortly after birth.
- Symptoms of bacterial meningitis include fever, neck stiffness, headache, confusion, convulsions, coma, and death.
- Diagnosis of bacterial meningitis is made through observations and culture of organisms in CSF. Bacterial meningitis is treated with antibiotics. *H. influenzae* and *N. meningitidis* have vaccines available.
- *Clostridium* species cause neurological diseases, including **botulism** and **tetanus**, by producing potent neurotoxins that interfere with neurotransmitter release. The PNS is typically affected. Treatment of *Clostridium* infection is effective only through early diagnosis with administration of antibiotics to control the infection as well as antitoxins to neutralize the endotoxin before they enter cells.
- *Listeria monocytogenes* is a foodborne pathogen that can infect the CNS, causing meningitis. The infection can be spread through the placenta to a fetus. Diagnosis is through culture of blood or CSF. This infection is treated with antibiotics. There is no vaccine.
- **Hansen's disease** (**leprosy**) is caused by the intracellular parasite *Mycobacterium leprae*. Infections cause demylenation of neurons, resulting in decreased sensation in peripheral appendages and body sites. Treatment is with multidrug antibiotic therapy, and there is no universally recognized vaccine.

Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs	Vaccine
Botulism	*Clostridium botulinum*	Blurred vision, drooping eyelids, difficulty swallowing and breathing, nausea, vomiting, often fatal	Ingestion of preformed toxin in food, ingestion of endospores in food by infants or immunocompromised adults, bacterium introduced via wound or injection	Antitoxin; penicillin (for wound botulism)	None

Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs	Vaccine
Hansen's disease (leprosy)	*Mycobacterium leprae*	Hypopigmented skin, skin lesions, and nodules, loss of peripheral nerve function, loss of fingers, toes, and extremities	Inhalation, possible transmissible from armadillos to humans	Dapsone, rifampin, clofazimin	None
Haemophilus influenzae type b meningitis	*Haemophilus influenza*	Nausea, vomiting, photophobia, stiff neck, confusion	Direct contact, inhalation of aerosols	Doxycycline, fluoroquinolones , second- and third-generation cephalosporins, and carbapenems	Hib vaccine
Listeriosis	*Listeria monocytogenes*	Initial flu-like symptoms, sepsis and potentially fatal meningitis in susceptible individuals, miscarriage in pregnant people	Bacterium ingested with contaminated food or water	Ampicillin, gentamicin	None
Meningococcal meningitis	*Neisseria meningitidis*	Nausea, vomiting, photophobia, stiff neck, confusion; often fatal	Direct contact	Cephalosporins or penicillins	Meningococcal conjugate
Neonatal meningitis	*Streptococcus agalactiae*	Temperature instability, apnea, bradycardia, hypotension, feeding difficulty, irritability, limpness, seizures, bulging fontanel, stiff neck, opisthotonos, hemiparesis, often fatal	Direct contact in birth canal	Ampicillin plus gentamicin, cefotaxime, or both	None

Disease	Pathogen	Signs and Symptoms	Transmission	Antimicrobial Drugs	Vaccine
Pneumococcal meningitis	*Streptococcus pneumoniae*	Nausea, vomiting, photophobia, stiff neck, confusion, often fatal	Direct contact, aerosols	Cephalosporins, penicillin	Pneumococcal vaccines
Tetanus	*Clostridium tetani*	Progressive spasmatic paralysis starting with the jaw, often fatal	Bacterium introduced in puncture wound	Penicillin, antitoxin	DTaP, Tdap

Table 8.5: Bacterial infections of the nervous system

ACELLULAR DISEASES OF THE NERVOUS SYSTEM

- **Viral meningitis** is more common and generally less severe than bacterial meningitis. It can result from secondary sequelae of many viruses or be caused by infections of arboviruses.
- Various types of **arboviral encephalitis** are concentrated in particular geographic locations throughout the world. These mosquito-borne viral infections of the nervous system are typically mild, but they can be life-threatening in some cases.
- **Zika virus** is an emerging arboviral infection with generally mild symptoms in most individuals, but infections of pregnant women can cause the birth defect microcephaly.
- **Polio** is typically a mild intestinal infection but can be damaging or fatal if it progresses to a neurological disease.
- **Rabies** is nearly always fatal when untreated and remains a significant problem worldwide.
- **Transmissible spongiform encephalopathies** such as **Creutzfeldt-Jakob disease** and **kuru** are caused by prions. These diseases are untreatable and ultimately fatal. Similar prion diseases are found in animals.

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs	Vaccine
Arboviral encephalitis (eastern equine, western equine, St. Louis, West Nile, Japanese)	EEEV, WEEV, SLEV, WNV, JEV	In mild cases, fever, chills, headaches, and restlessness; in serious cases, encephalitis leading to convulsions, coma, and death	From bird reservoirs to humans (and horses) by mosquito vectors of various species	Serologic testing of serum or CSF	None	Human vaccine available for JEV only; no vaccines available for other arboviruses

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs	Vaccine
Creutzfeldt-Jacob Disease and other TSEs	Prions	Memory loss, confusion, blurred vision, uncoordinated movement, insomnia, coma, death	Exposure to infected nerve tissue via consumption or transplant, inherited;	Tissue biopsy	None	None
Poliomyelitis	Poliovirus	Asymptomatic or mild nausea, fever, headache in most cases; in neurological infections, flaccid paralysis and potentially fatal respiratory paralysis	Fecal-oral route or contact with droplets or aerosols	Culture of poliovirus, PCR	None	Attenuated vaccine (Sabin), killed vaccine (Salk)
Rabies	Rabies virus (RV)	Fever, headaches, hyperactivity, hydrophobia, excessive salivation, terrors, confusion, spreading paralysis, coma, always fatal if not promptly treated	From bite of infected mammal	Viral antigen in tissue, antibodies to virus	Attenuated vaccine, rabies immunoglobulin	Attenuated vaccine
Viral meningitis	HSV-1, HSV-2, varicella zoster virus, mumps virus, influenza virus, measles virus	Nausea, vomiting, photophobia, stiff neck, confusion, symptoms generally resolve within 7–10 days	Sequela of primary viral infection	Testing of oral, fecal, blood, or CSF samples	Varies depending on cause	Varies depending on cause

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs	Vaccine
Zika virus infection	Zika virus	Fever, rash, conjunctivitis; in pregnant people, can cause fetal brain damage and microcephaly	Between humans by *Aedes* spp. mosquito vectors, also may be transmitted sexually or via blood transfusion	Zika virus RNA assay, Trioplex RT-PCR, Zika MAC-ELISA test	None	None

Table 8.6: Acellular infections of the nervous system

FUNGAL AND PARASITIC DISEASES OF THE NERVOUS SYSTEM

- **Neuromycoses** are uncommon in immunocompetent people, but immunocompromised individuals with fungal infections have high mortality rates. Treatment of neuromycoses require prolonged therapy with antifungal drugs at low doses to avoid side effects and overcome the effect of the blood-brain barrier.
- Some protist infections of the nervous systems are fatal if not treated, including **primary amoebic meningitis**, **granulomatous amoebic encephalitis**, **human African trypanosomiasis**, and **neurotoxoplasmosis**.
- The various forms of amoebic encephalitis caused by the different amoebic infections are typically fatal even with treatment, but they are rare.
- **African trypanosomiasis** is a serious but treatable disease endemic to two distinct regions in sub-Saharan Africa. Infections are caused by the insect-borne hemoflagellate *Trypanosoma brucei*.
- **Neurocysticercosis** is treated using antihelminthic drugs or surgery to remove the large cysts from the CNS.

Disease	Pathogen	Signs and Symptomes	Transmission	Diagnostic Tests	Antimicrobial Drugs
Aspergillosis	*Aspergillus fumigatus*	Meningitis, brain abscesses	Dissemination from respiratory infection	CSF, routine culture	Amphotericin B, voriconazole
Candidiasis	*Candida albicans*	Meningitis	Oropharynx or urogenital	CSF, routine culture	Amphotericin B, flucytosine
Coccidioidomycosis (Valley fever)	*Coccidioides immitis*	Meningitis (in about 1% of infections)	Dissemination from respiratory infection	CSF, routine culture	Amphotericin B, azoles
Cryptococcosis	*Cryptococcus neoformans*	Meningitis, granuloma formation in brain	Inhalation	Negative stain of CSF, routine culture	Amphotericin B, flucytosine

Disease	Pathogen	Signs and Symptomes	Transmission	Diagnostic Tests	Antimicrobial Drugs
Histoplasmosis	*Histoplasma capsulatum*	Meningitis, granulomas in the brain	Dissemination from respiratory infection	CSF, routine culture	Amphotericin B, itraconazole
Mucormycosis	*Rhizopus arrhizus*	Brain abscess	Nasopharynx	CSF, routine culture	Amphotericin B, azoles

Table 8.7: Neuromycoses

Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Granulomatous amoebic encephalitis (GAE)	*Acanthamoeba* spp., *Balamuthia mandrillaris*	Inflammation, lesions in CNS, almost always fatal	Freshwater ameobae invade CNS via breaks in skin or sinuses	CT scan, MRI, CSF	Fluconazole, miltefosine, voriconazole
Human African trypanosomiasis	*Trypanosoma brucei gambiense, T. brucei rhodesiense*	Chancre, Winterbottom's sign, undulating fever, lethargy, insomnia, usually fatal if untreated	Protozoan transmitted via bite of tsetse fly	Blood smear	Pentamidine and suramine (initial phase); melarsoprol and eflornithine (final phase)
Neurocysticercosis	*Taenia solium*	Brain cysts, epilepsy	Ingestion of tapeworm eggs in fecally contaminated food or surfaces	CT scan, MRI	Albendazole, praziquantel, dexamethasone
Neurotoxoplasmosis	*Toxoplasma gondii*	Brain abscesses, chronic encephalitis	Protozoan transmitted via contact with oocytes in cat feces	CT scan, MRI, CSF	Pyrimethamine, sulfadiazine, folinic acid
Primary amoebic meningoencephalitis (PAM)	*Naegleria fowleri*	Headache, seizures, coma, almost always fatal	Freshwater ameobae invade brain via nasal passages	CSF, IFA, PCR	Miltefosine (experimental)

Table: 8.8: Parasitic diseases of the nervous system

Figure Descriptions

Figure 8.1: a) Photo of brain. B) Photo of thin layer on top of brain being pulled back by forceps.

Figure 8.2: Micrograph of small red circles in pairs next to larger red cells.

Figure 8.3: a) Micrograph of green closing circles in pairs on a dark background. Photograph of a red plate with brown colonies. Clearing is seen around the colonies. b) Red plate with many small brown colonies.

Figure 8.4: Photo of a person in an arched position.

Figure 8.5: a) Micrograph of a rod-shaped cell. b) Diagram of infection. Step 1: Listeria monocytogenes enters cell via phagocytosis. Diagram shows rod-shaped cell (Listeria monocytogenes) in a phagosome. 2: Pathogen escapes when phagosome is lysed. 3: Pathogen reproduces. 4: Pathogen produces actin filaments from host cytoskeleton components. The diagram shows tails on the cell labeled actin filaments. 5: Actin pushes the pathogen from one cell to another through a protrusion of the host membrane. 6: The protrusion is engulfed by another cell. This forms a vesicle with the pathogen inside. 7: cycle repeats.

Figure 8.6: a) Black tissue on end of nose. B) Small purple cells, labeled M leprae, next to larger blue ones.

Figure 8.7: a) electron micrograph showing small red dots next to larger cellular structures. B) brain scans with arrows pointing to dark regions in the brain.

Figure 8.8: a) Electron micrograph of brown circles on a purple background. B) drawing of an infant with a smaller head than average; this is especially apparent in the back of the cranial vault.

Figure 8.9: Micrograph of bullet-shaped structures.

Figure 8.10: a) Photo of a person in a large machine with only their head exposed; a nurse stands next to them. B) Photo of children held between two bars holding a ball.

Figure 8.11: a) Micrograph of many circles. B) Photos of Albert Sabin and Jonas Salk.

Figure 8.12: Endogenous PrPC interacts with mutant version PrPSC. This converts PrPC into PrPSC. This leads to an accumulation of PRPSC. Each PRPSC can convert more PRPC. The options are: spontaneous generation of PRPSC, conversion of mutant PRP into PRPSC, and inoculation of PRPSC.

Figure 8.13: Micrograph of circles with rings around them.

Figure 8.14: Micrograph of white blood cells and a large cell with a small circle in the center labeled N. fowleri.

Figure 8.15: a) Photo of brain section with red granules in the center. b) Close-up of granules.

Figure 8.16: Micrograph of red circles labeled red blood cells and worm-shaped cells labeled Trypanosoma brucei.

Figure 8.17: Micrograph of a sphere with many smaller spheres inside.

Figure 8.18: Brain scans with small lumps (look like pimples) indicated by arrows.

Figure References

Figure 8.1: A normal human brain removed during an autopsy. Left: Suseno. Public Domain. https://commons.wikimedia.org/wiki/File:Human_Brain.jpg. Right: Modification of work by Centers for Disease Control and Prevention. Public Domain.

Figure 8.2: N. meningitidis (arrows) associated with neutrophils (the larger stained cells) in a gram-stained CSF sample. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 8.3: Digitally colorized fluorescent antibody stained micrograph of Streptococcus pneumoniae in CSF. Left: Centers for Disease Control and Prevention. Public Domain. Right: modification of work (c) Nathan Reading. CC BY 2.0. https://commons.wikimedia.org/wiki/File:Streptococcus_pneumoniae_columbia_agar.jpg.

Figure 8.4: A tetanus patient exhibiting the rigid body posture known as opisthotonos. Centers for Disease Control and Prevention. Public domain.

Figure 8.5: An electron micrograph of Listeria monocytogenes infecting a host cell. Left: Modification of work by Centers for Disease Control and Prevention. Public Domain. Right: Modification of work (c) Keith Ireton. CC BY 4.0.

Figure 8.6: (a) The nose of a patient with Hansen's disease. Modifications of work by Centers for Disease Control and Prevention. Public domain.

Figure 8.7: A false color TEM of a mosquito salivary gland cell shows an infection of the eastern equine encephalitis virus (red). Credits: a and b, modifications of works by Centers for Disease Control and Prevention. Public Domain.

Figure 8.8: This colorized electron micrograph and microcephaly. Modifications of work by Centers for Disease Control and Prevention. Public Domain

Figure 8.9: Virions of the rabies virus have a characteristic bullet-like shape. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 8.10: An Emerson respiratory (or iron lung) that was used to help some polio victims to breathe. Left: By Arthur John Faithful. Public Domain. https://commons.wikimedia.org/wiki/File:Both_Cabinet_Respirator_in_WWII.jpg. Right: modification of work by Centers for Disease Control and Prevention. Public Domain. https://commons.wikimedia.org/wiki/File:Polio_physical_therapy.jpg

Figure 8.11: Polio is caused by the poliovirus. Left: Centers for Disease Control and Prevention. Public Domain. https://commons.wikimedia.org/wiki/File:Polio_EM_PHIL_1875_lores.PNG. Right: Photo of Sabin. U.S. Army. Public Domain. https://commons.wikimedia.org/wiki/File:Albert_Sabin.jpg and Photo of Salk. SAS Scandinavian Airlines. Public Domain in the U.S. https://commons.wikimedia.org/wiki/File:Jonas_Salk_candid.jpg.

Figure 8.12: The replicative cycle of misfolded prion proteins. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 8.13: An India ink-negative stain of C. neoformans showing the thick capsules around the spherical yeast cells. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 8.14: Free-living amoeba in human brain tissue from a patient suffering from PAM. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 8.15: (a) Brain tissue from a patient who died of granulomatous amebic encephalitis (GAE) caused by Balamuthia mandrillaris. Modifications of work by Centers for Disease Control and Prevention. Public domain.

Figure 8.16: Trypanosoma brucei, the causative agent of African sleeping sickness, in a human blood smear. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 8.17: This Toxoplasma gondii cyst, observed in mouse brain tissue, contains thousands of inactive parasites. Modification of work by USDA. Public domain.

Figure 8.18: Brain CT scans of sagittal (left) and axial (right) sections of a brain with neurocysticercosis. (c) modification of work by Innocent Lule Segamwenge. https://commons.wikimedia.org/wiki/File:Neurocysticercosis_brain_CT.jpg. CC BY 4.0.

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CHAPTER 9.

FOUNDATIONS OF DISEASE MANAGEMENT

9.1 FUNDAMENTALS OF ANTIMICROBIAL CHEMOTHERAPY

Several factors are important in choosing the most appropriate antimicrobial drug therapy, including bacteriostatic versus bactericidal mechanisms, spectrum of activity, dosage and route of administration, the potential for adverse side effects, and the potential interactions between drugs. The following discussion will focus primarily on antibacterial drugs, but the concepts translate to other antimicrobial classes.

BACTERIOSTATIC VERSUS BACTERICIDAL

Antibacterial drugs can be classified as either bacteriostatic or bactericidal in their interactions with target bacteria. It is important to note that both types, such as tetracyclines and macrolides (bacteriostatic), and beta-lactams and fluoroquinolones (bactericidal) kill bacteria. However, bacteriostatic drugs require a higher concentration than bactericidal agents to achieve specific bacterial reduction. A literature review utilizing data from randomized control trials demonstrated there is no superiority of bactericidal agents to bacteriostatic agents. Alternative factors such as drug pharmacokinetics, therapeutic dosing, and tissue penetration, serve as more significant efficacy determinants.[1]

SPECTRUM OF ACTIVITY

The spectrum of activity of an antibacterial drug relates to diversity of targeted bacteria. A narrow-spectrum antimicrobial targets only specific subsets of bacterial pathogens. For example, some narrow-spectrum drugs only target gram-positive bacteria, whereas others target only gram-negative bacteria. If the pathogen causing an infection has been identified, it is best to use a narrow-spectrum antimicrobial and minimize collateral damage to the normal microbiota. A broad-spectrum antimicrobial targets a wide variety of bacterial pathogens, encompassing both gram-positive and gram-negative species. They are often employed as empiric therapy to cover a wide range of potential pathogens while waiting for the laboratory identification of the infecting pathogen. Broad-spectrum antimicrobials are also used for polymicrobial infections (mixed infection with multiple bacterial species), or as prophylactic measures prior to surgery or invasive procedures. Finally, broad-spectrum antimicrobials may be selected to treat an infection when a narrow-spectrum drug fails because of development of drug resistance by the target pathogen.

The risk associated with using broad-spectrum antimicrobials is that they will also target a broad spectrum of the normal microbiota, increasing the risk of a superinfection, a secondary infection in a patient having a preexisting infection. Superinfections develop when the antibacterial intended for the preexisting infection kills the protective microbiota, allowing another pathogen resistant to the antibacterial to proliferate and cause a secondary infection (figure 9.1). Common superinfections that develop from appropriate and inappropriate antimicrobial utilization include yeast infections (candidiasis) and pseudomembranous colitis caused by *Clostridium difficile*.

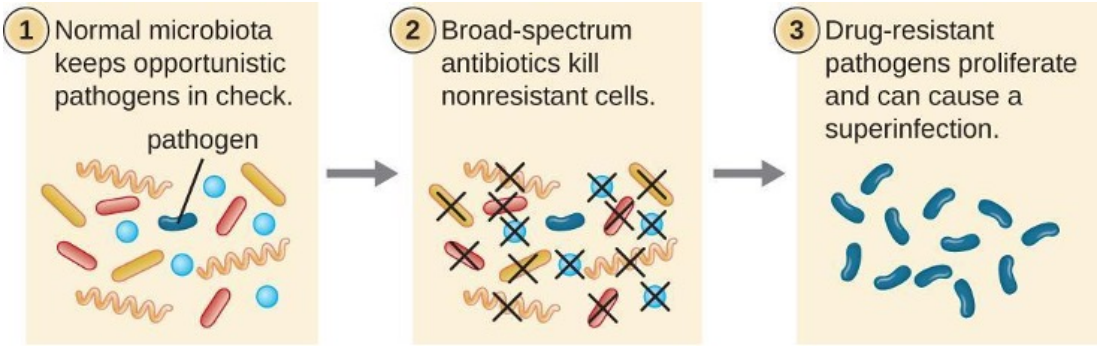


Figure 9.1: Broad-spectrum antimicrobial use may lead to the development of a superinfection. *Figure description available at the end of the chapter.*

DOSAGE AND ROUTE OF ADMINISTRATION

The dosage of a medication, defined as the amount administered within a specific time interval, requires careful determination to achieve optimal therapeutic drug levels at the infection site while minimizing potential toxicity (side effects) for the patient. Each drug class is associated with a variety of potential side effects, and some of these are described for specific drugs later in this chapter. Despite best efforts to optimize dosing, allergic reactions and other potentially serious side effects are not uncommon. Therefore, the goal is to select the optimum dosage that will minimize the risk of side effects while still achieving clinical cure, and there are important factors to consider when selecting the best dose and dosage interval.

For example, in pediatric patients, the dose is typically based upon the patient's mass. In adult patients, antimicrobial dosing varies depending on the specific agent used, and it may not always follow standard dosing protocols. Factors such as total, ideal, or adjusted body weight may be considered in determining the dose for some antimicrobials, while others may involve flat dosing. With the great variability in adult body mass, some experts have argued that mass should be considered for all patients when determining appropriate dosage.[2] Additionally, renal and hepatic function play a crucial role in determining the appropriate dose and frequency of the dosing interval. In general, patients with a history of liver or kidney dysfunction may experience reduced drug metabolism or clearance from the body, resulting in increased drug levels that may lead to toxicity and increase their susceptibility to side effects. Furthermore, certain antimicrobial agents, such as vancomycin and aminoglycosides, may require serum concentration monitoring to accurately guide subsequent dosing regimens.

There are also some factors specific to the drugs themselves that influence appropriate dose and time interval between doses. For example, the half-life, or rate at which 50% of a drug is eliminated from the plasma, can vary significantly between drugs. Some drugs have a short half-life of only 1 hour and must be given multiple times a day, whereas other drugs have half-lives exceeding 12 hours and can be given as a single dose every 24 hours. While a longer half-life may offer the advantage of convenient dosing intervals, it can be a concern for drugs with serious side effects, as elevated drug levels may persist for an extended period of time. Additionally, some drugs are dose dependent, meaning they are more effective when administered in large doses to provide high levels for a short time at the site of infection. Others are time dependent, meaning they are more effective when lower drug levels are maintained over the minimum inhibitory concentration for a longer period of time.

The route of administration, the method used to introduce a drug into the body, is also an important consideration for drug therapy. Oral administration is generally preferred for its convenience, allowing patients to self-administer at home. However, some drugs are poorly absorbed from the gastrointestinal (GI) tract into the bloodstream. These drugs are often useful for treating diseases of the intestinal tract, such as tapeworms treated

with praziquantel, or for ulcerative colitis, as with sulfasalazine. Some drugs that are not absorbed easily, such as bacitracin, polymyxin, and several antifungals, are available as topical preparations for treatment of superficial skin infections. In cases where oral administration is initially challenging due to illness or other factors, parenteral routes (intravenous or intramuscular) are preferred in healthcare settings. Intravenous administration can result in higher plasma levels compared to other routes of administration and oftentimes is a consideration for therapy selection. It is important to recognize that certain oral formulations exhibit identical bioavailability to their intravenous counterparts (e.g. linezolid, metronidazole, levofloxacin) and this should be taken into account when selecting the formulation (figure 9.2).

DRUG INTERACTIONS

For the optimal treatment of certain infections, a combination of two antibacterial drugs may be administered together to achieve a synergistic interaction that surpasses the efficacy of either drug alone. A classic example of synergistic combinations is trimethoprim and sulfamethoxazole (Bactrim®). Individually, these two drugs provide only bacteriostatic inhibition of bacterial growth, but combined, the drugs are bactericidal.

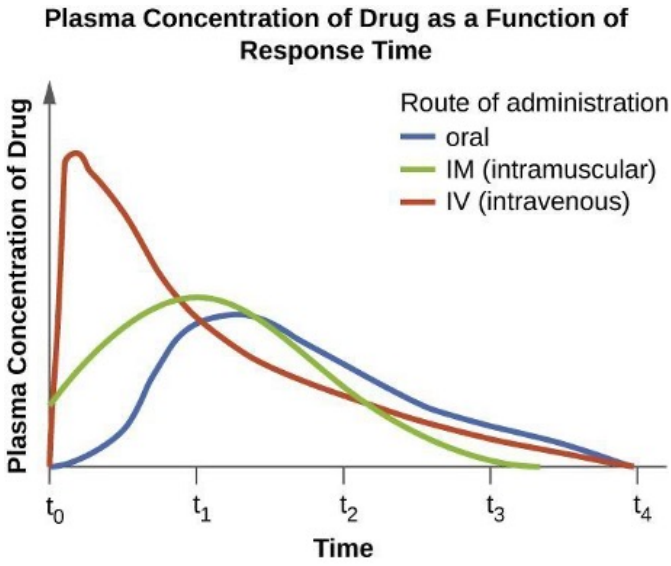


Figure 9.2: On this graph, t0 represents the time at which a drug dose is administered. The curves illustrate how plasma concentration of the drug changes over specific intervals of time (t1 through t4). As the graph shows, when a drug is administered intravenously, the concentration peaks very quickly and then gradually decreases. When drugs are administered orally or intramuscularly, it takes longer for the concentration to reach its peak. *Figure description available at the end of the chapter.*

Whereas synergistic drug interactions provide a benefit to the patient, antagonistic interactions produce harmful effects. Antagonism can occur between two antimicrobials or between antimicrobials and non-antimicrobials being used to treat other conditions. The effects vary depending on the drugs involved, but antagonistic interactions may cause loss of drug activity, decreased therapeutic levels due to increased metabolism and elimination, or increased potential for toxicity due to decreased metabolism and elimination. As an example, some antibacterials are absorbed most effectively from the acidic environment of the stomach. If a patient takes antacids, however, this increases the pH of the stomach and negatively impacts the absorption of these antimicrobials, decreasing their effectiveness in treating an infection.[3]

9.2 MECHANISMS OF ANTIBACTERIAL DRUGS

An essential attribute for an antimicrobial drug is selective toxicity, meaning that it selectively kills or inhibits the growth of microbial targets while causing minimal or no harm to the host. Most antimicrobial drugs currently in clinical use are antibacterial because the prokaryotic cell provides a greater variety of unique targets for selective toxicity, in comparison to fungi, parasites, and viruses. Each class of antibacterial drugs has a unique mode of action (the way in which a drug affects microbes at the cellular level), and these are summarized in figure 9.3 and table 9.1.

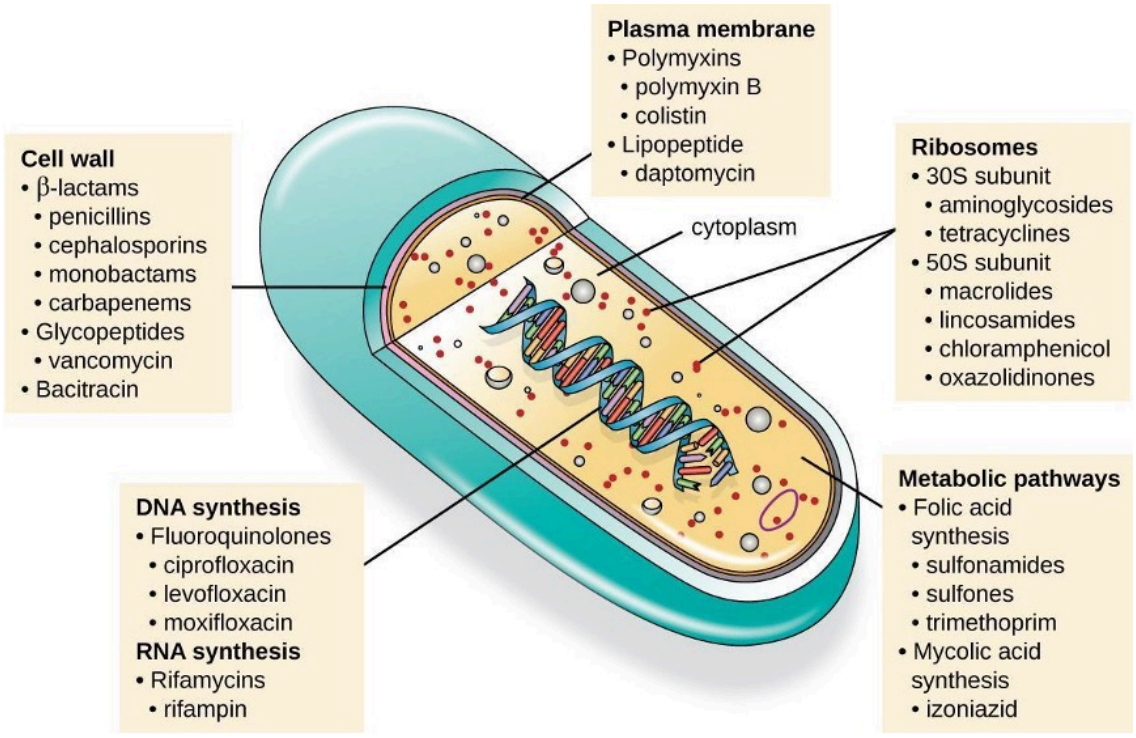


Figure 9.3: There are several classes of antibacterial compounds that are typically classified based on their bacterial target. *Figure description available at the end of the chapter.*

Mode of Action	Target	Drug Class
Inhibit cell wall biosynthesis	Penicillin-binding proteins	β-lactams: penicillins, cephalosporins, monobactams, carbapenems
	Peptidoglycan subunits	Glycopeptides
	Peptidoglycan subunit transport	Bacitracin
Inhibit biosynthesis of proteins	30S ribosomal subunit	Aminoglycosides, tetracyclines
	50S ribosomal subunit	Macrolides, lincosamides, chloramphenicol, oxazolidinones
Disrupt membranes	Lipopolysaccharide, inner and outer membranes	Polymyxin B, colistin, daptomycin
Inhibit nucleic acid synthesis	RNA	Rifamycin
	DNA	Fluoroquinolones
Antimetabolites	Folic acid synthesis enzyme	Sulfonamides, trimethoprim
	Mycolic acid synthesis enzyme	Isoniazid

Table 9.1: Common antibacterial drugs by mode of action

INHIBITORS OF CELL WALL BIOSYNTHESIS

Several classes of antibacterials, such as beta-lactam antibiotics (e.g., penicillins, cephalosporins) and glycopeptide antibiotics (e.g., vancomycin), target specific steps in the biosynthesis of peptidoglycan, a crucial component of bacterial cell walls. By inhibiting peptidoglycan formation, these antibacterials render bacterial cells more susceptible to osmotic lysis (table 9.2). Because human cells do not make peptidoglycan, this mode of action is an excellent example of selective toxicity.

Penicillin, the first antibiotic discovered, is one of several antibacterials within a class called β-lactams. This group of compounds includes the penicillins, cephalosporins, monobactams, and carbapenems, and is characterized by the presence of a β-lactam ring found within the central structure of the drug molecule (figure 9.4). The β-lactam antibacterials block the crosslinking of peptide chains during the biosynthesis of new peptidoglycan in the bacterial cell wall. They can block this process because the β-lactam structure is similar to the structure of the peptidoglycan subunit component that is recognized by the crosslinking transpeptidase enzyme, also known as a penicillin-binding protein (PBP). Although the β-lactam ring must remain unchanged for these drugs to retain their antibacterial activity, strategic chemical changes to the R groups have allowed for development of a wide variety of semisynthetic β-lactam drugs with increased potency, expanded spectrum of activity, and longer half-lives for better dosing, among other characteristics.

Penicillin G and penicillin V are natural antibiotics from fungi and are primarily active against gram-positive bacterial pathogens, and a few gram-negative bacterial pathogens. Penicillinase-resistant penicillins, including dicloxacillin and oxacillin, are a subgroup of antibiotics designed to withstand the enzymatic degradation by beta-lactamases produced by certain bacteria. These antibiotics maintain efficacy against penicillinase-producing strains of bacteria, making them valuable in the treatment of staphylococcal infections, including skin and soft tissue infections.

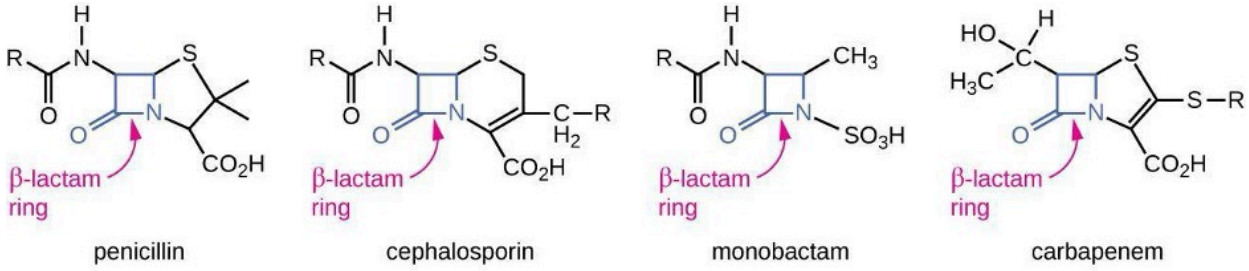
Similar to the penicillins, cephalosporins contain a β-lactam ring (figure 9.4) and block the transpeptidase activity of penicillin-binding proteins. However, the β-lactam ring of cephalosporins is fused to a six-member ring, rather than the five-member ring found in penicillins. This chemical difference provides cephalosporins with an increased resistance to enzymatic inactivation by β-lactamases. The family of semisynthetic cephalosporins is much larger than the penicillins, and these drugs have been classified into generations based primarily on their spectrum of activity, increasing in spectrum from the narrow-spectrum, first-generation cephalosporins to the broad-spectrum, fourth generation cephalosporins. Ceftaroline and ceftolozane are new fifth-generation cephalosporins. Ceftaroline has activity against methicillin-resistant *Staphylococcus aureus* (MRSA) while ceftolozane provides extended *Pseudomonas aeruginosa* coverage.

The carbapenems and monobactams also have a β-lactam ring as part of their core structure, and they inhibit the transpeptidase activity of penicillin-binding proteins. The only monobactam used clinically is aztreonam. It has activity only against gram-negative bacteria, including *Pseudomonas aeruginosa.* In contrast, the carbapenem family includes a variety of semisynthetic drugs (imipenem, meropenem, and doripenem) that provide very broad-spectrum activity against gram-positive and gram-negative bacterial pathogens.

The drug vancomycin, a member of a class of compounds called glycopeptides, was discovered in the 1950s as a natural antibiotic from the actinomycete *Amycolatopsis orientalis*. Similar to the β-lactams, vancomycin inhibits cell wall biosynthesis and is bactericidal. However, in contrast to the β-lactams, the structure of vancomycin is not similar to that of cell-wall peptidoglycan subunits and does not directly inactivate penicillin-binding proteins. Rather, vancomycin is a very large, complex molecule that binds to the end of the peptide chain of cell wall precursors. This creates a structural blockage that prevents the cell wall subunits from being incorporated into the growing N-acetylglucosamine and N-acetylmuramic acid (NAM-NAG) backbone of the peptidoglycan

structure (transglycosylation). Vancomycin also structurally blocks transpeptidation. Vancomycin is bactericidal against gram-positive bacterial pathogens, but it is not active against gram-negative bacteria because of its inability to penetrate the protective outer membrane.

The drug bacitracin consists of a group of structurally similar peptide antibiotics originally isolated from *Bacillus subtilis*. Bacitracin blocks the activity of a specific cell-membrane molecule that is responsible for the movement of peptidoglycan precursors from the cytoplasm to the exterior of the cell, ultimately preventing their incorporation into the cell wall. Bacitracin is effective against a wide range of bacteria, including gram-positive organisms found on the skin, such as *Staphylococcus* and *Streptococcus*. Although it may be administered orally or intramuscularly in some circumstances, bacitracin has been shown to be nephrotoxic (damaging to the kidneys). Therefore, it is more commonly combined with neomycin and polymyxin in topical ointments such as Neosporin®.



penicillin | cephalosporin | monobactam | carbapenem

R group	$-CH_2-$	CH_2-O-	$-CH(NH_2)-$	$-CH(NH_2)-C_6H_4-OH$	$3,5-(CH_3O)_2C_6H_3-$
Drug name	penicillin G	penicillin V	ampicillin	amoxicillin	methicillin
Spectrum of activity	G+ and a few G−	similar to penicillin G	G+ and more G− than penicillin	similar to ampicillin	G+ only, including β-lactamase producers
Route of administration	parenteral	oral	parenteral and oral	oral (better than ampicillin)	parenteral

Figure 9.4: Penicillins, cephalosporins, monobactams, and carbapenems all contain a β-lactam ring, the site of attack by inactivating β-lactamase enzymes. Although they all share the same nucleus, various penicillins differ from each other in the structure of their R groups. Chemical changes to the R groups provided increased spectrum of activity, acid stability, and resistance to β-lactamase degradation. *Figure description available at the end of the chapter.*

Mechanism of Action	Drug Class	Specific Drugs	Spectrum of Activity
Interact directly with PBPs and inhibit transpeptidase activity	Penicillins	Penicillin G, penicillin V	Streptococci, basic anaerobes (e.g.,non-difficile Clostridioides spp), syphilis (Treponema pallidum)
		Ampicillin, amoxicillin	Enterococci, Streptococci, basic anaerobes, Listeria monocytogenes,
	Cephalosporins	First generation: cefazolin cephalexin cefadroxil	Coverage against most gram-positive cocci, methicillin susceptible *Staphylococcus aureus* (MSSA) as well as some gram-negative (*E. Coli, P. mirabilis, K. pneumononiae*)
		Second generation: cefuroxime cefoxitin cefotetan cefaclor cefprozil	Coverage against streptococci and MSSA with increased gram-negative coverage *Haemophilus influenzae, Neisseria, Proteus, E. coli, Klebsiella*
		Third generation: ceftriaxone ceftazidime cefdinir cefpodoxime cefixime cefotaxime ceftibuten Fourth generation: cefepime	Streptococci (better than 1st or 2nd gen), MSSA (not as good as 1st or 2nd gen), increased gram-negative coverage (ceftazidime has Pseudomonas activity) Streptococci (better than 1st or 2nd gen), MSSA, increased Gram-negative coverage (better than 3rd gen) and has Pseudomonas activity
		Fifth generation cephalosporin: ceftaroline ceftolozane	Activity against gram-positive and gram-negative bacteria Ceftaroline provides MRSA coverage Ceftolozane provides extended *Pseudomonas aeruginosa* coverage
	Monobactams	Aztreonam	Gram-negative coverage including *Pseudomonas aeruginosa*
	Carbapenems	Imipenem, meropenem, doripenem, ertapenem	MSSA, streptococci, anaerobes (including B. fragilis) ertapenem lacks coverage for Acinetobacter, Pseudomonas, Enterococcus

Mechanism of Action	Drug Class	Specific Drugs	Spectrum of Activity
Large molecules that bind to the peptide chain of peptidoglycan subunits, blocking transglycosylation and transpeptidation	Glycopeptides	Vancomycin	Gram-positive bacteria streptococci, MRSA, enterococci
Block transport of peptidoglycan subunits across cytoplasmic membrane	Bacitracin	Bacitracin	Gram-positive and gram-negative bacteria

Table 9.2: Drugs that inhibit bacterial cell wall synthesis

INHIBITORS OF PROTEIN BIOSYNTHESIS

The cytoplasmic ribosomes found in animal cells (80S) are structurally distinct from those found in bacterial cells (70S), making protein biosynthesis a good selective target for antibacterial drugs. Several types of protein biosynthesis inhibitors are discussed in this section and are summarized in figure 9.5.

Protein Synthesis Inhibitors That Bind the 30S Subunit

Aminoglycosides are large, highly polar antibacterial drugs that bind to the 30S subunit of bacterial ribosomes, impairing the proofreading ability of the ribosomal complex. This impairment causes mismatches between codons and anticodons, resulting in the production of proteins with incorrect amino acids and shortened proteins that insert into the cytoplasmic membrane. Disruption of the cytoplasmic membrane by the faulty proteins kills the bacterial cells. The aminoglycosides, which include drugs such as streptomycin, gentamicin, neomycin, amikacin and tobramycin, are potent broad-spectrum antibacterials. However, aminoglycosides have been shown to be nephrotoxic (damaging to kidney), neurotoxic (damaging to the nervous system), and ototoxic (damaging to the ear).

Another class of antibacterial compounds that bind to the 30S subunit are the tetracyclines. In contrast to aminoglycosides, these drugs are bacteriostatic and inhibit protein synthesis by blocking the association of tRNAs with the ribosome during translation. Naturally occurring tetracyclines produced by various strains of *Streptomyces* were first discovered in the 1940s, and several semisynthetic tetracyclines, including doxycycline, and minocycline have also been produced. Although the tetracyclines are broad spectrum in their coverage of bacterial pathogens, side effects that can limit their use include phototoxicity, permanent discoloration of developing teeth, and liver toxicity with high doses or in patients with kidney impairment.

Protein Synthesis Inhibitors That Bind the 50S Subunit

There are several classes of antibacterial drugs that work through binding to the 50S subunit of bacterial ribosomes. The macrolide antibacterial drugs have a large, complex ring structure and are part of a larger class of naturally produced secondary metabolites called polyketides, complex compounds produced in a stepwise fashion through the repeated addition of two-carbon units by a mechanism similar to that used for fatty acid synthesis. Macrolides are broad-spectrum, bacteriostatic drugs that block elongation of proteins by inhibiting peptide bond formation between specific combinations of amino acids. The first macrolide was erythromycin. It was isolated in 1952 from *Streptomyces erythreus* and prevents translocation. Semisynthetic macrolides include azithromycin and clarithromycin. Compared with erythromycin, azithromycin has a broader spectrum of activity, fewer side effects, and a significantly longer half-life (1.5 hours for erythromycin versus 68 hours for

azithromycin) that allows for once-daily dosing and a shorter course of therapy (i.e., Z-Pak® formulation) for most infections. The lincosamides include the naturally produced lincomycin and semisynthetic clindamycin. Although structurally distinct from macrolides, lincosamides are similar in their mode of action to the macrolides through binding to the 50S ribosomal subunit and preventing peptide bond formation. Lincosamides are particularly active against streptococcal and staphylococcal infections.

The drug chloramphenicol represents yet another structurally distinct class of antibacterials that also bind to the 50S ribosome, inhibiting peptide bond formation. Chloramphenicol, produced by *Streptomyces venezuelae*, was discovered in 1947; in 1949, it became the first broad-spectrum antibiotic that was approved by the FDA. Although it is a natural antibiotic, it is also easily synthesized and was the first antibacterial drug synthetically mass produced. As a result of its mass production, broad-spectrum coverage, and ability to penetrate tissues efficiently, chloramphenicol was historically used to treat a wide range of infections, from meningitis to typhoid fever to conjunctivitis. Unfortunately, serious side effects, such as the lethal gray baby syndrome, and suppression of bone marrow production, have limited its clinical role. Chloramphenicol also causes anemia in two different ways. One mechanism involves the targeting of mitochondrial ribosomes within hematopoietic stem cells, causing a reversible, dose-dependent suppression of blood cell production. Once chloramphenicol dosing is discontinued, blood cell production returns to normal. This mechanism highlights the similarity between 70S ribosomes of bacteria and the 70S ribosomes within our mitochondria. The second mechanism of anemia is idiosyncratic (i.e., the mechanism is not understood), and involves an irreversible lethal loss of blood cell production known as aplastic anemia. This mechanism of aplastic anemia is not dose dependent and can develop after therapy has stopped. Because of toxicity concerns, chloramphenicol usage in humans is now rare in the United States and is limited to severe infections unable to be treated by less toxic antibiotics. Because its side effects are much less severe in animals, it is used in veterinary medicine.

The oxazolidinones, including linezolid and tedizolid, are a newer broad-spectrum class of synthetic protein synthesis inhibitors that bind to the 50S ribosomal subunit of gram-positive bacteria. However, their mechanism of action seems somewhat different from that of the other 50S subunit-binding protein synthesis inhibitors already discussed. Instead, they seem to interfere with formation of the initiation complex (association of the 50S subunit, 30S subunit, and other factors) for translation, and they prevent translocation of the growing protein from the ribosomal A site to the P site. Table 9.3 summarizes the protein synthesis inhibitors.

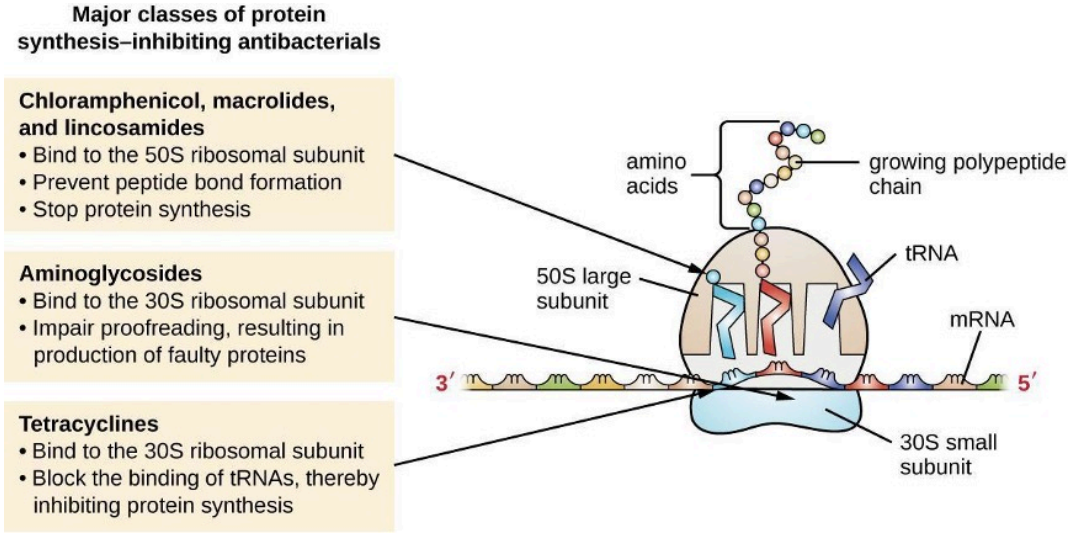


Figure 9.5: The major classes of protein synthesis inhibitors target the 30S or 50S subunits of cytoplasmic ribosomes. *Figure description available at the end of the chapter.*

Molecular Target	Mechanism of Action	Drug Class	Specific Drugs	Spectrum of Activity
30S subunit	Causes mismatches between codons and anticodons, leading to faulty proteins that insert into and disrupt cytoplasmic membrane	Aminoglycosides	Streptomycin, gentamicin, neomycin, amikacin, tobramycin	Extensive gram-negative coverage; some gram-positive coverage (not adequate as monotherapy)
	Blocks association of tRNAs with ribosome	Tetracyclines	Tetracycline, doxycycline, minocycline	Gram-positive and gram-negative with activity against many atypical pathogens (e.g. *Rickettsia* spp, *Chlamydia* spp.)
50S subunit	Blocks peptide bond formation between amino acids	Macrolides	Erythromycin, azithromycin, clarithromycin	Many gram-negative, atypical, and mycobacterial organisms as well as gram-positive organisms
		Lincosamides	Lincomycin, clindamycin	staphylococci, viridans group streptococci, *Streptococcus pyogenes,* and *Streptococcus pneumoniae,* anaerobes (e.g. *Clostridium perfringens*)
		Not applicable	Chloramphenicol	gram-positive and gram-negative
	Interferes with the formation of the initiation complex between 50S and 30S subunits and other factors.	Oxazolidinones	Linezolid, tedizolid	Gram-positive bacteria including streptococci, enterococci (including vancomycin-resistant enterococci [VRE]), coagulase-negative staphylococci, MSSA, MRSA, *Bacillus* species, *Corynebacterium* species, and *Listeria monocytogenes*

Table 9.3: Drugs that inhibit bacterial protein synthesis

INHIBITORS OF MEMBRANE FUNCTION

A small group of antibacterials target the bacterial membrane as their mode of action (table 9.4). The polymyxins are natural polypeptide antibiotics that were first discovered in 1947 as products of *Bacillus polymyxa*; only polymyxin B and polymyxin E (colistin) have been used clinically. They are lipophilic with detergent-like properties and interact with the lipopolysaccharide component of the outer membrane of gram-negative bacteria, ultimately disrupting both their outer and inner membranes and killing the bacterial cells. Unfortunately, the membrane-targeting mechanism is not a selective toxicity, and these drugs also target and damage the membrane of cells in the kidney and nervous system when administered systemically. Because of these serious side

effects and their poor absorption from the digestive tract, polymyxin B is used in over-the-counter topical antibiotic ointments (e.g., Neosporin®).

Oralcolistin was historically used only for bowel decontamination to prevent infections originating from bowel microbes in immunocompromised patients or for those undergoing certain abdominal surgeries. However, the emergence and spread of multidrug-resistant pathogens has led to increased use of intravenous colistin in hospitals, often as a drug of last resort to treat serious infections. The antibacterial daptomycin is a cyclic lipopeptide produced by *Streptomyces roseosporus* that seems to work like polymyxins, inserting in the bacterial cell membrane and disrupting it. However, in contrast to polymyxin B and colistin, which target only gram-negative bacteria, daptomycin specifically targets gram-positive bacteria. It is typically administered intravenously and seems to be well tolerated, showing reversible toxicity in skeletal muscles.

Mechanism of Action	Drug Class	Specific Drugs	Spectrum of Activity	Clinical Use
Interacts with lipopolysaccharide in the outer membrane of gram-negative bacteria, killing the cell through the eventual disruption of the outer membrane and cytoplasmic membrane	Polymyxins	Polymyxin B	Gram-negative bacteria, including multidrug-resistant strains	Topical preparations to prevent infections in wounds
		Polymyxin E (colistin)	Gram-negative bacteria, including multidrug-resistant strains	Oral dosing to decontaminate bowels to prevent infections in immunocompromised patients or patients undergoing invasive surgery/procedures.
				Intravenous dosing to treat serious systemic infections caused by multidrug-resistant pathogens
Inserts into the cytoplasmic membrane of gram-positive bacteria, disrupting the membrane and killing the cell	Lipopeptide	Daptomycin	Gram-positive bacteria, including multidrug-resistant strains	Complicated skin and skin-structure infections and bacteremia caused by gram-positive pathogens, including MRSA

Table 9.4: Drugs that inhibit bacterial membrane function

INHIBITORS OF NUCLEIC ACID SYNTHESIS

Some antibacterial drugs work by inhibiting nucleic acid synthesis (table 9.5). For example, metronidazole is a semisynthetic member of the nitroimidazole family that is also antiprotozoal. It interferes with DNA replication in target cells. The drug rifampin is a semisynthetic member of the rifamycin family and functions by blocking RNA polymerase activity in bacteria. The RNA polymerase enzymes in bacteria are structurally different from those in eukaryotes, providing selective toxicity against bacterial cells. It is used for the treatment of a variety of infections, but its primary use, often in a cocktail with other antibacterial drugs, is against mycobacteria that cause tuberculosis. Despite the selectivity of its mechanism, rifampin can induce liver enzymes (CYP450 3A4) to increase metabolism of other drugs being administered, leading to hepatotoxicity and negatively influencing the bioavailability and therapeutic effect of the companion drugs.

One member of the quinolone family, a group of synthetic antimicrobials, is nalidixic acid. It was discovered in 1962 as a byproduct during the synthesis of chloroquine, an antimalarial drug. Nalidixic acid selectively inhibits the activity of bacterial DNA gyrase, blocking DNA replication. Chemical modifications to the original quinolone backbone have resulted in the production of fluoroquinolones, like ciprofloxacin and levofloxacin, which also inhibit the activity of DNA gyrase. Ciprofloxacin and levofloxacin are effective against a broad spectrum gram-positive or gram-negative bacterium and are prescribed to treat a wide range of infections, including urinary tract infections, respiratory infections, abdominal infections, and skin infections. However, despite their selective toxicity against DNA gyrase, side effects associated with fluoroquinolones include phototoxicity, neurotoxicity, cardiotoxicity, glucose metabolism dysfunction, and increased risk for tendon rupture.

Mechanisms of Action	Drug Class	Specific Drugs	Spectrum of activity	Clinical Use
Inhibits bacterial RNA polymerase activity and blocks transcription, killing the cell	Rifamycin	Rifampin	Gram-positive and limited gram-negative bacteria , active against *Mycobacterium tuberculosi*s.	Combination therapy for treatment of tuberculosis
Inhibits the activity of DNA gyrase and blocks DNA replication, killing the cell	Fluoroquinolones	Ciprofloxacin, ofloxacin, moxifloxacin, levofloxacin	Activity against aerobic, enteric gram-negative bacilli, some are active against *Pseudomonas* species, selected gram-positive organisms, anaerobes, and mycobacteria.	Wide variety of skin and systemic infections

Table 9.5: Drugs that inhibit bacterial nucleic acid synthesis

INHIBITORS OF METABOLIC PATHWAYS

Some synthetic drugs control bacterial infections by functioning as antimetabolites, competitive inhibitors for bacterial metabolic enzymes (table 9.6). The sulfonamides (sulfa drugs) are the oldest synthetic antibacterial agents and are structural analogues of *para*-aminobenzoic acid (PABA), an early intermediate in folic acid synthesis (figure 9.6). By inhibiting the enzyme involved in the production of dihydrofolic acid, sulfonamides block bacterial biosynthesis of folic acid and, subsequently, pyrimidines and purines required for nucleic acid synthesis. This mechanism of action provides bacteriostatic inhibition of growth against a wide spectrum of gram-positive and gram-negative pathogens. Because humans obtain folic acid from food instead of synthesizing it intracellularly, sulfonamides are selectively toxic for bacteria. However, allergic reactions to sulfa drugs are common. The sulfones are structurally similar to sulfonamides but are not commonly used today except for the treatment of Hansen's disease (leprosy).

Trimethoprim is a synthetic antimicrobial compound that serves as an antimetabolite within the same folic acid synthesis pathway as sulfonamides. However, trimethoprim is a structural analogue of dihydrofolic acid and inhibits a later step in the metabolic pathway (figure 9.6). Trimethoprim is used in combination with the sulfa drug sulfamethoxazole to treat urinary tract infections, ear infections, and bronchitis. As discussed, the combination of trimethoprim and sulfamethoxazole is an example of antibacterial synergy. When used alone, each antimetabolite only decreases production of folic acid to a level where bacteriostatic inhibition of growth occurs. However, when used in combination, inhibition of both steps in the metabolic pathway decreases folic acid synthesis to a level that is lethal to the bacterial cell. Because of the importance of folic acid during fetal development, sulfa drugs and trimethoprim use should be carefully considered during early pregnancy.

The drug isoniazid is an antimetabolite with specific toxicity for mycobacteria and has long been used in combination with rifampin or streptomycin in the treatment of tuberculosis. It is administered as a prodrug, requiring activation through the action of an intracellular bacterial peroxidase enzyme, forming isoniazid-nicotinamide adenine dinucleotide (NAD) and isoniazid-nicotinamide adenine dinucleotide phosphate (NADP). This ultimately prevents the synthesis of mycolic acid, which is essential for mycobacterial cell walls. Possible side effects of isoniazid use include hepatotoxicity, neurotoxicity, and hematologic toxicity (anemia).

INHIBITOR OF ATP SYNTHASE

Bedaquiline, representing the synthetic antibacterial class of compounds called the diarylquinolines, uses a novel mode of action that specifically inhibits mycobacterial growth. The drug acts by blocking the ion-binding sites of mycobacterial ATP synthase, resulting in ATP depletion. This damages the energy-producing capacity of mycobacteria, disrupts pH homeostasis, and ultimately inhibits the growth of the strain. Due to its side effects, including hepatotoxicity and potentially lethal heart arrhythmia, its use is reserved for serious, otherwise untreatable cases of tuberculosis.

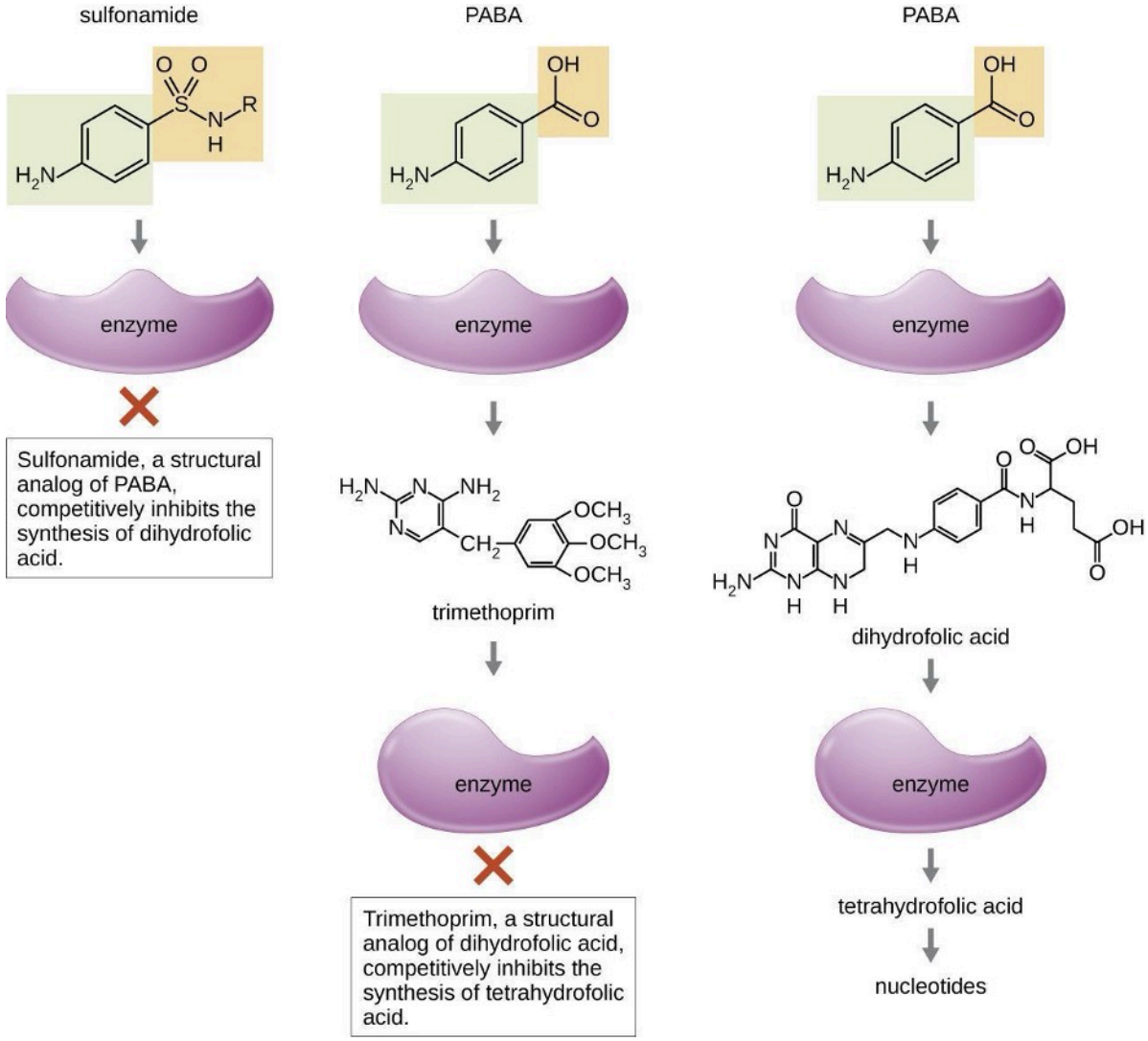


Figure 9.6: Sulfonamides and trimethoprim are examples of antimetabolites that interfere in the bacterial synthesis of folic acid by blocking purine and pyrimidine biosynthesis, thus inhibiting bacterial growth. *Figure description available at the end of the chapter.*

Metabolic Pathway Target	Mechanism of Action	Drug Class	Specific Drugs	Spectrum of Activity
Folic acid synthesis	Inhibits the enzyme involved in production of dihydrofolic acid	Sulfonamides	Sulfamethoxazole	Gram-positive and gram-negative bacterim
		Sulfones	Dapsone	Mycobacterium leprae
	Inhibits the enzyme involved in the production of tetrahydrofolic acid	Not applicable	Trimethoprim	Gram-positive and gram-negative bacteria
Mycolic acid synthesis	Interferes with the synthesis of mycolic acid	Not applicable	Isoniazid	*Mycobacterium* spp., including *M. tuberculosis*

Table 9.6: Antimetabolite drugs

Drugs to consider utilizing for certain bacterial infections	
GRAM POSITIVES	
MSSA	**Oral**: cephalexin, cefadroxil, dicloxacillin, amox/clav; **IV**: cefazolin, oxacillin, nafcillin
MRSA	**Oral**: SMX/TMP, doxycycline, clindamycin, linezolid, tedizolid, omadacycline, delafloxacin; **IV**: vancomycin, daptomycin, telavancin, dalbavancin, oritavancin, ceftaroline, tigecycline, eravacycline; all oral options also come as IV
Enterococci	Ampicillin, then vancomycin; if VRE: linezolid, daptomycin, oritavancin, tigecycline, omadacycline, eravacycline, nitrofurantoin (cystitis only), Fosfomycin (cystitis only)
Strep. pyogenes or *Strep. agalactiae*	Penicillin, amoxicillin, clindamycin
Strep. pneumonia or Viridans group *Streptococci*	Ceftriaxone, levofloxacin, amoxicillin, amoxicillin-clavulanic acid (beware penicillin & macrolide resistance)
Listeria monocytogenes	Ampicillin
GRAM NEGATIVES	
Pseudomonas aeruginosa	**Oral**: ciprofloxacin, levofloxacin, delafloxacin; **IV**: pip/taz, ceftazidime, ceftazidime-avibactam, cefepime, ceftolozane-tazobactam, cefiderocol, imipenem-cilastatin +/- relebactam, meropenem, aztreonam, aminoglycosides, polymyxins
E. coli, Klebsiella	**Oral**: cephalexin, amoxicillin-clavulanic acid, SMX/TMP, nitrofurantoin (cystitis only), fosfomycin, ciprofloxacin, levofloxacin, delafloxacin **IV**: ceftriaxone, ampicillin-sulbactam (beware resistance), cefepime, piperacillin-tazobactam, carbapenems.
ESBL-producer / Drug-Resistant GNR	Carbapenems, ceftolozane-tazobactam, ceftazidime- avibactam, meropenem-vaborbactam, imipenem- cilastatin-relebactam, cefiderocol, polymyxins, aminoglycosides, fosfomycin, nitrofurantoin (cystitis only), tigecycline, omadacycline, eravacycline
Carbapenem resistant GNR	ESBL-producer drug list minus carbapenem alone

Drugs to consider utilizing for certain bacterial infections	
NDM producer	Aztreonam + (MERO/VAB or CAZ/AVI), cefiderocol
Stenotrophomonas	SMX/TMP, levofloxacin, minocycline
Salmonella typhi	Fluoroquinolone, SMX/TMP, ceftriaxone

Table 9.7: Drugs to consider utilizing for certain bacterial infections

9.3 MECHANISMS OF OTHER ANTIMICROBIAL DRUGS

Because fungi, protozoa, and helminths are eukaryotic, their cells are very similar to human cells, making it more difficult to develop drugs with selective toxicity. Additionally, viruses replicate within human host cells, making it difficult to develop drugs that are selectively toxic to viruses or virus-infected cells. Despite these challenges, there are antimicrobial drugs that target fungi, protozoa, helminths, and viruses, and some even target more than one type of microbe. Table 9.8, table 9.9, table 9.10, and table 9.11 provide examples for antimicrobial drugs in these various classes.

ANTIFUNGAL DRUGS

The most common mode of action for antifungal drugs is the disruption of the cell membrane. Antifungals take advantage of small differences between fungi and humans in the biochemical pathways that synthesize sterols. Sterols are important in maintaining proper membrane fluidity and, hence, proper function of the cell membrane. For most fungi, the predominant membrane sterol is ergosterol. Because human cell membranes use cholesterol, instead of ergosterol, antifungal drugs that target ergosterol synthesis are selectively toxic (figure 9.7).

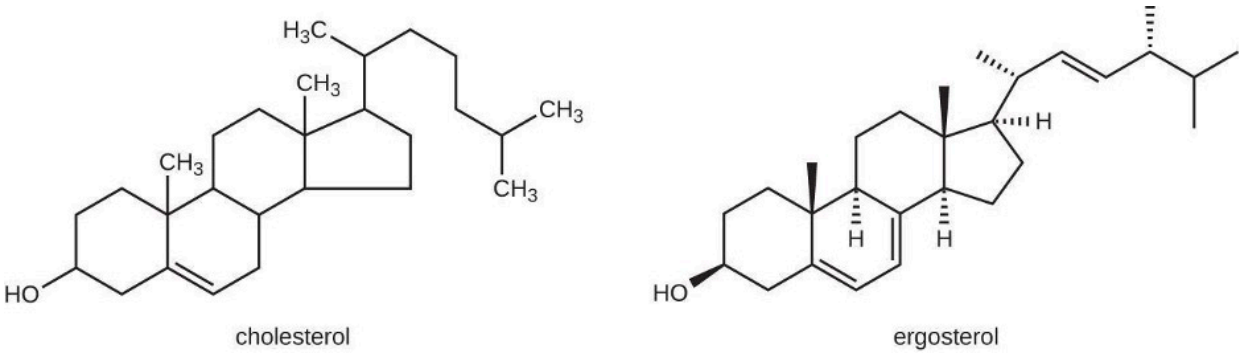


Figure 9.7: The predominant sterol found in human cells is cholesterol, whereas the predominant sterol found in fungi is ergosterol, making ergosterol a good target for antifungal drug development. *Figure description available at the end of the chapter.*

The imidazoles are synthetic fungicides that disrupt ergosterol biosynthesis; they are commonly used in medical applications and also in agriculture to keep seeds and harvested crops from molding. Examples include miconazole, ketoconazole, and clotrimazole, which are used to treat fungal skin infections such as tinea corporis (ringworm), specifically tinea pedis (athlete's foot), and tinea cruris (jock itch). These infections are commonly caused by dermatophytes of the genera *Trichophyton*, *Epidermophyton*, and *Microsporum*. Miconazole is also used predominantly for the treatment of vaginal yeast infections caused by the fungus *Candida*, and ketoconazole is used for the treatment of tinea versicolor and dandruff, which both can be caused by the fungus *Malassezia*.

The triazole drugs, including fluconazole, itraconazole, and voriconazole, also inhibit ergosterol biosynthesis. However, they can be administered orally or intravenously for the treatment of several types of systemic yeast infections, including oral thrush and cryptococcal meningitis, both of which are prevalent in immunocompromised patients. The triazoles also exhibit more selective toxicity, compared with the imidazoles, and are associated with fewer side effects.

The allylamines, a structurally different class of synthetic antifungal drugs, inhibit an earlier step in ergosterol biosynthesis. Examples include naftifine (Naftin®) and terbinafine (Lamisil®) which can be used topically for the treatment of dermatophytic skin infections like athlete's foot, ringworm, and jock itch. Oral treatment with terbinafine is also used for the treatment of fingernail and toenail fungus, but it can be associated with the rare side effect of hepatotoxicity.

Polyenes are a class of antifungal agents naturally produced by certain actinomycete soil bacteria and are structurally related to macrolides. These large, lipophilic molecules bind to ergosterol in fungal cytoplasmic membranes, thus creating pores. Common examples include nystatin, amphotericin B, and natamycin. Nystatin is typically used as a topical treatment for yeast infections of the skin, mouth, and vagina. The drug amphotericin B is used for systemic fungal infections like aspergillosis, cryptococcal meningitis, histoplasmosis, blastomycosis, and candidiasis. Amphotericin B was the only antifungal drug available for several decades, but its use is associated with some serious side effects, including nephrotoxicity.

Amphotericin B is often used with flucytosine, a fluorinated pyrimidine analog converted by a fungal-specific enzyme into a toxic product that interferes with DNA replication and protein synthesis in fungi. Flucytosine is also associated with hepatotoxicity and bone marrow depression.

Beyond targeting ergosterol in fungal cell membranes, there are a few antifungal drugs that target other fungal structures (figure 9.8). The echinocandins, (caspofungin, anidulafungin, micafungin, & rezafungin) are a group of naturally produced antifungal compounds that block the synthesis of β (1→3) glucan found in fungal cell walls but not found in human cells. Caspofungin is used for the treatment of invasive aspergillosis as well as candidemia.

The naturally produced antifungal griseofulvin is thought to specifically disrupt fungal cell division by interfering with microtubules involved in spindle formation during mitosis. It was one of the first antifungals, but its use is associated with hepatotoxicity, photosensitivity, and severe skin reactions. It is typically administered orally to treat various types of dermatophytic skin infections when other topical antifungal treatments are ineffective.

There are a few drugs that act as antimetabolites against fungal processes. For example, atovaquone, a representative of the naphthoquinone drug class, is a semisynthetic antimetabolite for fungal and protozoal versions of a mitochondrial cytochrome important in electron transport. Structurally, it is an analog of coenzyme Q, with which it competes for electron binding. It is particularly useful for the treatment of *Pneumocystis* pneumonia caused by *Pneumocystis jirovecii*. The antibacterial sulfamethoxazole-trimethoprim combination also acts as an antimetabolite against *P. jirovecii*.

Table 9.8 shows the various therapeutic classes of antifungal drugs, categorized by mode of action, with examples of each.

Mechanism of Action	Drug Class	Specific Drugs	Clinical Uses
Inhibit ergosterol synthesis	Imidazoles	miconazole, ketoconazole, clotrimazole	Fungal skin infections and vaginal yeast infections
	Triazoles	fluconazole, Itraconazole, voriconazole	Systemic yeast infections, oral thrush, and cryptococcal meningitis
	Allylamines	terbinafine	Dermatophytic skin infections (athlete's foot, ringworm, jock itch), and infections of fingernails and toenails
Bind ergosterol in the cell membrane and create pores that disrupt the membrane	Polyenes	nystatin	Used topically for yeast infections of skin, mouth, and vagina; also used for fungal infections of the intestine
		amphotericin B	Variety systemic fungal infections
Inhibit cell wall synthesis	Echinocandins	caspofungin, anidulafungin, micafungin, rezafungin	Systemic yeast infections
Inhibit microtubules and cell division	Not applicable	Griseofulvin	Dermatophytic skin infections

Table 9.8: Common antifungal drugs

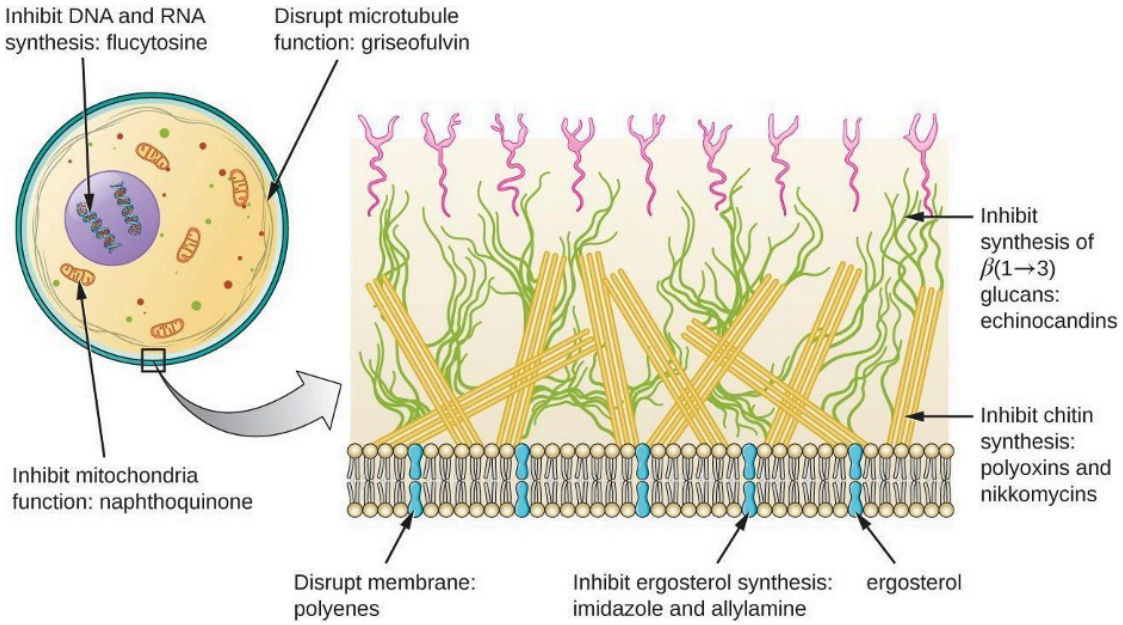


Figure 9.8: Antifungal drugs target several different cell structures. *Figure description available at the end of the chapter.*

ANTIPROTOZOAL DRUGS

There are a few mechanisms by which antiprotozoal drugs target infectious protozoans (table 9.10). Some are antimetabolites, such as atovaquone, proguanil, and artesunate. Atovaquone, in addition to being antifungal, blocks electron transport in protozoans and is used for treating protozoan infections including malaria, babesiosis, and toxoplasmosis. Proguanil is another synthetic antimetabolite that is processed in parasitic cells into its active form, which inhibits protozoan folic acid synthesis. It is often used in combination with atovaquone, and the combination is marketed as Malarone® for both malaria treatment and prevention.

Artesunate, an artemisinin derivative, is a semisynthetic derivative of artemisinin that is more water soluble than the natural version, which makes it more bioavailable for severe malaria. Artesunate is a prodrug that is metabolized to its active form, dihydroartemisinic (DHA). DHA contains an endoperoxide bridge activated by heme iron binding, resulting in oxidative stress, inhibition of protein and nucleic acid synthesis, thereby decreasing parasite growth and survival. Due to the rise in resistance to antimalarial drugs, artemisinins are used in combination with other antimalarial compounds in artemisinin-based combination therapy (ACT).

Several antimetabolites are used for the treatment of toxoplasmosis caused by the parasite *Toxoplasma gondii*. The synthetic sulfa drug sulfadiazine competitively inhibits the enzyme para-aminobenzoic acid (PABA) inhibiting folic acid synthesis and can be used to treat toxoplasmosis. Pyrimethamine is a synthetic drug that inhibits the enzyme dihydrofolate reductase resulting in inhibition of the folic acid production pathway. Side effects of pyrimethamine include decreased bone marrow activity that may cause increased bruising and low red blood cell counts. Two classes of antiprotozoal drugs interfere with nucleic acid synthesis: nitroimidazoles and quinolines.

Nitroimidazoles, including synthetic tinidazole and semisynthetic metronidazole, which was discussed previously as an antibacterial drug, are useful in combating a wide variety of protozoan pathogens, such as *Giardia lamblia*, *Entamoeba histolytica*, and *Trichomonas vaginalis*. Upon introduction into these cells in low-oxygen environments, nitroimidazoles become activated and introduce DNA strand breakage, interfering with DNA replication in target cells. Unfortunately, carcinogenicity has been seen in mice and rats treated chronically with metronidazole, and it is recommended that unnecessary use be avoided.

The aminoquinolines such as chloroquine (CQ) are a class of synthetic compounds related to quinine, which has a long history of use against malaria. CQ increases pH and accumulates in the food vacuole of parasites leading to the release of toxic products including hematin. CQ inhibits the polymerization and detoxification of hematin and interferes with the degradation of host erythrocyte hemoglobin, leading to the accumulation of free hematin that is highly toxic to *Plasmodium*, resulting in dissolution of the cell membrane and, ultimately, death of the parasites. Additionally, CQ can insert into the DNA double helix structure of *Plasmodium* impacting DNA replication and RNA transcription, to inhibit growth and reproduction of *Plasmodium.*[4]

Long-term prophylactic use of chloroquine may result in serious side effects, including hallucinations or cardiac issues. Patients with glucose-6-phosphate dehydrogenase deficiency experience severe anemia when treated with chloroquine.

Mechanism of Action	Drug Class	Specific Drugs	Clinical Uses
Inhibit electron transport in mitochondria	Naphthoquinone	Atovaquone	Malaria, babesiosis, and toxoplasmosis
Inhibit folic acid synthesis	Not applicable	Proguanil	Combination therapy with atovaquone for malaria treatment and prevention
	Sulfonamide	Sulfadiazine	Toxoplasmosis
	Not applicable	Pyrimethamine	Treatment of toxoplasmosis when used in combination with sulfonamide
Produces damaging reactive oxygen species	Not applicable	Artemisinin	Combination therapy to treat malaria
Inhibit DNA synthesis	Nitroimidazoles	Metronidazole, tinidazole	Infections caused by *Giardia lamblia*, *Entamoeba histolytica*, and *Trichomonas vaginalis*
Inhibit heme detoxification	Aminoquinolines	Chloroquine	Malaria

Table 9.9: Common antiprotozoal drugs

ANTIHELMINTHIC DRUGS

Because helminths are multicellular eukaryotes like humans, developing drugs with selective toxicity against them is extremely challenging. Despite this, several effective classes have been developed (table 9.10). Synthetic benzimidazoles, like mebendazole and albendazole, bind to helminthic β-tubulin, preventing microtubule formation. Microtubules in the intestinal cells of the worms seem to be particularly affected, leading to a reduction in glucose uptake. Besides their activity against a broad range of helminths, benzimidazoles are also active against many protozoans, fungi, and viruses. Their use for inhibiting mitosis and cell cycle progression in cancer cells is also under study.[5] Possible side effects of their use include gastrointestinal (abdominal pain, anorexia, diarrhea, nausea, vomiting) and bone marrow suppression (when used at higher doses or for prolonged duration).

The avermectins are members of the macrolide family that were first discovered from a Japanese soil isolate, *Streptomyces avermectinius*. A more potent semisynthetic derivative of avermectin is ivermectin, which binds to glutamate-gated chloride channels specific to invertebrates including helminths, blocking neuronal transmission and causing starvation, paralysis, and death of the worms. Ivermectin is used to treat roundworm diseases, including onchocerciasis (also called river blindness, caused by the worm *Onchocerca volvulus*) and strongyloidiasis (caused by the worm *Strongyloides stercoralis* or *S. fuelleborni*). Ivermectin can also treat parasitic insects like mites, lice, and bed bugs, and is nontoxic to humans.[6][7][8]

Another synthetic antihelminthic drug is praziquantel which is particularly useful for the treatment of schistosomiasis (caused by blood flukes from three genera of *Schistosoma*). Its mode of action is binding to a TRPM2-like channel causing the influx of calcium into the worm, resulting in intense spasm and paralysis of the worm.[9]

Mechanism of Action	Drug Class	Specific Drugs	Clinic Uses
Inhibit microtubule formation, reducing glucose uptake	Benzimidazoles	Mebendazole, albendazole	*Ancylostoma duodenale* or *Necator americanus* (hookworms), *Ascaris lumbricoides* (roundworms), *Enterobius vermicularis* (pinworms), and *Trichuris trichiura* (whipworms)
Block neuronal transmission, causing paralysis and starvation	Avermectins	Ivermectin	Onchocerciasis (river blindness) due to the immature form of *Onchocerca volvulus*. intestinal (e.g., non-disseminated) strongyloidiasis due to *Strongyloides stercoralis*.
Induce calcium influx	Not applicable	Praziquantel	Schistosomiasis (blood flukes)

Table 9.10: Common antihelminthic drugs

ANTIVIRAL DRUGS

Unlike the complex structure of fungi, protozoa, and helminths, viruses have a simpler composition consisting of nucleic acid, a protein coat, viral enzymes, and, sometimes, a lipid envelope. Furthermore, viruses are obligate intracellular pathogens that use the host's cellular machinery to replicate. These characteristics make it difficult to develop drugs with selective toxicity against viruses.

Many antiviral drugs are nucleoside analogs and function by inhibiting nucleic acid biosynthesis. For example, acyclovir (marketed as Zovirax®) is a synthetic analog of the nucleoside guanosine (figure 9.9). It is activated by virus-specific thymidine kinase and then further converted to acyclovir triphosphate by other cellular enzymes. Acyclovir triphosphate inhibits DNA synthesis and viral replication by competing with deoxyguanosine triphosphate for viral DNA polymerase and being incorporated into viral DNA. Acyclovir and its derivatives are frequently used to treat herpes virus infections, including genital herpes, chickenpox, and shingles infections. Acyclovir can be administered either topically or systemically, depending on the infection. One possible side effect of its use includes nephrotoxicity.

Ribavirin, another synthetic guanosine analog, works by a mechanism of action that is not entirely clear. It appears to interfere with both DNA and RNA synthesis, perhaps by reducing intracellular pools of guanosine triphosphate (GTP). Ribavirin also appears to inhibit the RNA polymerase of hepatitis C virus. It is primarily used for the treatment of the RNA viruses like hepatitis C (in combination therapy with interferon) and respiratory syncytial virus. Possible side effects of ribavirin use include anemia and developmental effects on unborn children in pregnant patients. In recent years, another nucleotide analog, sofosbuvir (Solvaldi®), has also been developed for the treatment of hepatitis C. Sofosbuvir is a uridine analog that interferes with viral polymerase activity. It is commonly co-administered with ribavirin, with and without interferon.

Inhibition of nucleic acid synthesis is not the only target of synthetic antivirals. Although the mode of action of amantadine and its relative rimantadine are not entirely clear, these drugs appear to bind to a transmembrane protein that is involved in the escape of the influenza virus from endosomes. Blocking escape of the virus also prevents viral RNA release into host cells and subsequent viral replication. Increasing resistance has limited the use of amantadine and rimantadine in the treatment of influenza A. Use of amantadine can result in neurological side effects, but the side effects of rimantadine seem less severe. Interestingly, because of their effects on

brain chemicals such as dopamine and NMDA (N-methyl D-aspartate), amantadine and rimantadine are also used for the treatment of Parkinson's disease.

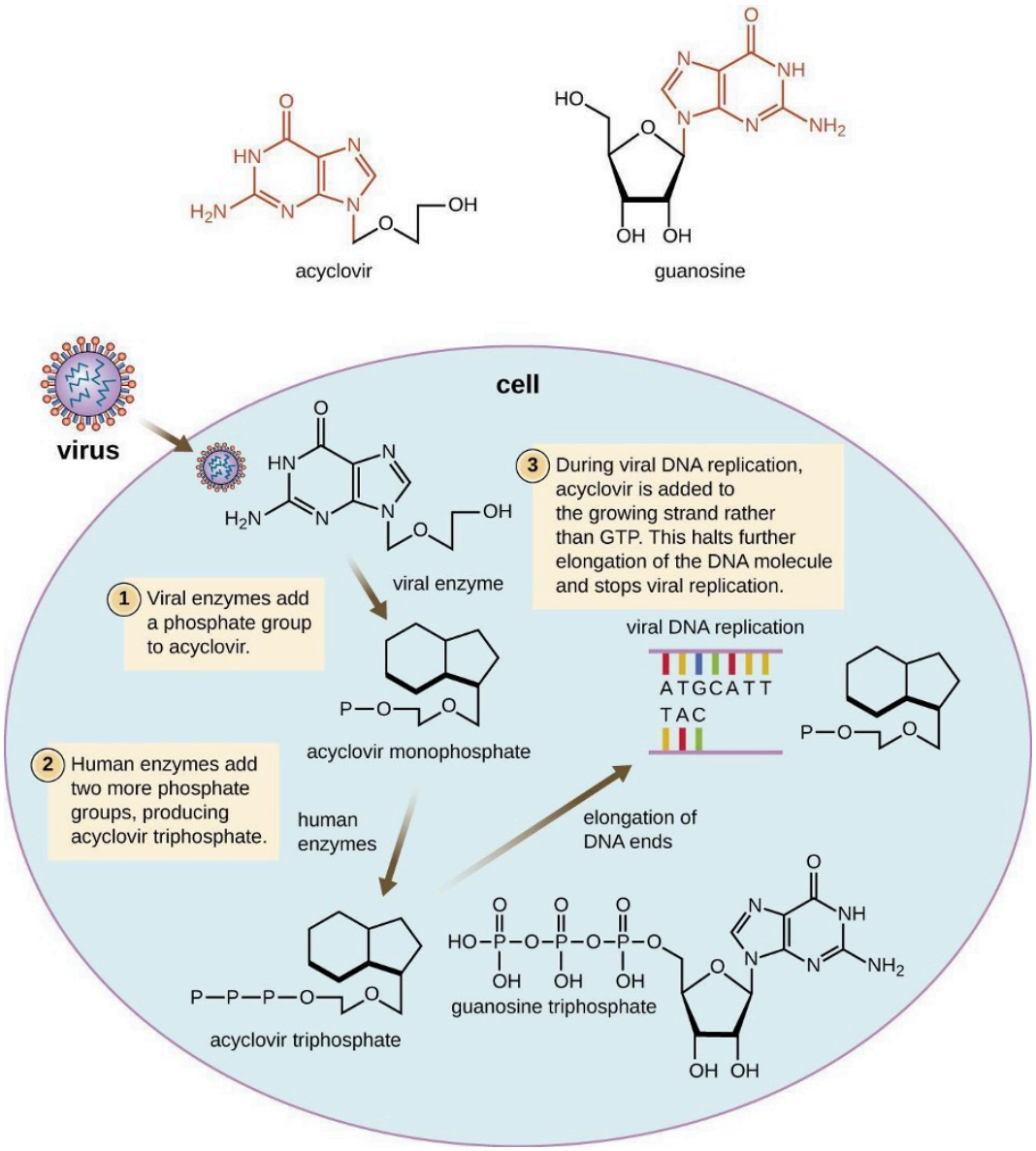


Figure 9.9: Acyclovir is a structural analog of guanosine. It is specifically activated by the viral enzyme thymidine kinase and then preferentially binds to viral DNA polymerase, leading to chain termination during DNA replication. *Figure description available at the end of the chapter.*

Neuraminidase inhibitors, including oseltamivir (Tamiflu®) and zanamivir (Relenza®), specifically target influenza viruses by blocking the activity of influenza virus neuraminidase, preventing the release of the virus from infected cells. These three antivirals can decrease flu symptoms and shorten the duration of illness, but they differ in their modes of administration: oseltamivir is administered orally and zanamivir is inhaled. Resistance to these neuraminidase inhibitors still seems to be minimal.

Viruses with complex life cycles, such as HIV, can be more difficult to treat. First, HIV targets CD4-positive white blood cells, which are necessary for a normal immune response to infection. Second, HIV is a retrovirus, meaning that it converts its RNA genome into a DNA copy that integrates into the host cell's genome, thus hiding within host cell DNA. Third, the HIV reverse transcriptase lacks proofreading activity and introduces

mutations that allow for rapid development of antiviral drug resistance. To help prevent the emergence of resistance, a combination of specific synthetic antiviral drugs is typically used in ART for HIV (figure 9.10). Table 9.11 shows the various therapeutic classes of antiviral drugs, categorized by mode of action, with examples of each.

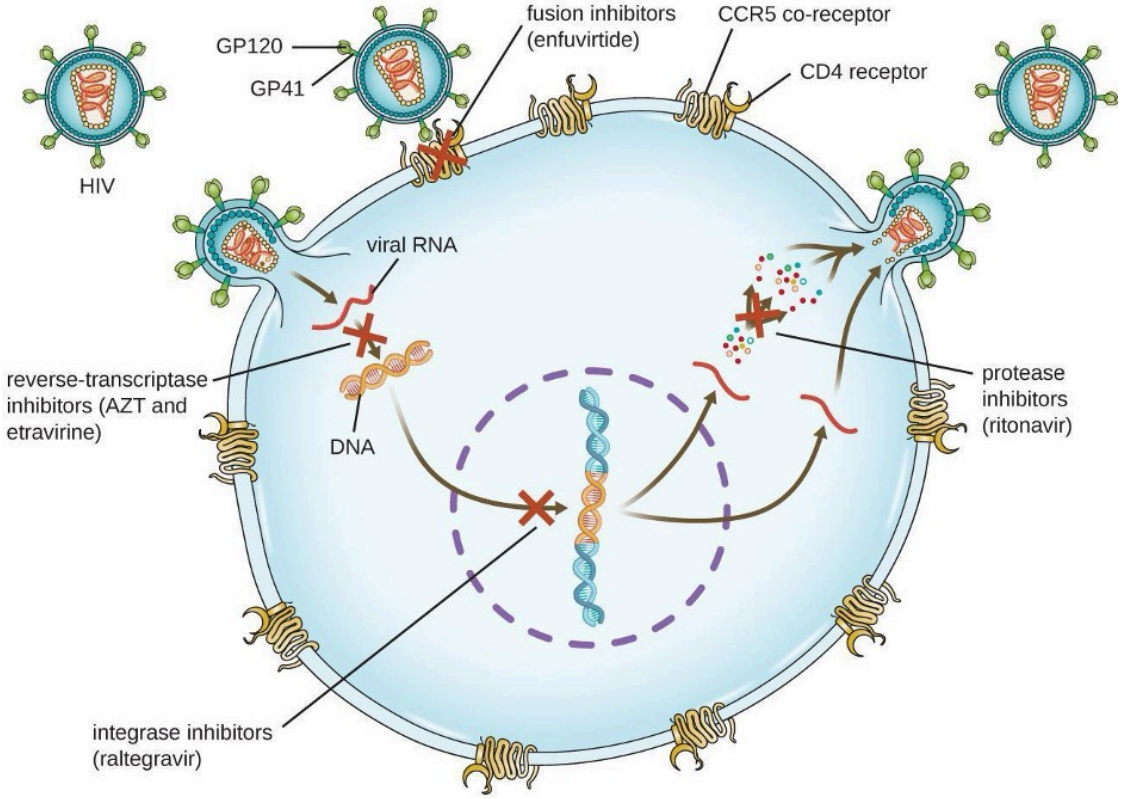


Figure 9.10: Antiretroviral therapy (ART) is typically used for the treatment of HIV. The targets of drug classes currently in use are shown here. *Figure description available at the end of the chapter.*

Targeted therapy for HIV

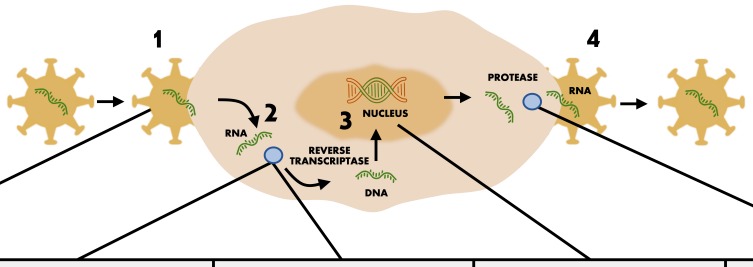
Fusion/entry inhibitors are a class of drugs employed in HIV treatment that specifically target the initial stages of the virus life cycle blocking the fusion of HIV with the host cell membrane preventing viral entry into host cells (figure 9.11).

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are a class of antiretroviral drugs used in the treatment of HIV. They work by binding to the viral enzyme reverse transcriptase, disrupting its function, preventing the conversion of viral RNA into DNA, thereby hindering the replication of the virus.

Nucleoside reverse transcriptase inhibitors (NRTIs) act by mimicking the building blocks of DNA. Once incorporated into the growing viral DNA chain, NRTIs terminate the process, preventing further replication and inhibiting the progression of HIV infection.

Integrase inhibitors are a class of antiretroviral drugs that specifically target the viral enzyme integrase. By blocking the integration of viral DNA into the host cell genome, these inhibitors disrupt the virus's ability to establish a permanent infection, ultimately suppressing HIV replication.

TARGETING HIV REPLICATION



Fusion/Entry Inhibitors	Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI)	Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTI)	Integrase Inhibitors	Protease Inhibitors
Maravi**roc** (MCV) "Maravi**roc** inhibits d**oc**king" En**fu**virtide (T-20) "En**fu**viratide inhibits **fu**sion" Ibalizumab	Dela**vir**dine (DLV) Efa**vir**enz (EFV) Etra**vir**ine (ETR) Ne**vir**apine (NVP) Rilpi**vir**ine (RPV)	Abacavir (ABC) Didanosine (ddi) Emtricitabine (FTC) Lamivudine (3TC) Stavudine (d4T) **T**enofovir (TDF) Zidovudine (ZDV)	Ral**tegr**avir (DRV) Elvi**tegr**avir (EVG) Dolu**tegr**avir (RAL)	Atazana**vir** (ATV) Daruna**vir** (DRV) Fosamprena**vir** (FPV) Indina**vir** (IDV) Lopina**vir** (LPV) Nelfina**vir** (NFV) Ritona**vir** (RTV) Saquina**vir** (SQV) Tiprana**vir** (TPV)
• **Maraviroc**: CCR5 antagonist; need to check "co-receptor tropism" since only effective against CCR5-using virions • **Enfuvirtide**: gp41 fusion inhibitor • **Ibalizumab**: monoclonal antibody against CD4 receptor	• Rash & hepatotoxicity common to all • **Delavirdine** & **efavirenz** contraindicated in pregnancy • Vivid dreams/CNS symptoms with **efavirenz**	• **Abacavir** contraindicated for HLA-B*5701 due to increased risk of hypersensitivity • **Emtricitabine/tenofovir** used in combination for pre-exposure & treatment (Travuda) • **Zidovudine** used during delivery to prevent perinatal transmission • **Tenofovir** is the only nucelo**t**ide	• Increase creatine kinase • AKA – integrase strand transfer inhibitor (INSTIs)	• Hyperglycemia, nausea, diarrhea, lipodystrophy with all • **Indinavir** – nephropathy, hematuria, thrombocytopenia • Rifampin in an inducer and decreases protease inhibitor concentrations – use rifabutin instead

- HAART (highly active antiretroviral therapy) is initiated at time of HIV diagnosis. Current Guidelines for the use of Antiretroviral Agents can be found here: https://clinicalinfo.hiv.gov/en/guidelines/hiv-clinical-guidelines-adult-and-adolescent-arv/what-start-initial-combination?view=full
- Miscellaneous agent: **Cobicistat** – CYP3A4 inhibitor increasing concentrations of other antiretrovirals - no antiretroviral activity

Figure 9.11: Targeting HIV replication. *Figure description available at the end of the chapter.*

Protease inhibitors are designed to block the activity of the viral enzyme protease. By inhibiting protease, these drugs prevent the maturation of newly formed viral particles, ultimately curtailing the production of infectious HIV and slowing the progression of the disease.

Targeted therapy for Hepatitis C

Glecaprevir and pibrentasvir (Mavyret®) are antiviral drugs used in combination for the treatment of chronic hepatitis C infections. Glecaprevir is an NS3/4A protease inhibitor, while pibrentasvir is an NS5A inhibitor. Together, they work synergistically to disrupt the viral life cycle, inhibiting viral replication and assembly. The mechanism of action involves glecaprevir inhibiting the NS3/4A protease, a key enzyme in the viral polyprotein processing, and pibrentasvir targeting the NS5A protein, crucial for viral RNA replication and assembly. This dual mechanism results in a high barrier to resistance and improved efficacy against a broad range of hepatitis C virus genotypes. Common adverse effects of the glecaprevir/pibrentasvir combination therapy include fatigue, headache, and nausea.

Mechanism of Action	Drug	Clinical Uses
Nucleoside analog inhibition of nucleic acid synthesis	Acyclovir	Herpes virus infections
	zidovudine	HIV infections
	Ribavirin	Hepatitis C virus and respiratory syncytial virus infections
Non-nucleoside noncompetitive inhibition	Etravirine	HIV infections
Inhibit escape of virus from endosomes	Amantadine, rimantadine	Infections with influenza virus
Inhibit neuraminidase	Oseltamivir, zanamivir	Infections with influenza virus
Inhibition of protease	Ritonavir	HIV infections
Inhibition of integrase	Raltegravir	HIV infections
Inhibition of membrane fusion	Enfuvirtide	HIV infections

Table 9.11: Antiviral drugs

9.4 DRUG RESISTANCE

Antimicrobial resistance is not a new phenomenon. In nature, microbes are constantly evolving to overcome the antimicrobial compounds produced by other microorganisms. Human development of antimicrobial drugs and their widespread clinical use has simply provided another selective pressure that promotes further evolution. Several important factors can accelerate the evolution of drug resistance. These include the overuse and misuse of antimicrobials, inappropriate use of antimicrobials, subtherapeutic dosing, and patient noncompliance with the recommended course of treatment.

Some organisms, such as *Pseudomonas* with porin and permeability changes, are more likely to have the intrinsic resistance machinery necessary to have reduced susceptibility to certain agents. Other organisms expressing resistance to antimicrobials may be more likely due to acquired resistance mechanisms. Many genes responsible for drug resistance are found on plasmids or in transposons that can be transferred easily between microbes through horizontal gene transfer. Transposons can also move resistance genes between plasmids and chromosomes to promote the spread of resistance. *E. coli* is a bacterial example that has acquired genes for ESBL production via plasmid exchange.

MECHANISMS FOR DRUG RESISTANCE

There are several common mechanisms for drug resistance, which are summarized in figure 9.12. These mechanisms include enzymatic modification of the drug, modification of the antimicrobial target, and prevention of drug penetration or accumulation.

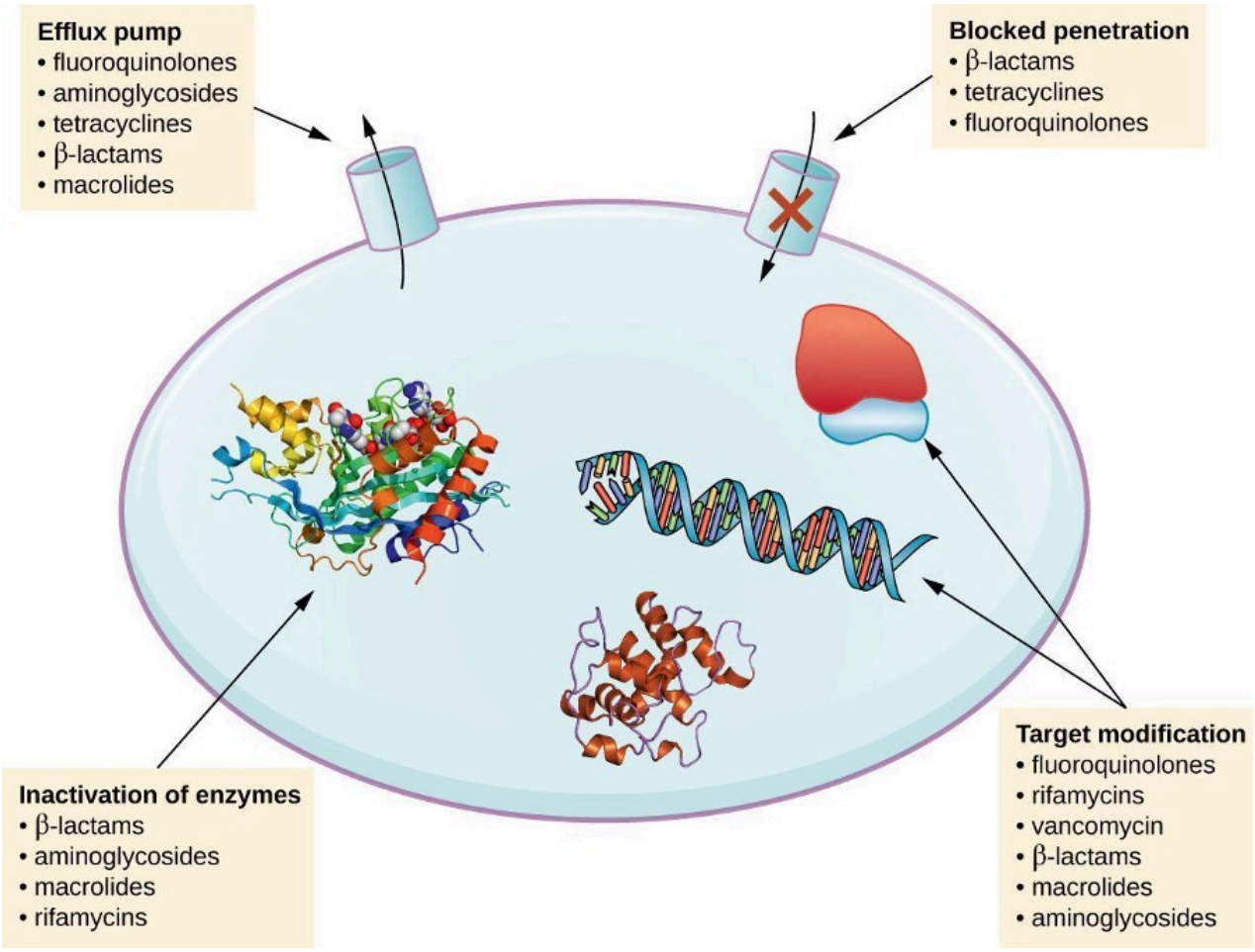


Figure 9.12: There are multiple strategies that microbes use to develop resistance to antimicrobial drugs. (Not shown: target overproduction, target mimicry, and enzymatic bypass). *Figure description available at the end of the chapter.*

Drug Modification or Inactivation

Resistance genes may code for enzymes that chemically modify an antimicrobial, thereby inactivating it, or destroy an antimicrobial through hydrolysis. Resistance to many types of antimicrobials occurs through this mechanism. For example, aminoglycoside resistance can occur through enzymatic transfer of chemical groups to the drug molecule, impairing the binding of the drug to its bacterial target. For β-lactams, bacterial resistance can involve the enzymatic hydrolysis of the β-lactam bond within the β-lactam ring of the drug molecule. Once the β-lactam bond is broken, the drug loses its antibacterial activity. This mechanism of resistance is mediated by β-lactamases, which are the most common mechanism of β-lactam resistance. Inactivation of rifampin commonly occurs through glycosylation, phosphorylation, or adenosine diphosphate (ADP) ribosylation, and resistance to macrolides and lincosamides can also occur due to enzymatic inactivation of the drug or modification.

Prevention of Cellular Uptake or Efflux

Microbes may develop resistance mechanisms that involve inhibiting the accumulation of an antimicrobial drug, which then prevents the drug from reaching its cellular target. This strategy is common among gram-negative pathogens and can involve changes in outer membrane lipid composition, porin channel selectivity, and/or porin channel concentrations. For example, a common mechanism of carbapenem resistance among *Pseudomonas aeruginosa* is to decrease the amount of its OprD porin, which is the primary portal of entry for

carbapenems through the outer membrane of this pathogen. Additionally, many gram-positive and gram-negative pathogenic bacteria produce efflux pumps that actively transport an antimicrobial drug out of the cell and prevent the accumulation of drug to a level that would be antibacterial. For example, resistance to β-lactams, tetracyclines, and fluoroquinolones commonly occurs through active efflux out of the cell, and it is rather common for a single efflux pump to have the ability to translocate multiple types of antimicrobials.

Target Modification

Because antimicrobial drugs have very specific targets, structural changes to those targets can prevent drug binding, rendering the drug ineffective. Through spontaneous mutations in the genes encoding antibacterial drug targets, bacteria have an evolutionary advantage that allows them to develop resistance to drugs. This mechanism of resistance development is quite common. Genetic changes impacting the active site of penicillin-binding proteins (PBPs) can inhibit the binding of β-lactam drugs and provide resistance to multiple drugs within this class. This mechanism is very common among strains of *Streptococcus pneumoniae*, which alter their own PBPs through genetic mechanisms. In contrast, strains of *Staphylococcus aureus* develop resistance to methicillin (MRSA) through the acquisition of a new low-affinity PBP, rather than structurally altering their existing PBPs. Not only does this new low-affinity PBP provide resistance to methicillin, but it provides resistance to virtually all β-lactam drugs, with the exception of the newer fifth generation cephalosporins designed specifically to kill MRSA. Other examples of this resistance strategy include alterations in

- ribosome subunits, providing resistance to macrolides, tetracyclines, and aminoglycosides;
- lipopolysaccharide (LPS) structure, providing resistance to polymyxins;
- RNA polymerase, providing resistance to rifampin;
- DNA gyrase, providing resistance to fluoroquinolones;
- metabolic enzymes, providing resistance to sulfa drugs, sulfones, and trimethoprim; and
- peptidoglycan subunit peptide chains, providing resistance to glycopeptides.

Target Overproduction or Enzymatic Bypass

When an antimicrobial drug functions as an antimetabolite, targeting a specific enzyme to inhibit its activity, there are additional ways that microbial resistance may occur. First, the microbe may overproduce the target enzyme such that there is enough antimicrobial-free enzyme to do the proper enzymatic reaction. Second, the bacterial cell may develop a bypass that circumvents the need for the functional target enzyme. Both strategies have been found as mechanisms of sulfonamide resistance. Vancomycin resistance among *S. aureus* has been shown to involve the decreased cross-linkage of peptide chains in the bacterial cell wall, which provides an increase in targets for vancomycin to bind to in the outer cell wall. Increased binding of vancomycin in the outer cell wall provides a blockage that prevents free drug molecules from penetrating to where they can block new cell wall synthesis.

Target Mimicry

A recently discovered mechanism of resistance called target mimicry involves the production of proteins that prevent drugs from binding to their bacterial cellular targets. For example, fluoroquinolone resistance by Mycobacterium tuberculosis can involve producing a protein that resembles DNA. This protein is called MfpA (Mycobacterium fluoroquinolone resistance protein A). The mimicry of DNA by MfpA results in DNA gyrase binding to MfpA, preventing the binding of fluoroquinolones to DNA gyrase.

MULTIDRUG-RESISTANT MICROBES AND CROSS RESISTANCE

From a clinical perspective, our greatest concerns are multidrug-resistant microbes (MDRs) and cross resistance. MDRs are colloquially known as "superbugs" and carry one or more resistance mechanism(s), making them resistant to multiple antimicrobials. In cross-resistance, a single resistance mechanism confers resistance to multiple antimicrobial drugs. For example, having an efflux pump that can export multiple antimicrobial drugs is a common way for microbes to be resistant to multiple drugs by using a single resistance mechanism. In recent years, several clinically important superbugs have emerged, and the CDC reports that superbugs are responsible for more than 2 million infections in the US annually, resulting in at least 23,000 fatalities.[10] Several of the superbugs have been dubbed the ESKAPE pathogens. This acronym refers to the names of the pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) but it is also fitting in that these pathogens are able to "escape" many conventional forms of antimicrobial therapy. As such, infections by ESKAPE pathogens can be difficult to treat and cause many nosocomial infections.

METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

Methicillin, a semisynthetic penicillin, was designed to resist inactivation by β-lactamases. Unfortunately, soon after the introduction of methicillin to clinical practice, methicillin-resistant strains of *S. aureus* appeared and started to spread. The mechanism of resistance, acquisition of a new low-affinity PBP, provided *S. aureus* with resistance to all available β-lactams. Strains of methicillin-resistant *S. aureus* (MRSA) are widespread opportunistic pathogens and a particular concern for skin and other wound infections. These strains may also cause pneumonia and septicemia. Although originally a problem in healthcare settings (hospital-acquired MRSA [HA-MRSA]), MRSA infections are now also acquired through contact with contaminated members of the general public, called community-associated MRSA (CA-MRSA). Approximately one-third of the population carries *S. aureus* as a member of their normal nasal microbiota without illness, and about 6% of these strains are methicillin resistant.[1112]

VANCOMYCIN-RESISTANT ENTEROCOCCI AND *STAPHYLOCOCCUS AUREUS*

Vancomycin is only effective against gram-positive organisms, and it is used to treat wound infections, septic infections, endocarditis, and meningitis that are caused by pathogens resistant to other antibiotics. It is considered one of the last lines of defense against such resistant infections, including MRSA. With the rise of antibiotic resistance in the 1970s and 1980s, vancomycin use increased, and it is not surprising that we saw the emergence and spread of vancomycin-resistant enterococci (VRE), vancomycin-resistant *S. aureus* (VRSA), and vancomycin-intermediate *S. aureus* (VISA). The mechanism of vancomycin resistance among enterococci is target modification involving a structural change to the peptide component of the peptidoglycan subunits, preventing vancomycin from binding. These strains are typically spread among patients in clinical settings by contact with healthcare workers and contaminated surfaces and medical equipment.

VISA and VRSA strains differ from each other in the mechanism of resistance and the degree of resistance each mechanism confers. VISA strains exhibit intermediate resistance, with a minimum inhibitory concentration (MIC) of 4–8 μg/mL, and the mechanism involves an increase in vancomycin targets. VISA strains decrease the crosslinking of peptide chains in the cell wall, providing an increase in vancomycin targets that trap vancomycin in the outer cell wall. In contrast, VRSA strains acquire vancomycin resistance through horizontal transfer of resistance genes from VRE, an opportunity provided in individuals co-infected with both VRE and

MRSA. VRSA exhibits a higher level of resistance, with MICs of 16 μg/mL or higher.[13] In the case of all three types of vancomycin-resistant bacteria, rapid clinical identification is necessary so proper procedures to limit spread can be implemented. Oxazolidinones, like linezolid, are useful for the treatment of these vancomycin-resistant, opportunistic pathogens, as well as MRSA.

EXTENDED-SPECTRUM **B**-LACTAMASE–PRODUCING GRAM-NEGATIVE PATHOGENS

Gram-negative pathogens that produce extended-spectrum β-lactamases (ESBLs) show resistance well beyond just penicillins. The spectrum of β-lactams inactivated by ESBLs provides for resistance to all penicillins, cephalosporins, monobactams, and the β-lactamase-inhibitor combinations, but not the carbapenems. An even greater concern is that the genes encoding for ESBLs are usually found on mobile plasmids that also contain genes for resistance to other drug classes (e.g., fluoroquinolones, aminoglycosides, tetracyclines), and may be readily spread to other bacteria by horizontal gene transfer. These multidrug-resistant bacteria are members of the intestinal microbiota of some individuals, but they are also important causes of opportunistic infections in hospitalized patients, from whom they can be spread to other people.

CARBAPENEM-RESISTANT GRAM-NEGATIVE BACTERIA

The occurrence of carbapenem-resistant Enterobacterales (CRE) and carbapenem resistance among other gram-negative bacteria (e.g., *P. aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophila*) is a growing healthcare concern. These pathogens develop resistance to carbapenems through a variety of mechanisms, including production of carbapenemases (broad-spectrum β-lactamases that inactivate all β-lactams, including carbapenems), active efflux of carbapenems out of the cell, and/or prevention of carbapenem entry through porin channels. Similar to concerns with ESBLs, carbapenem-resistant, gram-negative pathogens are usually resistant to multiple classes of antibacterials, and some have even developed pan-resistance (resistance to all available antibacterials). Infections with carbapenem-resistant, gram-negative pathogens commonly occur in healthcare settings through interaction with contaminated individuals or medical devices, or as a result of surgery.

MULTIDRUG-RESISTANT *MYCOBACTERIUM TUBERCULOSIS*

The emergence of multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) and extensively drug-resistant *Mycobacterium tuberculosis* (XDR-TB) is also of significant global concern. MDR-TB strains are resistant to both rifampin and isoniazid, the drug combination typically prescribed for treatment of tuberculosis. XDR-TB strains are additionally resistant to any fluoroquinolone and at least one of three other drugs (amikacin, kanamycin, or capreomycin) used as a second line of treatment, leaving these patients very few treatment options. Both types of pathogens are particularly problematic in immunocompromised persons, including those suffering from HIV infection. The development of resistance in these strains often results from the incorrect use of antimicrobials for tuberculosis treatment, selecting for resistance.

Figure Descriptions

Figure 9.1: Diagram of process of superinfection. 1: Normal microbiota keeps opportunistic pathogens in check. The image shows many different bacteria, only 1 of which is labeled pathogen. 2: Broad-spectrum antibiotics kill nonresistant cells. The image shows all cells but the pathogen being killed. 3: Drug-resistant pathogens proliferate and can cause a superinfection. The image shows many of the pathogens.

Figure 9.2: Graph with time on the X axis and Plasma Concentration of Drug on the Y axis. IV route increases plasma concentration very quickly and then tapes off. The intramuscular route and oral route increase concentrations more slowly, with the intramuscular route being a bit faster than oral but also dropping off more quickly.

Figure 9.3: An illustration of a cell is shown with a view inside. The double helix is visible in the center, and a label points to it indicating DNA synthesis, fluoroquinolones, ciprofloxacin, levofloxacin, moxifloxacin, RNA synthesis, Rifamycins, and rifampin. Another label points to the cell wall and indicates beta-lactams, penicillins, cephalosporins, monobactams, carbapenems, glycopeptides, vancomycin, and bacitracin. A third label points to the plasma membrane and indicates polymyxins, polymyxin B, colistin, lipopeptide, and daptomycin. Within the cytoplasm, another label points to ribosomes, which include 30s subunit, aminoglycosides, tetracyclines, 50s subunit, macrolides, lincosamides, chloramphenicol, and oxazolidinones. The final label points to the metabolic pathways and indicates folic acid synthesis, sulfonamides, sulfones, trimethoprim, mycolic acid synthesis, and isoniazid.

Figure 9.4: The top of the image shows diagrams of various antibiotics. All have a beta-lactam ring, which is a square made of 3 carbons and nitrogen; one of the carbons has a double-bonded O. The antibiotics shown are penicillin, cephalosporin, monobactam, and carbapenem. Below is a table with the rows: R group, Drug name, spectrum of activity, and route of administration. Penicillin G has an R group of carbon linked to a 6-carbon ring; it is active on G+ and a few G- cells and has a parenteral route of administration. Penicillin V has an R group of a carbon linked to an oxygen linked to a carbon ring. IT affects G+ and a few G- and is administered orally. Ampicillin has an R group of Carbon attached to both an amino group and a carbon ring. It is effective against G+ and more G- than penicillin. It is administered orally and parenterally. Amoxicillin has an R group similar to ampicillin, but the carbon ring has an additional OH. It has similar activity to ampicillin and is administered orally (better than ampicillin). Methicillin has an R group of carbon right with 2 CH3O attached to the ring. It is effective against G+ only, including B-lactam producers. It is administered parenterally.

Figure 9.5: Major classes of protein synthesis-inhibiting antibacterials. Chloramphenicol, macrolides, and lincosamides: bind to the 50S ribosomal subunit, preventing peptide bond formation, and stop protein synthesis. Aminoglycosides: bind to the 30S ribosomal subunit, implant proofreading, resulting in production of faulty proteins. Tetracyclines: bind to the 30S ribosomal subunit, block the binding of tRNAs, thereby inhibiting protein synthesis.

Figure 9.6: PABA binds to an enzyme to produce dihydrofolic acid, which binds to another enzyme to produce tetrahydrofolic acid and nucleotides. Trimethoprim, a structural analog of dihydrofolic acid, completely inhibits the synthesis of tetrahydrofolic acid. Sulfonamide, a structural analog of PABA, competitively inhibits the synthesis of dihydrofolic acid.

Figure 9.7: Cholesterol and ergosterol both have 4 fused carbon rings with a chain of carbons off the top ring. The differences are the placements of a few double bonds.

Figure 9.8: Targets of antifungal drugs: Inhibits mitochondria function: naphthoquinone. Disrupt membrane: polyenes. Inhibit ergosterol synthesis: imidazole and allylamine. Inhibit synthesis of beta(1-3) glucans: echinocandins. Inhibit chitin synthesis: polyoxins and nikko mycins.

Figure 9.9: Acyclovir looks similar to guanosine except that the sugar is replaced with a short carbon chain. Step 1: Viral enzyme adds a phosphate group to acyclovir. Step 2: Human enzymes add two more phosphate groups, producing acyclovir triphosphate. Step 3: During viral DNA replication, acyclovir is added to the growing strand rather than GTP. This halts further elongation of the DNA molecule and stops viral replication.

Figure 9.10: Diagram showing HIV infection and locations where drugs can stop the infection. GP120 and G(42 are proteins that are on the surface of the virus and bind to CD4 receptor and CCR5. Enfuvirtide is a fusion inhibitor that blocks this process. When the virus enters, it produces DNA from RNA, this can be blocked by AZT and etravirine which are reverse-transcriptase inhibitors. Next, the viral DNA integrates into the host DNA. Raltegravir is an integrase inhibitor and blocks this step. Finally the virus is rebuilt. Ritonavir is a protease inhibitor and blocks this step.

Figure 9.11: An intricate diagram detailing the targeted therapy for HIV is represented by a series of grey columns arranged in an accompanying arrow table. The descriptions of the columns are given left to right. Starting leftmost, an arrow points to RNA contained within a vesicle, indicating the location of Fusion/Entry Inhibitors. Within this section, Maraviroc (MCV) is described as inhibiting docking, Enfuvirtide (T-20) inhibits fusion, and Ibalizumab is mentioned as a monoclonal antibody against the CD4 receptor. Next, an arrow extends towards RNA after its entry into the cell, representing Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI). The drugs listed include Delavirdine (DLV), Efavirenz (EFV), Etravirine (ETR), Nevirapine (NVP), and Rilpivirine (RPV), with common adverse effects such as rash and hepatotoxicity. Next, accompanied by arrows pointing towards a blue sphere in the diagram, signify Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTI). Listed drugs include Abacavir (ABC), Didanosine (ddi), Emtricitabine (FTC), Lamivudine (3TC), Stavudine (d4T), Tenofovir (TDF), and Zidovudine (ZDV), with specific considerations for each drug, such as contraindications and preferred usage scenarios. The next column, with an arrow extending to the nucleus denote Integrase Inhibitors. Raltegravir (DRV), Elvitegravir (EVG), and Dolutegravir (RAL) are listed, along with a note on increased creatine kinase and their alternative name as integrase strand transfer inhibitors (INSTIs). Lastly, an arrow pointing towards a second blue sphere represent Protease Inhibitors. Atazanavir (ATV), Darunavir (DRV), Fosamprenavir (FPV), Indinavir (IDV), Lopinavir (LPV), Nelfinavir (NFV), Ritonavir (RTV), Saquinavir (SQV), and Tipranavir (TPV) are listed, along with common adverse effects and considerations for concurrent medication usage.

Figure 9.12: Mechanisms of resistance. Efflux pump (pumping drugs out of the cell): fluoroquinolones, aminoglycosides, tetracyclines, Beta-lactams, macrolides. Blocked penetration (not letting drugs into the cell): beta-lactams, tetracyclines, fluoroquinolones. Target modification (changing the target of the drug, such as ribosomes or DNA): fluoroquinolones, rifamycins, vancomycin, beta-lactams, macrolides, aminoglycosides. Inactivating enzyme (enzyme that breaks down the drug): beta-lactams, aminoglycosides, macrolides, rifamycins.

Figure References

Figure 9.1: Broad-spectrum antimicrobial use may lead to the development of a superinfection. Modification of work by Centers for Disease Control and Prevention. Public domain.

Figure 9.2: On this graph, t0 represents the time at which a drug dose is administered. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 9.3: There are several classes of antibacterial compounds that are typically classified based on their bacterial target. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 9.4: Penicillins, cephalosporins, monobactams, and carbapenems all contain a β-lactam ring, the site of attack by inactivating β-lactamase enzymes. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 9.5: The major classes of protein synthesis inhibitors target the 30S or 50S subunits of cytoplasmic ribosomes. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 9.6: Sulfonamides and trimethoprim are examples of antimetabolites that interfere in the bacterial synthesis of folic acid by blocking purine and pyrimidine biosynthesis, thus inhibiting bacterial growth. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 9.7: The predominant sterol found in human cells is cholesterol, whereas the predominant sterol found in fungi is ergosterol, making ergosterol a good target for antifungal drug development. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 9.8: Antifungal drugs target several different cell structures. Modification of work by (c) Maya and Rike. CC BY 3.0 Unported. https://commons.wikimedia.org/wiki/File:Cell_wall_structure_of_Fungi.png

Figure 9.9: Acyclovir is a structural analog of guanosine. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 9.10: Antiretroviral therapy (ART) is typically used for the treatment of HIV. The targets of drug classes currently in use are shown here. Modification of work (c) Thomas Splettstoesser. CC BY SA 4.0. https://commons.wikimedia.org/wiki/File:HI-virion-structure_en.svg

Figure 9.11: Targeting HIV replication. (c) Rice University. OpenStax Microbiology. CC BY 4.0. https://openstax.org/details/books/microbiology.

Figure 9.12: There are multiple strategies that microbes use to develop resistance to antimicrobial drugs. Modification of work (c) Gerard D Wright. CC BY 4.0.

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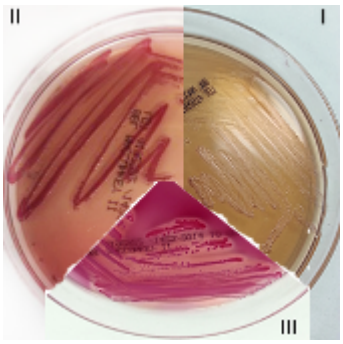
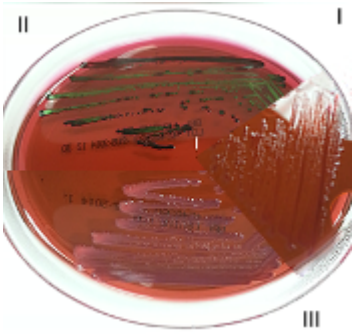
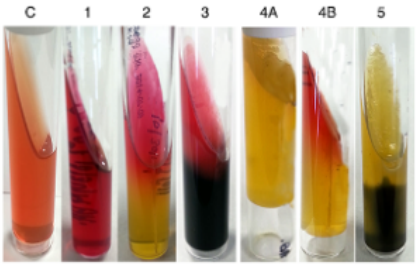
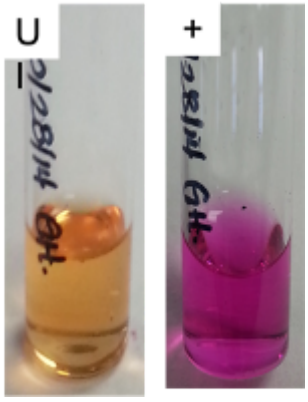
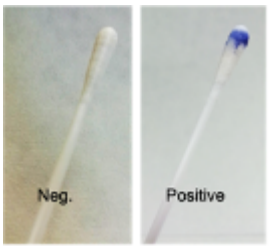
APPENDIX A: BACTERIOLOGY RESOURCE

Test	Visual	Notes
MacConkey (MAC) Media		**MacConkey Media:** Bile salts and crystal violet are added to inhibit gram-positives; the fermentation of lactose produces acidic products that turn the crystal violet (also a pH indicator) pink.
Eosin Methylene Blue (EMB) Media		**EMB Media:** Eosin blue and methylene blue inhibits the growth of gram-positives; the fermentation of lactose produces acidic products that turn the methylene blue to purple or creates a green metabolic sheen with high lactose fermentation.
Kligler's Iron Agar (KIA)		**Contents of media:** (C) control, (1) glucose, (2) lactose, (3) peptone, (4A) phenol red, (4B) sodium thiosulfate, and (5) iron All Enterobacteriaceae will initially ferment glucose, changing the color from red to yellow. If the microorganism can ferment lactose, it will be fermented second, also producing acid end products and the media will stay yellow. If lactose cannot be fermented, the peptone will be utilized and basic products will be produced, changing the slant back to red. The black color is ferrous sulfide produced from the reaction of H_2S with the iron in the media.

Test	Visual	Notes
Urease Test		If a microorganism produces urease, it can break-down urea in the media. Ammonia is an alkaline product of this enzymatic reaction and changes the color of the pH indicator (phenol red) to pink.
Citrate Test		If a microorganism produces citrase, citrate in the media is broken-down to oxaloacetic acid and acetic acid. The oxaloacetic acid is further oxidized to pyruvic acid and CO_2. The CO_2 combines with Na in the media to form sodium bicarbonate. This is an alkaline product that changes the pH indicator (bromophenol blue) from green to blue.
SIM Test		**S-H_2S production:** similar process to KIA (above). **I-Indole production:** tryptophan is broken down to ammonia, pyruvic acid, and indole. The indole reacts with Kovac's reagent to produce a red color. **M-motility:** motility is observed as growth radiating outward along the length of the stab line.
Oxidase Test		If the microorganism has cytochrome c then they oxidize the TMPD to a dark purple. Microorganisms that lack cytochrome c can still use oxygen as a terminal electron acceptor but simply do not use cytochrome c.

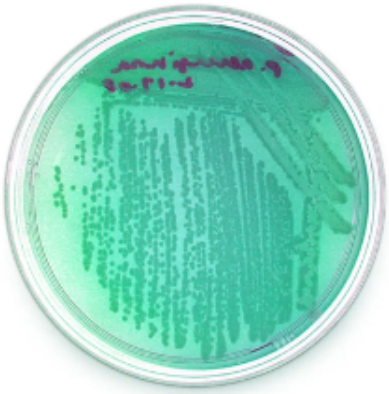
Test	Visual	Notes
Phenylethyl alcohol (PEA) agar		Allows the growth of gram-positive organisms, particularly cocci Inhibits most gram-negative bacteria and fungi PEA agar is used to inhibit common contaminants such as Escherichia coli and Proteus species.
Tryptic Soy Agar (TSA)		Allows the growth of gram-positive organisms, particularly cocci Inhibits most gram-negative bacteria and fungi PEA agar is used to inhibit common contaminants such as Escherichia coli and Proteus species.
Mannitol salt agar or MSA		Mannitol salt agar or MSA contains a high concentration (about 7.5–10%) of salt (NaCl) which is inhibitory to most bacteria - making MSA selective against most Gram-negative and selective for some Gram-positive bacteria (Staphylococcus, Enterococcus and Micrococcaceae) that tolerate high salt concentrations.
Blood Agar Plate (BAP)		BAP tests the ability of an organism to produce hemolysins. The degree of hemolysis by these hemolysins is helpful in differentiating members of the genera Staphylococcus, Streptococcus and Enterococcus.

Table A.1: Biochemical tests and media used to differentiate Enterobacteriaceae

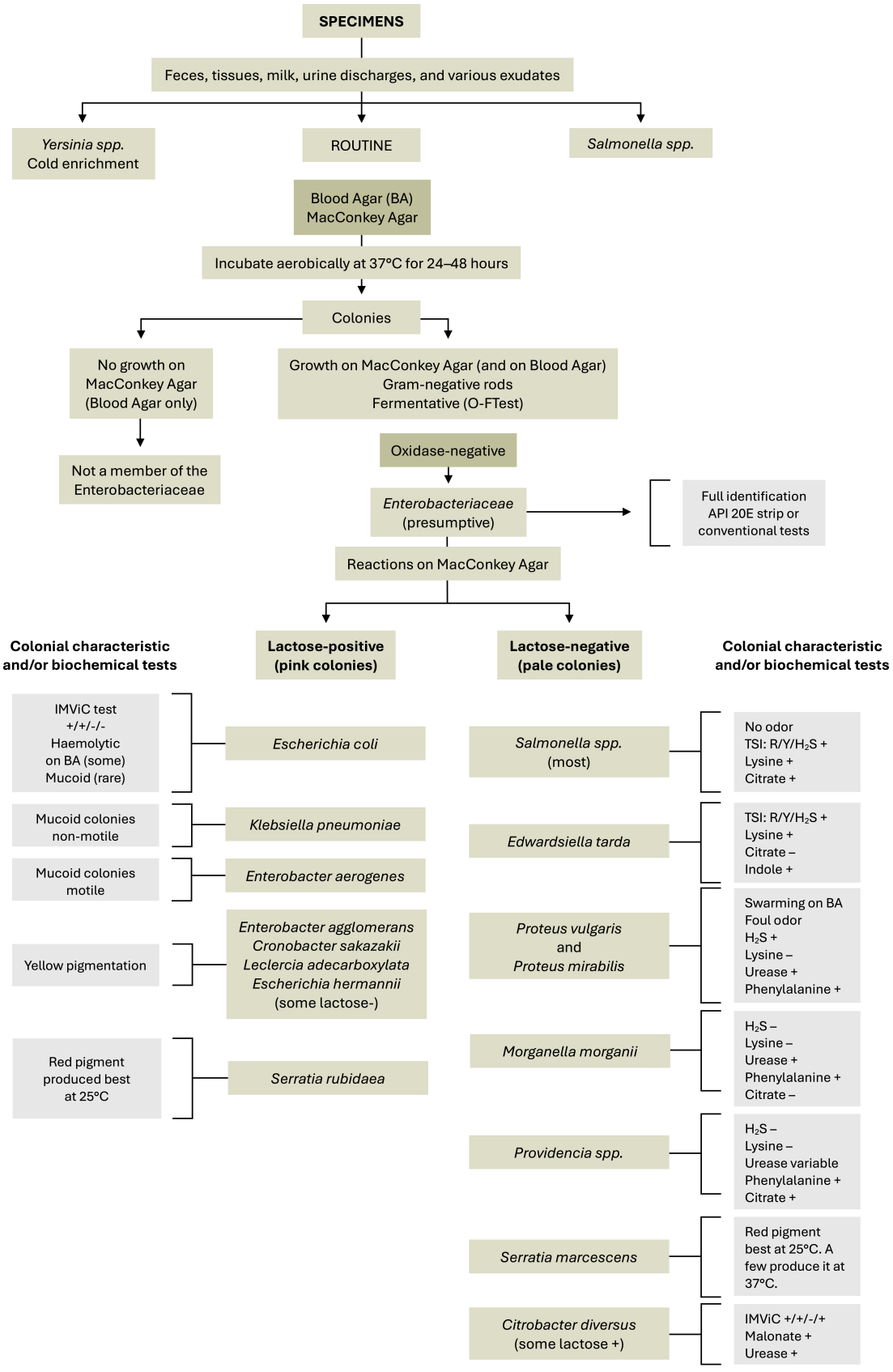


Figure A.1: Specimens. Figure description available below.

Figure Description

Figure A.1: Flow chart. Specimens. Points to feces, tissues, milk, urine discharges, and various exudates. Points to (1) *yersinia spp.* cold enrichment, (2) routine, and (3) *salmonella spp.* Blood agar (BA) / MacConkey Agar points to incubate aerobically at 37°C for 24–48 hours. Points to colonies. Points to (1) no growth on MacConkey Agar (blood agar only) / not a member of the enterobacteriaceae, and (2) growth on MacConkey Agar and on Blood Agar, gram-negative rods, fermentative (O-FTest). Oxidase negative. Points to *enterobacteriaceae* (presumptive). Points to full identification, API 20E strip or conventional tests. Also points to reactions on MacConkey Agar, which can be either lactose-positive (pink colonies) or lactose-negative (pale colonies). Colonial characteristics and/or biochemical tests of lactose positive: (1) *Esterichia coli* – IMViC test +/+/-/-, haemolytic on BA (some), mucoid (rare), (2) *Klebsiella pneumoniae* – Mucoid colonies non-motile, (3) *Enterobacter aerogenes* – Mucoid colonies motile, (4) *Enterobacter agglomerans, Cronbacter sakazakii, Leclercia adecarboxylata, Escherichia hermannii* (some lactose-) – yellow pigmentation, (5) *Serratia rubidaea* – red pigment produced best at 25°C. Colonial characteristics and/or biochemical tests of lactose negative: (1) *Salmonella spp.* (most) – no odor, TSI R/Y/H_2S +, lysine +, citrate +, (2) *Edwardsiella tarda* – TSI R/Y/H_2S +, lysine +, citrate -, indole +, (3) *Proteus vulgaris* and *proteus mirabilis* – swarming on BA, foul odor, H_2S +, lysine -, urease +, phenylalanine +, (4) *Morganella morganii* – H_2S -, lysine -, urease +, phenylalanine +, citrate -, (5) *Providencia spp.* – H_2S -, lysine -, urease variable, phenylalanine +, citrate +, (6) *Serratia marcescens* – red pigment best at 25°C, a few produce it at 37°C, (7) *Citrobacter diversus* (some lactose +) – IMViC +/+/-/+, malonate +, urease +.

Figure Reference

Figure A.1: Specimens. Under fair use and redrawn. Visit https://veteriankey.com/enterobacteriaceae to access the figure, resources, and other information.

APPENDIX B: QUICK REFERENCES—MEDICALLY IMPORTANT BACTERIA

Organism	Characteristics	Diseases	Habitat/ Pathogenesis	Pathogenesis	Laboratory Diagnosis	Treatment/ Prevention
Gram positive cocci						
Staphylococcus aureus	Gram positive cocci in clusters. **Coagulase positive.** **Catalase positive.** Most isolates produce lactamase. Some isolates have an altered penicillin binding protein (PBP) making it resistant to methicillin and nafcillin. (e.g MRSA strains).	Abscesses of many organs; skin and soft tissue infections/ impetigo, endocarditis, osteomyelitis, septic arthritis, sepsis and wound site infections. Also, hospital acquired pneumonia. Also, exotoxin-mediated diseases such as gastroenteritis (food poisoning), toxic shock syndrome, and scalded skin syndrome. Methicillin resistant *Staphylococcus aureus* (MRSA) is the most common cause of skin abscesses, pneumonia, necrotizing fasciitis, and sepsis in immunocompetent patients and users of intravenous drugs.	Main habitat is human nose; also found on human skin. Transmission is via hands.	Abscess containing pus; pyogenic lesions that enter bloodstream; Predisposing factors: suture, skin breaks, IV drug use. **Endotoxins:** **Toxic shock syndrome toxin** is a superantigen and causes toxic shock syndrome by stimulating many helper T-cells to release large amounts of lymphokines, especially interleukin (IL)2. **Enterotoxin**, which causes food poisoning, is also a superantigen. Food poisoning has a short incubation period because it is preformed in food. **Scalded skin syndrome toxin** is a protease that cleaves desmoglein in tight junctions in the skin. **Protein A** binds to the heavy chain of IgG. Reduces phagocytosis. Gamma heavy chain cannot bind to its receptor on the surface of neutrophils and macrophages.	Gram stained, smear and culture. **Blood agar:** Yellow or gold colonies **Coagulase positive:** *S. aureus* **Coagulase negative:** *S. epidermidis*	Penicillin G for sensitive isolates --> β-lactamase --> Vancomycin Vancomycin-resistant strains exist. Cefazolin is used to prevent surgical wound infections. No vaccine. Handwashing reduces transmission.

Organism	Characteristics	Diseases	Habitat/ Pathogenesis	Pathogenesis	Laboratory Diagnosis	Treatment/ Prevention
Staphylococcus epidermidis	Gram positive cocci in clusters. **Coagulase negative. Catalase positive.**	Endocarditis on prosthetic heart valves, prosthetic hip infection, intravascular catheter infection, cerebrospinal fluid shunt infection, neonatal sepsis.	Normal flora of the human skin and mucous membranes. Patient's own strains cause infection, but transmission from person to person via hands may occur.	**Glycocalyx producing** strains adhere well to foreign bodies such as prosthetic implants and catheters. Low virulence organism that causes disease in immunocompromised patients and in those with implants. **It is a major cause of hospital acquired infections.** **No exotoxins** have been identified.	Gram stained smear **Blood agar:** Whitish, nonhemolytic colonies **Coagulase negative** *S. epidermidis* is sensitive to novobiocin, whereas the other coagulase negative *Staphylococcus, Staphylococcus saprophyticus*, is resistant	Vancomycin plus either rifampin or an aminoglycoside. It produces β-lactamases and is resistant to many antibiotics. No vaccine.
Streptococcus pneumoniae (Pneumococcus)	Gram positive "lancet shaped" cocci in pairs (diplococci) or short chains. α Hemolytic colonies. **Catalase negative.** Growth is inhibited by optochin in contrast to *viridans streptococci*, which are resistant. Colonies are bile-soluble. Prominent polysaccharide capsule. One of the three classical encapsulated pyogenic bacteria (*Neisseria meningitidis* and *Haemophilus influenzae* are the other two).	Pneumonia and meningitis in adults Otitis media and sinusitis in children.	Human upper respiratory tract. Transmission is via respiratory droplets.	Induces pyogenic inflammatory response. No known exotoxins. Polysaccharide capsule retards phagocytosis. **Antipolysaccharide antibody** opsonizes the organism and provides type-specific immunity. **IgA protease** degrades secretory IgA on respiratory mucosa, allowing colonization. Viral respiratory infection predisposes to pneumococcal pneumonia by damaging mucociliary elevator; splenectomy predisposes to sepsis. Skull fracture with spinal fluid leakage from nose predisposes to meningitis.	Gram stained smear and culture. **Blood agar:** α-Hemolytic colonies. Growth **inhibited by bile and optochin**. Quellung reaction occurs (swelling of capsule with type-specific antiserum). Serologic tests for antibody not useful. Tests for capsular antigen in spinal fluid and C polysaccharide in urine can be diagnostic.	Penicillin G. Low-level and high-level resistance to penicillin is caused by alterations in penicillin binding proteins. **No β-lactamase is made.** **Two vaccines are available.** Oral penicillin is used in immunocompromised children.

Organism	Characteristics	Diseases	Habitat/ Pathogenesis	Pathogenesis	Laboratory Diagnosis	Treatment/ Prevention
Streptococcus pyogenes (Group A Streptococcus)	Gram positive cocci in chains. β-Hemolytic colonies. **Catalase negative.** **Bacitracin sensitive.** β- *Hemolytic streptococci* are subdivided into group A, B, etc., by differences in the antigenicity of their cell wall carbohydrate.	Pharyngitis and cellulitis; toxigenic diseases, for example necrotizing fasciitis ("flesheating" streptococci) and scarlet fever; immunologic (antibodymediated) diseases (e.g., rheumatic fever and acute glomerulonephritis).	Human throat and skin. Transmission is via respiratory droplets.	**Suppurative Diseases: Hyaluronidase** ("spreading factor") mediates subcutaneous spread seen in cellulitis. **Toxigenic infections: Erythrogenic toxin** and **pyrogenic exotoxin A** act as superantigens and cause scarlet fever and streptococcal toxic shock syndrome, respectively. **Exotoxin B** (a protease) causes necrotizing fasciitis. M protein that forms the pilus impedes phagocytosis. **Immunologic diseases:** Rheumatic fever is caused by immunologic cross-reaction between bacterial antigen and human heart and joint tissue. M protein reacts with myosin in cardiac muscle, and acute glomerulonephritis is caused by immune complexes formed between streptococcal antigens and antibodies to those antigens. The immune complexes are trapped by glomeruli, complement is activated, neutrophils are attracted to the site by C5a, and proteases produced by neutrophils damage glomeruli.	**Suppurative infections:** Gram stained smear and culture. **Blood agar:** β-Hemolytic colonies (Hemolysis due to streptolysins O and S.) If isolate is sensitive to bacitracin, it is identified as *S. pyogenes*. **ELISA** tests for group A streptococcal antigens in throat swabs. **Immunological disease:** If rheumatic fever is suspected, patient's antistreptolysin O (ASO) antibody titer is tested to determine whether previous exposure to *S. pyogenes* has occurred. If acute glomerulonephritis is suspected, antibody to streptococcal **DNase B** is used as evidence of a previous skin infection by *S. pyogenes*.	Penicillin G (no significant resistance). Oral penicillin V or amoxicillin is often used. Prevention—Penicillin is used in patients with rheumatic fever to prevent recurrent *S. pyogenes* pharyngitis. This prevents additional damage to heart valves. No vaccine.

Organism	Characteristics	Diseases	Habitat/ Pathogenesis	Pathogenesis	Laboratory Diagnosis	Treatment/ Prevention
Streptococcus agalactiae (Group B Streptococcus)	Gram positive cocci in chains. β-Hemolytic colonies subdivided into group A, B, etc., by differences in the antigenicity of their cell wall carbohydrate. **Catalase negative.** **Bacitracin resistant.**	Neonatal meningitis and sepsis.	Human vagina. Transmission occurs during birth.	Pyogenic organism. **No exotoxins** identified. Predisposing factors to neonatal infection include rupture of membranes more than 18 hours before delivery, labor prior to 37 weeks (infant is premature), absence of maternal antibody, and heavy colonization of the genital tract by the organism.	Gram stained smear and culture. **Blood agar:** β-Hemolytic colonies that are resistant to bacitracin. Organisms **hydrolyze hippurate** and are CAMP test positive.	Penicillin G. No vaccine. Penicillin or ampicillin should be given to mothers if prolonged rupture of membranes occurs, if mother has a fever, or if the neonate is premature.
Enterococcus faecalis	Gram positive cocci in chains. **Catalase negative.**	Urinary tract and biliary tract infections are most frequent. Endocarditis is rare but life threatening.	Human colon; urethra and female genital tract can be colonized. May enter bloodstream during gastrointestinal (GI) or genitourinary tract procedures. May infect other sites (e.g., endocarditis).	Pyogenic organism. **No exotoxins** identified.	Gram stained smear and culture. αor **Blood agar:** β-Hemolytic or nonhemolytic colonies Grows in 6.5% NaCl and hydrolyzes esculin in the presence of 40% bile. Serologic tests not useful.	Penicillin or vancomycin plus an aminoglycoside. Organism is resistant to either drug given individually, but given together, they have a synergistic effect. Aminoglycoside alone is ineffective because it cannot penetrate. Penicillin or vancomycin weakens the cell wall, allowing the aminoglycoside to penetrate. Vancomycin resistant enterococci (VRE) are important causes of nosocomial infections. Linezolid can be used to treat VRE. Penicillin and gentamicin should be given to patients with damaged heart valves prior to intestinal or urinary tract procedures. No vaccine is available.

Organism	Characteristics	Diseases	Habitat/ Pathogenesis	Pathogenesis	Laboratory Diagnosis	Treatment/ Prevention
Viridans Group Streptococci (e.g., *Streptococcus sanguis*, *Streptococcus mutans*)	Gram-positive cocci in chains. αHemolytic colonies. **Catalase negative.** Growth is resistant to optochin in contrast to pneumococci, which are inhibited. Colonies are not dissolved by bile.	Endocarditis is the most important disease. Also brain abscess, especially in mixed infections with mouth anaerobes. *S. mutans* implicated in dental caries.	Habitat is the human oropharynx. Organism enters bloodstream during dental procedures.	Low virulence organism. Bacteremia from dental procedures spreads organism to damaged heart valves. Organism is protected from host defenses within vegetations. No known toxins. Glycocalyx composed of polysaccharide enhances adhesion to heart valves.	Gram stained smear and culture. **Blood agar:** α-Hemolytic colonies. Growth **not inhibited by bile or optochin**, in contrast to pneumococci. Serologic tests not useful.	Penicillin G with or without an aminoglycoside. Penicillin to prevent endocarditis in patients with damaged or prosthetic heart valves who undergo dental procedures.

Organism	Characteristics	Diseases	Habitat/ Pathogenesis	Pathogenesis	Laboratory Diagnosis	Treatment/Prevention
Gram negative cocci						
Neisseria meningitidis (Meningococcus)	Gram negative "kidney bean" diplococci. **Oxidase positive.** Large polysaccharide capsule. One of the three classic encapsulated pyogenic bacteria (*S. pneumoniae* and *H. influenzae* are the other two).	Meningitis and meningococcemia.	Human upper respiratory tract; transmission is via respiratory droplets.	After colonizing the upper respiratory tract, the organism reaches the meninges via the bloodstream. Endotoxin in cell wall causes symptoms of septic shock seen in meningococcemia. **No known exotoxins**; IgA protease produced. Polysaccharide capsule is antiphagocytic and is the main virulence factor of this aggressive pathogen. Deficiency in late complement components predisposes to recurrent meningococcal infections.	Gram stained smear and culture. **Chocolate agar:** Oxidase positive colonies. **Ferments maltose** in contrast to gonococci, which do not.	Penicillin G (no significant resistance). The vaccines against groups A, C, Y, and W135 meningococci contain the polysaccharide capsule as the immunogen. The vaccine against group B meningococci contains factor H-binding protein as the immunogen. The polysaccharide vaccine exists in two forms: the conjugate vaccine contains the polysaccharides coupled to a carrier protein such as diphtheria toxoid, and the non-conjugate vaccine contains only the polysaccharides. Rifampin or ciprofloxacin is given to close contacts to decrease oropharyngeal carriage.
Neisseria gonorrhoeae (Gonococcus)	Gram negative "kidney bean" diplococci. **Oxidase positive.**	Gonorrhea. Neonatal conjunctivitis and pelvic inflammatory disease.	Human genital tract. Transmission in adults is by sexual contact. Transmission to neonates is during birth.	Organism invades mucous membranes and causes inflammation. Endotoxin present but weaker than that of meningococcus, so less severe disease when bacteremia occurs. No exotoxins identified. IgA protease and pili are virulence factors.	Gram stained smear and culture. Organism visible intracellularly within neutrophils in urethral exudate. **Thayer-Martin medium:** Oxidase positive colonies Gonococci do not ferment maltose, whereas meningococci do. Nucleic acid amplification tests (NAATs) are used as a screening test in urogenital infections.	Ceftriaxone for uncomplicated cases. Doxycycline or azithromycin for urethritis caused by coinfection with *Chlamydia trachomatis*. High/low-level resistance to penicillin is caused by penicillinase or reduced permeability and altered binding proteins. No vaccine. Condoms offer protection. Treat eyes of newborns with erythromycin ointment or silver nitrate to prevent conjunctivitis.

Organism	Characteristics	Diseases	Habitat/ Pathogenesis	Pathogenesis	Laboratory Diagnosis	Treatment/ Prevention
Gram positive rods						
Bacillus anthracis	Aerobic, gram positive, **Spore forming rods.** Capsule composed of poly-D-glutamate. ***B. anthracis* is the only medically important organism that has a capsule composed of amino acids rather than polysaccharides.**	Anthrax.	Soil. Transmission is by contact with infected animals or inhalation of spores from animal hair and wool.	Anthrax toxin consists of three proteins: **edema factor**, which is an adenylate cyclase; **lethal factor**, which kills cells by inhibiting a signal transduction protein involved in cell division; **protective antigen**, which mediates the entry of the other two components into the cell. The capsule is antiphagocytic.	Gram stained **Blood agar:** aerobic culture *B. anthracis* is **non-motile**, in contrast to other Bacillus species. Rise in antibody titer in indirect hemagglutination test is diagnostic.	Penicillin G (no significant resistance). Vaccine consisting of protective antigen is given to individuals in high risk occupations.
Bacillus cereus	Aerobic, gram positive, **Spore forming rod.**	Gastroenteritis (food poisoning).	Grains, such as rice. Spores survive boiling during preparation of rice, then germinate when rice is held at warm temperature.	**Two enterotoxins** are produced: 1) one acts like cholera toxin (i.e., cyclic adenosine monophosphate [AMP] is increased within enterocytes); 2) the other acts like staphylococcal enterotoxin (i.e., it is a superantigen).	None.	Symptomatic only. No vaccine.
Clostridium tetani	Anaerobic, gram positive **Spore forming rods.** Spore is at one end ("terminal spore"), organism looks like a tennis racket.	Tetanus.	Soil. Organism enters through breaks in the skin.	Spores germinate under anaerobic conditions in the wound. Organism produces exotoxin, which blocks release of inhibitory neurotransmitters (glycine and γaminobutyric acid [GABA]) from spinal neurons. Excitatory neurons are unopposed, and extreme muscle spasm (tetanus, spastic paralysis) results. Tetanus toxin (tetanospasmin) is a protease that cleaves proteins involved in the release of the inhibitory neurotransmitters.	Primarily a clinical diagnosis. Rarely isolated.	Hyperimmune human globulin to neutralize toxin. Also penicillin G and diazepam. No significant resistance to penicillin. Toxoid vaccine, usually given to children in combination with diphtheria toxoid and acellular pertussis vaccine (DTaP). Give tetanus toxoid booster every 10 years.

Organism	Characteristics	Diseases	Habitat/ Pathogenesis	Pathogenesis	Laboratory Diagnosis	Treatment/ Prevention
Clostridium botulinum	Anaerobic, gram positive	Botulism.	Soil. Organism and botulinum toxin transmitted in improperly preserved food.	Botulinum toxin is a protease that cleaves proteins involved in the release of acetylcholine at the myoneural junction, causing flaccid paralysis. Failure to sterilize food during preservation allows spores to survive. Spores germinate in anaerobic environment and produce toxin. The toxin is heat labile; foods eaten without proper cooking are usually implicated.	Detection of toxin involves either antitoxin in serologic tests or production of the disease in mice.	Antitoxin to types A, B, and E made in horses. Respiratory support may be required.
Clostridium perfringens	Anaerobic, gram positive **Spore forming rods.**	Gas gangrene (necrotizing fasciitis, myonecrosis) and food poisoning.	Soil and human colon. Myonecrosis results from contamination of wound with soil or feces. Food poisoning --> ingestion of contaminated food.	Gas gangrene in wounds is caused by germination of spores under anaerobic conditions and the production of cytotoxic factors: **alpha toxin**, a lecithinase that cleaves cell membranes. Gas in tissue (CO2 and H2) is produced by organism's anaerobic metabolism. Food poisoning is caused by production of **enterotoxin**; acts as a superantigen, similar to that of *S. aureus*.	Gram stained smear plus anaerobic culture. Production of **lecithinase** is detected on egg yolk agar and identified by enzyme inhibition with specific antiserum.	Penicillin G plus debridement of the wound in gas gangrene/and to prevent. Only symptomatic treatment needed in food poisoning. No vaccine.
Clostridium difficile	Anaerobic, gram positive, **Spore forming rods.**	Pseudomembranous colitis.	Human colon. Transmission is fecal–oral.	Antibiotics suppress normal flora of colon, allowing *C. difficile* to overgrow and produce large amounts of exotoxins. **Exotoxins A and B inhibit GTPases**, causing inhibition of signal transduction and depolymerization of actin filaments. --> Apoptosis and death of enterocytes.	Exotoxin in the stool is typically detected by ELISA test or PCR assay. Exotoxin in stool can also be detected by cytopathic effect on cultured cells. A screening test that detects the glutamine dehydrogenase of the organism in stool is available.	Oral vancomycin or fidaxomicin can be used. If life threatening, use vancomycin plus metronidazole. No vaccine.

Organism	Characteristics	Diseases	Habitat/ Pathogenesis	Pathogenesis	Laboratory Diagnosis	Treatment/ Prevention
Corynebacterium diphtheriae	Club-shaped gram positive **Rods arranged in V or L shape.** Granules stain metachromatically. Aerobic, **non–spore forming**	Diphtheria.	Human throat. Transmission is via respiratory droplets.	Organism secretes an **exotoxin** that inhibits protein synthesis by adding ADPribose to elongation factor2 (EF2). Toxin has two components: **subunit A** --> ADPribosylating activity **subunit B** --> binds the toxin to cell surface receptors. Pseudomembrane in throat caused by death of mucosal epithelial cells.	Gram stained smear and culture. **Tellurite plate:** Black colonies Document toxin production with precipitin test or by disease produced in laboratory animals.	Antitoxin made in horses neutralizes the toxin. Penicillin G kills the organism. Toxoid vaccine (toxoid is formaldehyde treated toxin), usually given to children in combination with tetanus toxoid and acellular pertussis vaccine (DTaP).
Listeria monocytogenes	Small gram positive rods. Aerobic, **non–spore forming**	Meningitis and sepsis in newborns and immunocompromised adults. Gastroenteritis.	Organism colonizes the GI and female genital tracts; in nature, it is widespread in animals, plants, and soil. Transmission is across the placenta or by contact during delivery. Outbreaks of sepsis in neonates and gastroenteritis in the general population are related to ingestion of unpasteurized milk products.	**Listeriolysin** is an exotoxin that degrades cell membranes. Reduced cell mediated immunity and immunologic immaturity as in neonates predispose to disease. Intracellular pathogen that moves from cell to cell via "actin rockets."	Gram stained smear and culture. **Blood agar:** β-Hemolytic colonies; small Tumbling motility.	Ampicillin with or without gentamicin. Pregnant women and immunocompromised patients should not ingest unpasteurized milk products or raw vegetables. Trimethoprim/sulfamethoxazole given to immunocompromised patients to prevent Pneumocystis pneumonia can also prevent listeriosis. No vaccine

Organism	Characteristics	Diseases	Habitat/ Pathogenesis	Pathogenesis	Laboratory Diagnosis	Treatment/ Prevention
Gram negative rods related to the enteric tract						
Escherichia coli	Facultative gram negative rods Ferments lactose.	Urinary tract infection (UTI), sepsis, neonatal meningitis, and "traveler's diarrhea" Hemolytic-uremic syndrome.	Human colon, vagina and urethra. Acquired during birth in neonatal meningitis and by the fecal–oral route in diarrhea.	**Endotoxin** in cell wall causes septic shock. **Heat labile toxin (LT)** stimulates adenylate cyclase by ADPribosylation --> Increased cyclic AMP causes outflow of chloride ions and water, resulting in diarrhea. **Heat stable toxin (ST)** causes diarrhea, perhaps by stimulating guanylate cyclase. Virulence factors include pili for attachment to mucosal surfaces and a capsule that impedes phagocytosis. **Shiga toxin (verotoxin)** is an enterotoxin produced by E. coli strains O157:H7 serotype. It inhibits protein synthesis by removing adenine from the 28S rRNA of human ribosomes. Predisposing factors to UTI in women include the proximity of the anus to the vagina and urethra. Abnormalities (e.g., strictures, valves, and stones) Indwelling urinary catheters and intravenous lines predispose to UTI and sepsis, respectively. Colonization of the vagina leads to neonatal meningitis The main virulence factor for neonatal meningitis is the K1 capsular polysaccharide.	Gram stained smear and culture. **Eosin–methylene blue (EMB) or MacConkey's agar:** Lactose fermenting colonies Green sheen on EMB agar. **Triple sugar iron (TSI) agar:** Acid slant and acid butt with gas but no H2S. Differentiate from other lactose positive organisms by biochemical reactions. For epidemiologic studies, type organism by O and H antigens by using known antisera.	Ampicillin or sulfonamides for UTIs. Third generation cephalosporins for meningitis and sepsis. Rehydration is effective in traveler's diarrhea; trimethoprimsulfamethoxazole may shorten duration of symptoms. Antibiotic resistance mediated by plasmid encoded enzymes (e.g., β-lactamase and aminoglycoside modifying enzymes). Prevention of UTI involves limiting the frequency and duration of urinary catheterization. Prevention of sepsis involves promptly removing or switching sites of intravenous lines. Traveler's diarrhea is prevented by eating only cooked food and drinking boiled water in certain countries. Prophylactic doxycycline or bismuth subsalicylate may prevent traveler's diarrhea.

Organism	Characteristics	Diseases	Habitat/ Pathogenesis	Pathogenesis	Laboratory Diagnosis	Treatment/ Prevention
Salmonella typhi	Facultative gram negative rods. **Non–lactose fermenting.** Produces H2S.	Typhoid fever.	Human colon only. Transmission is by the fecal–oral route.	Infects the cells of the reticuloendothelial system, especially in the liver and spleen. **Endotoxin** in cell wall causes fever. **Capsule (Vi antigen)** is a virulence factor. **No exotoxins known.** Decreased stomach acid resulting from ingestion of antacids or gastrectomy predisposes to Salmonella infections. Chronic carrier state established in gallbladder. Organism excreted in bile results in fecal–oral spread to others.	Gram stained smear and culture. **EMB or MacConkey's agar:** Non–lactose fermenting **TSI agar:** Alkaline slant and acid butt, with no gas and a small amount of H2S. Biochemical and serologic reactions used to identify species. Identity can be determined by using known antisera against O, H, and Vi antigens in **agglutination test**. **Widal test** detects agglutinating antibodies to O and H antigens in patient's serum	Most effective drug is ceftriaxone. Ampicillin and trimethoprim/ sulfamethoxazole can be used in patients who are not severely ill. Resistance to chloramphenicol and ampicillin is mediated by plasmid encoded acetylating enzymes and βlactamase, respectively. Public health measures. Two vaccines are in common use; one vaccine contains purified Vi polysaccharide capsule as the immunogen and the other contains live, attenuated S. typhi as the immunogen.
Shigella species (e.g., *Shigella dysenteriae, Shigella sonnei*)	Facultative gram negative rods. **Non–lactose fermenting.** **Non-motile**, in contrast to Salmonella.	Enterocolitis (dysentery)	Human colon only; no animal carriers for Shigella. Transmission is by the fecal–oral route.	Invades the mucosa of the ileum and colon but does not penetrate farther; therefore, sepsis is rare. **Endotoxin** in cell wall. Infectious dose is much lower (1–10 organisms) than that of Salmonella because it is resistant to stomach acid. Children in psychiatric hospitals and day care centers experience outbreaks of shigellosis. No chronic carrier state.	Gram stained smear and culture. **EMB or MacConkey's agar:** Non–lactose fermenting **TSI agar:** Alkaline slant with an acid butt and no gas or H2S. Identified by biochemical reactions or by serology with antiO antibody in **agglutination test**.	Fluid and electrolyte replacement only. In severe cases, ciprofloxacin. Resistance is mediated by plasmid encoded enzymes (e.g., βlactamase, which degrades ampicillin, and a mutant pteroate synthetase, which reduces sensitivity to sulfonamides). Public health measures.

Organism	Characteristics	Diseases	Habitat/ Pathogenesis	Pathogenesis	Laboratory Diagnosis	Treatment/ Prevention
Salmonella enterica (often called Salmonella enteritidis)	Facultative gram negative rods. **Non–lactose fermenting.** Produces H2S **Motile**, in contrast to Shigella.	Enterocolitis. Sepsis with metastatic abscesses occasionally.	Enteric tract of humans and animals (e.g., poultry and domestic livestock). Fecal–oral route - transmission	Invades the mucosa of the small and large intestines. Can enter blood, causing sepsis. Infectious dose is at least 100,000 organism is inactivated by stomach acid. **Endotoxin in cell wall; no exotoxin.** Predisposing factors include lowered stomach acidity from either antacids or gastrectomy. Sickle cell anemia predisposes to Salmonella osteomyelitis.	Gram stained smear and culture. **EMB or MacConkey's agar:** Non–lactose fermenting **TSI agar:** Alkaline slant and acid butt, with gas and H2S. Biochemical and serologic reactions used to identify species. Can identify the organism by using known antisera in **agglutination assay**. **Widal test** detects antibodies in patient's serum to the O and H antigens of the organism but is not widely used.	Antibiotics usually not recommended for uncomplicated enterocolitis. Ceftriaxone or other drugs are used for sepsis, depending on organisms, much greater than the infectious dose of Shigella. Infectious dose is high because sensitivity tests. Resistance to ampicillin and chloramphenicol is mediated by plasmid encoded β-lactamases and acetylating enzymes, respectively. Public health measures. Do not eat raw eggs or meat. No vaccine is available.
Vibrio cholerae	Comma shaped gram negative rods **Oxidase-positive** (distinguishes them from Enterobacteriaceae)	Cholera	Human colon and shellfish. Transmission is by the fecal–oral route.	Massive, watery diarrhea caused by enterotoxin that activates adenylate cyclase by adding ADPribose to the stimulatory G protein. Increase in cyclic AMP activates cyclic AMP dependent kinase that phosphorylates a membrane ion channel. This causes an outflow of chloride ions and water. Toxin has two components: **subunit A**, which has the ADPribosylating activity; **subunit B**, which binds the toxin to cell surface receptors. Organism produces **mucinase**, which enhances attachment to the intestinal mucosa. Role of endotoxin is unclear.	Gram stained smear and culture. **Agglutination** of the isolate with known antisera confirms the identification.	Fluid and electrolyte replacement. Tetracycline is not necessary but shortens duration. Public health measures. For travelers to endemic areas, oral vaccine containing live, attenuated bacteria is available in United States. Tetracycline used for close contacts.

Organism	Characteristics	Diseases	Habitat/ Pathogenesis	Pathogenesis	Laboratory Diagnosis	Treatment/ Prevention
Campylobacter jejuni	Comma shaped gram negative rods **Microaerophilic** Grows well at 42°C	Enterocolitis	Human and animal feces. Transmission is by the fecal–oral route.	Invades mucosa of the colon but does not penetrate. No enterotoxin known.	Gram stained smear **Culture on special agar** (e.g., Skirrow's agar) at 42°C in high CO2, low O2	Usually symptomatic treatment only; erythromycin for severe disease. Public health measures.
Helicobacter pylori	Curved gram negative rod	Gastritis and peptic ulcer. Risk factor for gastric carcinoma.	Human stomach. Transmission is by ingestion.	Organisms synthesize **urease**, which produces ammonia that damages gastric mucosa. Ammonia also neutralizes acid pH in stomach, which allows the organism to live in gastric mucosa.	Gram stain and culture. **Urease positive.** Serologic tests for antibody and the "urea breath" test are useful.	One regimen is amoxicillin, clarithromycin, metronidazole, and a proton pump inhibitor such as omeprazole. No vaccine.
Klebsiella pneumoniae	Facultative gram negative rods Large polysaccharide capsule	Pneumonia, UTI, and sepsis	Human upper respiratory and enteric tracts. Organism is transmitted to the lungs by aspiration from upper respiratory tract and by inhalation of respiratory droplets. It is transmitted to the urinary tract by ascending spread of fecal flora.	**Endotoxin** causes fever and shock associated with sepsis. **No exotoxin known.** Organism has large capsule, which impedes phagocytosis. Chronic pulmonary disease predisposes to pneumonia; catheterization predisposes to UTI.	Gram stained smear and culture. **MacConkey's agar:** Lactose fermenting Characteristic mucoid colonies due to abundant polysaccharide capsule.	Cephalosporins alone or with aminoglycosides, but antibiotic sensitivity testing must be done. Resistance is mediated by plasmid encoded enzymes, especially β-lactamase. No vaccine.
Enterobacter cloacae	Enteric gram negative rod Similar to *K. pneumoniae*.	Causes hospital-acquired pneumonia, UTI, and sepsis.				Highly antibiotic resistant.
Serratia marcescens	Enteric gram negative rod Similar to *K. pneumoniae*.	Causes hospital-acquired pneumonia, UTI, and sepsis.			Red pigmented colonies.	Highly antibiotic resistant.

Organism	Characteristics	Diseases	Habitat/ Pathogenesis	Pathogenesis	Laboratory Diagnosis	Treatment/ Prevention
Proteus species (e.g., *Proteus vulgaris, Proteus mirabilis*)	Facultative gram negative rods. **Non–lactose fermenting.** **Highly motile.** Produce urease. Antigens of OX strains of P. vulgaris cross-react with many rickettsiae.	UTI and sepsis.	Human colon and the environment (soil and water). Transmission to urinary tract is by ascending spread of fecal flora.	**Endotoxin** causes fever and shock associated with sepsis. **No exotoxins known.** **Urease** is a virulence factor because it degrades urea to produce ammonia, which raises the pH. This leads to "struvite" stones, which can obstruct urine flow, damage urinary epithelium, and serve as a nidus for recurrent infection by trapping bacteria within the stone. Predisposing factors: colonization of the vagina, urinary catheters, and abnormalities of the urinary tract such as strictures, valves, and stones.	Gram stained smear and culture. **Blood Agar:** "Swarming" (spreading) as a consequence of the high motility. **EMB or MacConkey's agar:** Non–lactose fermenting **TSI agar:** Alkaline slant and acid butt with H2S. Organism produces urease, whereas Salmonella, which can appear similar on TSI agar, does not. Note: *P. mirabilis* is **indole negative**, whereas *P. vulgaris*, *M. morganii*, and *Providencia* species are **indole positive**.	Trimethoprim/ sulfamethoxazole nitrofurantoin, ciprofloxacin for uncomplicated UTIs Third generation cephalosporin used for serious infections. *P. mirabilis* is more likely to be sensitive to antibiotics such as ampicillin Antibiotic sensitivities should be tested. Resistance is mediated by plasmid encoded enzymes. No vaccine. Prompt removal of urinary catheters helps prevent UTIs.
Pseudomonas aeruginosa	Aerobic gram negative rods. **Non–lactose fermenting** **Pyocyanin** (blue-green) pigment produced **Oxidase positive**	Wound infection, UTI, pneumonia, and sepsis. Nosocomial infections, in burn patients and those with cystic fibrosis. Causes endocarditis in intravenous drug users.	Water sources (e.g., in hospital respirators and humidifiers). Also inhabits the skin, upper respiratory tract, and colon of about 10% of people. Transmission is via water aerosols, aspiration, and fecal contamination.	**Endotoxin** is responsible for fever and shock associated with sepsis. **Exotoxin A**, which acts like diphtheria toxin (inactivates EF2). **Pili and capsule** are virulence factors that mediate attachment and inhibit phagocytosis, respectively. **Glycocalyx** producing strains predominate in chronic infections in cystic fibrosis patients. Severe burns and neutropenia are important predisposing factors.	Gram stained smear and culture. **EMB or MacConkey's agar:** Non–lactose fermenting **TSI agar:** Alkaline slant and alkaline butt because the sugars are not fermented. **Oxidase positive.**	Antibiotics chosen on the basis of antibiotic sensitivities; resistance is common. Antipseudomonal penicillin and aminoglycoside are often used. Resistance is mediated by a variety of plasmid encoded enzymes (e.g., βlactamases and acetylating enzymes). Disinfection of water related equipment in the hospital, handwashing, and prompt removal of urinary and intravenous catheters. No vaccine.

Organism	Characteristics	Diseases	Habitat/ Pathogenesis	Pathogenesis	Laboratory Diagnosis	Treatment/ Prevention
Gram negative rods related to the respiratory tract						
Haemophilus influenzae	Small gram negative (coccobacillary) rods. Requires factors X (hemin) and V (NAD) for growth. Of the six capsular polysaccharide types, type b causes 95% of invasive disease. Type b capsule is polyribitol phosphate.	Sinusitis, otitis media, and pneumonia are common. Epiglottitis is uncommon, but *H. influenzae* is the most important cause. *H. influenzae* used to be a leading cause of meningitis, but the vaccine has greatly reduced the number of cases.	Upper respiratory tract. Transmission is via respiratory droplets.	**Polysaccharide capsule** is the most important determinant of virulence. **Unencapsulated** ("untypeable") strains cause mucosal infections but not invasive infections. **IgA protease** is produced. Immune response of the child to capsular polysaccharides can be inadequate. **No exotoxins** identified.	Gram stained smear **Chocolate agar:** Growth requires both factors X and V. Determine serotype by using antiserum in various tests (e.g., latex agglutination). Capsular antigen can be detected in serum or cerebrospinal fluid.	Ceftriaxone for meningitis.Approximately 25% of strains produce β-lactamase. Vaccine containing the type b capsular polysaccharide conjugated to diphtheria toxoid or other protein is given between 2 and 18 months of age. Rifampin can prevent meningitis in close contacts.
Legionella pneumophila	Gram negative rods, but stain poorly with standard Gram stain. Require increased iron and cysteine for growth in culture. Sixteen serogroups; most cases caused by serogroup 1.	Legionnaires' disease ("atypical" pneumonia).	Environmental water sources. Transmission is via aerosol from the water source. Person-to-person transmission does not occur.	Aside from endotoxin, no toxins, enzymes, or virulence factors are known. Predisposing factors include being older than 55 years, smoking, and having a high alcohol intake. Immunosuppressed patients are highly susceptible. The organism replicates intracellularly; therefore, cell mediated immunity is an important host defense. Smoking damages alveolar macrophages, it predisposes to pneumonia.	Microscopy with silver impregnation stain or fluorescent antibody. Culture on **charcoal yeast extract agar** containing increased amounts of iron and cysteine. Urinary antigen provides rapid diagnosis for serogroup 1 bacteria only. Diagnosis can be made serologically by detecting rise in antibody titer in patient's serum.	Azithromycin or erythromycin. Rifampin can be added in severe cases. No vaccine or prophylactic.

Organism	Characteristics	Diseases	Habitat/ Pathogenesis	Pathogenesis	Laboratory Diagnosis	Treatment/ Prevention
Bordetella pertussis	Small gram negative rods.	Whooping cough (pertussis).	Human respiratory tract. Transmission is via respiratory droplets.	Pertussis toxin stimulates adenylate cyclase by adding ADPribose onto the inhibitory G protein. Toxin has two components: **subunit A**, which has the ADPribosylating activity **subunit B**, which binds the toxin to cell surface receptors. **Pertussis toxin** causes lymphocytosis in the blood by inhibiting chemokine receptors. Inhibition of these receptors prevents lymphocytes from entering tissue, resulting in large numbers being retained in the blood. Inhibition of chemokine receptors occurs because pertussis toxin ADPribosylates the inhibitory G protein, which prevents signal transduction within the cell. In addition, extracellular adenylate cyclase is produced, which can inhibit killing by phagocytes. Tracheal cytotoxin damages ciliated epithelium of respiratory tract.	Gram stained smear plus culture on **BordetGengou agar**. Identified by biochemical reactions and slide agglutination with known antisera. PCR tests, if available, are both sensitive and specific.	Azithromycin. The acellular vaccine containing pertussis toxoid and four other purified proteins is recommended rather than the killed vaccine, which contains whole organisms. Usually given to children in combination with diphtheria and tetanus toxoids (DTaP). Azithromycin is useful in unimmunized people who are known to be exposed.
Pasteurella multocida	Small gram negative rods.	Wound infection (e.g., cellulitis).	Reservoir is the mouth of many animals, especially cats and dogs. Transmission is by animal bites.	Spreads rapidly in skin and subcutaneous tissue. **No exotoxins.**	Gram stained smear and culture.	Penicillin G. amoxicillin-clavulanate should be given to individuals with cat bites. No vaccine.
Bartonella henselae	Small gram negative rod.	Cat Scratch disease (CSD) and bacillary angiomatosis (BA).	Reservoir is the cat's mouth and transmitted by scratch or bite.	Low virulence organism. CSD is self limited in immunocompetent individuals, but BA occurs in immunocompromised individuals.	Diagnosis of CSD made by serologic tests. Biopsy of BA lesion shows pleomorphic rods using Warthin-Starry stain.	None for CSD. Doxycycline or erythromycin for BA. No vaccine.

Organism	Characteristics	Diseases	Habitat/ Pathogenesis	Pathogenesis	Laboratory Diagnosis	Treatment/Prevention
Mycobacteria						
Mycobacterium tuberculosis	Aerobic, acid fast rods. High lipid content of cell wall, which prevents dyes used in Gram stain from staining organism. Lipids include mycolic acids and wax D. Grows very slowly, which requires that drugs be present for long periods (months). **Catalase positive**, which is required to activate isoniazid to the active drug.	Tuberculosis.	Human lungs. Transmission is via respiratory droplets produced by coughing.	Granulomas and caseation mediated by cellular immunity (i.e., macrophages and CD4 positive T-cells [delayed hypersensitivity]). **Cord factor** (trehalose mycolate) correlates with virulence. **No exotoxins or endotoxin.** Suppression of cell mediated immunity increases risk of reactivation and dissemination.	Acid fast rods seen with **Ziehl-Neelsen (or Kinyoun) stain.** Slow growing (3–6 weeks) colony on Löwenstein- Jensen medium. Organisms produce **niacin** and are **catalase positive**. **Skin Test—**Purified protein derivative (PPD) skin test is positive if induration measuring 10 mm or more appears 48 hours after inoculation. Induration is caused by a delayed hypersensitivity response. Positive skin test indicates that the person has been infected but not necessarily that the person has the disease tuberculosis.	**Long-term therapy** (6–9 months) with three drugs: isoniazid, rifampin, and pyrazinamide. A fourth drug, ethambutol, is used in severe cases, in immunocompromised patients (e.g., those with acquired immunodeficiency syndrome [AIDS]), and where the chance of isoniazid resistant organisms is high, as in Southeast Asians. Most patients become noninfectious within 2 weeks of adequate therapy. **Treatment of latent (asymptomatic)** infections consists of isoniazid taken for 6 to 9 months or isoniazid plus rifampin for 3 months. Multidrug-resistant (MDR) strains have emerged and require other drug combinations. Vaccine used rarely in the United States but widely used in parts of Europe and Asia.

Organism	Characteristics	Diseases	Habitat/Pathogenesis	Pathogenesis	Laboratory Diagnosis	Treatment/ Prevention
Actinomycetes						
Actinomyces israelii	Anaerobic, gram positive filamentous, branching rods	Actinomycosis (abscesses with draining sinus tracts)	Human mouth, especially anaerobic crevices around the teeth. Transmission into tissues occurs during dental disease or trauma. Organism also aspirated into lungs, causing thoracic actinomycosis. Retained intrauterine device (IUD) predisposes to pelvic actinomycosis.	**No toxins or virulence factors known.** Organism forms sinus tracts that open onto skin and contain "sulfur granules," mats of intertwined filaments of bacteria.	Gram stained smear **Blood agar plate:** anaerobic "Sulfur granules" visible in the pus.	Penicillin G and surgical drainage. No vaccine available.
Nocardia asteroides	Aerobic, gram positive filamentous, branching rods. Weakly acidfast.	Nocardiosis (especially lung and brain abscesses).	Soil. Transmission is via airborne particles	**No toxins or virulence factors known.** Immunosuppression and cancer predispose to infection.	Gram stained smear and modified ZiehlNeelsen stain. **Blood agar plate:** anaerobic No serologic tests.	Sulfonamides. No vaccine available.
Mycoplasmas						
Mycoplasma pneumoniae	Smallest free living organisms. Not seen on Gram stained smear--> no cell wall, so dyes are not retained. Penicillins and cephalosporins are not effective because there is no cell wall (peptidoglycan). **The only bacteria with cholesterol in cell membrane.**	"Atypical" pneumonia.	Human respiratory tract. Transmission is via respiratory droplets.	No exotoxins	Gram stain not useful. Can be cultured on special bacteriologic media but takes at least 10 days to grow. **Positive cold–agglutinin test** is presumptive evidence. Complement fixation test for antibodies to Mycoplasma pneumoniae is more specific.	Azithromycin or doxycycline. No vaccine available.

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Spirochetes						
Treponema pallidum	Spirochetes. Not seen on Gram stained smear because organism is too thin. Not cultured in vitro.	Syphilis.	Human genital tract. Transmission is by sexual contact and from mother to fetus across the placenta.	Organism multiplies at site of inoculation and then spreads widely via the bloodstream. Many features of syphilis are attributed to blood vessel involvement causing vasculitis. Primary and secondary lesions heal spontaneously. Tertiary lesions consist of gummas (granulomas in bone, muscle, and skin), aortitis, or central nervous system inflammation. **No toxins or virulence factors known.**	Seen by darkfield microscopy or immunofluorescence. Serologic tests important: VDRL and RPR are nontreponemal (nonspecific) tests used for screening; FTAABS is the most widely used specific test for *Treponema pallidum*. Antigen in VDRL and RPR is beef heart cardiolipin; antigen in FTAABS is killed *T. pallidum*. VDRL declines with treatment, whereas FTAABS remains positive for life.	Penicillin is effective in the treatment of all stages of syphilis. In primary and secondary syphilis, use benzathine penicillin G (a depot preparation) because *T. pallidum* grows slowly, so drug must be present for a long time. There is no resistance. Benzathine penicillin given to contacts. No vaccine available.
Borrelia burgdorferi	Spirochetes. Gram stain not useful. Can be cultured *in vitro* but not usually done.	Lyme disease.	The main reservoir is the white-footed mouse. Very small nymph stage of ixodid tick (deer tick) is the most common vector. Eighty percent of cases are in the northeastern states of Connecticut, New York, and New Jersey. Very small nymph stage of ixodid tick (deer tick) is the most common vector. Tick must feed on person for at least 24 hours to deliver an infectious dose of *B. burgdorferi*.	Organism invades skin, causing a rash called erythema migrans. It then spreads via the bloodstream to involve primarily the heart, joints, and central nervous system. **No toxins or virulence factors identified.**	Diagnosis is usually made serologically (i.e., by detecting IgM antibody). Confirm positive serologic test with Western blot assay.	Doxycycline for early stages; penicillin G for late stages. No vaccine available. Avoid tick bite. Can give doxycycline or amoxicillin to people who are bitten by a tick in endemic areas.

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Chlamydiae						
Chlamydia trachomatis	Obligate intracellular parasites. Not seen on Gram stained smear. Exists as inactive elementary body extracellularly and as metabolically active, dividing reticulate body intracellularly.	Nongonococcal urethritis, cervicitis, inclusion conjunctivitis, lymphogranuloma venereum, and trachoma. Also pneumonia in infants.	Human genital tract and eyes. Transmission is by sexual contact and during passage of neonate through birth canal. Transmission in trachoma is chiefly by hand to eye contact.	**No toxins or virulence factors known.**	Nucleic acid amplification test (NAAT) using the patient's urine is used to diagnose chlamydial sexually transmitted disease. Gram stain of urethral exudates that show neutrophils but no gram negative diplococci (gonococci) is presumptive evidence for chlamydial infection. Cytoplasmic inclusions seen on Giemsa stained or fluorescent antibody–stained smear of exudate. Organism grows in cell culture and embryonated eggs, but these are not often used.	A tetracycline (e.g., doxycycline) or a macrolide (e.g., azithromycin). Erythromycin effective in infected mother to prevent neonatal disease. No vaccine available.
Chlamydia pneumoniae	Same as *C. trachomatis*.	Atypical pneumonia.	Human respiratory tract. Transmission is by respiratory aerosol.	**No toxins or virulence factors known.**	NAAT and serologic tests for antibody in patient's serum.	A tetracycline, such as doxycycline. No vaccine available.
Rickettsiae						
Rickettsia rickettsii	Obligate intracellular parasites. Not seen well on Gram stained smear. Antigens cross-react with OX strains of *P. vulgaris* (WeilFelix reaction).	Rocky Mountain spotted fever.	Dermacentor (dog) ticks are both the vector and the main reservoir. Transmission is via tick bite. Dogs and rodents can be reservoirs as well.	Organism invades endothelial lining of capillaries, causing vasculitis. **No toxins or virulence factors identified.**	Diagnosis made by detecting antibody in serologic tests such as the ELISA test. Weil-Felix test is no longer used. Stain and culture rarely done.	Doxycycline. Protective clothing and prompt removal of ticks. Tetracycline effective in exposed persons. No vaccine available.