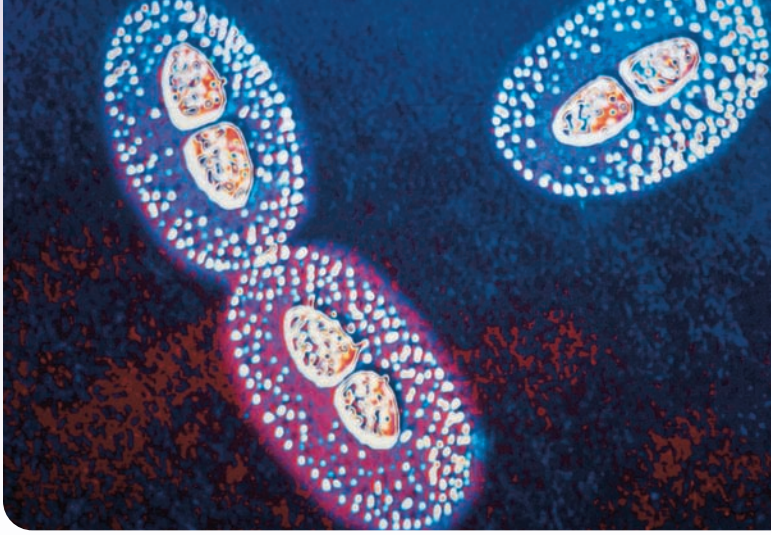
33 Pathogenicity of Microorganisms



Three *Streptococcus pneumoniae*, each surrounded by a slippery mucoid capsule (shown as a layer of white spheres around the diplococcus bacteria). The polysaccharide capsule is vital to the pathogenicity of this bacterium because it prevents phagocytic cells from accomplishing phagocytosis.

Preview

- If a microorganism (symbiont) either harms or lives at the expense of another organism, it is called a parasitic organism and the relationship is termed parasitism. In this relationship the infected organism is referred to as the host.
- Those organisms capable of causing disease are called pathogens. Disease is any change in the host from a healthy to an unhealthy, abnormal state in which part or all of the host's body is not capable of carrying on its normal functions.
- The steps for the infectious process involving viral diseases include the following: entry into a potential host, attachment to a susceptible cell, penetration of viral nucleic acid, replication of virus particles within the host cell, and ultimate release from the host cell. Newly replicated virus particles are available to infect other susceptible cells. Viral infection can result in cellular injury, stimulation of immune responses, or evasion of the virus from immune detection resulting in chronic infection.
- The steps of the infectious process involving bacterial diseases usually include the following: the bacterium is transmitted to a suitable host, attaches to and/or colonizes the host, grows and multiplies within or on the host, and interferes with or impairs the normal physiological activities of the host.
- During coevolution with human hosts, pathogenic bacteria have evolved complex signal transduction pathways to regulate the genes necessary for virulence.
- The genes that encode virulence factors are often located on large segments of DNA within the bacterial genome, called pathogenicity islands, that carry genes responsible for virulence.
- Two distinct categories of disease can be recognized based on the role bacteria play in the disease causing process: infections (invasion and growth) and intoxications.
- Toxins produced by pathogenic bacteria are either exotoxins or endotoxins.
- Viruses and bacteria are continuously evolving and producing unique mechanisms that enable them to escape the host's arsenal of defenses.

Chapter 30 introduces the concept of symbiosis and deals with several of its subordinate categories, including commensalism and mutualism. In this chapter the process of parasitism is presented along with one of its possible consequences—pathogenicity. The parasitic way of life is so successful, that it has evolved independently in nearly all groups of organisms. In recent years concerted efforts to understand organisms and their relationships with their hosts have developed within the disciplines of virology, bacteriology, mycology, parasitology (protozoology and helminthology), entomology, and zoology. This chapter examines the parasitic way of life in terms of health and disease and emphasizes viral and bacterial disease mechanisms. We conclude the chapter with some viral and bacterial mechanisms used to evade host defenses.

33.1 Host-Parasite Relationships

Relationships between two organisms can be very complex. A larger organism that supports the survival and growth of a smaller organism is called the host. The interaction of the two is symbiotic. **Symbiosis** refers to the "living together" of organisms and includes

Pathogenicity is not the rule. Indeed, it occurs so infrequently and involves such a relatively small number of species, considering the huge population of bacteria on earth, that it has a freakish aspect. Disease usually results from inconclusive negotiations for symbiosis, an overstepping of the line by one side or the other, a biological misinterpretation of borders.

—*Lewis Thomas*

commensalism, mutualism, and parasitism (*see figure 30.1*). A commensalistic relationship is demonstrated by the microflora of the cecum of mammals. The mammal provides food and shelter for the microflora, while the microorganisms enzymatically break down complex nutrients to be utilized by the mammal. In addition to relationships between two living organisms, many microorganisms are saprophytic. These organisms obtain nutrients from dead or decaying organic matter. Although some saprophytes are capable of causing disease, most are not parasites; rather they can be thought of as scavengers. Technically, **parasites** are those organisms that live on or within a host organism and are metabolically dependent on the host. Unfortunately, the term parasite has other meanings. It is often used to mean a protozoan or helminth organism living within a host. However, any organism that causes disease is a parasite. Microbiologists can also define infectious disease by the **host-parasite relationship.** A small number of microorganisms can exist as either saprophytes or parasites. Furthermore, commensals, like those associated with the gut, can become parasites when they are present in a location within the host other than the site they normally colonize. These organisms are often referred to as opportunists.

Several types of parasitism are recognized. If an organism lives on the surface of its host, it is an **ectoparasite;** if it lives internally, it is an **endoparasite.** Some parasites, especially those with complex life cycles, inhabit multiple hosts. The host on or in which the parasitic organism either attains sexual maturity or reproduces is the **final host.** A host that serves as a temporary but essential environment for some stages of development is an **intermediate host.** In contrast, a **transfer host** is not necessary for the completion of the organism's life cycle but is used as a vehicle for reaching a final host. A host infected with a parasitic organism that also can infect humans is called a **reservoir host.**

The host-parasite relationship is complex and dynamic. When a parasite is growing and multiplying within or on a host, the host is said to have an **infection.** The nature of an infection can vary widely with respect to severity, location, and number of organisms involved (**table 33.1**). An infection may or may not result in overt disease. An **infectious disease** is any change from a state of health in which part or all of the host body is not capable of carrying on its normal functions due to the presence of a parasite or its products. Any organism or agent that produces such a disease is also known as a **pathogen** [Greek *patho*, disease, and *gennan*, to produce]. Its ability to cause disease is called **pathogenicity.** A **primary (frank) pathogen** is any organism that causes disease in a healthy host by direct interaction. Conversely, an **opportunistic pathogen** refers to an organism that is part of the host's normal microbiota, but is able to cause disease when the host is immunocompromised or when it has gained access to other tissue sites.

At times an infectious organism can enter a latent state in which there is no shedding of the organism (that is, the organism is not infectious at that time) and no symptoms present within the host. This latency can be either intermittent or quiescent. Intermittent latency is exemplified by the herpesvirus that causes cold sores (fever blisters). After an initial infection, the symptoms subside. However, the virus remains in nerve tissue and can be cyclically activated weeks or years later by factors such as stress or sunlight. In a quiescent latency the organism persists but remains inactive for long periods of time, usually for years. For example, the varicella-zoster virus causes chickenpox in children and remains after the disease has subsided. In adulthood, under certain conditions, the same virus may erupt into a disease called shingles. Direct contact diseases: Cold sores (section 37.3); Airborne diseases: Chickenpox and shingles (section 37.1)

The outcome of most host-parasite relationships is dependent on three main factors: (1) the number of microorganisms infecting the host, (2) the degree of pathogenicity (or virulence) of the organism, and (3) the host's defenses or degree of resistance (**figure 33.1**). Usually the greater the number of organisms within a given host, the greater the likelihood of disease. However, a few organisms can cause disease if they are extremely virulent or if the host's resistance is low. Such infections can be a serious problem among hospitalized patients with very low resistance.

The term **virulence** [Latin *virulentia*, from *virus*, poison] refers to the degree or intensity of pathogenicity. As mentioned previously, pathogenicity is a general term that refers to an organism's potential to cause disease. Various physical and chemical characteristics (such as structures that facilitate attachment and molecules that bypass host defenses) contribute to pathogenicity, and thus virulence. Individual characteristics that confer virulence are called **virulence factors** (e.g., capsules, pili, toxins). Virulence is determined by three characteristics of the pathogen: invasiveness, infectivity, and pathogenic potential. **Invasiveness** is the ability of the organism to spread to adjacent or other tissues. **Infectivity** is the ability of the organism to establish a focal point of infection. **Pathogenic potential** refers to the degree that the pathogen causes damage. A major aspect of pathogenic potential is toxigenicity. **Toxigenicity** is the pathogen's ability to produce toxins, chemical substances that damage the host and produce disease. Virulence is often meas-

$$\text{Infection (infectious disease)} = \frac{\text{No. of organisms} \times \text{Virulence}}{\text{Host resistance}}$$

Figure 33.1 Mathematical Expression of Infection. As a mathematical expression, infection or infectious disease can be evaluated by determining the relative contributions of the number of organisms, their virulence, and the host resistance. Organism number reflects the infectious dose and the rate at which the organism can reproduce. Virulence reflects the total number of virulence factors encoded by the genome and expressed in the host. Host resistance is a function of immune status (immunizations, nutrition, previous exposure, etc.) or the effects of chemotherapeutic intervention.

Table 33.1 Various Types of Infections Associated with Parasitic Organisms

Type	Definition
Abscess	A localized infection with a collection of pus surrounded by an inflamed area
Acute	Short but severe
Bacteremia	Presence of viable bacteria in the blood
Chronic	Persisting over a long time
Covert	Subclinical, with no symptoms
Cross	Transmitted between hosts infected with different organisms
Focal	Existing in circumscribed areas
Fulminating	Infectious agent multiplying with great intensity
Iatrogenic	Caused as a result of health care
Latent	Persisting in tissues for long periods, during most of which there are no symptoms
Localized	Restricted to a limited region or to one or more anatomical areas
Nosocomial	Developed during a stay at a hospital or other clinical care facility
Opportunistic	Resulting from endogenous microbiota, especially when host resistance is very low
Overt	Symptomatic
Phytogenic	Caused by plant pathogens
Polymicrobial	More than one organism present simultaneously
Primary	First infection that often allows other organisms to invade host at that site
Pyogenic	Resulting in pus formation
Secondary	Caused by an organism following an initial or primary infection
Sepsis	(1) The condition resulting from the presence of bacteria or their toxins in blood or tissues; the presence of pathogens or their toxins in the blood or other tissues (2) Systemic response to infection; this systemic response is manifested by two or more of the following conditions as a result of infection: temperature, >38 or <36°C; heart rate, >90 beats per min; respiratory rate, >20 breaths per min, or pCO_2, <32 mm Hg; leukocyte count, >12,000 cells per ml^3, or >10% immature (band) forms
Septicemia	Blood poisoning associated with persistence of pathogenic organisms or their toxins in the blood
Septic shock	Sepsis with hypotension despite adequate fluid resuscitation, along with the presence of perfusion abnormalities that may include, but are not limited to, lactic acidosis, oliguria, or an acute alteration in mental status
Severe sepsis	Sepsis associated with organ dysfunction, hypoperfusion, or hypotension; hypoperfusion and perfusion abnormalities may include, but are not limited to, lactic acidosis, oliguria, or an acute alteration in mental status
Sporadic	Occurring only occasionally
Subclinical	No detectable symptoms or manifestations occurring (covert)
Systemic	Spread throughout the body
Toxemia	Condition arising from toxins in the blood
Zoonotic	Caused by a parasitic organism that is normally found in animals other than humans

ured experimentally by determining the **lethal dose 50 (LD_{50})** or the **infectious dose 50 (ID_{50})**. These values refer to the dose or number of pathogens that either kill or infect, respectively, 50% of an experimental group of hosts within a specified period (**figure 33.2**).

It should be noted that disease can result from causes other than toxin production. Sometimes a host triggers exaggerated immunological responses (**immunopathology**) upon a second exposure or chronic exposure to a microbial antigen. These hypersensitivity reactions damage the host even though the pathogen doesn't produce a toxin. Tuberculosis is a good example of the involvement of hypersensitivity reactions in disease. Some diseases also might be due to autoimmune responses. For instance, a viral or bacterial pathogen may stimulate the immune system to attack host tissues because it carries antigens that resembled those of the host, a phenomenon known as molecular mimicry. Streptococcal infections may cause rheumatic fever in this way. Immune disorders: Hypersensitivities (section 32.11)

1. Define parasitic organism, parasitism, infection, infectious disease, pathogenicity, virulence, invasiveness, infectivity, pathogenic potential, and toxigenicity.
2. What factors determine the outcome of most host-parasite relationships?

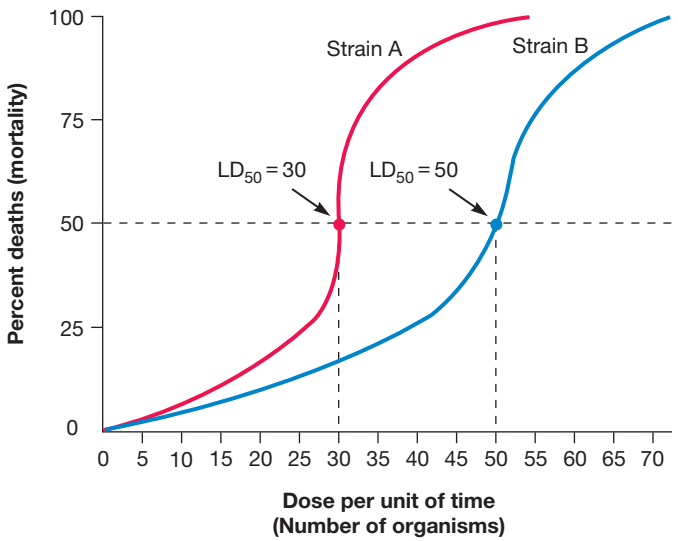


Figure 33.2 Determination of the LD_{50} of a Pathogenic Microorganism. Various doses of a specific pathogen are introduced into experimental host animals. Deaths are recorded and a graph constructed. In this example, the graph represents the susceptibility of host animals to two different strains of a pathogen—strain A and strain B. For strain A the LD_{50} is 30, and for strain B it is 50. Hence strain A is more virulent than strain B.

33.2 Pathogenesis of Viral Diseases

The fundamental process of viral infection is the expression of the viral replicative cycle (*see section 18.2*) in a host cell. The steps for the infectious process involving viruses usually include the following:

1. Maintain a reservoir
2. Enter a host
3. Contact and enter susceptible cells
4. Replicate within the cells
5. Release from host cells (immediate or delayed)
6. Spread to adjacent cells
7. Virus-host interactions engender host immune response
8. Be either cleared from the body of the host, establish a persistent infection, or kill the host
9. Be shed back into the environment

The determinants of pathogenicity are now discussed in more detail. Reproduction of verebrate viruses (section 18.2)

Maintaining a Reservoir

Like other infectious agents, viruses must reside somewhere before they are transmitted to a specific host or tissue site. Because most viruses are limited to the type of host that they can infect (animal viruses infect animals, plant viruses infect plants, and so on), they must gain access to a susceptible host so that they can replicate. Thus the most common **reservoirs** of human viruses are humans and other animals. Some viruses are acquired early in the life of a host, only to cause disease at some later time. More often, however, viruses are transmitted from reservoir (a human host) to host (another human), to cause noticable infection in a relatively short time frame. Because viruses require viable host cells in which to replicate, the source and/or reservoir may harbor large numbers of viral particles that can infect equally large numbers of new hosts upon their release. However, some viruses may not be able to leave their reservoirs or may leave at a very slow rate. Because the source and/or reservoir of a pathogen are part of the infectious disease cycle, this aspect of pathogenicity is discussed in detail in chapter 36, which covers the epidemiology of infectious diseases. Microbial Diversity & Ecology 18.1: SARS: Evolution of a virus

Contact, Entry, and Primary Replication

The first step in the infectious process is the attachment and entrance of the virus into a susceptible host and the host's cells. Entrance may be accomplished through one of the body surfaces (skin, respiratory system, gastrointestinal system, urogenital system, or the conjunctiva of the eye). Other viruses enter the host by sexual contact, needle sticks, blood transfusions and organ transplants, or by insect **vectors** (organisms that transmit the pathogen from one host to another).

Regardless of the method of entry into the host organism, viral infection begins when the viral particle penetrates a host cell to gain access to the cell's replicative machinery. This process is called adsorption, or the attachment to the cell surface. Recall that adsorption occurs because viruses produce specific protein ligands that bind to host cell receptors embedded within their plasma membranes. Host specificity for the virus is a function of viral gene expression; the virus must express the ligand so as to dock with a specific host cell. Protein ligands are usually positioned on the virus to maximize contact with the cell. Enveloped viruses use spikes—viral proteins that protrude from their membrane. Naked viruses have their ligands as part of their capsid proteins.

Each viral ligand only binds to a complementary receptor on the host cell surface. Binding of a virus to its receptor typically results in penetration of the cell or the delivery of virus nucleic acid to the cytoplasm of the cell. In the case of human viruses, nucleic acid enters the host cell by (1) direct entry of just the nucleic acid, as with poliovirus; (2) endocytosis and the release of nucleic acid from the capsid (uncoating), as with the poxviruses; or (3) fusion of the viral envelope with the cell membrane and subsequent uncoating, as with influenza virus (*see figure 18.4*).

Some viruses replicate at the site of entry, cause disease at the same site (e.g., respiratory and gastrointestinal infections), and do not spread throughout the body. Others spread to sites distant from the point of entry and replicate at these sites. For example, the poliovirus enters through the gastrointestinal tract but produces disease in the central nervous system. Food-borne and waterborne diseases: Poliomyelitis (section 37.4)

Release from Host Cells

The details by which various viruses exit their host cells are described in chapter 18. Briefly, there are two distinct release mechanisms that viruses use. The first mechanism is very dramatic and results in relatively large numbers of virions leaving the host cell at the same time and host cell death. This mechanism is called host cell lysis. Replication of viral particles increases within the host cell until the cell membrane can no longer contain all that is within its boundaries. The cell simply expands beyond a size that the cell membrane can maintain its integrity—it lyses. The virions are then free to infect other susceptible cells.

The second general release mechanism is called budding or "blebbing." Here a newly formed nucleocapsid pushes against the host cell membrane until the membrane evaginates and pinches off behind the virus. The released virus is coated with host cell membrane, now called the viral envelope. Release of viral particles by budding is a slower process than lysis; exiting viral particles take relatively small amounts of host cell membrane. The host cell can replenish its membrane permitting continued virus release over the life of the infected cell (*see figure 18.11*).

Viral Spread and Cell Tropism

Mechanisms of viral spread vary, but the most common routes are the bloodstream and lymphatic system. The presence of viruses in the blood is called **viremia.** In some instances, spread is by way of nerves (e.g., rabies, herpes simplex, and varicella-zoster viruses).

Viruses exhibit cell, tissue, and organ specificities. These specificities are called **tropisms** (Greek *trope,* turning). A tropism by a specific virus usually reflects the presence of specific cell surface receptors on the eucaryotic host cell for that virus (*see figure 37.14*).

Virus-Host Interactions

The interaction between a virus and its host cell can result in a variety of effects. Viruses can be either cytopathic or noncytopathic. **Cytopathic viruses** are those that ultimately kill the host cell; the result is often local necrosis. Alternatively, cytopathic viruses can trigger **apoptosis,** or programmed cell death, which culminates in the death of the host cell, often before viral replication can occur (*see figure 16.15*). Although both involve death of host cells, necrosis and apoptosis are very different phenomena. Apoptosis is a normal process in multicellular organisms. It is used during development to remove cells or tissues that are longer needed. It involves nuclear degeneration, the partial digestion of many cell proteins by proteolytic enzymes called capsases, and the formation of apoptotic bodies (membrane-enclosed cell components), which are subsequently phagocytosed by macrophages. Unlike necrosis, the apoptotic cell does not lyse and release its contents. Rather, apoptosis is a controlled dismantling of the cell that results in cell death. In the case of viral infection, apoptosis also prevents virus replication and spread. Some viruses stimulate apoptosis but use special viral proteins to prolong the process long enough to complete viral replication. This ensures that the virus can continue to infect new host cells.

Noncytopathic viruses do not immediately produce cell death and result in latent or persistent infections. As a result, noncytopathic viruses can be subdivided into productive and nonproductive. Noncytopathic viruses that produce persistent infection with the release of only a few new viral particles at a time are said to be productive. Noncytopathic viruses that do not actively make virus at detectable levels for a period of time (latent infection) are considered nonproductive. However, these viruses may be triggered to a reactivated (productive) state by environmental stressors or other factors. Persistent, latent, and slow virus infections (section 18.4)

As anyone who has ever had the flu or a cold knows, clinical illness may be a result of virus-host cell interactions. Some tissues, such as intestinal epithelium, can quickly regenerate when damaged by viruses. Thus they are easily repaired following cellular damage. In contrast, tissues of the nervous system are limited in their ability to regenerate and are thus difficult to repair following damage by viruses. Moreover, infection of cells with some viruses can result in the integration of viral DNA. In a few cases, this can cause them to transform into cancerous cells. This is the result of viral DNA interference with host DNA growth cycle regulation. Viruses and cancer (section 18.5)

Host Immune Response

Both humoral and cellular components of the immune response are involved in the control of viral infections and are discussed in detail in chapters 31 and 32 and summerized in section 32.9.

Recovery from Infection

The host will either succumb to or recover from a viral infection. Recovery processes involve nonspecific defense mechanisms and specific humoral and cellular immunity. The relative importance of each of these factors varies with the virus and the disease, and is covered in chapter 37.

Virus Shedding

The last step in the infectious process is shedding of the virus back into the environment. This is necessary to maintain a source of viruses in a population of hosts. Shedding often occurs from the same body surface used for entry. During this period, an infected host is infectious (contagious) and can spread the virus. In some viral infections, such as a rabies infection, the infected human is the final host because virus shedding does not occur.

1. For a virus to cause disease, certain steps are usually accomplished. Briefly describe each of these steps.
2. If you were to design an antiviral drug, which step or steps in the viral life cycle would you target? Explain your answer.
3. What are the four most common patterns of viral infections? Describe apoptosis and its role in viral infections.

33.3 Overview of Bacterial Pathogenesis

The steps for infections by pathogenic bacteria usually include the following:

1. Maintain a reservoir. A reservoir is a place to live and multiply before and after causing an infection.
2. Initial transport to and entry into the host.
3. Adhere to, colonize, and/or invade host cells or tissues.
4. Evade host defense mechanisms.
5. Multiply (grow) or complete its life cycle on or in the host or the host's cells.
6. Damage the host.
7. Leave the host and return to the reservoir or enter a new host.

The first five factors influence the degree of infectivity and invasiveness. Toxigenicity plays a major role in the sixth. These determinants are now discussed in more detail.

Maintaining a Reservoir of the Bacterial Pathogen

All bacterial pathogens must have at least one reservoir. The most common reservoirs for human pathogens are other humans, animals, and the environment. Since the source and/or reservoir of the pathogen is part of the infectious disease cycle, this aspect of pathogenicity is discussed in detail in chapter 36, which covers the epidemiology of infectious diseases. The infectious disease cycle (section 36.5)

Transport of the Bacterial Pathogen to the Host

An essential feature in the development of an infectious disease is the initial transport of the bacterial pathogen to the host. The most obvious means is direct contact—from host to host (coughing, sneezing, body contact). Bacteria also are transmitted indirectly in a variety of ways. Infected hosts shed bacteria into their surroundings. Once in the environment bacteria can be deposited on various surfaces, from which they can be either resuspended into the air or indirectly transmitted to a host. Soil, water, and food are indirect vehicles that harbor and transmit bacteria to hosts. Arthropod vectors and **fomites** (inanimate objects that harbor and transmit pathogens) also are involved in the spread of many bacteria.

Attachment and Colonization by the Bacterial Pathogen

After being transmitted to an appropriate host, the bacterial pathogen must be able to adhere to and colonize host cells or tissues. In this context **colonization** means the establishment of a site of microbial reproduction on or within a host. It does not necessarily result in tissue invasion or damage. Colonization depends on the ability of the bacteria to survive in the new (host) environment and to compete successfully with the host's normal microbiota for essential nutrients. Specialized structures that allow bacteria to compete for surface attachment sites also are necessary for colonization.

Bacterial pathogens adhere with a high degree of specificity to particular tissues. Adherence structures such as pili and fimbriae (**table 33.2**), and specialized adhesion molecules on the bacterium's cell surface that bind to complementary receptor sites on the host cell surface (**figure 33.3**), facilitate bacterial attachment to host cells. They are one type of virulence factor. Recall that virulence factors are bacterial products or structural components (e.g., capsules and adhesins) that contribute to virulence or pathogenicity. Components external to the cell wall (section 3.9)

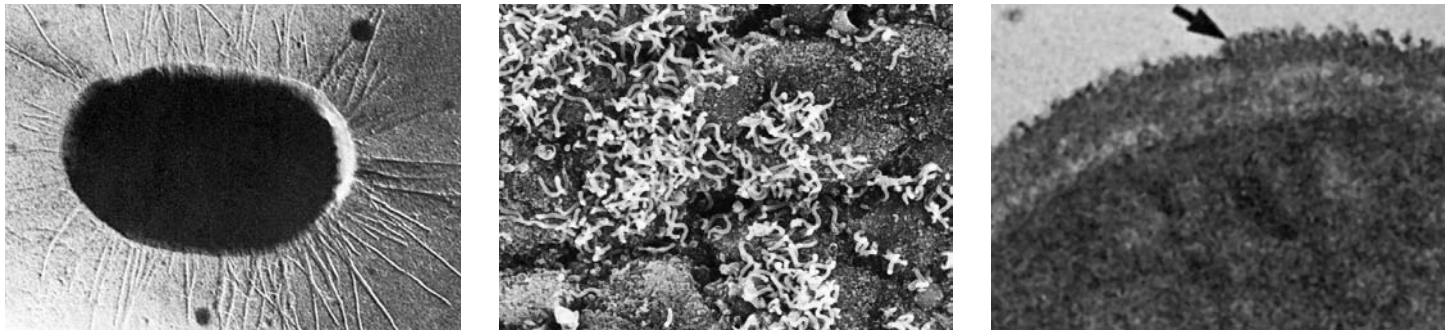
Invasion of Host Tissues

Entry into host cells and tissues is a specialized strategy used by many bacterial pathogens for survival and multiplication. Pathogens often actively penetrate the host's mucous membranes and epithelium after attachment to the epithelial surface. This may be accomplished through production of lytic substances that alter the host tissue by (1) attacking the extracellular matrix and basement membranes of integuments and intestinal linings, (2) degrading carbohydrate-protein complexes between cells or on the cell surface (the glycocalyx), or (3) disrupting the cell surface.

At times a bacterial pathogen can penetrate the epithelial surface by passive mechanisms not related to the pathogen itself. Examples include (1) small breaks, lesions, or ulcers in a mucous membrane that permit initial entry; (2) wounds, abrasions, or burns on the skin's surface; (3) arthropod vectors that create small wounds while feeding; (4) tissue damage caused by other organisms; (e.g., a dog bite) and (5) existing eucaryotic internalization pathways (e.g., endocytosis and phagocytosis. Phagocytosis (section 31.3)

Table 33.2 Bacterial Adherence Factors That Play a Role in Infectious Diseases

Adherence Factor	Description
Fimbriae	Filamentous structures that help attach bacteria to other bacteria or to solid surfaces
Glycocalyx or capsule	A layer of exopolysaccharide fibers with a distinct outer margin that surrounds many cells; it inhibits phagocytosis and aids in adherence; when the layer is well organized and not easily washed off it is called a capsule
Pili	Filamentous structures that bind procaryotes together for the transfer of genetic material
S layer	The outermost regularly structured layer of cell envelopes of some bacteria that may promote adherence to surfaces
Slime layer	A bacterial film that is less compact than a capsule and is removed easily
Teichoic and lipoteichoic acids	Cell wall components in gram-positive bacteria that aid in adhesion



(a) (b) (c)

Figure 33.3 Microbial Adherence. **(a)** Transmission electron micrograph of fimbriated *Escherichia coli* (×16,625). **(b)** Scanning electron micrograph of epithelial cells with adhering vibrios (×1,200). **(c)** *Candida albicans* fimbriae (arrow) are used to attach the fungus to vaginal epithelial cells.

Once under the mucous membrane, the bacterial pathogen may penetrate to deeper tissues and continue disseminating throughout the body of the host. One way the pathogen accomplishes this is by producing specific structures and/or enzymes that promote spreading (**table 33.3**). These products represent other types of virulence factors. Bacteria may also enter the small terminal lymphatic capillaries that surround epithelial cells. These capillaries merge into large lymphatic vessels that eventually drain into the circulatory system. Once the circulatory system is reached, the bacteria have access to all organs and systems of the host.

Bacterial invasiveness varies greatly among pathogens. For example, *Clostridium tetani* (cause of tetanus) produces a variety of virulence factors but is considered noninvasive. *Bacillus anthracis* (cause of anthrax) and *Yersinia pestis* (cause of plague) also produce substantial virulence factors and are highly invasive. Members of the genus *Streptococcus* span the spectrum of virulence factors and invasiveness.

Growth and Multiplication of the Bacterial Pathogen

For a bacterial pathogen to be successful in growth and reproduction (colonization), it must find an appropriate environment (e.g., nutrients, pH, temperature, redox potential) within the host. Those areas of the host's body that provide the most favorable conditions will harbor the pathogen and allow it to grow and multiply to produce an infection. Some bacteria can actively grow and multiply in the blood plasma. The presence of viable bacteria in the bloodstream is called **bacteremia.** The presence of bacteria or their toxins in the blood often is termed **septicemia** [Greek *septikos,* produced by putrefaction, and *haima,* blood].

Some bacteria are able to grow and multiply within various cells of a host. Organisms with this ability to live intracellularly are subdivided into two groups. Facultative intracellular pathogens are those organisms that can reside within the cells of the host or in the environment. An example of a facultative intracellular pathogen is *Brucella abortus,* which is capable of growth and replication within macrophages, neutrophils, and trophoblast cells. However, facultative intracellular pathogens can also be grown in pure culture without host cell support. In contrast, obligate intracellular pathogens are incapable of growth and multiplication outside a host cell. Examples of obligate intracellular pathogens include viruses and the rickettsia. These microbes cannot be grown in the laboratory outside of their host cells.

Leaving the Host

The last determinant of a successful bacterial pathogen is its ability to leave the host and enter either a new host or a reservoir. Unless a successful escape occurs, the disease cycle will be interrupted and the microorganism will not be perpetuated. Most bacteria employ passive escape mechanisms. Passive escape occurs when a pathogen or its progeny leave the host in feces, urine, droplets, saliva, or desquamated cells.

Regulation of Bacterial Virulence Factor Expression

As noted in many chapters, some pathogenic bacteria have adapted to both the free-living state and to an environment within a human host. In the adaptive process, these pathogens have evolved complex signal transduction pathways to regulate the genes necessary for virulence. A virulence factor may be present simply because the bacterium has been infected by a phage—that is, the genes for virulence factors reside on a lysogenic phage genome (prophage). Often environmental factors control the expression of the virulence genes. Common signals include temperature, osmolality, available iron, pH, specific ions, and other nutrient factors. Several examples are now presented.

The gene for diphtheria toxin from *Corynebacterium diphtheriae* (the pathogen that causes diphtheria) is carried on the temperate bacteriophage β, and its expression is regulated by iron. The toxin is produced only by strains lysogenized by the phage. Expression of the virulence genes of *Bordetella pertussis* (the pathogen that causes whooping cough) is enhanced when the bacteria grow at body temperature (37°C) and suppressed when grown at a lower temperature. Finally, the virulence factors of *Vibrio cholerae* (the pathogen that causes cholera) are carried on a temperate phage and regulated at various levels by many environmental factors. Expression of the cholera toxin is higher at pH 6 than at pH 8 and higher at 30 than at 37°C.

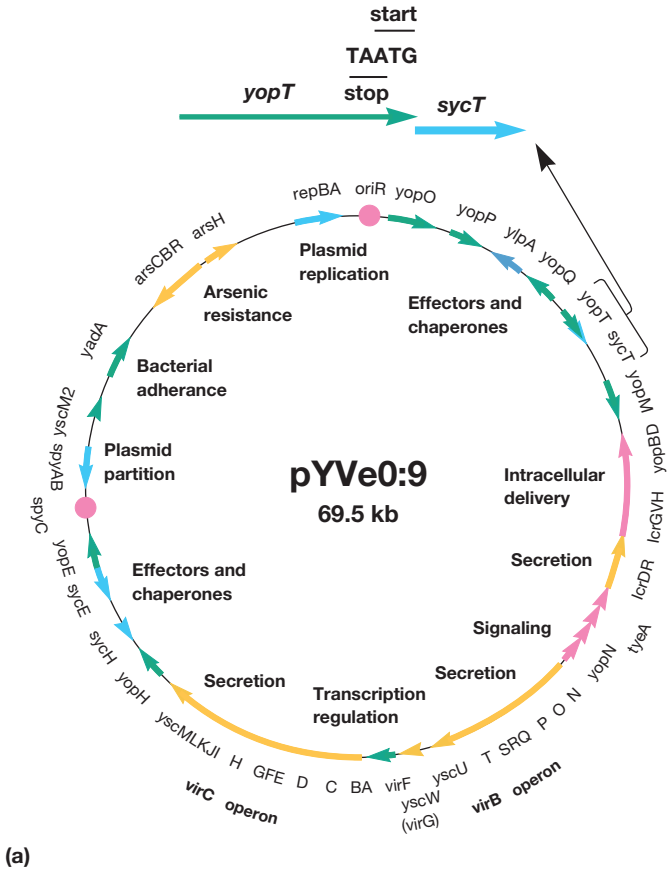
Table 33.3 Microbial Products (Virulence Factors) Involved in Bacterial Pathogen Dissemination Throughout a Mammalian Host

Product	Organism Involved	Mechanism of Action
Coagulase	*Staphylococcus aureus*	Coagulates (clots) the fibrinogen in plasma. The clot protects the pathogen from phagocytosis and isolates it from other host defenses.
Collagenase	*Clostridium* spp.	Breaks down collagen that forms the framework of connective tissues; allows the pathogen to spread.
Deoxyribonuclease (along with calcium and magnesium)	Group A streptococci, staphylococci, *Clostridium perfringens*	Lowers viscosity of exudates, giving the pathogen more mobility.
Elastase and alkaline protease	*Pseudomonas aeruginosa*	Cleaves laminin associated with basement membranes.
Hemolysins	Staphylococci, streptococci, *Escherichia coli*, *Clostridium perfringens*	Lyse erythrocytes; make iron available for microbial growth.
Hyaluronidase	Groups A, B, C, and G streptococci, staphylococci, clostridia	Hydrolyzes hyaluronic acid, a constituent of the extracellular matrix that cements cells together and renders the intercellular spaces amenable to passage by the pathogen.
Hydrogen peroxide (H_2O_2) and ammonia (NH_3)	*Mycoplasma* spp., *Ureaplasma* spp.	Are produced as metabolic wastes. These are toxic and damage epithelia in respiratory and urogenital systems.
Immunoglobulin A protease	*Streptococcus pneumoniae*	Cleaves immunoglobulin A into Fab and Fc fragments.
Lecithinase or phospholipase	*Clostridium* spp.	Destroys the lecithin (phosphatidylcholine) component of plasma membranes, allowing pathogen to spread.
Leukocidins	Staphylococci, pneumococci, streptococci	Pore-forming exotoxins that kill leukocytes; cause degranulation of lysosomes within leukocytes, which decreases host resistance.
Porins	*Salmonella enterica* serovar Typhimurium	Inhibit leukocyte phagocytosis by activating the adenylate cyclase system.
Protein A Protein G	*Staphylococcus aureus* *Streptococcus pyogenes*	Located on cell wall. Immunoglobulin G (IgG) binds to protein A by its Fc end, thereby preventing complement from interacting with bound IgG.
Pyrogenic exotoxin B (cysteine protease)	Group A streptococci, *(Streptococcus pyogenes)*	Degrades proteins.
Streptokinase (fibrinolysin, staphylokinase)	Group A, C, and G streptococci, staphylococci	A protein that binds to plasminogen and activates the production of plasmin, thus digesting fibrin clots; this allows the pathogen to move from the clotted area.

Pathogenicity Islands

The genes that encode major virulence factors in many bacteria (e.g., *Yersinia* spp., *Pseudomonas aeruginosa*, *Shigella flexneri*, *Salmonella*, enteropathogenic *E. coli*) are found on large segments of DNA, called **pathogenicity islands,** which carry genes responsible for virulence. Pathogenicity islands have been acquired during evolution by horizontal gene transfer. A pathogen may have more than one pathogenicity island. They have several common sequence characteristics. The 3′ and 5′ ends of the islands contain insertion-like elements, suggesting their promiscuity as mobile genetic elements. The G + C nucleotide content of pathogenicity islands differs significantly from the G + C content of the remaining bacterial genome. The pathogenicity island DNA also exhibits several open reading frames, suggesting other putative genes. Interestingly, pathogenicity islands are typically associated with genes that encode tRNA. An excellent example of virulence genes carried in a pathogenicity island are those involved in protein secretion. So far, five pathways of protein secretion (types I to V) have been described in gram-negative bacteria. A set of approximately 25 genes encodes a pathogenicity mechanism termed the **type III secretion system (TTSS)** that enables gram-negative bacteria to secrete and inject virulence proteins into the cytoplasm of eucaryotic host cells. Protein secretion in procaryotes (section 3.8)

Many gram-negative bacteria that live in close relationships with host organisms are able to modulate host activities by secreting proteins directly into the interior of the host cell using the TTSS. Perhaps the best studied TTSS is that of *Yersinia pestis* and *Y. enterocolitica*, which cause bubonic plague and gastroenteritis, respectively. Both bacteria use the same plasmid-encoded TTSS consisting of the Yop (*Yersinia* outer protein) secretion (Ysc) injectisome and secreted Yop products (**figure 33.4*a***). The TTSS injectisome is composed of a basal body and a needle. The



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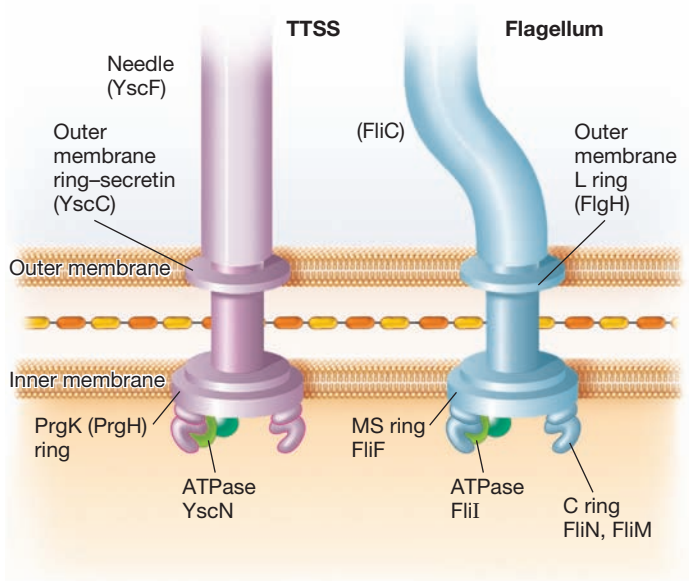

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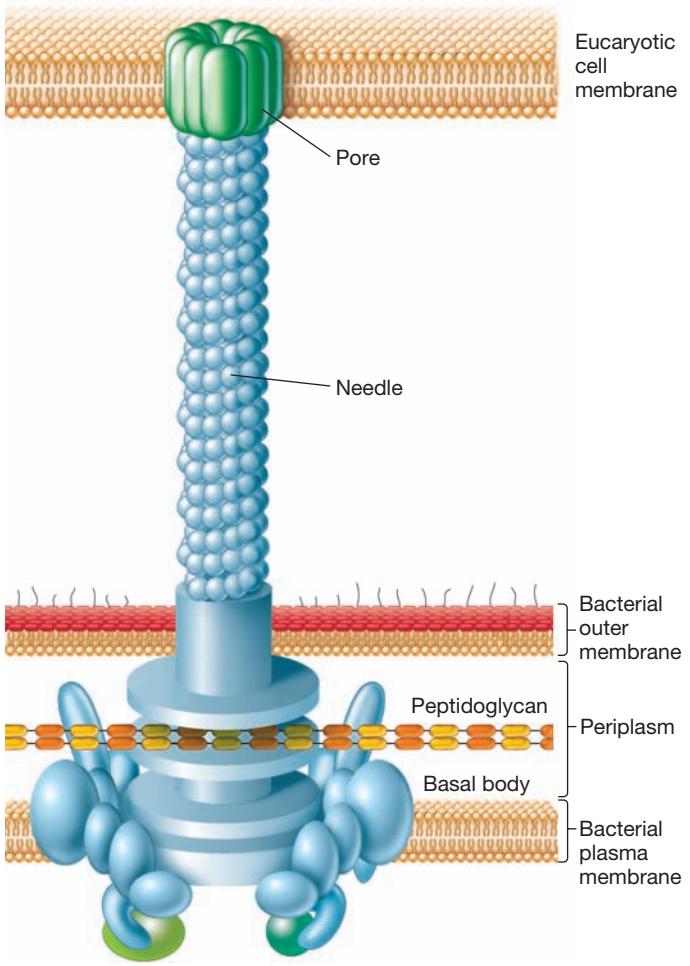

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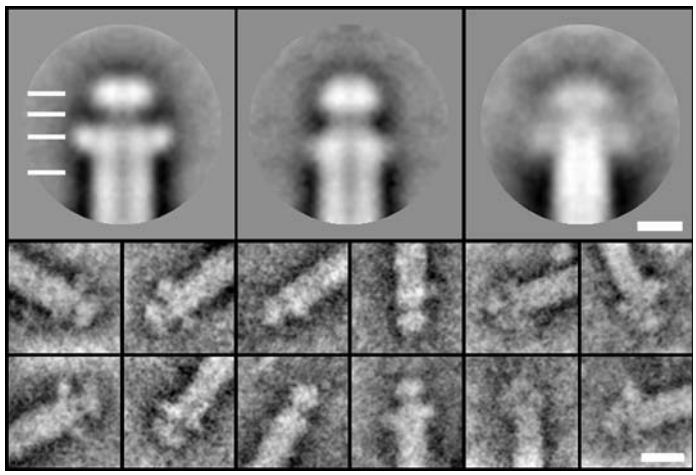


(d)

Figure 33.4 Type III Secretion System. **(a)** The type III secretion system (TTSS) and other virulence genes of *Yersinia* are encoded on the pYV plasmid. The TTSS genes encoding the *Yersinia* outer proteins (Yop) are homologous to many of the genes encoding flagellar proteins. **(b)** Both the TTSS injectisome and the flagella are anchored in the plasma membrane by similar basal body structures. **(c)** X-ray fiber diffraction resolves the injectisome as a helical structure. **(d)** Scanning tunneling electron microscopy reveals the injectisome tip, indicating how it may lock into the translocator pore on the target cell.

basal body is made from a number of proteins that are homologous (having similar amino acid sequences) to proteins that make up the basal body of bacterial flagella. This suggests that the injectisome is held in the bacterial envelope, similar to the ring system that holds a flagellum (figure 33.4*b*). The injectisome employs an ATPase that "energizes" the transport of other TTSS proteins, called "effectors," through "translocator pores" (formed by YopB and YopD proteins), into the host cell. Another protein, LcrV (also known as V antigen), is required for the correct assembly of the translocator pores. X-ray fiber diffraction of TTSS of *Shigella* demonstrates that the needle component of the TTSS has a helical arrangement (figure 33.4*c*). Scanning tunneling electron microscopy of the injectisome needle reveals a characteristic tip (head, neck, and base) through which a central channel is seen (figure 34.4*d*). LcrV localizes at the tip of the needle, which may explain its critical role in facilitating transport of TTSS-mediated proteins and their role in *Yersinia* virulence.

Unlike other bacterial secretory systems, the type III system is triggered specifically by contact with host cells, which helps avoid inappropriate activation of host defenses. Secretion of these virulence proteins into a host cell allows the pathogen to subvert the host cell's normal signal transduction system. Redirection of cellular signal transduction can disarm host immune responses or reorganize the cytoskeleton, thus establishing subcellular niches for bacterial colonization and facilitating "stealth and interdiction" of host defense communication lines.

Pathogenicity islands generally increase microbial virulence and are absent in nonpathogenic members of the same genus or species. One specific example is found in *E. coli*. The enteropathogenic *E. coli* strains possess large DNA fragments, 35 to 170 kilobases in size, that contain several virulence genes absent from commensal *E. coli* strains. Some of these genes code for proteins that alter actin microfilaments within a host intestinal cell. As a consequence, the host cell surface bulges and develops into a cuplike pedestal to which the bacterium tightly binds.

1. What seven steps are involved in the infection process and pathogenesis of bacterial diseases?
2. What are some ways in which bacterial pathogens are transmitted to their hosts? Define vector and fomite.
3. Describe several specific adhesins by which bacterial pathogens attach to host cells.
4. Once under the mucus and epithelial surfaces, what are some mechanisms that bacterial pathogens possess to promote their dissemination throughout the body of a host?
5. What are virulence factors? Pathogenicity islands?

33.4 Toxigenicity

Two distinct categories of disease can be recognized based on the role of the bacteria in the disease-causing process: infections and intoxications. An infectious disease results partly from the pathogen's growth and reproduction (or invasiveness) that often produce tissue alterations.

Intoxications are diseases that result from a specific toxin (e.g., botulinum toxin) produced by bacteria. Some toxins are only produced during host infection. Toxins can even induce disease in the absence of the organism that produced them. A **toxin** [Latin *toxicum*, poison] is a substance, such as a metabolic product of the organism, that alters the normal metabolism of host cells with deleterious effects on the host. The term **toxemia** refers to the condition caused by toxins that have entered the blood of the host. Some toxins are so potent that even if the bacteria that produced them are eliminated (for instance, by antibiotic chemotherapy), the disease conditions persist. Toxins produced by bacteria can be divided into two main categories: exotoxins and endotoxins. The primary characteristics of the two groups are compared in **table 33.4.**

Exotoxins

Exotoxins are soluble, heat-labile, proteins (a few are enzymes) that usually are released into the surroundings as the bacterial pathogen grows. In general, exotoxins are produced by gram-positive bacteria, although some gram-negative bacteria also make exotoxins. Often exotoxins may travel from the site of infection to other body tissues or target cells in which they exert their effects.

Exotoxins are usually synthesized by specific bacteria that often have plasmids or prophages bearing the toxin genes. They are associated with specific diseases and often are named for the disease they produce (e.g., the diphtheria toxin). Exotoxins are among the most lethal substances known; they are toxic in microgram-per-kilogram concentrations (e.g., botulinum toxin), but are typically heat-labile (inactivated at 60 to 80°C). Exotoxins are proteins that exert their biological activity by specific mechanisms. As proteins, the toxins are highly immunogenic and can stimulate the production of neutralizing antibodies called **antitoxins.** The toxin proteins can also be inactivated by formaldehyde, iodine, and other chemicals to form immunogenic **toxoids** (tetanus toxoid, for example). In fact, the tetanus vaccine is a solution of tetanus toxoid.

Exotoxins can be grouped into four types based on their structure and physiological activities. (1) One type is the AB toxin, which gets its name from the fact that the portion of the toxin (B) that binds to a host cell receptor is separate from the portion (A) that has the enzyme activity that causes the toxicity (**figure 33.5*a***). (2) A second type, which also may be an AB toxin, consists of those toxins that affect a specific host site (nervous tissue [neurotoxins], the intestines [enterotoxins], general tissues [cytotoxins]) by acting extracellularly or intracellularly on the host cells. (3) A third type does not have separable A and B portions and acts by disorganizing host cell membranes. Examples include the leukocidins, hemolysins, and phospholipases. (4) A fourth type is the superantigen that acts by stimulating T cells directly to release cytokines. Examples of these types are now discussed. The general properties of some AB exotoxins are presented in **table 33.5.**

AB Toxins

AB toxins are composed of an enzymatic subunit (A) that is responsible for the toxic effect once inside the host cell and a binding subunit (B) (figure 33.5). Isolated A subunits are enzymatically active but lack binding and cell entry capability, whereas isolated

Table 33.4 Characteristics of Exotoxins and Endotoxins

Characteristic	Exotoxins	Endotoxins
Chemical composition	Protein, often with two components (A and B)	Lipopolysaccharide complex on outer membrane; lipid A portion is toxic
Disease examples	Botulism, diphtheria, tetanus	Gram-negative infections, meningococcemia
Effect on host	Highly variable between different toxins	Similar for all endotoxins
Fever	Usually do not produce fever	Produce fever by induction of interleukin-1 and TNF
Genetics	Frequently carried by extrachromosomal genes such as plasmids	Synthesized directly by chromosomal genes
Heat stability	Most are heat sensitive and inactivated at 60-80°C	Heat stable to 250°C
Immune response	Antitoxins provide host immunity; highly antigenic	Weakly immunogenic; immunogenicity associated with polysaccharide
Location	Usually excreted outside the living cell	Part of outer membrane of gram-negative bacteria
Production	Produced by both gram-positive and gram-negative bacteria	Found only in gram-negative bacteria; Released on bacterial death and some liberated during growth
Toxicity	Highly toxic and fatal in nanogram quantities	Less potent and less specific than exotoxin; causes septic shock
Toxoid production	Converted to antigenic, nontoxic toxoids; toxoids are used to immunize (e.g., tetanus toxoid)	Toxoids cannot be made

B subunits bind to target cells but are nontoxic and biologically inactive. The B subunit interacts with specific receptors on the target cell or tissue such as the gangliosides GM1 for cholera toxin, GT1 and/or GD1 for tetanus toxin, and SV2 for botulinum toxin. The B subunit therefore determines what cell type the toxin will affect.

Several mechanisms for the entry of A subunits or fragments into target cells have been proposed. In one mechanism the B subunit inserts into the plasma membrane and creates a pore through which the A subunit enters (figure 33.5*a*). In another mechanism entry is by receptor-mediated endocytosis (figure 33.5*b*).

The mechanism of action of an AB toxin can be quite complex, as shown by the example of diphtheria toxin (figure 33.5*b*). The diphtheria toxin is a protein of about 62,000 Daltons. It binds to cell surface receptors by the B subunit and is taken into the cell through the formation of a clathrin-coated vesicle. The toxin then enters the vesicle membrane and the two subunits are separated; the A subunit escapes into the cytosol. The A subunit is an enzyme that catalyzes the addition of an ADP-ribose group to the eucaryotic elongation factor EF2 that aids in translocation during protein synthesis. The substrate for this reaction is the coenzyme NAD^+.

$$NAD^+ + EF2 \rightarrow \text{ADP-ribosyl-EF2} + \text{nicotinamide}$$

The modified EF2 protein cannot participate in the elongation cycle of protein synthesis, and the cell dies because it can no longer synthesize proteins. ADP-ribosylation is a common mechanism for the A subunit of a number of toxins; however, the specific host molecule to which the ADP-ribose group is attached differs. AB exotoxins vary widely in their relative contribution to the disease process with which they are associated.

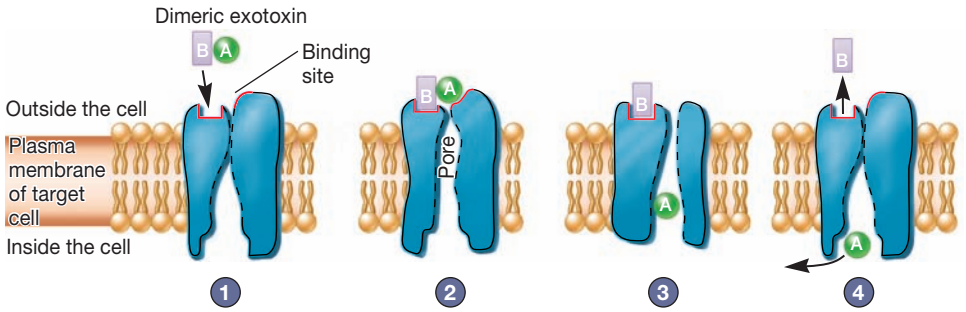
A variation of this AB toxin is the cytolethal distending toxin (CDT) produced by *Campylobacter* spp. Discovered in 1987, CDT is a tripartite holotoxin complex encoded by three tandem genes; *cdtA, cdtB, and cdtC.* CDT binding and internalization appear to be encoded by the *cdtA* and *cdtC* genes, while the active component of the holotoxin is encoded within the *cdtB* gene. The predicted amino acid sequence of CdtB is homologous to proteins having deoxyribonuclease (DNase) I activity. However, the mechanism of CDT in disease is unclear. *C. jejuni* has all three genes. In culture with epithelial cells, the CDT of *C. jejuni* induces a progressive epithelial cell distension resulting from an irreversible blockage of the cell cycle at the G2/M phase. This leads to oversized cells without cell division (distension) and cell death.

Specific Host Site Exotoxins

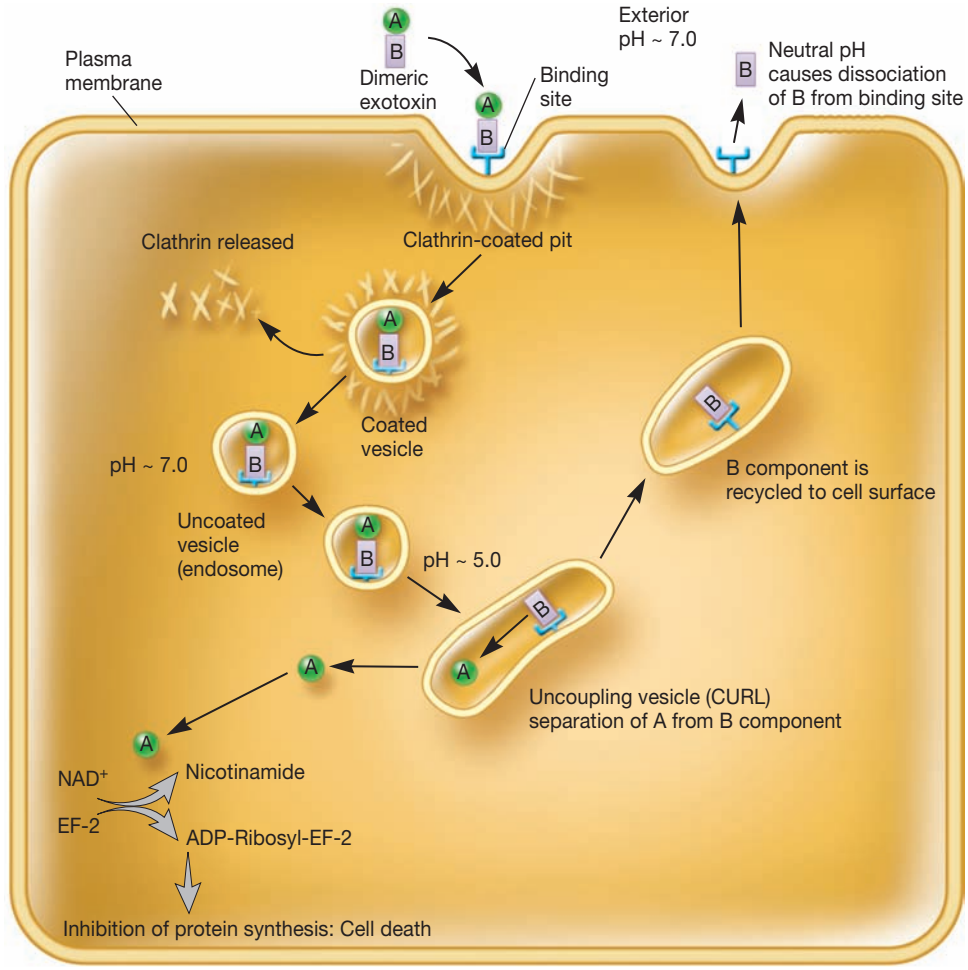
The second type of exotoxin is categorized on the basis of the site affected: **neurotoxins** (nerve tissue), **enterotoxins** (intestinal mucosa), and **cytotoxins** (general tissues). Some of the bacterial pathogens that produce these exotoxins are presented in table 33.5: neurotoxins (botulinum toxin and tetanus toxin), enterotoxins (cholera toxin, *E. coli* heat labile toxins), and cytotoxins (diphtheria toxin, Shiga toxin). Note that many AB toxins are also host site specific, thus these categories are not mutually exclusive.

Neurotoxins usually are ingested as preformed toxins that affect the nervous system and indirectly cause enteric (pertaining to the small intestine) symptoms. Examples include staphylococcal enterotoxin B, *Bacillus cereus* emetic toxin [Greek *emetos,* vomiting], and botulinum toxin.

True enterotoxins [Greek *enter,* intestine] have a direct effect on the intestinal mucosa and elicit profuse fluid secretion (diarrhea). The classic enterotoxin, cholera toxin (choleragen), has been studied extensively. It is an AB toxin. The B subunit is made of five parts arranged as a donut-shaped ring. The B subunit ring anchors itself to the epithelial cell's plasma membrane and then inserts the smaller A subunit into the cell. The A subunit ADP-ribosylates and thereby activates tissue adenylate cyclase to increase intestinal cyclic AMP (cAMP) concentrations. High concentrations of cAMP provoke the movement of massive quantities of water and electrolytes across the intestinal cells into



(a)



(b)

Figure 33.5 Two AB Exotoxin Transport Mechanisms **(a)** Subunit B of the dimeric exotoxin (AB) binds to a specific membrane receptor of a target cell [1]. A conformational change [2] generates a pore [3] through which the A subunit crosses the membrane and enters the cytosol, followed by re-creation [4] of the binding site. **(b)** Receptor-mediated endocytosis of the diphtheria toxin involves the dimeric exotoxin binding to a receptor-ligand complex that is internalized in a clathrin-coated pit that pinches off to become a coated vesicle. The clathrin coat depolymerizes resulting in an uncoated endosome vesicle. The pH in the endosome decreases due to the H^+-ATPase activity. The low pH causes A and B components to separate. An endosome in which this separation occurs is sometimes called a CURL (*c*ompartment of *u*ncoupling of *r*eceptor and *l*igand). The B subunit is then recycled to the cell surface. The A subunit moves through the cytosol, catalyzes the ADP-ribosylation of EF-2 (elongation factor 2) and inhibits protein synthesis, leading to cell death.

Table 33.5 Properties of Some AB Model Bacterial Exotoxins

Toxin	Organism	Gene Location	Subunit Structure	Target Cell Receptor	Enzymatic Activity	Biologic Effects
Anthrax toxins	*Bacillus anthracis*	Plasmid	Three separate proteins (EF, LF, PA)[a]	Capillary morphogenesis protein 2 (CMP-2) and tumor endothelium marker 8 (TEM8)	EF is a calmodulin-dependent adenylate cyclase; LF is a zinc-dependent protease that cleaves a host signal transduction molecule (MAPKK)	EF + PA: increase in target cell cAMP level, localized edema; LF + PA: altered cell signaling; death of target cells
Bordetella adenylate cyclase toxin	*Bordetella* spp.	Chromosomal	A-B[b]	CR3 intergrin (CD11–CD18)	Calmodulin-activated adenylate cyclase	Increase in target cell cAMP level; decrease ATP production; modified cell function or cell death
Botulinum toxin	*Clostridium botulinum*	Phage	A-B[c]	Synaptic vesicle 2 (SV2)	Zinc-dependent endoprotease cleavage of presynaptic protein (SNARE)	Decrease in peripheral, presynaptic acetylcholine release; flaccid paralysis
Cholera toxin	*Vibro cholera*	Phage	A-5B[d]	Ganglioside (GM_1)	ADP ribosylation of adenylate cyclase regulatory protein, G_S	Activation of adenylate cyclase, increase in cAMP level; secretory diarrhea
Diphtheria toxin	*Corynebacterium diphtheriae*	Phage	A-B[e]	Heparin-binding, EGF-like growth factor precursor	ADP ribosylation of elongation factor 2	Inhibition of protein synthesis; cell death
Heat-labile enterotoxins[f]	*E. coli*	Plasmid	——— Similar or Identical to Cholera Toxin ———			
Pertussis toxin	*Bordetella pertussis*	Chromosomal	A-5B[g]	Asparagine-linked oligosaccharide and lactosylceramide sequences	ADP ribosylation of signal-transducing G proteins	Block of signal transduction mediated by target G proteins
Pseudomonas exotoxin A	*P. aeruginosa*	Chromosomal	A-B	α_2-Macroglobulin/LDL receptor	— Similar or Identical to Diphtheria Toxin —	
Shiga toxin	*Shigella dysenteriae*	Chromosomal	A-5B[h]	Globotriaosylceramide (Gb_3)	RNA *N*-glycosidase	Inhibition of protein synthesis, cell death
Shiga-like toxin 1	*Shigella* spp., *E. coli*	Phage	——— Similar or Identical to Shiga Toxin ———			
Tetanus toxin	*C. tetani*	Plasmid	A-B[c]	Ganglioside (GT_1 and/or GD_{1b})	Zinc-dependent endopeptidase cleavage of synaptobrevin	Decrease in neurotransmitter release from inhibitory neurons; spastic paralysis

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[a]The binding component (known as protective antigen [PA]) catalyzes/facilitates the entry of either edema factor (EF) or lethal factor (LF).

[b]Apparently synthesized as a single polypeptide with binding and catalytic (adenylate cyclase) domains.

[c]Holotoxin is apparently synthesized as a single polypeptide and cleaved proteolytically as diphtheria toxin; subunits are referred to as L: light chain, A equivalent; H: heavy chain, B equivalent.

[d]The A subunit is proteolytically cleaved into A1 and A2, with A1 possessing the ADP-ribosyl transferase activity; the binding component is made up of five identical B units.

[e]Holotoxin is synthesized as a single polypeptide and cleaved proteolytically into A and B components held together by disulfide bonds.

[f]The heat-labile enterotoxins of *E. coli* are now recognized to be a family of related molecules with identical mechanisms of action.

[g]The binding portion is made up of two dissimilar heterodimers labeled S2-S3 and S2-S4 that are held together by a bridging peptide, SS.

[h]Subunit composition and structure similar to cholera toxin.

the lumen of the gut. To maintain osmotic homeostasis, the cell then relases this water; this results in severe dirarrhea (cholera victims can lose 20% of their water per day). The genes for this enterotoxigenicity are encoded on a filamentous phage within *Vibrio cholerae.* Food-borne and waterborne diseases: Cholera (section 38.4)

Cytotoxins have a specific toxic action upon cells/tissues of special organs and are named according to the type of cell/tissue or organ for which they are specific. Examples include nephrotoxin (kidney), hepatotoxin (liver), and cardiotoxin (heart).

Membrane-Disrupting Exotoxins

The third type of exotoxin lyses host cells by disrupting the integrity of the plasma membrane. There are two subtypes of **membrane-disrupting exotoxins.** The first, is a protein that binds to the cholesterol portion of the host cell plasma membrane, inserts itself into the membrane, and forms a channel (pore) (**figure 33.6*a***). This causes the cytoplasmic contents to leak out. Also, because the osmolality of the cytoplasm is higher than the extracellular fluid, this causes a sudden influx of water into the cell, causing it to swell and rupture. Two specific examples of this type of membrane-disrupting exotoxin are now presented.

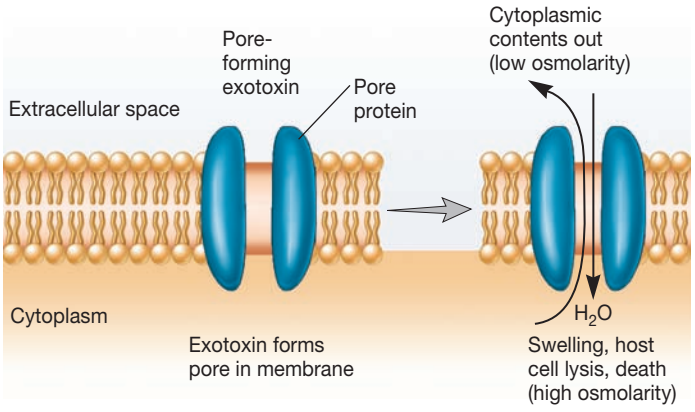


Figure 33.6 Two Subtypes of Membrane-Disrupting Exotoxins. **(a)** A channel-forming (pore-forming) type of exotoxin inserts itself into the normal host cell membrane and makes an open channel (pore). Formation of multiple pores causes cytoplasmic contents to leave the cell and water to move in, leading to cellular lysis and death of the host cell. **(b)** A phospholipid-hydrolyzing phospholipase exotoxin destroys membrane integrity. The exotoxin removes the charged polar head groups from the phospholipid part of the host cell membrane. This destabilizes the membrane and causes the host cell to lyse.

Some pathogens produce membrane-disrupting toxins that kill phagocytic leukocytes. These are termed **leukocidins** [*leuko*cyte and Latin *caedere,* to kill]. Most leukocidins are produced by pneumococci, streptococci, and staphylococci. Since the pore-forming exotoxin produced by these bacteria destroys leukocytes, this in turn decreases host resistance. Other toxins, called **hemolysins** [*haima,* blood, and Greek *lysis,* dissolution], also can be secreted by pathogenic bacteria. Many hemolysins probably form pores in the plasma membrane of erythrocytes through which hemoglobin and/or ions are released (the erythrocytes lyse or, more specifically, hemolyze). **Streptolysin-O (SLO)** is a hemolysin, produced by *Streptococcus pyogenes,* that is inactivated by O_2 (hence the "O" in its name). SLO causes beta hemolysis of erythrocytes on agar plates incubated anaerobically. A complete zone of clearing around the bacterial colony growing on blood agar is called **beta hemolysis,** and a partial clearing of the blood (leaving a greenish halo of hemoglobin) is called **alpha hemolysis. Streptolysin-S (SLS)** is also produced by *S. pyogenes* but is insoluble and bound to the bacterial cell. It is O_2 stable (hence the "S" in its name) and causes beta hemolysis on aerobically incubated blood-agar plates. In addition to hemolysins, SLO and SLS are also leukocidins and kill leukocytes. It should also be noted that hemolysins attack the plasma membranes of many cells, not just erythrocytes and leukocytes.

The second subtype of membrane-disrupting toxins are the **phospholipase** enzymes. Phospholipases remove the charged head group (figure 33.6*b*) from the lipid portion of the phospholipids in the host-cell plasma membrane. This destabilizes the membrane so that the cell lyses and dies. One example of the pathogenesis caused by phospholipases is observed in the disease gas gangrene. In this disease, the *Clostridium perfringens* α-toxin almost completely destroys the local population of white blood cells (that are drawn in by inflammation to fight the infection) through phospholipase activity.

Superantigens

As discussed in chapter 32, superantigens are bacterial and viral proteins that can provoke as many as 30% of a person's T cells to release massive concentrations of cytokines. The best-studied superantigen is also a staphylococcal enterotoxin. Staphylococcal entertoxin B (SEB) exhibits biological activity as a superantigen at nanogram concentrations and is therefore classified as a select agent; it has the potential to be misused as a bioterror agent. SEB exerts its superantigen activity by bridging the unfilled class II MHC molecules of antigen-presenting cells to T-cell receptors. Because no processed antigen is involved, many T cells are activated at once. This activation of T cells results in normal cytokine release; however, the sum total of the combined cytokines overwhelms cells and tissues. Cytokines stimulate endothelial damage, circulatory shock, and multiorgan failure. T-cell biology: Superantigens (section 32.5)

Roles of Exotoxins in Disease

Humans are exposed to bacterial exotoxins in three main ways: (1) ingestion of preformed exotoxin, (2) colonization of a mucosal surface followed by exotoxin production, and (3) colonization of a wound or abscess followed by local exotoxin production. Each of these is now briefly discussed.

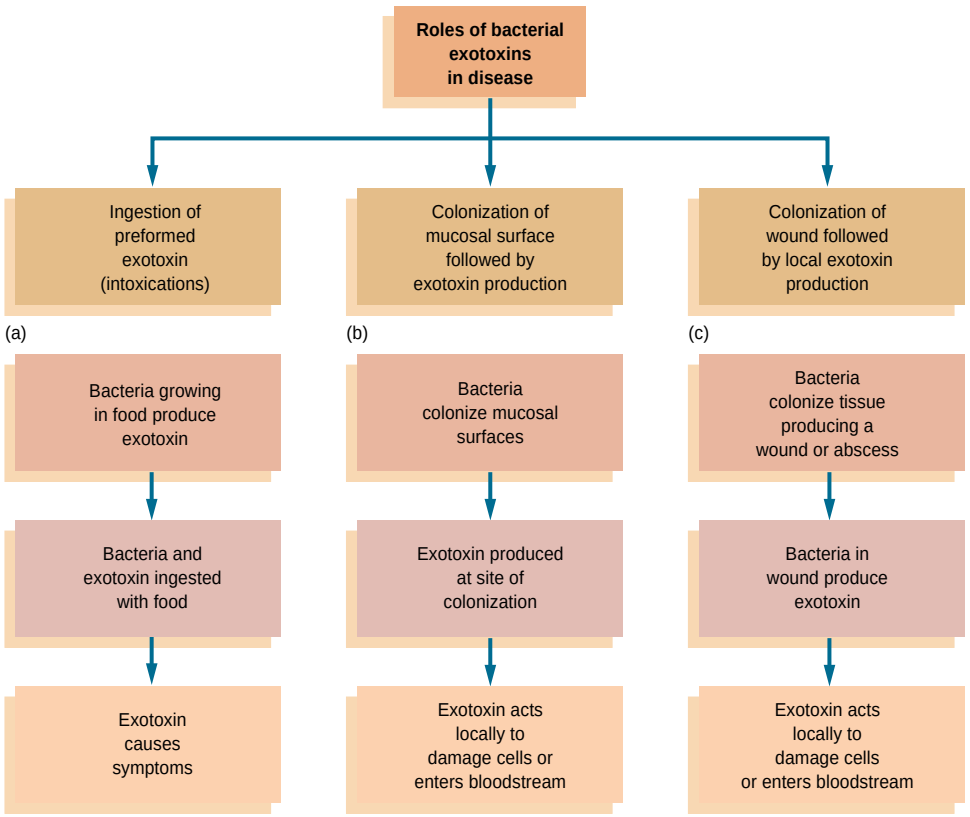


Figure 33.7 Roles of Exotoxins in Disease Pathogenesis. Three ways **(a, b, c)** in which bacterial exotoxins can contribute to the progression of disease in a human.

In the first example (**figure 33.7*a***), the exotoxin is produced by bacteria growing in food. When food is consumed, the preformed exotoxin is also consumed. The classical example is staphylococcal food poisoning caused solely by the ingestion of preformed enterotoxin. Since the bacteria (*Staphylococcus aureus*) cannot colonize the gut, they pass through the body without producing any more exotoxin; thus, this type of bacterial disease is self-limiting.

In the second example (figure 33.7*b*), bacteria colonize a mucosal surface but do not invade underlying tissue or enter the bloodstream. The toxin either causes disease locally or enters the bloodstream and is distributed systemically where it can cause disease at distant sites. The classical example here is the disease cholera caused by *Vibrio cholerae*. Once the bacteria enter the body, they adhere to the intestinal mucosa. They are not invasive but secrete the cholera toxin. As a result, cholera toxin stimulates hypersecretion of water and chloride ions and the patient loses massive quantities of water through the gastrointestinal tract.

The third example of exotoxins in disease pathogenesis occurs when bacteria grow in a wound or abscess (figure 33.7*c*). The exotoxin causes local tissue damage or kills phagocytes that enter the infected area. A disease of this type is gas gangrene in which the exotoxin (α-toxin) of *Clostridium perfringens* lyses red blood cells, induces edema, and causes tissue destruction in the wound.

1. What is the difference between an infectious disease and an intoxication? Define toxemia.
2. Describe some general characteristics of exotoxins.
3. How do exotoxins get into host cells?
4. Describe the biological effects of several bacterial exotoxins.
5. Discuss the mechanisms by which exotoxins can damage cells.
6. What are the four types of exotoxins?
7. What is the mode of action of a leukocidin? Of a hemolysin?
8. Name two specific hemolysins.
9. What are the three main roles exotoxins have in human disease pathogenesis?

Endotoxins

Gram-negative bacteria have lipopolysaccharide (LPS) in the outer membrane of their cell wall that, under certain circumstances, is toxic to specific hosts. This LPS is called an **endotoxin** because it is bound to the bacterium and is released when the microorganism lyses (**Techniques & Applications 33.1**). Some is also released during bacterial multiplication. The toxic component of the LPS is the lipid portion, called lipid A. Lipid A is not a single macromolecular structure but appears to be a complex array of lipid residues. The lipid A component exhibits all the properties associated with endotoxicity and gram-negative bacteremia. The bacterial cell wall: Gram-negative cell walls (section 3.6)

Besides the preceding characteristics, bacterial endotoxins are

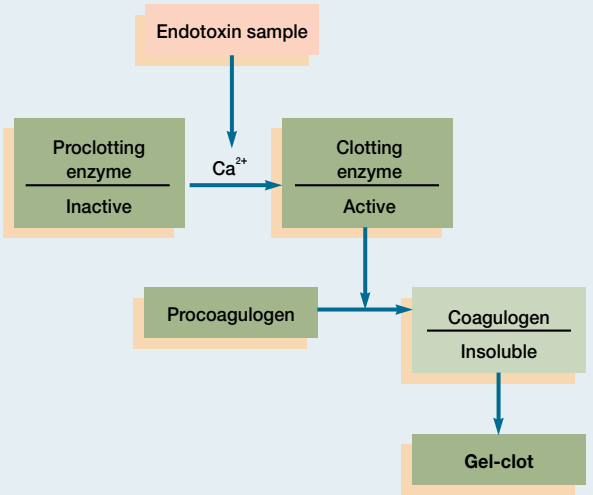
1. Heat stable
2. Toxic (nanogram amounts)
3. Weakly immunogenic
4. Generally similar, despite source
5. Usually capable of producing general systematic effects: fever (are pyrogenic), shock, blood coagulation, weakness, diarrhea, inflammation, intestinal hemorrhage, and fibrinolysis (enzymatic breakdown of fibrin, the major protein component of blood clots)

Techniques & Applications

33.1 Detection and Removal of Endotoxins

Bacterial endotoxins plagued the pharmaceutical industry and medical device producers for years. For example, administration of drugs contaminated with endotoxins resulted in complications—even death—to patients. In addition, endotoxins can be problematic for individuals and firms working with cell cultures and genetic engineering. The result has been the development of sensitive tests and methods to identify and remove these endotoxins. The procedures must be very sensitive to trace amounts of endotoxins. Most firms have set a limit of 0.25 **endotoxin units (E.U.),** 0.025 ng/ml, or less as a release standard for their drugs, media, or products.

One of the most accurate tests for endotoxins is the in vitro *Limulus* amoebocyte lysate (LAL) assay. The assay is based on the observation that when an endotoxin contacts the clot protein from circulating amoebocytes of the horseshoe crab (*Limulus*), a gel-clot forms. The assay kits available today contain calcium, proclotting enzyme, and procoagulogen. The proclotting enzyme is activated by bacterial endotoxin (lipopolysaccharide) and calcium to form active clotting enzyme (see **Box figure**). Active clotting enzyme then catalyzes the cleavage of procoagulogen into polypeptide subunits (coagulogen). The subunits join by disulfide bonds to form a gel-clot. Spectrophotometry is then used to measure the protein precipitated by the lysate. The LAL test is sensitive at the nanogram level but must be standardized against Food and Drug Administration Bureau of Biologics endotoxin reference standards. Results are reported in endotoxin units per milliliter and reference made to the particular reference standards used.



Removal of endotoxins presents more of a problem than their detection. Those present on glassware or medical devices can be inactivated if the equipment is heated at 250°C for 30 minutes. Soluble endotoxins range in size from 20 kDa to large aggregates with diameters up to 0.1 μm. Thus they cannot be removed by conventional filtration systems. Manufacturers have developed special filtration systems and filtration cartridges that retain these endotoxins and help alleviate contamination problems.

The characteristics of endotoxins and exotoxins are contrasted in table 33.4. The main biological effect of lipid A is an indirect one, being mediated by host molecules and systems rather than by lipid A directly. For example, endotoxins can initially activate Hageman Factor (blood clotting factor XII), which in turn activates up to four humoral systems: coagulation, complement, fibrinolytic, and kininogen systems (*see figure 38.26*).

Gram-negative endotoxins also indirectly induce a fever in the host by causing macrophages to release **endogenous pyrogens** that reset the hypothalamic thermostat. One important endogenous pyrogen is the cytokine interleukin-1 (IL-1). Other cytokines released by macrophages, such as the tumor necrosis factor, also produce fever.

Evidence indicates that LPS affects macrophages, monocytes, and neutrophils by binding to the soluble pattern-recognition receptor (formerly LPS-binding protein) for transfer to the membrane-bound CD14 on these cells. LPS-bound CD14 then complexes with toll-like receptor (TLR) 4 to initiate a signaling process that upregulates the phagocyte response to LPS. Part of this response is the synthesis and release of cytokines IL-1, IL-6, IL-8, tumor necrosis factor α, and platelet-activating factor. These and other proinflammatory mediators signal target cells resulting in fever, complement activation, prostaglandin synthesis, and activation of the coagulation cascade. Phagocytosis: Toll-like receptors (section 31.3); Chemical mediators in nonspecific resistance: Cytokines (section 31.6)

1. Describe the chemical structure of the LPS endotoxin.
2. List some general characteristics of endotoxins.
3. How do gram-negative endotoxins induce fever in a mammalian host?

33.5 Host Defense Against Microbial Invasion

In host-pathogen relationships, the balance between host integrity and resource utilization results in the co-evolution of survival strategies (**figure 33.8**). Competition, harsh environments, and cellular biowarfare have demanded unique solutions from host and pathogen alike. The complex, albeit "sneaky," methods employed by microbes to gain access to host resources have invariably been met with equally complex countermeasures. The host-pathogen relationship is indeed defined by ecological principles. The host provides a myriad of niches for microbes that have adapted to various temperature optima, nutrient content, oxygen concentration, and tolerance for host, as well as other microorganisms (antagonism).

Chapters 31 and 32 detail the innate and adaptive responses, respectively, available to the human host in preventing or limiting infection. A variety of physical, chemical, and biological barriers establish a formidable defense against microbial invasion.

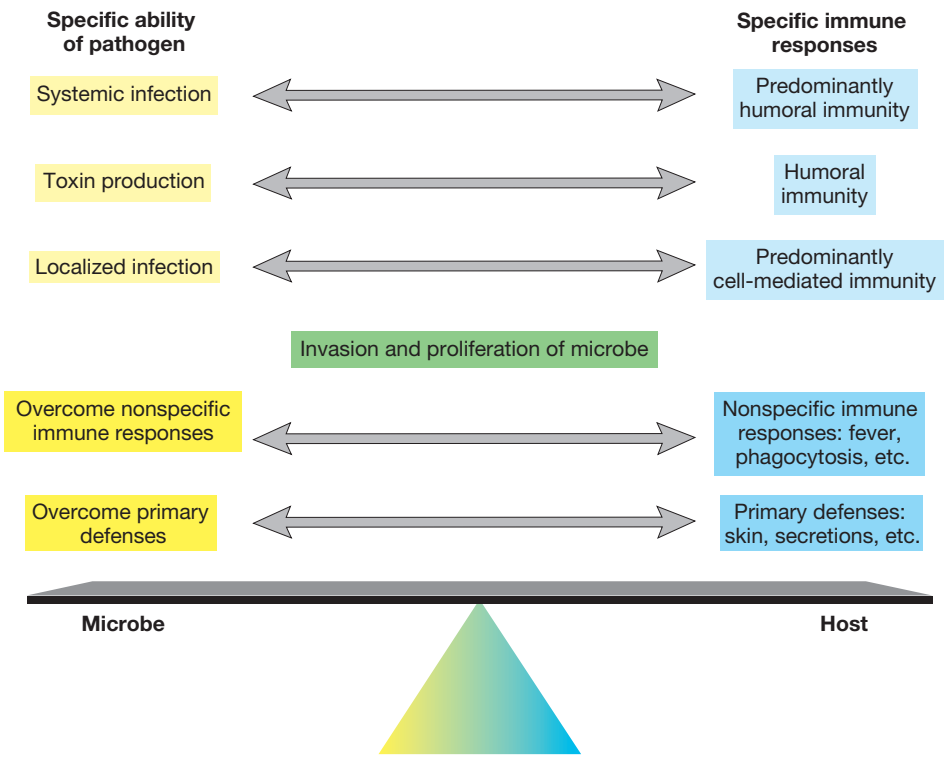


Figure 33.8 The Balance Between Microorganism Activity and Host Immunity. The interaction between the two determines the final host-parasite balance.

Nonetheless, microorganisms often breech these sophisticated barriers, occasionally prevailing. However, more recent evidence suggests that host cells are equipped with sensitive inter- and intracellular surveillance systems that recognize unique pathogen-associated patterns. As in medical diagnostics, early immune detection usually leads to early clearance of disease. Coupled with the ability to produce highly specific receptors and antibodies, the host prevents many more microbial invasions than those that are noticed.

Primary Defenses

Recall that the host has evolved several strategies to prevent pathogen entry. This is intuitively the most logical mechanism to develop. In other words, there would be no need for secondary defenses if the primary systems were 100% effective. An impenetrable suit of armor might restrict microbial entry, but limits other essential functions. Thus a multilayered skin, speckled with glands producing antimicrobial substances and an army of formidable microbial allies, is a sound compromise. Mucous membranes with cilia, pH regulation, flushing mechanisms, and additional antimicrobial products are efficient transition sites where the host tissues interface with their environment.

Secondary Defenses

Because the defenses of the skin and mucous membranes can be overcome, a secondary system has evolved. Composed of soluble antimicrobial products and cells capable of sensing and responding to invading microbes, the secondary system is quite effective in controlling infection. The host blood and interstitial fluids contain evolutionarily conserved, antimicrobial proteins that are very efficacious. These proteins include a variety of low-molecular-weight, "pore-forming" peptides and the ubiquitous lysozyme. Additionally, host cells have evolved strategies to exploit pathogen sensitivities to toxic oxygen radicals. Finally, sophisticated, soluble receptors police the host in search of microbial ligands. Binding of such ligands to their receptors initiates processes designed to amplify microbial detection and destruction. Examples of these processes include complement activation, inflammation, fever, and phagocytosis.

Factors Influencing Host Defenses

There are a variety of factors that influence the primary and secondary immune responses of the host. Age, stress, nutritional deficiencies, and genetic background all play substantial roles in host defense. The very young and the very old tend to be more susceptible to diseases. Some individuals are inherently (genetically) more resistant or more susceptible to particular diseases. Nutrition also influences these factors. In fact, historians have discovered a link between times of famine and times of disease. For example, nutritional deficiencies can decrease epithelial integrity, weaken antibody responses, and facilitate changes in the normal flora. Stress too can inhibit the immune response by stimulating the production of corticosteroids, which depress immune responses. Lastly, long-term exposure to environmental pollutants, drug abuse, or certain prescribed medicines may also inhibit normal host defenses against infection.

33.6 Microbial Mechanisms For Escaping Host Defenses

So far, we have discussed some of the ways viral and bacterial pathogens cause disease in a host. During the course of microbe and human evolution, these same pathogens have evolved ways for evading host defenses. Many of these mechanisms are found throughout the microbial world and several are now discussed.

Evasion of Host Defenses by Viruses

As noted earlier in this chapter, the pathology arising from a viral infection is due to either (1) the host's immune response, which attacks virus-infected cells or produces hypersensitivity reactions, or (2) the direct consequence of viral multiplication within host cells. Viruses have evolved a variety of ways to suppress or evade the host's immune response. These mechanisms are now becoming recognized through genomics and the functional analysis of specific gene products. Immune disorders: Hypersensitivities (section 32.11)

Some viruses may mutate and change antigenic sites (antigenic drift) on the virion proteins (e.g., the influenza virus) or may down-regulate the level of expression of viral cell surface proteins (e.g., the herpesvirus). Other viruses (HIV) may infect cells (T cells) of the immune system and diminish their function. HIV as well as the measles virus and cytomegalovirus cause the fusion of host cells. This allows these viruses to move from an infected cell to an uninfected cell without exposure to the antibody-containing fluids of the host. The herpesvirus may infect neurons that express little or no major histocompatibility complex molecules. The adenovirus produces proteins that inhibit major histocompatibility complex function. Finally, hepatitis B virus-infected cells produce large amounts of antigens not associated with the complete virus. These antigens bind the available neutralizing antibody so that there is insufficient free antibody to bind with the complete virion. Airborne diseases: Influenza (section 37.1); Recognition of foreignness (section 32.4)

Evasion of Host Defenses by Bacteria

Bacteria also have evolved many mechanisms to evade host defenses. Because bacteria would not be well served either by the death of their host or their own death, their survival strategy is protection against host defenses rather than host destruction.

Evading the Complement System

To evade the activity of complement, some bacteria have capsules (see chapter opening figure) that prevent complement activation. Some gram-negative bacteria can lengthen the O chains in their lipopolysaccharide to prevent complement activation. Others such as *Neisseria gonorrhoeae* generate **serum resistance.** These bacteria have modified lipooligosaccharides on their surface that interfere with proper formation of the membrane attack complex (*see figure 31.23*) during the complement cascade. The virulent forms of *N. gonorrhoeae* that possess serum resistance are able to spread throughout the body of the host and cause systemic disease, whereas those *N. gonorrhoeae* that lack serum resistance remain localized in the genital tract. Chemical mediators in nonspecific resistance: Complement (section 31.6)

Resisting Phagocytosis

As noted previously, before a phagocytic cell can engulf a bacterium, it must first directly contact the bacterium's surface. Some bacteria such as *Streptococcus pneumoniae, Neisseria meningitidis,* and *Haemophilus influenzae* can produce a slippery mucoid capsule that prevents the phagocyte from effectively contacting the bacterium. Other bacteria evade phagocytosis by producing specialized surface proteins such as the M protein on *S. pyogenes.* Like capsules, these proteins interfere with adherence between a phagocytic cell and the bacterium.

Bacterial pathogens use other mechanisms to resist phagocytosis. For example, *Staphylococcus* produces leukocidins that destroy phagocytes before phagocytosis can occur. *S. pyogenes* releases a protease that cleaves the C5a complement factor and thus inhibits complement's ability to attract phagocytes to the infected area.

Survival Inside Phagocytic Cells

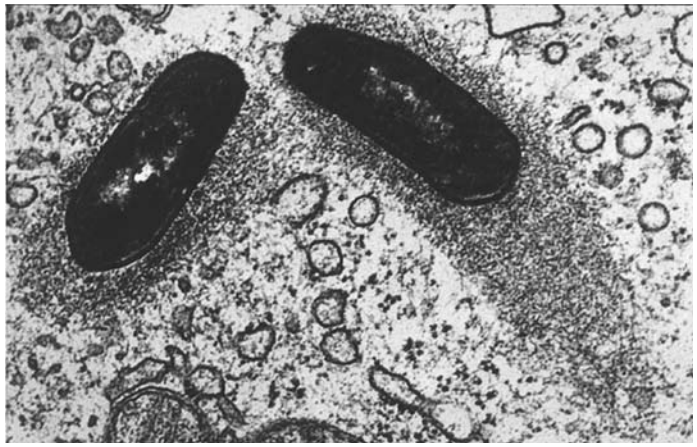
Some bacteria have evolved the ability to survive inside neutrophils, monocytes, and macrophages. They are very pathogenic because they are impervious to a most important host protective mechanism. One method of evasion is to escape from the phagosome before it merges with the lysosome, as seen with *Listeria monocytogenes, Shigella,* and *Rickettsia.* These bacteria use actin-based motility to move within mammalian host cells and spread between them. Upon lysing the phagosome, they gain access to the cytoplasm. Each bacterium then recruits to its surface host cell actin and other cytoskeletal proteins and activates the assembly of an actin tail (**figure 33.9*a***). The actin tails propel the bacteria through the cytoplasm of the infected cell to its surface where they push out against the plasma membrane and form protrusions (figure 33.9*b*). The protrusions are engulfed by adjacent cells, and the bacteria once again enter phagosomes and escape into the cytoplasm. In this way the infection spreads to adjacent cells. The lysosomes never have a chance to merge with the phagosomes. Another approach is to resist the toxic products released into the phagolysosome after fusion occurs. A good example of a bacterium that is resistant to the lysosomal enzymes is *Mycobacterium tuberculosis,* probably at least partly because of its waxy external layer. Still other bacteria prevent fusion of phagosomes with lysosomes (e.g., *Chlamydia*). Phagocytosis (section 31.3)

Evading the Specific Immune Response

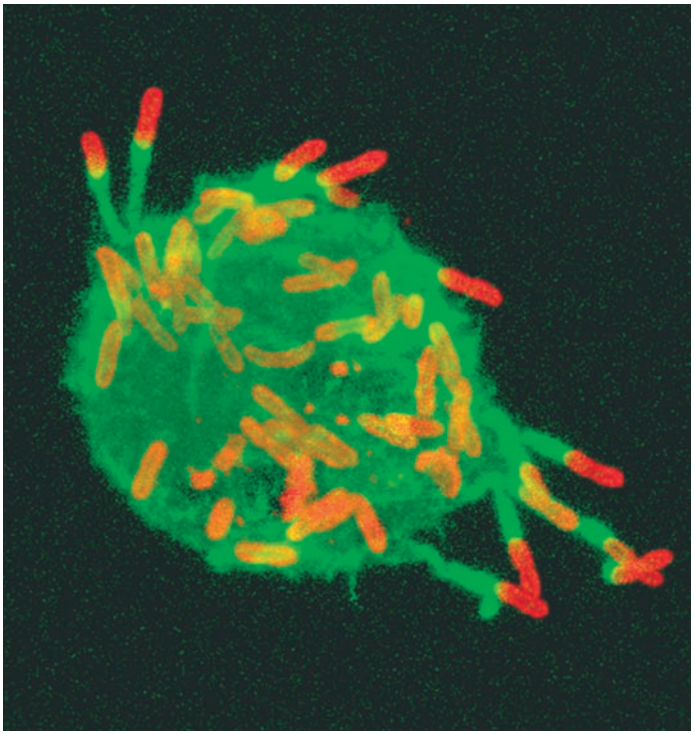
To evade the specific immune response, some bacteria (e.g., *S. pyogenes*) produce capsules that are not antigenic because they resemble host tissue components. *N. gonorrhoeae* can evade the specific immune response by two mechanisms: (1) it makes genetic variations in its pili (phase variation) so that specific antibodies are useless against the new pili and adherence to host tissue occurs, and (2) it produces IgA proteases that destroy secretory IgA and allow adherence. Finally, some bacteria produce proteins (such as staphylococcal protein A and protein G of *S. pyogenes*) that interfere with antibody-mediated opsonization by binding to the Fc portion of immunoglobulins.

1. What are some mechanisms viruses use to evade host defenses?
2. How do bacteria evade each of the following host defenses: the complement system, phagocytosis, and the specific immune response?

Figure 33.9 Formation of Actin Tails by Intracellular Bacterial Pathogens. **(a)** Transmission electron micrograph of *Lysteria monocytogenes* in a host macrophage. The bacterium has polymerized host actin into a long tail that it uses for intracellular propulsion and to move from one host cell to another. **(b)** *Burkholderia pseudomallei* (stained red) also forms actin tails (stained dark green) as shown in this confocal micrograph. Note that the actin tails enable the bacterial cells to be propelled out of the host cell.



(a)



(b)

Summary

33.1 Host-Parasite Relationships

a. Parasitism is a type of symbiosis between two species in which the smaller organism is physiologically dependent on the larger one, termed the host. The parasitic organism usually harms its host in some way.

b. An infection is the colonization of the host by a parasitic organism. An infectious disease is the result of the interaction between the parasitic organism and its host, causing the host to change from a state of health to one of a diseased state. Any organism that produces such a disease is a pathogen (**figure 33.1**).

c. Pathogenicity refers to the quality or ability of an organism to produce pathological changes or disease. Virulence refers to the degree or intensity of pathogenicity of an organism and is measured experimentally by the LD_{50} or ID_{50} (**figure 33.2**).

33.2 Pathogenesis of Viral Diseases

a. The fundamental process of viral infection is the expression of the viral replicative cycle in a host cell. To produce disease a virus enters a host, comes into contact with susceptible cells, and reproduces.

b. Viruses spread to adjacent cells when they are released by either host cell lysis or budding.

c. Host cell damage caused by viruses stimulates a host immune response involving neutralizing antibodies for free virions and activated killer cells for intracellular viruses.

d. Recovery from infection results when the virus has either been cleared from the body of the host, establishes a persistent infection, or kills the host.

e. The viral infection cycle is complete when the virus is shed back into the environment, to be acquired by another host.

33.3 Overiew of Bacterial Pathogenesis

a. Pathogens or their products can be transmitted to a host by either direct or indirect means. Transmissibility is the initial requisite in the establishment of an infectious disease.

b. Special adherence factors allow pathogens to bind to specific receptor sites on host cells and colonize the host (**table 33.2** and **figure 33.3**).

c. Pathogens can enter host cells by both active and passive mechanisms. Once inside, they can produce specific products and/or enzymes that promote dissemination throughout the body of the host. These are termed virulence factors (**table 33.3**).

d. The pathogen generally is found in the area of the host's body that provides the most favorable conditions for its growth and multiplication.

e. During coevolution with human hosts, some pathogenic bacteria have evolved complex signal transduction pathways to regulate the genes necessary for virulence.

f. Many bacteria are pathogenic because they have large segments of DNA called pathogenicity islands that carry genes responsible for virulence.

33.4 Toxigenicity

a. Intoxications are diseases that result from the entrance of a specific toxin into a host. The toxin can induce the disease in the absence of the toxin-producing organism. Toxins produced by pathogens can be divided into two main categories: exotoxins and endotoxins (**table 33.4**).

b. Exotoxins are soluble, heat-labile, potent, toxic proteins produced by the pathogen. They have very specific effects and can be categorized as neurotoxins, cytotoxins, or enterotoxins. Most exotoxins conform to the AB model in which the A subunit or fragment is enzymatic and the B subunit or fragment, the binding portion (**table 33.5**). Several mechanisms exist by which the A component enters target cells (**figure 33.5**).

c. Exotoxins can be divided into four types: (1) the AB toxins, (2) specific host site toxins (neurotoxins, enterotoxins, cytotoxins), (3) toxins that disrupt plasma membranes of host cells (leukocidins, hemolysins, and phospholipases), and (4) superantigens.

d. Bacterial exotoxins cause disease in a human host in three main ways: (1) ingestion of preformed exotoxin, (2) colonization of a mucosal surface followed