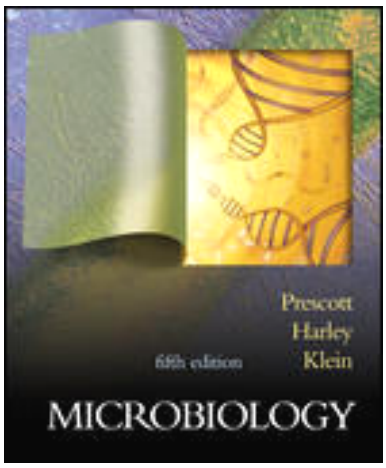

Microbiology

5th Edition

Lansing M. Prescott

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Overview

Prescott, Harley and Klein's 5th edition provides a balanced, comprehensive introduction to all major areas of microbiology. Because of this balance, *Microbiology, 5/e* is appropriate for students preparing for careers in medicine, dentistry, nursing, and allied health, as well as research, teaching, and industry. Biology and chemistry are prerequisites. The Fifth Edition has been updated extensively to reflect the latest discoveries in the field.

New to This Edition

- Every chapter in the book has been updated to reflect the latest discoveries in microbiology, including information on genomics, biofilms, mechanisms of toxins, classification, and emerging diseases. The most extensive revision has occurred in the areas of genetics, microbial ecology, and immunology where material has been updated and reorganized to allow for easier use.
- New Genomics chapter: Chapter 15. The genetics coverage has been reorganized for clarity and ease of teaching. The genetics section now ends with a completely new chapter on genomics. New Chapter 28 on microorganism interactions and microbial ecology!
- Newly developed art program--much of the art is new or revised! It incorporates color and style consistency throughout so students will easily identify certain topics.
- New critical thinking questions have been added to provide practice in analyzing data, predicting outcomes, and to teach students how to think logically.
- The general organization of the text has been modified to provide a more logical flow of topics and give greater emphasis to microbial ecology

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Features

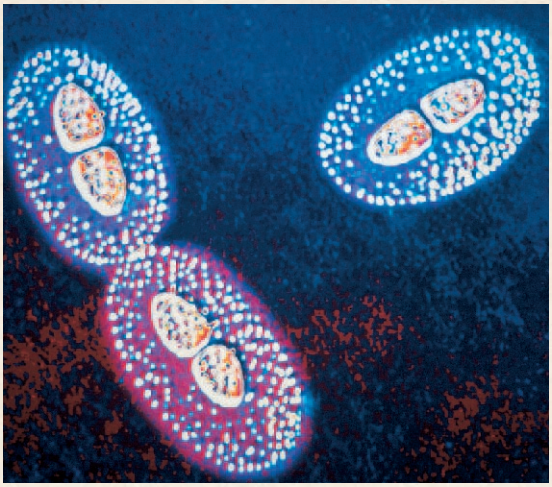
- Prescott's textbook contains briefer chapters than most books, but more of them (42). Students will find the concise chapters more palatable and less intimidating. Short chapters give the instructor the opportunity to fit the text more closely to the instructor's syllabus. Topic flexibility is allowed.
- There is an outstanding pedagogical system including outlines, concepts, key terms, cross-referencing, readings, new critical thinking questions, etc., to help students understand difficult material.

PART X

Microbial Diseases and Their Control

CHAPTER 34

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 since it prevents phagocytic cells from accomplishing phagocytosis.

Outline

Concepts

1. 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 body of an animal is referred to as the host.
2. 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.
3. The obligatory steps for the infectious process involving viral diseases are: a virus must enter a host, come into contact with susceptible cells, replicate within the cells, spread to adjacent cells, cause cellular injury, engender a host immune response, be cleared from the body of the host or establish a persistent infection, and be shed back into the environment.
4. The obligatory steps for the infectious process involving bacterial diseases are: the bacterium must be transmitted to a suitable host, attach to and/or colonize the host, grow and multiply within or on the host, and interfere with or impair the normal physiological activities of the host.
5. One important result of the conservation of chromosomal genes is that bacteria are clonal. Only one or a few clonal types of some bacterial pathogens exist in the environment.
6. During coevolution with human hosts, some pathogenic bacteria have evolved complex signal transduction pathways to regulate the genes necessary for virulence.
7. Many bacteria are pathogenic because they have large segments of DNA, called pathogenicity islands, that carry genes responsible for virulence.
8. Two distinct categories of disease can be recognized based on the role bacteria play in the disease causing process: infections (invasions) and intoxications.
9. Toxins produced by pathogenic bacteria are either exotoxins or endotoxins.
10. Viruses and bacteria are continuously evolving and producing unique mechanisms that enable them to escape the host's arsenal of defenses.

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

Chapter 28 introduces the concept of symbiosis and deals with two of its subordinate categories: commensalism and mutualism. In this chapter the third category, 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, rickettsiology, chlamydiology, bacteriology, mycology, parasitology (protozoology and helminthology), entomology, and zoology. This chapter examines the parasitic way of life in terms of health and disease in the animal body and emphasizes viral and bacterial disease mechanisms. The chapter concludes with some viral and bacterial mechanisms used to escape host defenses.

34.1 Host-Parasite Relationships

If a symbiont either harms or lives at the expense of another organism (the **host**), it is a **parasitic organism,** and the relationship is called **parasitism.** In this relationship the body of the host can be viewed as a microenvironment that shelters and supports the growth and multiplication of the parasitic organism. The parasitic organism is usually the smaller of the two partners and is metabolically dependent on the host. There are many parasitic agents or organisms among the viruses, bacteria, fungi, plants, and animals (**table 34.1**). By convention, when the word **parasite** is used without qualification, it refers specifically to a protozoan or helminthic (nematode, trematode, cestode) organism.

Several types of parasitism are recognized. If an organism lives on the surface of its host, it is an **ectoparasite;** if it lives in-

Table 34.1 Categorization of Parasitic Organisms and Agents by Size

Discipline		Parasitic Group		Approximate Size
Virology		Prions	Agents 25–400 nm	350 kDa
		Viroids		130 kDa
		Viruses		
Bacteriology		Chlamydiae	Microorganisms (microbiota)	0.2–1.5 µm
		Mycoplasmas		0.3–0.8 µm
		Rickettsias		0.5–2 µm
		Other bacteria		1–10 µm
Mycology		Fungi		3–15 µm diameter (hyphae)
Protozoology	Parasitology	Protozoa		1–150 µm
Helminthology		Nematodes	Parasites	3 mm–30 cm
		Platyhelminthes (cestodes, trematodes)		1 mm–10 m
Entomology		Ticks and mites	Ectoparasites	0.1–15 mm
Zoology		Horsehair worms		10–20 cm
		Mesozoa		Up to 100 cm
		Leeches		1–5 cm

Table 34.2 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 course
Bacteremia	Presence of viable bacteria in the blood
Chronic	Persists over a long time
Covert	Subclinical, no symptoms
Cross	Transmitted between hosts infected with different organisms
Focal	Exists in circumscribed areas
Fulminating	Infectious agent multiplies with great intensity
Iatrogenic	Caused as a result of health care
Latent	Persists 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
Mixed	More than one organism present simultaneously
Nosocomial	Develops during a stay at a hospital or other clinical care facility
Opportunistic	Due to an agent that does not harm a healthy host but takes advantage of an unhealthy one
Overt	Symptomatic
Phytogenic	Caused by plant pathogens
Primary	First infection that often allows other organisms to appear on the scene
Pyogenic	Results 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	Occurs only occasionally
Subclinical (inapparent or covert)	No detectable symptoms or manifestations
Systemic	Spread throughout the body
Toxemia	Condition arising from toxins in the blood
Zoonosis	Caused by a parasitic organism that is normally found in animals other than humans

ternally, it is an **endoparasite.** 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.**

Because, by definition, parasitic organisms are dependent on their hosts, the symbiotic relationship between the host and parasite is a dynamic one (**figure 34.1**). 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 34.2**). 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 an organism or its products. Any organism or agent that produces such a disease is 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 a direct interaction. Conversely, an **opportunistic pathogen** is an organism that is either normally free-living, or a part of the host's normal microbiota, but which may adopt a pathogenic role under certain circumstances, such as when the immune system is compromised.

At times an infectious organism can enter a latent state in which there is no shedding of the organism and no symptoms present within the host. This latency can be either intermittent or quiescent. Intermittent latency is exemplified by the herpes virus that causes cold sores (fever blisters). After an initial infection, the symptoms subside. However, the virus remains in nerve tissue and can be 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

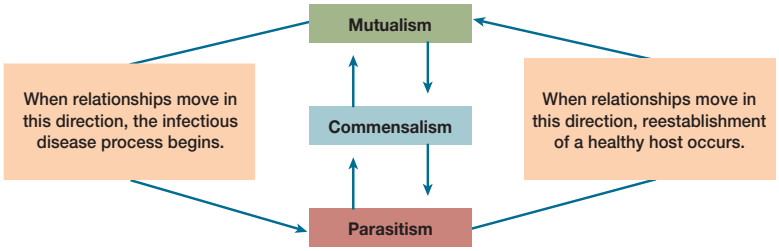


Figure 34.1 Symbiosis. All symbiotic relationships are dynamic, and shifts among them can occur as indicated by the arrows. The most beneficial relationship is mutualism; the most destructive is parasitism. Host susceptibility, virulence of the parasitic organism, and number of parasites are factors that influence these relationships. Disease can result from a shift from either mutualism or commensalism to parasitism. Health may be regained by the reestablishment of mutualism or commensalism.

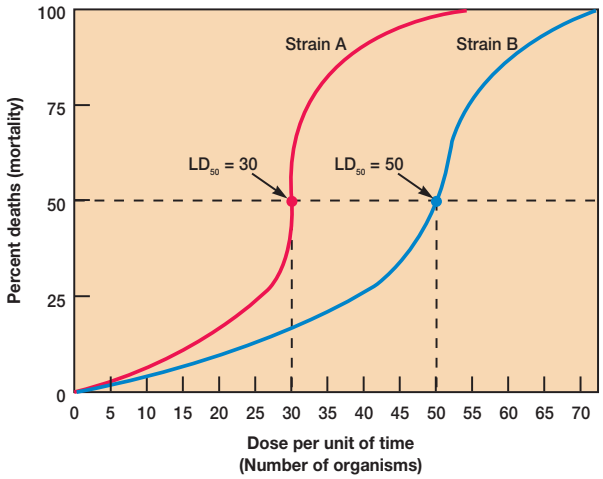


Figure 34.2 Determination of the LD_{50} of a Pathogenic Microorganism. Various doses of a specific pathogen are injected into experimental host animals. Deaths are recorded and a graph constructed. In this example, the graph represents the susceptibility of host animals to two different strains of a 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.

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. Cold sores (p. 884); Chickenpox (varicella) and shingles (herpes zoster) (pp. 871–72)

The outcome of most host-parasite relationships is dependent on three main factors: (1) the number of organisms present in or on the host, (2) the virulence of the organism, and (3) the host's defenses or degree of resistance. 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. A host's resistance can drop so much that its own microbiota may cause disease. Such a disease is sometimes called an endogenous disease because the agent originally comes from within the host's own body. Endogenous diseases 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. It 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 will damage the host and produce disease. Virulence is often measured 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 will either kill or infect, respectively, 50% of an experimental group of hosts within a specified period (**figure 34.2**).

It should be noted that disease can result from causes other than toxin production. Sometimes a host will trigger 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 (*see pp. 906–8*). 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 (*see p. 905*). Hypersensitivity reactions (pp. 768–71)

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?

34.2 Pathogenesis of Viral Diseases

The fundamental process of viral infection is the expression of the viral replicative cycle (*see figures 17.5, 18.4–18.7*) in a host cell. The steps for the infectious process involving viruses are that a virus must

1. Enter a host
2. Contact and enter susceptible cells

3. Replicate within the cells
4. Spread to adjacent cells
5. Cause cellular injury
6. Engender a host immune response
7. Be either cleared from the body of the host, establish a persistent infection, or kill the host
8. Be shed back into the environment

The determinants of pathogenicity are now discussed in more detail.

Entry, Contact, 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 needle sticks, blood transfusions and organ transplants, or by insect **vectors** (organisms that transmit the pathogen from one host to another). 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 enteroviruses enter through the gastrointestinal tract but produce disease in the central nervous system. Mucous membranes (pp. 710–12)

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 virus, herpes simplex, and varicella-zoster viruses; *see figure 38.2*).

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 figures 18.4 and 38.14*).

Cell Injury and Clinical Illness

Destruction of the virus-infected cells in the target tissues and alterations in host physiology are responsible for the development of viral disease and clinical illness. Some tissue (e.g., intestinal epithelium) can rapidly regenerate after a viral attack and withstand extensive damage. Other tissues, such as nervous system tissues, are not able to regenerate and may never resume normal functioning after damage has occurred.

The potential effects of viruses on individual host cells are the result of a complex series of events. There are four generally accepted patterns of a viral infection. (1) In lytic infections the virus multiplies and kills the host cell immediately and new virions are released. (2) In persistent viral infections the virus lives in the host cell and releases small numbers of virions over a long period of time. This causes little damage to the host cell. (3) In latent infections, the virus resides in the cell but produces no virions. At some later time the virus can be activated and a lytic infection occurs (*see section 18.4*). (4) Some viruses can transform the host cell into a cancer cell that becomes the focal point for a tumor (*see section 18.5*).

Host Immune Response

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

Recovery from Infection

The host will either succumb or recover from a viral infection. Recovery mechanisms 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 will be covered in chapter 38.

Virus Shedding

The last step in the infectious process is shedding of the infectious 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 and can spread the virus. In some viral infections, such as a rabies infection, humans are dead-end hosts because virus shedding does not occur.

1. For a virus to cause disease, certain steps must be accomplished. Briefly describe each of these steps.
2. What are the four most common patterns of viral infections?

34.3 Pathogenesis of Bacterial Diseases

The steps for infections by pathogenic bacteria include

1. Maintain a reservoir. A **reservoir** is a place to live before and after causing an infection (*see p. 854*).
2. Initially be transported to the host.
3. Adhere to, colonize, and/or invade the host.
4. Multiply (grow) or complete its life cycle on or in the host or the host's cells.
5. Initially evade host defense mechanisms.
6. Possess the ability to damage the host.
7. Leave the host and return to the reservoir or enter a new host.

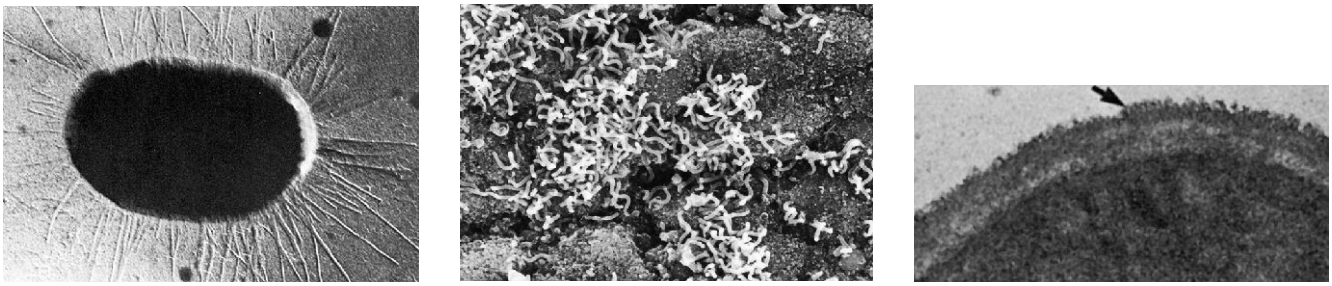
The first five factors influence the degree of infectivity and invasiveness. Toxigenicity plays a major role in the sixth. The determinants are now discussed in more detail.

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

Table 34.3 Bacterial Adherence Factors (Adhesins) 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 archaeobacteria and eubacteria 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 34.3 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.

the pathogen is part of the infectious disease cycle, this aspect of pathogenicity is discussed in detail in chapter 37, which covers the epidemiology of infectious diseases. The infectious disease cycle (pp. 852–58)

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 later. Soil, water, and food are indirect vehicles that harbor and transmit bacteria to hosts. 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 and 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 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 and many nonpathogens adhere with a high degree of specificity to particular tissues. Adherence factors called **adhesins** (**table 34.3**) are one reason for this specificity. Adhesins are specialized molecules or structures on the bacteria's cell surface that bind to complementary receptor sites on the host cell surface (**figure 34.3**). They are one type of virulence factor. **Virulence factors** are bacterial products or structural components (e.g., capsules and adhesins) that contribute to virulence or pathogenicity.

Invasion of the Bacterial Pathogen

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 ground substance 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 mu-

Table 34.4 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, causing anemia and weakened host defenses; make iron available for microbial growth.
Hyaluronidase	Groups A, B, C, and G streptococci, staphylococci, clostridia	Hydrolyzes hyaluronic acid, a constituent of the intercellular ground substance 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 (phosphatidycholine) 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 typhimurium*	Inhibit leukocyte phagocytosis by activating the adenylate cyclase system.
Protein A	*Staphylococcus aureus*	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.

cous 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; and (5) existing eucaryotic internalization pathways (e.g., endocytosis and phagocytoses; *see figure 31.16*).

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 products and/or enzymes that promote spreading (**table 34.4**). These products are 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.

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 (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 invade specific cells in which they grow and multiply. Many of these intracellular pathogens have evolved such elaborate nutrient-gathering mechanisms that they have become totally dependent on the host's cells. Finally, 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].

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.

The Clonal Nature of Bacterial Pathogens

The main mechanism bacteria use to exchange genetic information is the transfer of extrachromosomal genetic elements, plasmids, and phages (*see chapter 13*). Many genes that code for bacterial virulence factors are found on plasmids or phages. These mobile genetic elements can transfer virulence factors between members

of the same species or different species by horizontal gene transfer (*see section 13.1*). At times the genetic elements are part of highly mobile DNA (transposons, *see section 13.3*), and there is a recombination between the extrachromosomal DNA and the chromosome. When this recombination occurs, the genes coding for virulence may become chromosomal.

One important result of the conservation of these chromosomal genes is that the bacteria are clonal. Some bacterial pathogens have only one or a few clonal types existing in the environment. For example, the bacterium that causes typhoid fever (*Salmonella typhi*) has two clonal types, whereas there are over 100 clonal types of *Haemophilus influenzae,* but only a small number are associated with bacterial pneumonia.

Regulation of Bacterial Virulence Factors

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. 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 (figure 34.5*b*) 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 phages. Expression of the virulence gene 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 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. Osmolality and available amino acids are also important.

Pathogenicity Islands

Many bacteria (*Yersinia* spp., *Pseudomonas aeruginosa, Shigella flexneri, Salmonella typhimurium,* enteropathogenic *E. coli*) are pathogenic because they have large segments of DNA, called **pathogenicity islands,** that carry genes responsible for virulence. These pathogenicity islands have been acquired during evolution by horizontal gene transfer. A pathogen may have more than one pathogenicity island. 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 20 genes encode a pathogenicity mechanism termed the **type III secretion system** that enables gram-negative bacteria to secrete and inject virulence proteins into the cytoplasm of eucaryotic host cells (**figure 34.4**). 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 initiates sophisticated "biochemical crosstalk" between the pathogen and the host. The injected proteins resemble eucaryotic factors that signal transduction functions and they are capable of interfering with eucaryotic signaling pathways. Redirection of cellular signaling transduction may 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 not present in nonpathogenic members of the same genus or species. One specific example is found in *E. coli.* The enteropathogenic *E. coli* possesses large DNA fragments, 35 to 170 kilobases in size, that contain several virulence genes absent from commensal *E. coli.* 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 mucous 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?
6. What is the significance of the clonal nature of bacterial pathogens? The regulation of virulence factors?

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 the entrance of a specific preformed toxin (e.g., botulinum toxin) into the body of a host. 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. 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 34.5.**

Exotoxins

Exotoxins are soluble, heat-labile, proteins (a few are enzymes) that usually are released into the surroundings as the bacterial pathogen grows. Often exotoxins may travel from the site of infection to other body tissues or target cells in which they exert their effects. Exotoxins usually are

1. Synthesized by specific bacteria that often have plasmids or prophages bearing the exotoxin genes

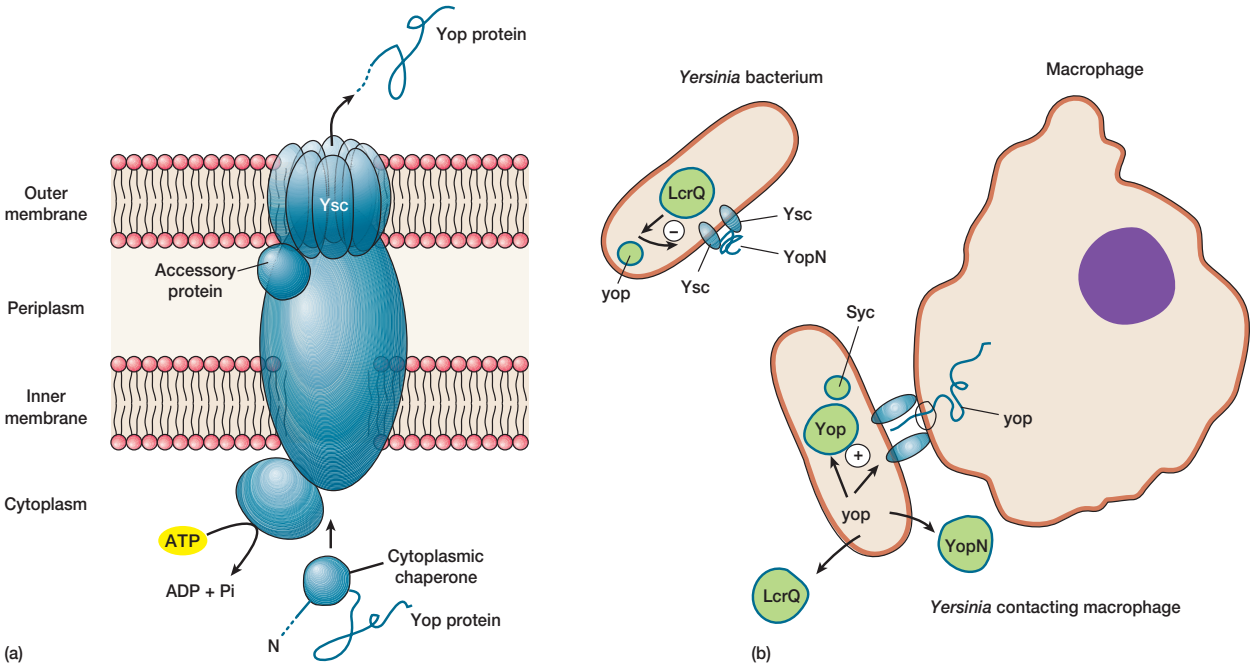


Figure 34.4 **Type III Secretion System and Mode of Action of Yop Virulence Protein.** (**a**) Schematic drawing of the type III secretion system and Yop (a specific virulence protein) secretion by *Yersinia* spp. (**b**) In this schematic, prior to cell contact, the type III secretion channels (Ysc) are kept shut by YopN, and cytoplasmic accumulation of the negative regulatory factor LcrQ leads to transcription repression of *yop* genes (negative sign in circle). After *Yersinia* contacts the macrophage, YopN is released, allowing rapid secretion of LcrQ, which in turn relieves the block of *yop* gene expression (positive sign in circle). Anti-host Yop proteins, protected by cognate chaperones (Syc), are translocated into the macrophage by the type III secretion machinery (as shown in part *a*). After contact of the *Yersinia* bacterium with a macrophage, Yop is injected into the cytoplasm of the target cell where it catalyzes a rapid and specific dephosphorylation of several macrophage proteins that are required for normal phagocytosis.

Table 34.5 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 release of interleukin-1
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
Immune response	Antitoxins provide host immunity; highly antigenic	Limited antibodies produced; weakly immunogenic
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 microgram quantities	Moderate toxicity
Toxoid production	Converted to antigenic, nontoxic toxoids; toxoids are used to immunize (e.g., tetanus toxoid)	Toxoids cannot be made

2. Heat-labile proteins inactivated at 60 to 80°C
3. Among the most lethal substances known (toxic in very small doses [microgram per kilogram amounts]; e.g., the botulinum toxin)
4. Associated with specific diseases and have specific mechanisms of action
5. Highly immunogenic and stimulate the production of neutralizing antibodies called **antitoxins**
6. Easily inactivated by formaldehyde, iodine, and other chemicals to form immunogenic **toxoids**
7. Unable to produce a fever in the host directly
8. Often given the name of the disease they produce (e.g., the diphtheria toxin)

Exotoxins can be divided 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. (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 to release cytokines. Examples of the first three types are now discussed. Superantigens are discussed in detail in section 32.2. The general properties of some AB exotoxins are presented in **table 34.6.**

Table 34.6 Properties of Some AB Model Bacterial Exotoxins

Toxin	Organism	Genetic Control	Subunit Structure	Target Cell Receptor	Enzymatic Activity	Biologic Effects
Anthrax toxins	*B. anthracis*	Plasmid	Three separate proteins (EF, LF, PA)[a]	Unknown, probably glycoprotein	EF is a calmodulin-dependent adenylate cyclase; LF enzyme activity is unknown	EF + PA: increase in target cell cAMP level, localized edema; LF + PA: death of target cells and experimental animals
Bordetella adenylate cyclase toxin	*Bordetella* spp.	Chromosomal	A-B[b]	Unknown, probably glycolipid	Calmodulin-activated cyclase	Increase in target cell cAMP level; modified cell function or cell death
Botulinum toxin	*C. botulinum*	Phage	A-B[c]	Possibly ganglioside (GD_{1b})	Zinc-dependent endopeptidase cleavage of synaptobrevin	Decrease in peripheral, presynaptic acetylcholine release; flaccid paralysis
Cholera toxin	*V. cholera*	Chromosomal	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	*C. 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	*B. pertussis*	Chromosomal	A-5B[g]	Unknown, probably glycoprotein	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	*S. 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 A_1 and A_2, with A_1 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.

AB Exotoxins. AB toxins are composed of an enzymatic subunit or fragment (A) that is responsible for the toxic effect once inside the host cell and a binding subunit or fragment (B). Isolated A subunits are enzymatically active but lack binding and cell entry capability, whereas isolated 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 GM_1 for cholera toxin, GT_1 and/or GD_1 for tetanus toxin, and GD_1 for botulinum toxin.

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 34.5*a***). In another mechanism entry is by receptor-mediated endocytosis (figure 34.5*b*).

The mechanism of action of an AB toxin can be quite complex, as shown by the example of diphtheria toxin (figure 34.5*b*). The diphtheria toxin is a protein of about 62,000 mol wt. It binds to cell surface receptors by the B fragment portion and is taken into the cell through the formation of a clathrin-coated vesicle (*see p. 403*). The toxin then enters the vesicle membrane and is cleaved into two parts, one of which, the A fragment, escapes into the cytosol. The A fragment 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 (*see pp. 270–71*). 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.

AB exotoxins vary widely in their relative contribution to the disease process with which they are associated.

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 34.6: neurotoxins (botulinum toxin and tetanus toxin), enterotoxins (cholera toxin, *E. coli* heat-labile toxins), and cytoxins (diphtheria toxin, Shiga toxin).

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. 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 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 the lumen of the gut. The genes for this enterotoxigenicity reside on the *Vibrio cholera* chromosome. Toxins in food (pp. 926–33, 975–76); Toxins as superantigens (p. 732)

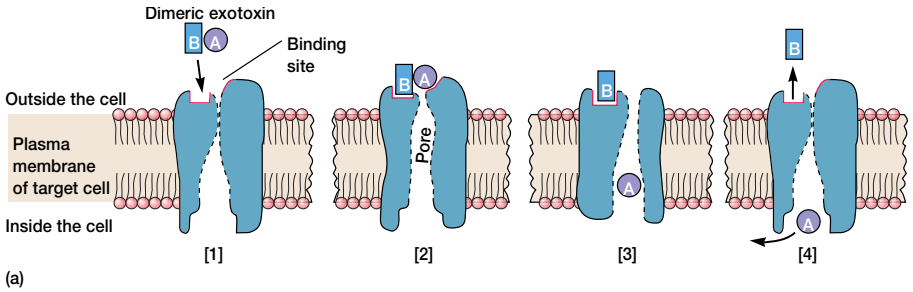
Cytotoxins have a specific toxic action upon cells/tissues of special organs and are named according to the type of cell/tissue or organ for which they are specific. Examples include nephrotoxin (kidney), hepatotoxin (liver), and cardiotoxin (heart).

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 (**figure 34.6*a***), 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). 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.

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 pneumonococci, 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 (*see figure 23.17*). A complete zone of clearing around the bacterial colony growing on blood agar is called **beta hemolysis,** and a partial clearing of the blood 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. SLO and SLS act as 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 sub-type of membrane-disrupting toxins are the **phospholipase** enzymes. Phospholipases remove the charged head group (figure 34.6*b*) from the lipid portion of the phospholipids in the host-cell plasma membrane. This destabilizes the membrane and the cell lyses and dies. One example of the pathogenesis caused by phospholipases is the disease gas gangrene. In this disease, the *Clostridium perfringens* α-toxin almost totally destroys the local population of white blood cells.

Roles of Exotoxins in Disease. Bacterial exotoxins affect a human host 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.



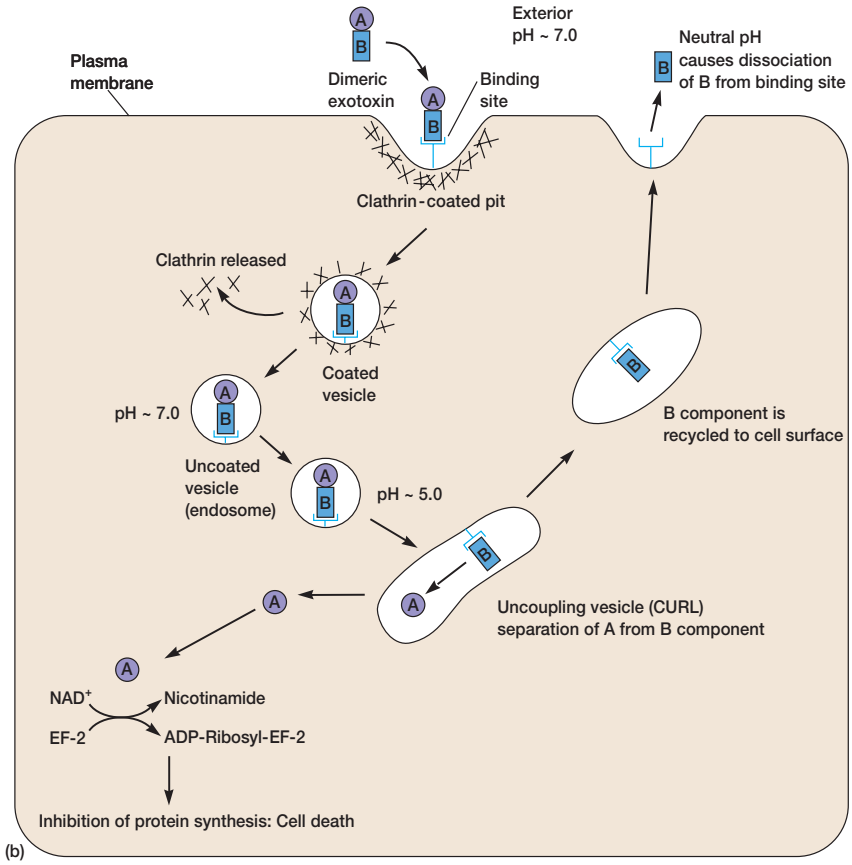


Figure 34.5 **Diagrammatic Representation of Two AB Exotoxin Transport Mechanisms.** (**a**) Domain B of the dimeric exotoxin (AB) binds to a specific membrane receptor of a target cell [*1*]. A conformational change [*2*] generates a pore [*3*] through which the A domain crosses the membrane and enters the cytosol, followed by recreation [*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 (compartment of *u*ncoupling of *r*eceptor and *l*igand). The B domain is then recycled to the cell surface. The A domain moves through the cytosol, catalyzes the ADP-ribosylation of EF-2 (elongation factor 2) and inhibits protein synthesis, leading to cell death.

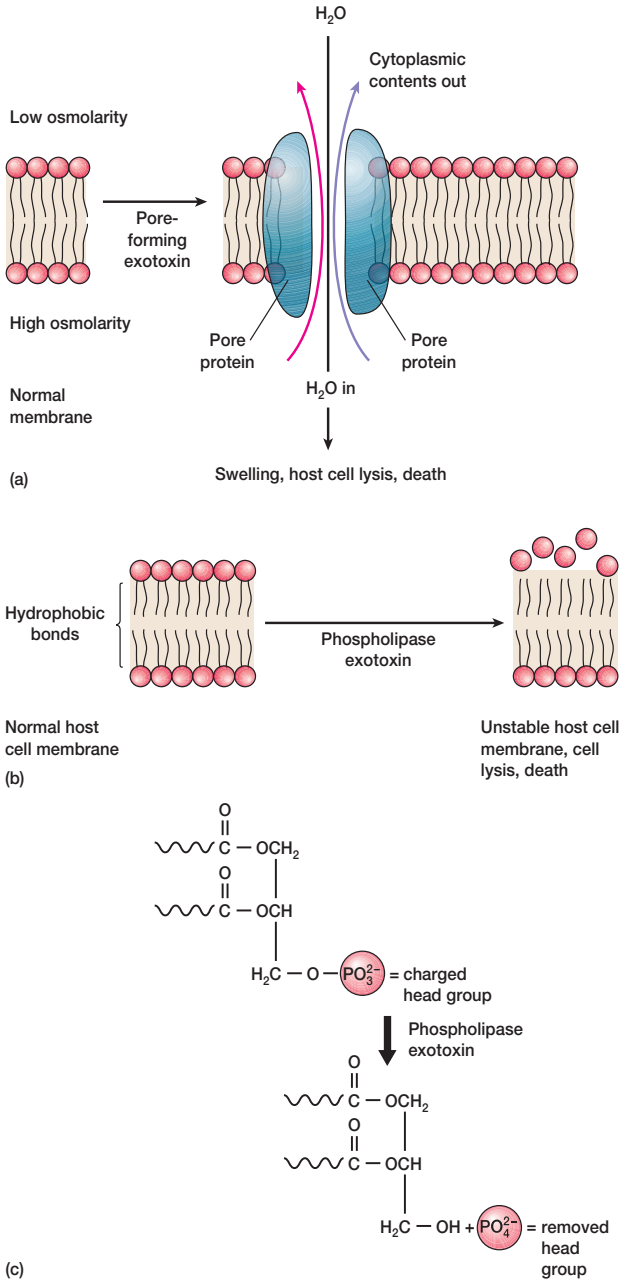


Figure 34.6 Two Subtypes of Membrane-Disrupting Exotoxins. (**a**) A channel-forming (pore-forming) type of exotoxin that inserts itself into the normal host cell membrane and makes an open channel (pore). Formation of multiple pores causes cytoplasmic contents to leave the cell and water to move in, leading to cellular lysis and death of the host cell. (**b**) A phospholipid-hydrolyzing phospholipase exotoxin destroys membrane integrity. (**c**) 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.

In the first example (**figure 34.7*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 (*see section 39.4*) 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 34.7*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* (*see section 39.4*). Once the bacteria enter the body, they adhere to the intestinal mucosa where they are not invasive but secrete the cholera toxin, which is an AB exotoxin that catalyzes an ADP– ribosylation similar to that of diphtheria exotoxin (figure 34.5*b*). As a result, cholera toxin stimulates hypersecretion of water and chloride ions and the patient loses massive quantities of water.

The third example of exotoxins in disease pathogenesis occurs when bacteria grow in a wound or abscess (figure 34.7*c*). The exotoxin causes local tissue damage or kills phagocytes that enter the infected area. A disease of this type is gas gangrene (*see section 39.3*) in which the exotoxin (α-toxin) of *Clostridium perfringens* causes the 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 (*see figures 3.23–3.25*) is called an **endotoxin** because it is bound to the bacterium and is released when the microorganism lyses (**Box 34.1**). 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 (see characteristic 5 on p. 801) associated with endotoxicity and gram-negative bacteremia. Gram-negative cell wall (pp. 58–60)

Box 34.1

Detection and Removal of Endotoxins

Bacterial endotoxins have plagued the pharmaceutical industry and medical device producers for years. For example, administration of drugs contaminated with endotoxins can result in complications—even death—to patients. Recently endotoxins have become a problem 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 *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 0.1μm. Thus they cannot be removed by conventional filtration systems. Manufacturers are currently developing special filtration systems and filtration cartridges that retain these endotoxins and help alleviate contamination problems.

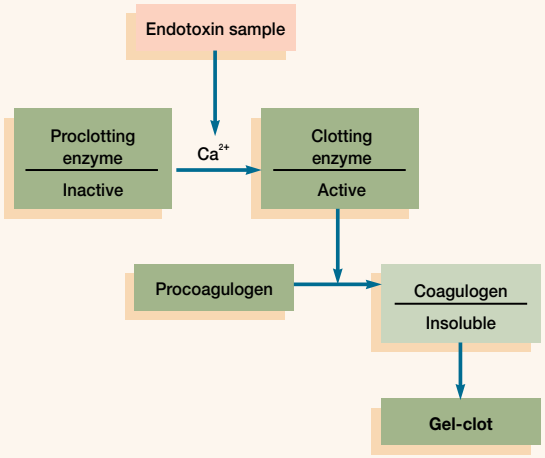
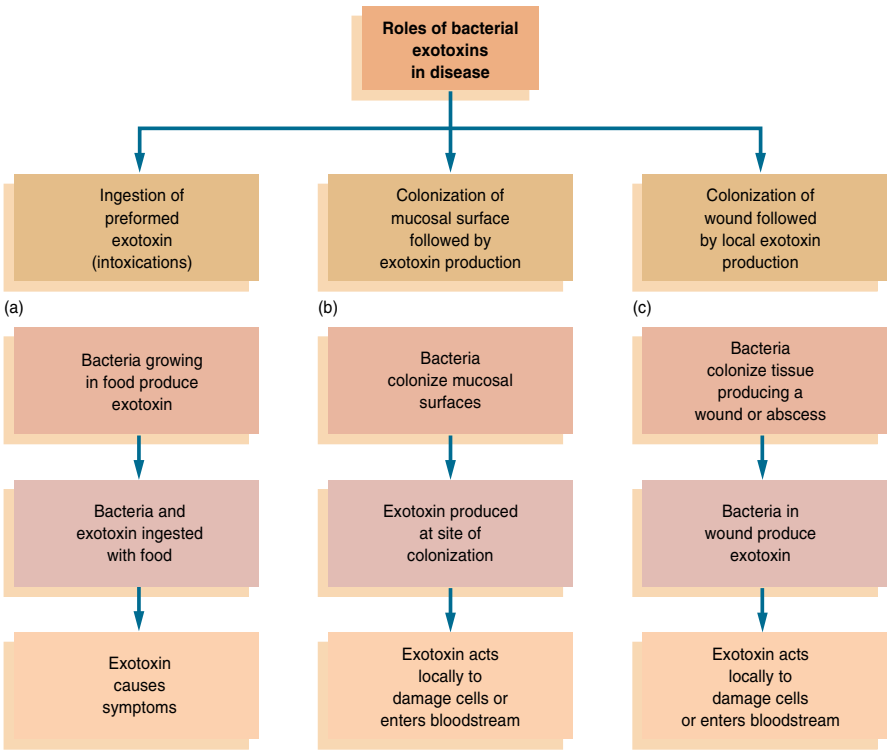


Figure 34.7 Roles of Exotoxins in Disease Pathogenesis. Three ways (**a**, **b**, **c**) in which bacterial exotoxins can contribute to the progression of disease in a human.



Besides the preceding characteristics, bacterial endotoxins are

1. Heat stable
2. Toxic only at high doses (milligram per kilogram 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)

The characteristics of endotoxins and exotoxins are contrasted in table 34.5.

The main biological effect of LPS is an indirect one, being mediated by host molecules and systems rather than by LPS 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.

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 (*see figure 31.19*). Other cytokines released by macrophages, such as the tumor necrosis factor, also produce fever.

Recent evidence indicates that LPS affects macrophages and monocytes by binding to special plasma proteins called **LPS-binding proteins.** The LPS-LPS-binding protein complex then attaches to receptors on monocytes, macrophages, and other cells. This triggers several events, including the production of cytokines IL-1, IL-6, and tumor necrosis factor. As mentioned previously, IL-1 and tumor necrosis factor induce fever. These cytokines also promote other endotoxin effects: complement activation, coagulation, prostaglandin formation, and so forth.

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?

34.4 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 escaping 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 (*see section 33.2*), 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 just now becoming recognized through genomics and the functional analysis of specific gene products. Several examples follow.

Some viruses may mutate and change antigenic sites (antigenic drift, *see section 37.5*) on the virion proteins (the influenza virus) or may down-regulate the level of expression of viral cell surface proteins (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 (*see section 32.4*). 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 (*see section 32.6*) so that there is insufficient free antibody to bind with the complete viral particle.

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. Several of these evasive mechanisms are now discussed.

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 31.14*) 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.

Complement activation (pp. 714–18, 758–59)

Resisting Phagocytosis

As noted several times previously, before a phagocytic cell can phagocytose 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 can resist phagocytosis in quite different ways. For example, *Staphylococcus* produces leukocidins

that destroy phagocytes before phagocytosis can occur. *Streptococcus 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. Such pathogens are very pathogenic because they are impervious to a most important host protective mechanism. One evading mechanism is to escape from the phagosome before it merges with the lysosome, as seen with *Listeria monocytogenes, Shigella,* and *Rickettsia.* 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 (*Chlamydia*). Phagocytosis (pp. 718–20)

Evading the Specific Immune Response

To evade the specific immune response, some bacteria (*Streptococcus pyogenes*) produce capsules that are not antigenic since they resemble host tissue components. *N. gonorrhoeae* can also 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 *Streptococcus pyogenes*) that interfere with antibody-mediated opsonization (*see figure 31.15*) 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?

Summary

1. 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.
2. An infection is the colonization of the host by a parasitic organism. An infectious disease is the result of the interaction (**figure 34.1**) between the parasitic organism and its host, causing the host to change from a state of health to a diseased state. Any organism that produces such a disease is a pathogen.
3. 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 34.2**).
4. The fundamental process of viral infection is the expression of the viral replicative cycle in a host cell. To produce disease a virus must enter a host; come into contact with susceptible cells; reproduce; spread to adjacent cells; cause cellular injury; engender a host immune response; be either cleared from the body of the host, establish a persistent infection, or kill the host; and be shed back into the environment.
5. 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.
6. Special adherence factors (**table 34.3**) allow pathogens to bind to specific receptor sites on host cells and colonize the host (**figure 34.3**).
7. 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 34.4**).
8. The pathogen generally is found in the area of the host's body that provides the most favorable conditions for its growth and multiplication.
9. In bacteria, one important result of the conservation of chromosomal genes is that bacteria are clonal. For most pathogenic bacteria, there are only a few clonal types that exist in the environment.
10. During coevolution with human hosts, some pathogenic bacteria have evolved complex signal transduction pathways to regulate the genes necessary for virulence.
11. Many bacteria are pathogenic because they have large segments of DNA called pathogenicity islands that carry genes responsible for virulence.
12. 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 34.5**).
13. Exotoxins are soluble, heat-labile, potent, toxic proteins produced by the pathogen as a result of its normal metabolism. 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 34.6**). Several mechanisms exist by which the A component enters target cells (**figure 34.5**).
14. Exotoxins can be divided into four types: (a) the AB toxins, (b) specific host site toxins (neurotoxins, enterotoxins, cytotoxins), (c) toxins that disrupt plasma membranes of host cells (leukocidins, hemolysins, and phospholipases), and (d) superantigens.
15. Bacterial exotoxins cause disease in a human host in three main ways: (a) ingestion of preformed exotoxin, (b) colonization of a mucosal surface followed by exotoxin production, and (c) colonization of a wound followed by local exotoxin production.
16. Endotoxins are heat-stable, toxic substances that are part of the cell wall lipopolysaccharide of some gram-negative bacteria. Most endotoxins function by initially activating Hageman Factor, which in turn activates one to four humoral systems. These include the intrinsic blood clotting cascade, complement activation, fibrinolytic system, and kininogen system. Endotoxins also stimulate macrophages to release cytokines such as IL-1, IL-6, and TNF-α.
17. During the course of microbe and human evolution, some pathogens have evolved ways for escaping host defenses. Viruses have mechanisms that either suppress or evade the host's immune response. Bacteria have evolved mechanisms to evade the complement system, phagocytosis, and the specific immune response.

Key Terms

AB toxins *797*
adhesin *792*
alpha hemolysis *797*
antitoxin *796*
bacteremia *793*
beta hemolysis *797*
colonization *792*
cytotoxin *797*
ectoparasite *788*
endogenous pyrogen *801*
endoparasite *789*
endotoxin *799*
endotoxin unit (E.U.) *800*
enterotoxin *797*
exotoxin *794*
final host *789*
fomite *792*
hemolysin *797*
host *788*
immunopathology *790*
infection *789*
infectious disease *789*
infectious dose 50 (ID_{50}) *790*
infectivity *790*
intermediate host *789*
intoxication *794*
invasiveness *790*
lethal dose 50 (LD_{50}) *790*
leukocidin *797*
LPS-binding protein *801*
membrane-disrupting exotoxin *797*
neurotoxin *797*
opportunistic pathogen *789*
parasite *788*
parasitic organism *788*
parasitism *788*
pathogen *789*
pathogenicity *789*
pathogenic potential *790*
pathogenicity island *794*
phospholipase *797*
primary (frank) pathogen *789*
reservoir *791*
reservoir host *789*
septicemia *793*
serum resistance *801*
streptolysin-O (SLO) *797*
streptolysin-S (SLS) *797*
toxemia *794*
toxigenicity *790*
toxin *794*
toxoid *796*
transfer host *789*
tropism *791*
type III secretion system *794*
vector *791*
viremia *791*
virulence *790*
virulence factor *792*

Questions for Thought and Review

1. Why does a parasitic organism not have to be a parasite?
2. In general, infectious diseases that are commonly fatal are newly evolved relationships between the parasitic organism and the host. Why is this so?
3. What does an organism require to be parasitic?
4. What are some bacterial determinants that provide the organism with the ability to colonize and invade the host?
5. What is the significance of the clonal nature of bacterial pathogens?
6. How do some bacteria regulate their virulence factors?
7. Describe a pathogenicity island.
8. What are four types of exotoxins based on their structural and physiological activities?
9. What is the difference between the general properties of endotoxins and exotoxins?
10. How do some viruses evade the defenses of a human host? How do some bacteria evade the defenses of a human host?

Critical Thinking Questions

1. Explain the observation that different pathogens infect different parts of the host.
2. Intracellular bacterial infections present a particular difficulty for the host. Why is it harder to defend against these infections than against viral infections and extracellular bacterial infections?

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