Bacterial Pathogenesis

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General Concepts

Host Susceptibility

Resistance to bacterial infections is enhanced by phagocytic cells and an intact immune system. Initial resistance is due to nonspecific mechanisms. Specific immunity develops over time. Susceptibility to some infections is higher in the very young and the very old and in immunosuppressed patients.

Bacterial Infectivity

Bacterial infectivity results from a disturbance in the balance between bacterial virulence and host resistance. The "objective" of bacteria is to multiply rather than to cause disease; it is in the best interest of the bacteria not to kill the host.

Host Resistance

Numerous physical and chemical attributes of the host protect against bacterial infection. These defenses include the antibacterial factors in secretions covering mucosal surfaces and rapid rate of replacement of skin and mucosal epithelial cells. Once the surface of the body is penetrated, bacteria encounter an environment virtually devoid of free iron needed for growth, which requires many of them to scavenge for this essential element. Bacteria invading tissues encounter phagocytic cells that recognize them as foreign, and through a complex signaling mechanism involving interleukins, eicosanoids, and complement, mediate an inflammatory response in which many lymphoid cells participate.

Genetic and Molecular Basis for Virulence

Bacterial virulence factors may be encoded on chromosomal, plasmid, transposon, or temperate bacteriophage DNA; virulence factor genes on transposons or temperate bacteriophage DNA may integrate into the bacterial chromosome.

Host-Mediated Pathogenesis

In certain infections (e.g., tuberculosis), tissue damage results from the toxic mediators released by lymphoid cells rather than from bacterial toxins.

Intracellular Growth

Some bacteria (e.g., Rickettsia species) can grow only within eukaryotic cells, whereas others (e.g., *Salmonella* species) invade cells but do not require them for growth. Most pathogenic bacteria multiply in tissue fluids and not in host cells.

Virulence Factors

Virulence factors help bacteria to (1) invade the host, (2) cause disease, and (3) evade host defenses. The following are types of virulence factors:

Adherence Factors: Many pathogenic bacteria colonize mucosal sites by using pili (fimbriae) to adhere to cells.

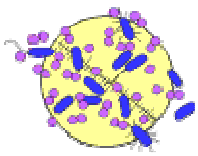
Invasion Factors: Surface components that allow the bacterium to invade host cells can be encoded on plasmids, but more often are on the chromosome.

Capsules: Many bacteria are surrounded by capsules that protect them from opsonization and phagocytosis.

Endotoxins: The lipopolysaccharide endotoxins on Gram-negative bacteria cause fever, changes in blood pressure, inflammation, lethal shock, and many other toxic events.

Exotoxins: Exotoxins include several types of protein toxins and enzymes produced and/or secreted from pathogenic bacteria. Major categories include cytotoxins, neurotoxins, and enterotoxins.

Siderophores: Siderophores are iron-binding factors that allow some bacteria to compete with the host for iron, which is bound to hemoglobin, transferrin, and lactoferrin.



INTRODUCTION

Infection is the invasion of the host by microorganisms, which then multiply in close association with the host's tissues. Infection is distinguished from disease, a morbid process that does not necessarily involve infection (diabetes, for example, is a disease with no known causative agent). Bacteria can cause a multitude of different infections,

ranging in severity from inapparent to fulminating. Table 7-1 lists these types of infections.

TABLE 7-1 Types of Bacterial Infections

Type of Infection	Description	Examples
Inapparent (subclinical)	No detectable clinical symptoms of infection	Asymptomic gonorrhea in women and men
Dormant (latent)	Carrier state	Typhoid carrier
Accidental	Zoonosis or environmental or inadvertent exposures	Anthrax, cryptococcal infection, and laboratory exposure, respectively
Opportunistic	Infection caused by normal flora or transient bacteria when normal host defenses are compromised	*Serrati* or *Candida* infection of the genitourinary tract
Primary	Clinically apparent (e.g., invasion and multiplication of microbes in body tissues, causing local tissue injury)	*Shigella* dysentery
Secondary	Microbial invasion subsequent to primary infection	Bacterial pneumonia following viral lung infection
Mixed	Two or more microbes infecting the same tissue	Anaerobic abscess (*E coli* and *Bacteroides fragilis*)
Acute	Rapid onset (hours or days); brief duration (days or weeks)	Diphtheria
Chronic	Prolonged duration (months or years)	Mycobacterial diseases (tuberculosis and leprosy)
Localized	Confined to a small area or to an organ	Staphylococcal boil
Generalized	Disseminated to many body regions (gonococcemia)	Gram-negative bacteremia
Pyogenic	Pus-forming	Staphyloccal and streptococcal infection
Retrograde	Microbes ascending in a duct or tube against the flow of secretions or excretions	*E coli* urinary tract infection
Fulminant	Infections that occur suddenly and intensely	Airborne *Yersinia pestis* (pneumonic plague)

The capacity of a bacterium to cause disease reflects its relative pathogenicity. On this basis, bacteria can be organized into three major groups. When isolated from a patient, frank or primary pathogens are considered to be probable agents of disease (e.g., when the cause of diarrheal disease is identified by the laboratory isolation of *Salmonella* spp from feces). Opportunistic pathogens are those isolated from patients whose host defense mechanisms have been compromised. They may be the agents of disease (e.g., in patients who have been predisposed to urinary tract infections with *Escherichia coli* by catheterization). Finally, some bacteria, such as Lactobacillus acidophilus, are considered to be nonpathogens, because they rarely or never cause human disease. Their

categorization as nonpathogens may change, however, because of the adaptability of bacteria and the detrimental effect of modern radiation therapy, chemotherapy, and immunotherapy on resistance mechanisms. In fact, some bacteria previously considered to be nonpathogens are now known to cause disease. Serratia marcescens, for example, is a common soil bacterium that causes pneumonia, urinary tract infections, and bacteremia in compromised hosts.

Virulence is the measure of the pathogenicity of an organism. The degree of virulence is related directly to the ability of the organism to cause disease despite host resistance mechanisms; it is affected by numerous variables such as the number of infecting bacteria, route of entry into the body, specific and nonspecific host defense mechanisms, and virulence factors of the bacterium. Virulence can be measured experimentally by determining the number of bacteria required to cause animal death, illness, or lesions in a defined period after the bacteria are administered by a designated route. Consequently, calculations of a lethal dose affecting 50 percent of a population of animals (LD_{50}) or an effective dose causing a disease symptom in 50 percent of a population of animals (ED_{50}) are useful in comparing the relative virulence of different bacteria.

Pathogenesis refers both to the mechanism of infection and to the mechanism by which disease develops. The purpose of this chapter is to provide an overview of the many bacterial virulence factors and, where possible, to indicate how they interact with host defense mechanisms and to describe their role in the pathogenesis of disease. It should be understood that the pathogenic mechanisms of many bacterial diseases are poorly understood, while those of others have been probed at the molecular level. The relative importance of an infectious disease to the health of humans and animals does not always coincide with the depth of our understanding of its pathogenesis. This information is best acquired by reading each of the ensuing chapters on specific bacterial diseases, infectious disease texts, and public health bulletins.

Host Susceptibility

Susceptibility to bacterial infections depends on the physiologic and immunologic condition of the host and on the virulence of the bacteria. Before increased amounts of specific antibodies or T cells are formed in response to invading bacterial pathogens, the "nonspecific" mechanisms of host resistance (such as polymorphonuclear neutrophils and macrophage clearance) must defend the host against the microbes. Development of effective specific immunity (such as an antibody response to the bacterium) may require several weeks (Fig. 7-1). The normal bacterial flora of the skin and mucosal surfaces also serves to protect the host against colonization by bacterial pathogens. In most healthy individuals, bacteria from the normal flora that occasionally penetrate the body (e.g., during tooth extraction or routine brushing of teeth) are cleared by the host's cellular and humoral mechanisms. In contrast, individuals with defective immune responses are prone to frequent, recurrent infections with even the least virulent bacteria. The best-known example of such susceptibility is acquired immune deficiency syndrome (AIDS), in which the $CD4^+$ helper lymphocytes are progressively decimated by human immunodeficiency virus (HIV). However, resistance mechanisms can be altered by many

other processes. For example, aging often weakens both nonspecific and specific defense systems so that they can no longer effectively combat the challenge of bacteria from the environment. Infants are also especially susceptible to certain pathogens (such as group B streptococci because their immune systems are not yet fully developed and cannot mount a protective immune response to important bacterial antigens. In addition, some individuals have genetic defects of the complement system or cellular defenses (e.g., inability of polymorphonuclear neutrophils to kill bacteria). Finally, a patient may develop granulocytopenia as a result of a predisposing disease, such as cancer, or immunosuppressive chemotherapy for organ transplants or cancer.

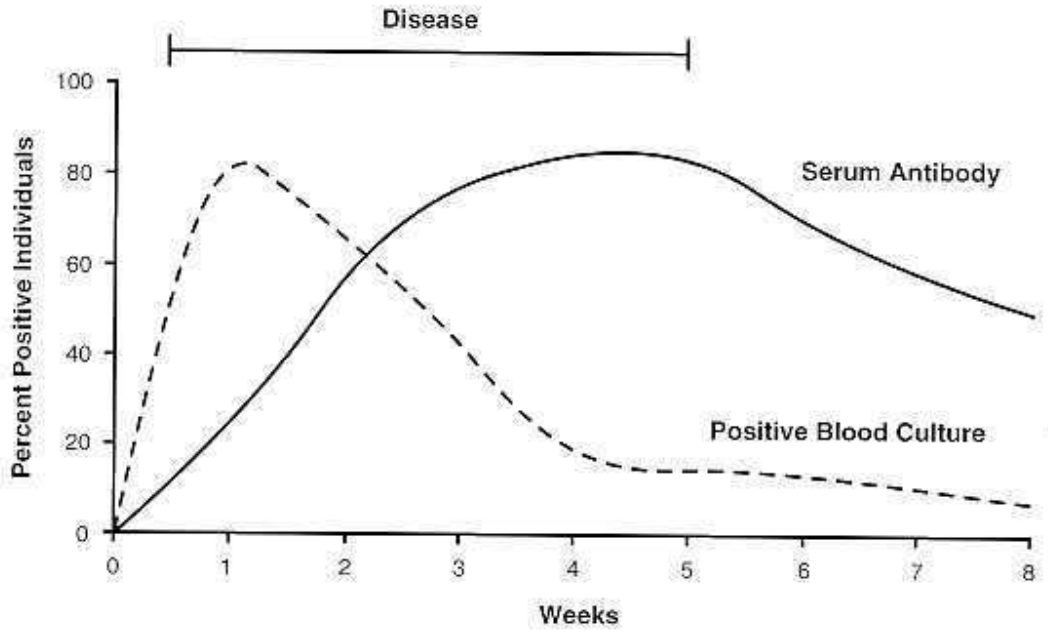


FIGURE 7-1 Serum antibody response to Salmonella typhi during typhoid fever and its relationship to septicemia.

Host resistance can be compromised by trauma and by some underlying diseases. An individual becomes susceptible to infection with a variety of bacteria if the skin or mucosa is breached, particularly in the case of severe wounds such as burns or contaminated surgical wounds. Cystic fibrosis patients, who have poor ciliary function and consequently cannot clear mucus efficiently from the respiratory tract, are abnormally susceptible to infection with mucoid strains of *Pseudomonas aeruginosa*, resulting in serious respiratory distress. Ascending urinary tract infections with *Escherichia coli* are common in women and are particularly troublesome in patients with urinary tract obstructions. A variety of routine medical procedures, such as tracheal intubation and catheterization of blood vessels and the urethra, increase the risk of bacterial infection. The plastic devices used in these procedures are readily colonized by bacteria from the skin, which migrate along the outside of the tube to infect deeper tissues

or enter the bloodstream. Because of this problem, it is standard practice to change catheters frequently (e.g., every 72 hours for peripheral intravenous catheters).

Many drugs have been developed to treat bacterial infections. Antimicrobial agents are most effective, however, when the infection is also being fought by healthy phagocytic and immune defenses. Some reasons for this situation are the poor diffusion of antibiotics into certain sites (such as the prostate gland), the ability of many bacteria to multiply or survive inside cells (where many antimicrobial agents have little or no effect), the bacteriostatic rather than bactericidal action of some drugs, and the capacity of some organisms to develop resistance to multiple antibiotics.

Many bacterial pathogens are transmitted to the host by a vector, usually an arthropod. For example, Rocky Mountain spotted fever and Lyme disease are both vectored by ticks, and bubonic plague is spread by fleas. Susceptibility to these diseases depends partly on the host's contact with the vector.

Pathogenic Mechanisms

Bacterial Infectivity

Factors that are produced by a microorganism and evoke disease are called virulence factors. Examples are toxins, surface coats that inhibit phagocytosis, and surface receptors that bind to host cells. Most frank (as opposed to opportunistic) bacterial pathogens have evolved specific virulence factors that allow them to multiply in their host or vector without being killed or expelled by the host's defenses. Many virulence factors are produced only by specific virulent strains of a microorganism. For example, only certain strains of *E coli* secrete diarrhea-causing enterotoxins.

Virulence factors should never be considered independently of the host's defenses; the clinical course of a disease often depends on the interaction of virulence factors with the host's response. An infection begins when the balance between bacterial pathogenicity and host resistance is upset. In essence, we live in an environment that favors the microbe, simply because the growth rate of bacteria far exceeds that of most eukaryotic cells. Furthermore, bacteria are much more versatile than eukaryotic cells in substrate utilization and biosynthesis. The high mutation rate of bacteria combined with their short generation time results in rapid selection of the best-adapted strains and species. In general, bacteria are much more resistant to toxic components in the environment than eukaryotes, particularly when the major barriers of eukaryotes (skin and mucous membranes) are breached.

From a practical standpoint, bacteria can be said to have a single objective: to multiply. Only a few of the vast number of bacterial species in the environment consistently cause disease in a given host. From a teleologic standpoint, it is not in the best interest of the pathogen to kill the host, because in most cases the death of the host means the death of the pathogen. The most highly evolved or adapted pathogens are the ones that acquire the necessary nutritional substances for growth and dissemination with the smallest

expenditure of energy and least damage to the host. For example, *Rickettsia akari*, the etiologic agent of rickettsialpox, causes a mild, self-limited infection consisting of headache, fever, and a papulovesicular rash. Other members of the rickettsial group, such as *R rickettsii*, the agent of Rocky Mountain spotted fever, elicit more severe, life-threatening infections. Some bacteria that are poorly adapted to the host synthesize virulence factors (e.g., tetanus and diphtheria toxin) so potent that they threaten the life of the host.

Host Resistance

Although easily damaged, the skin represents one of the most important barriers of the body to the microbial world, which contains a diverse array of bacteria in enormous numbers. Fortunately, most bacteria in the environment are relatively benign to individuals with normal immune systems. However, patients who are immunosuppressed, such as individuals receiving cancer chemotherapy or have AIDS, opportunistic microbial pathogens can establish life-threatening infections. Normally, microbes in the environment are prevented from entering the body by the skin and mucous membranes. The outermost surface of the skin consists of squamous cell epithelium, largely comprised of dead cells that are sloughed off as new cells are formed below them. In addition to the skin barrier, mucous membranes of the respiratory, gastrointestinal, and urogenital systems represent other portals through which bacteria can gain access to the body. Like the squamous epithelial cells of the skin, the mucosal epithelial cells divide rapidly, and as the cells mature, they are pushed laterally toward the intestinal lumen and shed. The entire process is reported to require only 36-48 hours for complete replacement of the epithelium, which diminishes the number of bacteria associated with the epithelium. The skin surface is a dry, acidic environment, and the temperature is less than 37° C. The pores and crevices of the skin also are colonized by the "normal bacterial flora", which ensure competition for pathogens to which the skin is exposed. Similarly, the mucous layer that covers the epithelia contains hostile substances to microbial colonization. Protective levels of lysozyme, lactoferrin, and lactoperoxidase in the mucus either kill bacteria or restrict their growth. In addition, the mucus contains secretory immunoglobulins (predominantly sIgA) synthesized by plasma cells resident in the submucosal tissue During the normal course of life, individuals develop local antibodies specific for a variety of intestinal bacteria that colonize mucosal surfaces.

Another mechanism of restricting growth of bacteria that penetrate the skin and mucous membranes is competition for iron. Typically, the amount of free iron in tissues and blood available to bacteria is very low, since plasma transferrin binds virtually all iron in the blood. Similarly, hemoglobin in the erythrocytes binds iron. Without free iron, bacterial growth is restricted unless the bacteria synthesize siderophores or receptors for iron containing molecules that compete for transferrin-bound iron. Such siderophores strip iron from transferrin and present it to the bacteria, which enables them to grow. The phagocytic cells of the body patrol the blood and tissues for foreign substances, including bacteria. This task is assumed predominantly by polymorphonuclear neutrophils; however, monocytes, macrophages, and eosinophils also participate. After phagocytosis, these bacterial cells usually are killed unless their numbers are excessive or they possess

virulence factors, that enable them to survive the lysosomal enzymes and acidic pH. In some instances, the bacteria kill the phagocyte or multiply within the macrophage, escaping the hostile extracellular environment. When inflammation occurs, phagocytic cells, along with lymphocytes, play an important role in innate immunity to bacterial infections. During the interaction of bacterial cells with macrophages, T cells, and B cells, specific antibody responses and/or cell-mediated immunity develop to protect against reinfection.

Genetic and Molecular Basis for Virulence

Virulence factors in bacteria may be encoded on chromosomal DNA, bacteriophage DNA, plasmids, or transposons in either plasmids or the bacterial chromosome (Fig. 7-2; Table 7-2). For example, the capacity of the Shigella species to invade cells is a property encoded in part on a 140-mega-dalton plasmid. Similarly, the heat-labile enterotoxin (LTI) of *E coli* is plasmid encoded, whereas the heat-labile toxin (LTII) is encoded on the chromosome. Other virulence factors are acquired by bacteria following infection by a particular bacteriophage, which integrates its genome into the bacterial chromosome by the process of lysogeny (Fig. 7-2). Temperate bacteriophages often serve as the basis of toxin production in pathogenic bacteria. Examples include diphtheria toxin production by Corynebacterium diphtheriae, erythrogenic toxin formation by Streptococcus pyogenes, Shiga-like toxin synthesis by *E coli*, and production of botulinum toxin (types C and D) by *Clostridium botulinum*. Other virulence factors are encoded on the bacterial chromosome (e.g., cholera toxin, *Salmonella enterotoxin*, and *Yersinia* invasion factors).

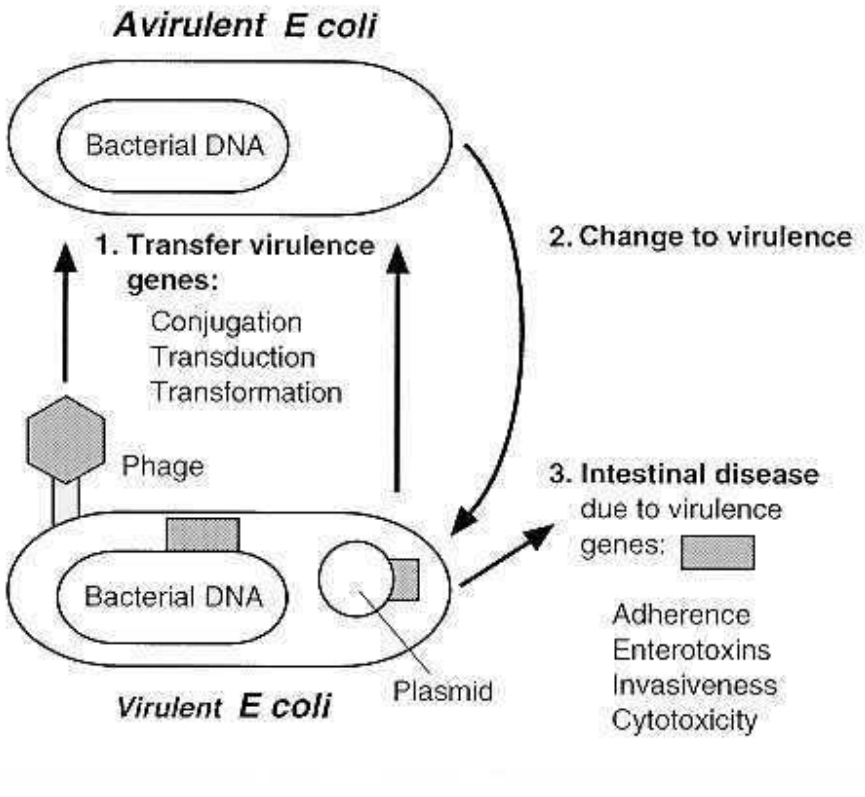


FIGURE 7-2 Mechanisms of acquiring bacterial virulence genes.

TABLE 7-2 Genetic Basis for Virulence of Selected Bacterial Pathogens

Gene(s) Encoded on	Bacterial Pathogen	Virulence Factor
Chromosome	*Vibrio cholerae*	Enterotoxin
	Salmonella typhimurium	Enterotoxin, invasion factors
	Shigella spp	Enterotoxin,invasion factors
	Aeromonas hydrophila	Enterotoxin, aerolysin
	Pseudomonas aeruginosa	Exotoxin A
	Staphylococcus aureus	Enterotoxin B
	Yersinia enterocolitica	Invasion factors
	Yersinia pseudotuberculosis	Invasion factors
	Escherichia coli	Enterotoxin (LTII)
Plasmid	*Shigella* spp	Invasion factors
	Escherichia coli	Invasion factors, colonization factor, and enterotoxin (LTI)
	Staphylococcus aureus	Exfoliative toxin
	Bacillus anthracis	Anthrax toxin
Bacteriophage	*Corynebacterium diphtheriae*	Diphtheria toxin
	Streptococcus pyogenes	Erythrogenic toxin
	Escherichia coli	Shiga-like enterotoxin
	Clostridium botulinum	Botulinum toxin (C,D)
Transposons*	*Escherichia coli*	Enterotoxins(STA and STB), iron acquisition,hemolysin

* Transposable genetic elements located on plasmids that often insert into the chromosome

The transfer of genes for antibiotic resistance among bacteria is a significant medical problem, although none of these properties actually confers increased virulence to the bacterium. Rather, they provide the opportunity for resistant bacteria to proliferate and produce other virulence factors in patients who are being treated with an inappropriate antibiotic. Resistance factors are discussed fully in Chapter 5.

An intriguing question regarding most bacterial protein toxins is the purpose they serve for the bacteriophage or the bacterium carrying them. Several bacterial toxins are enzymes. For example, cholera toxin, diphtheria toxin, Pseudomonas exotoxin A, and pertussis toxin all are NAD+ glycohydrolases that also act as ADP-ribosyltransferases. The toxic effect of these bacterial enzymes on the host is integral to the pathogenesis of the bacterial infections, but the function of the enzymes in the normal bacterial physiology is not known. Of all the protein toxins synthesized by pathogenic bacteria, there are few instances in which the function of the protein to the bacterium is known. It would be unlikely for the bacterium or infecting bacteriophage to expend the energy necessary to synthesize these relatively high-molecular-weight and complex molecules if they offered it no advantage. Frequently the toxicity of these substances is "unintentional" as far as the bacteria are concerned, considering that the primary goal of the microorganisms is to acquire nutrients and multiply rather than to harm the host.

Host-Mediated Pathogenesis

The pathogenesis of many bacterial infections cannot be separated from the host immune response, for much of the tissue damage is caused by the host response rather than by bacterial factors. Classic examples of host response-mediated pathogenesis are seen in diseases such as Gram-negative bacterial sepsis, tuberculosis, and tuberculoid leprosy. The tissue damage in these infections is caused by toxic factors released from the lymphocytes, macrophages, and polymorphonuclear neutrophils infiltrating the site of infection (Fig. 7-3). Often the host response is so intense that host tissues are destroyed, allowing resistant bacteria to proliferate. In lepromatous leprosy, in contrast, the absence of a cellular response to Mycobacterium leprae allows the bacteria to multiply to such large numbers in the skin that they become tightly packed and replace healthy tissue. The molecular basis for this specific immune anergy is poorly understood.

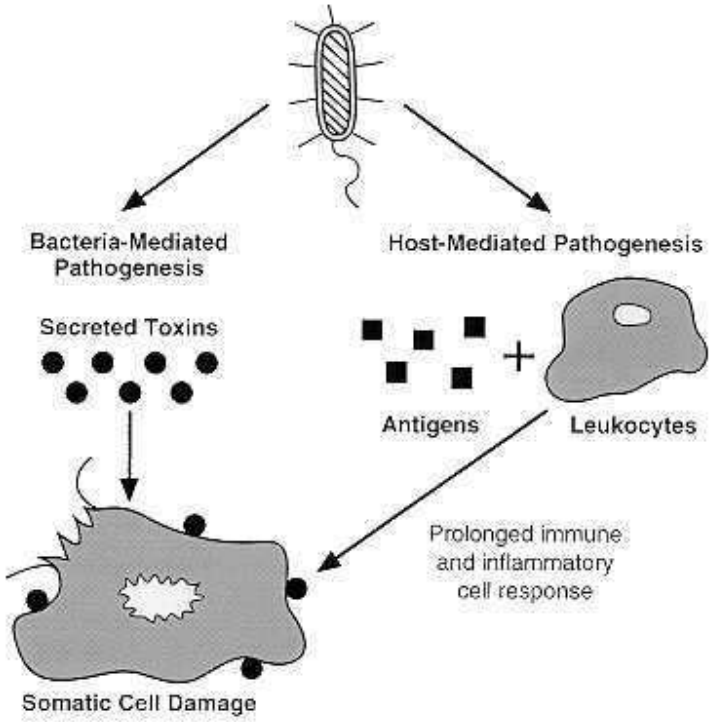


FIGURE 7-3 Generalized mechanisms of bacterial pathogenesis: bacteria-induced toxicity or host-mediated damage.

Intracellular Growth

In general, bacteria that can enter and survive within eukaryotic cells are shielded from humoral antibodies and can be eliminated only by a cellular immune response. However, these bacteria must possess specialized mechanisms to protect them from the harsh effects of the lysosomal enzymes encountered within the cell (see Ch. 1). Pathogenic

bacteria can be grouped into three categories on the basis of their invasive properties for eukaryotic cells (Fig. 7-4; Table 7-3). Although some bacteria (e.g., *Rickettsia, Coxiella,* and *Chlamydia*) grow only inside host cells, others (e.g., *Salmonella, Shigella,* and *Yersinia*) are facultative intracellular pathogens, invading cells when it gives them a selective advantage in the host.

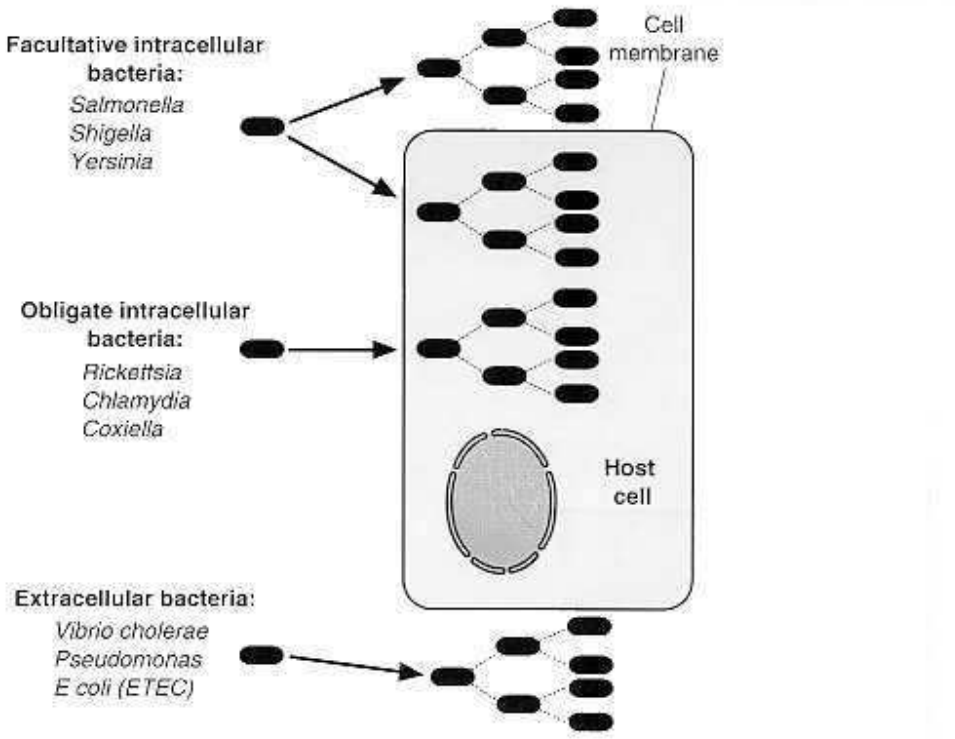


FIGURE 7-4 Examples of pathogenic bacteria, indicating their preferred growth phase within the host. (ETEC:enterotoxigenic *E coli*)

TABLE 7-3 Intracellular or Extracellular Growth Preference Relative to Eukaryotic Cells

Category	Bacterial Pathogen
Obligate intracellular	*Rickettsia* spp *Coxiella burnetii* *Chlamydia* spp
Facultative intracellular	*Salmonella* spp *Shigella* spp *Legionella pneumophila* Invasive *Escherichia coli* *Neisseria* spp *Mycobacterium* spp *Listeria monocytogenes* *Bordetella pertussis*
Predominantly extracellular	*Mycoplasma* spp *Pseudomonas aeruginosa* Enterotoxigenic *Escherichia coli* *Vibrio cholerae* *Staphylococcus aureus* *Streptococcus pyogenes* *Haemophilus influenzae* *Bacillus anthracis*

Some bacteria survive the intracellular milieu by producing phospholipases to dissolve the phagocytic vesicle surrounding them. This appears to be the case for R rickettsii, which destroys the phagosomal membrane with which the lysosomes fuse. Legionella pneumophila, which prefers the intracellular environment of macrophages for growth, appears to induce its own uptake and blocks lysosomal fusion by undefined mechanisms. Other bacteria have evolved to the point that they prefer the low-pH environment within the lysosomal granules, as may be the case for *Coxiella burnetii*, a highly resistant member of the rickettsial group. Salmonella and Mycobacterium species also appear to be very resistant to intracellular killing by phagocytic cells, but their mechanisms of resistance are not yet fully understood. Certainly, the capacity of bacteria to survive and multiply within host cells has great impact on the pathogenesis of the respective infections.

Most bacterial pathogens do not invade cells, proliferating instead in the extracellular environment enriched by body fluids. Some of these bacteria (e.g., V cholerae and Bordetella pertussis) do not even penetrate body tissues, but, rather, adhere to epithelial surfaces and cause disease by secreting potent protein toxins. Although bacteria such as *E coli* and *P aeruginosa* are termed noninvasive, they frequently spread rapidly to various tissues once they gain access to the body. All bacteria could at some point be considered intracellular once they become ingested by polymorphonuclear neutrophils and macrophages, but these organisms are not renowned for their capacity to survive the intracellular environment or to induce their own uptake by most host cells.

Specific Virulence Factors

The virulence factors of bacteria can be divided into a number of functional types. These are discussed in the following sections:

Adherence and Colonization Factors

To cause infection, many bacteria must first adhere to a mucosal surface. For example, the alimentary tract mucosa is continually cleansed by the release of mucus from goblet cells and by the peristaltic flow of the gut contents over the epithelium. Similarly, ciliated cells in the respiratory tract sweep mucus and bacteria upward. In addition, the turnover of epithelial cells at these surfaces is fairly rapid. The intestinal epithelial cell monolayer is continually replenished, and the cells are pushed from the crypts to the villar tips in about 48 hours. To establish an infection at such a site, a bacterium must adhere to the epithelium and multiply before the mucus and extruded epithelial cells are swept away. To accomplish this, bacteria have evolved attachment mechanisms, such as pili (fimbriae), that recognize and attach the bacteria to cells (see Ch. 2). Colonization factors (as they are often called) are produced by numerous bacterial pathogens and constitute an important part of the pathogenic mechanism of these bacteria. Some examples of piliated, adherent bacterial pathogens are *V cholerae, E coli, Salmonella* spp, *N gonorrheae, N meningitidis,* and *Streptococcus pyogenes*.

Invasion Factors

Mechanisms that enable a bacterium to invade eukaryotic cells facilitate entry at mucosal surfaces. Some of these invasive bacteria (such as Rickettsia and Chlamydia species) are obligate intracellular pathogens, but most are facultative intracellular pathogens (Fig. 7-4). The specific bacterial surface factors that mediate invasion are not known in most instances, and often, multiple gene products are involved. Some *Shigella* invasion factors are encoded on a 140 megadalton plasmid, which, when conjugated into *E coli,* gives these noninvasive bacteria the capacity to invade cells. Other invasion genes have also recently been identified in *Salmonella* and *Yersinia* pseudotuberculosis. The mechanisms of invasion of *Rickettsia,* and *Chlamydia* species are not well known.

Capsules and Other Surface Components

Bacteria have evolved numerous structural and metabolic virulence factors that enhance their survival rate in the host. Capsule formation has long been recognized as a protective mechanism for bacteria (see Ch. 2). Encapsulated strains of many bacteria (e.g., pneumococci) are more virulent and more resistant to phagocytosis and intracellular killing than are nonencapsulated strains. Organisms that cause bacteremia (e.g., *Pseudomonas*) are less sensitive than many other bacteria to killing by fresh human serum containing complement components, and consequently are called serum resistant. Serum resistance may be related to the amount and composition of capsular antigens as well as to the structure of the lipopolysaccharide. The relationship between surface structure and virulence is important also in *Borrelia* infections. As the bacteria encounter an increasing specific immune response from the host, the bacterial surface antigens are altered by mutation, and the progeny, which are no longer recognized by the immune

response, express renewed virulence. *Salmonella typhi* and some of the paratyphoid organisms carry a surface antigen, the *Vi antigen*, thought to enhance virulence. This antigen is composed of a polymer of galactosamine and uronic acid in 1,4-linkage. Its role in virulence has not been defined, but antibody to it is protective.

Some bacteria and parasites have the ability to survive and multiply inside phagocytic cells. A classic example is Mycobacterium tuberculosis, whose survival seems to depend on the structure and composition of its cell surface. The parasite *Toxoplasma gondii* has the remarkable ability to block the fusion of lysosomes with the phagocytic vacuole. The hydrolytic enzymes contained in the lysosomes are unable, therefore, to contribute to the destruction of the parasite. The mechanism(s) by which bacteria such as *Legionella pneumophila, Brucella abortus,* and *Listeria* monocytogenes remain unharmed inside phagocytes are not understood.

Endotoxins

Endotoxin is comprised of toxic lipopolysaccharide components of the outer membrane of Gram-negative bacteria (see Ch. 2). Endotoxin exerts profound biologic effects on the host and may be lethal. Because it is omnipresent in the environment, endotoxin must be removed from all medical supplies destined for injection or use during surgical procedures. The term endotoxin was coined in 1893 by Pfeiffer to distinguish the class of toxic substances released after lysis of bacteria from the toxic substances (exotoxins) secreted by bacteria. Few, if any, other microbial products have been as extensively studied as bacterial endotoxins. Perhaps it is appropriate that a molecule with such important biologic effects on the host, and one produced by so many bacterial pathogens, should be the subject of intense investigation.

Structure of Endotoxin

Figure 7-5 illustrates the basic structure of endotoxin. Endotoxin is a molecular complex of lipid and polysaccharide; hence, the alternate name lipopolysaccharide. The complex is secured to the outer membrane by ionic and hydrophobic forces, and its strong negative charge is neutralized by Ca^{2+} and Mg^{2+} ions.

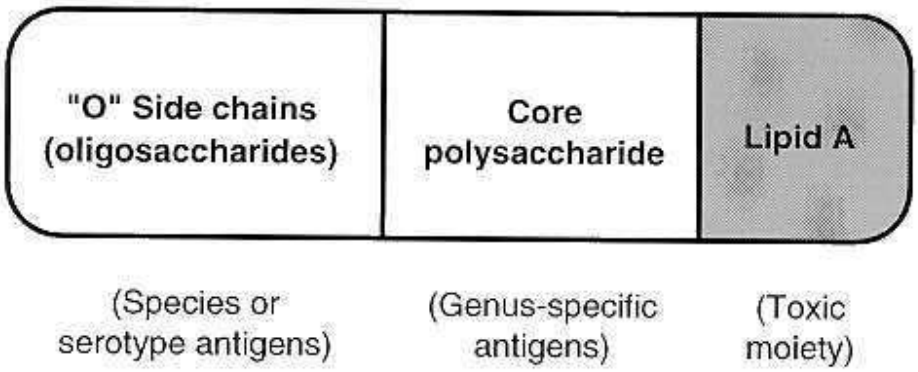


FIGURE 7-5 Basic structure of endotoxin (lipopolysaccharide) from Gram-negative bacteria.

The structure of endotoxin molecules from *Salmonella* spp and *E coli* is known in detail. Enough data on endotoxin from other Gram-negative organisms have been gathered to reveal a common pattern with genus and species diversity. Although all endotoxin molecules are similar in chemical structure and biologic activity, some diversity has evolved. Purified endotoxin appears as large aggregates. The molecular complex can be divided into three regions (Fig. 7-5): (1) the O-specific chains, which consist of a variety of repeating oligosaccharide residues, (2) the core polysaccharide that forms the backbone of the macromolecule, and (3) lipid A, composed usually of a glucosamine disaccharide with attached long-chain fatty acids and phosphate. The polysaccharide portions are responsible for antigenic diversity, whereas the lipid A moiety confers toxicity. Dissociation of the complex has revealed that the polysaccharide is important in solubilizing the toxic lipid A component, and in the laboratory it can be replaced by carrier proteins (e.g., bovine serum albumin).

Members of the family Enterobacteriaceae exhibit O-specific chains of various lengths, whereas *N gonorrhoeae, N meningitidis,* and *B pertussis* contain only core polysaccharide and lipid A. Some investigators working on the latter forms of endotoxin prefer to call them lipooligosaccharides to emphasize the chemical difference from the endotoxin of the enteric bacilli. Nevertheless, the biologic activities of all endotoxin preparations are essentially the same, with some being more potent than others.

Biologic Activity of Endotoxin

The biologic effects of endotoxin have been extensively studied. Purified lipid A (conjugated to bovine serum albumin) and endotoxin elicit the same biologic responses.

Table 7-4 lists some of the biologic effects of endotoxin. The more pertinent toxic effects include pyrogenicity, leukopenia followed by leukocytosis, complement activation, depression in blood pressure, mitogenicity, induction of prostaglandin synthesis, and hypothermia. These events can culminate in sepsis and lethal shock. However, it should be noted from Table 7-4 that not all effects of endotoxin are necessarily detrimental; several induce responses potentially beneficial to the host, assuming the stimulation is not excessive. These include:

1. mitogenic effects on B lymphocytes that increase resistance to viral and bacterial infections

2. induction of gamma interferon production by T lymphocytes, which may enhance the antiviral state, promote rejection of tumor cells, and activate macrophages and natural killer cells

3. activation of the complement cascade with the formation of C3a and C5a

4. induction of the formation of interleukin-1 by macrophages and interleukin-2 and other mediators by T lymphocytes.

TABLE 7-4 Multiple Biologic Activities Exhibited by the Lipid A Component of Endotoxin

Pyrogenicity
Leukopenia, leukocytosis
Complement activation
Depression of blood pressure
Hageman factor activation
Platelet activation
Induction of plasminogen activator
Bone marrow necrosis
Hypothermia in mice
Lethal toxicity in mice
Shwartzman reaction
Induction of prostaglandin synthesis
Limulus lysate gelation
*Induction of nonspecific resistance to infection
*Induction of endotoxin tolerance
*Adjuvant activity
*Mitogenic activity for lymphocytes
*Macrophage activation
*Induction of interferon synthesis
*Induction of tumor necrosis factor synthesis

*Potentially beneficial stimulatory effects of endotoxin in low doses.

Current research focuses on exploiting some of the potential beneficial effects of "nontoxic" endotoxin derivatives and holds promise for development of future treatment regimens for stimulating the immune response. For example, the toxicity of endotoxin is largely attributed to lipid A, attached to a polysaccharide carrier. The toxicity of lipid A is markedly reduced after hydrolysis of a phosphate group or deacylation of one or more

fatty acids from the lipid A molecule. Clinical trials are in progress to test a monophosphoryl lipid A for its potential of inducing low dose tolerance to endotoxin. Tolerance to endotoxin can be achieved by pretreatment of an animal with low doses of endotoxin or a detoxified lipid A derivative before challenge with high doses of endotoxin. Experimental studies have demonstrated that induction of tolerance to endotoxin reduces the dangerous effects of endotoxin. It is hoped that these relatively nontoxic lipid A derivatives may be useful in reducing the severity of bacterial sepsis in which bacterial endotoxin produces a life-threatening clinical course.

Endotoxin, which largely accumulates in the liver following injection of a sublethal dose by the intravenous route, can be devastating because of its ability to affect a variety of cell and host proteins. Kupffer cells, granulocytes, macrophages, platelets, and lymphocytes all have a cell receptor on their surface called CD14, which binds endotoxin. Endotoxin binding to the CD14 receptor on macrophages is enhanced by interaction with a host protein made in the liver (i.e., LPS-binding protein). The extent of involvement of each cell type probably depends on the level of endotoxin exposure. The effects of endotoxin on such a wide variety of host cells result in a complex array of host responses that can culminate in the serious condition gram-negative sepsis, which often leads to shock and death. The effects of endotoxin on host cells are known to stimulate prostaglandin synthesis and to activate the kallikrein system, the kinin system, the complement cascade via the alternative pathway, the clotting system, and the fibrinolytic pathways. When these normal host systems are activated and operate out of control, it is not surprising that endotoxin can be lethal. Although it is difficult to comprehend the mechanisms of all the cell responses and the myriad sequelae of the cell mediators released rather indiscriminately in the host following exposure to endotoxin, it does seem clear that the host cellular response to endotoxin, rather than a direct toxic effect of endotoxin, plays the major role in causing tissue damage (Fig. 7-3).

Detection of Endotoxin in Medical Solutions

Endotoxin is omnipresent in the environment. It is found in most deionized-water lines in hospitals and laboratories, for example, and affects virtually every biologic assay system ever examined. It tends to be a scapegoat for all biologic problems encountered in the laboratory, and, many times, this reputation is deserved. Because of its pyrogenic and destructive properties, extreme care must be taken to avoid exposing patients to medical solutions containing endotoxin. Even though all supplies should be sterile, solutions for intravenous administration can become contaminated with endotoxin-containing bacteria after sterilization as a result of improper handling. Furthermore, water used in the preparation of such solutions must be filtered through ion exchange resins to remove endotoxin, because it is not removed by either autoclave sterilization or filtration through bacterial membrane filters. If endotoxin-containing solutions were used in such medical procedures as renal dialysis, heart bypass machines, blood transfusions, or surgical lavage, the patient would suffer immediate fever accompanied by a rapid and possibly lethal alterations in blood pressure.

Solutions for human or veterinary use are prepared under carefully controlled conditions to ensure sterility and to remove endotoxin. Representative samples of every manufacturing batch are checked for endotoxin by one of two procedures: the Limulus lysate test or the rabbit pyrogenicity test. The rabbit pyrogenicity test is based on the exquisite sensitivity of rabbits to the pyrogenic effects of endotoxin. A sample of the solution to be tested usually is injected intravenously into the ear veins of adult rabbits while the rectal temperature of the animal is monitored. Careful monitoring of the temperature responses provides a sensitive and reliable indicator of the presence of endotoxin and, importantly, one measure of the safety of the solution for use in patients.

The Limulus lysate test is more common and less expensive. This test, which is based on the ability of endotoxin to induce gelation of lysates of amebocyte cells from the horseshoe crab Limulus polyphemus, is simple, fast, and sensitive (about 1 ng/ml). It is so sensitive, however, that trace quantities of endotoxin in regular deionized water often obscure the results. It can be used for rapid detection of certain Gram-negative infections (e.g., of cerebrospinal fluid); however, blood contains inhibitors that prevent gelation. Test kits are commercially available. The amebocyte is the sole phagocytic immune cell of the horseshoe crab, and the gelation reaction is believed to be involved in sequestering invading Gram-negative bacteria.

Exotoxins

Exotoxins, unlike the lipopolysaccharide endotoxin, are protein toxins released from viable bacteria. They form a class of poisons that is among the most potent, per unit weight, of all toxic substances. Most of the higher molecular-sized exotoxin proteins are heat labile; however, numerous low molecular-sized exotoxins are heat-stable peptides. Unlike endotoxin, which is a structural component of all Gram-negative cells, exotoxins are produced by some members of both Gram-positive and Gram-negative genera. The functions of these exotoxins for the bacteria are usually unknown, and the genes for most can be deleted with no noticeable effect on bacterial growth. In contrast to the extensive systemic and immune-system effects of endotoxin on the host, the site of action of most exotoxins is more localized and is confined to particular cell types or cell receptors. Tetanus toxin, for example, affects only internuncial neurons. In general, exotoxins are excellent antigens that elicit specific antibodies called antitoxins. Not all antibodies to exotoxins are protective, but some react with important binding sites or enzymatic sites on the exotoxin, resulting in complete inhibition of the toxic activity (i.e., neutralization).

Exotoxins can be grouped into several categories (e.g., neurotoxins, cytotoxins, and enterotoxins) based on their biologic effect on host cells. Neurotoxins are best exemplified by the toxins produced by *Clostridium* spp, for example, the botulinum toxin formed by *C botulinum*. This potent neurotoxin acts on motor neurons by preventing the release of acetylcholine at the myoneural junctions, thereby preventing muscle excitation and producing flaccid paralysis. The cytotoxins constitute a larger, more heterogeneous grouping with a wide array of host cell specificities and toxic manifestations. One cytotoxin is diphtheria toxin, which is produced by *Corynebacterium diphtheriae*. This cytotoxin inhibits protein synthesis in many cell types by catalyzing the ADP-

ribosylation of elongation factor II, which blocks elongation of the growing peptide chain.

Enterotoxins stimulate hypersecretion of water and electrolytes from the intestinal epithelium and thus produce watery diarrhea. Some enterotoxins are cytotoxic (e.g., shiga-like enterotoxin from *E coli*), while others perturb eukaryotic cell functions and are cytotonic (e.g., cholera toxin). Enterotoxins also can disturb normal smooth muscle contraction, causing abdominal cramping and decrease transit time for water absorption in the intestine. Enterotoxigenic *E coli* and *V cholerae* produce diarrhea after attaching to the intestinal mucosa, where they elaborate enterotoxins. Neither pathogen invades the body in substantial numbers, except in the case of *E coli* species that have acquired an invasion plasmid. Importantly, cholera toxin and *E coli* heat-labile enterotoxins I and II cause ADP-ribosylation of cell proteins in a manner similar to diphtheria toxin, except that the primary target is the regulatory protein (G_s_) of adenylate cyclase, resulting in increased levels of cyclic 3',5'-adenosine monophosphate (cAMP) (see Ch. 25). In contrast, the organisms responsible for shigellosis (*Shigella dysenteriae, S boydii, S flexneri,* and *S sonnei*) penetrate the mucosal surface of the colon and terminal ileum to proliferate and cause ulcerations that bleed into the intestinal lumen. Despite causing extensive ulceration of the mucosa, the pathogens rarely enter the bloodstream. The Shiga enterotoxin produced by *Shigella* species and the Shiga-like enterotoxin elaborated by many isolates of *E coli* inhibit protein synthesis in eukaryotic cells. It is not clear how this cytotoxic enterotoxin causes hypersecretion of water and electrolytes from the intestinal epithelium. These enterotoxins differ from those secreted by *V cholerae* and *E coli* in that the Shiga toxins are cytotoxic and lethal, whereas the cholera toxin-like enterotoxins are not. The latter enterotoxins cause no structural damage to cells, and are described as cytotonic. The ensuing inflammatory response to the invading bacteria and/or their toxins appears to activate neurologic control mechanisms (e.g., prostaglandins, serotonin) that normally regulate water and electrolyte transport.

Siderophores

Both animals and bacteria require iron for metabolism and growth, and the control of this limited resource is often used as a tactic in the conflict between pathogen and host. Animals have evolved mechanisms of "withholding" iron from tissue fluids in an attempt to limit the growth of invading bacteria. Although blood is a rich source of iron, this iron is not readily available to bacteria since it is not free in solution. Most of the iron in blood is bound either to hemoglobin in erythrocytes or to transferrin in plasma. Similarly, the iron in milk and other secretions (e.g., tears, saliva, bronchial mucus, bile, and gastrointestinal fluid) is bound to lactoferrin. Some bacteria express receptors for eukoyotic iron-binding proteins (e.g., transferrin-binding outer membrane proteins on the surface of *Neisserira* spp). Via these specialized receptors iron acquisition is facilitated, providing the esssential element for bacterial growth.

Other bacteria have evolved elaborate mechanisms to extract the iron from host proteins (Fig. 7-6). Siderophores are substances produced by many bacteria (and some plants) to capture iron from the host. The absence of iron triggers transcription of the genes coding

for the enzymes that synthesize siderophores, as well as for a set of surface protein receptors that recognize siderophores carrying bound iron. The binding constants of the siderophores for iron are so high that even iron bound to transferrin and lactoferrin is confiscated and taken up by the bacterial cells. An example of a bacterial siderophore is enterochelin, which is produced by *Escherichia* and *Salmonella* species. Classic experiments have demonstrated that Salmonella mutants that have lost the capacity to synthesize enterochelin lose virulence in an assay of lethality in mice. Injection of purified enterochelin along with the Salmonella mutants restores virulence to the bacteria. Therefore, siderophore production by many pathogenic bacteria is considered an important virulence mechanism.

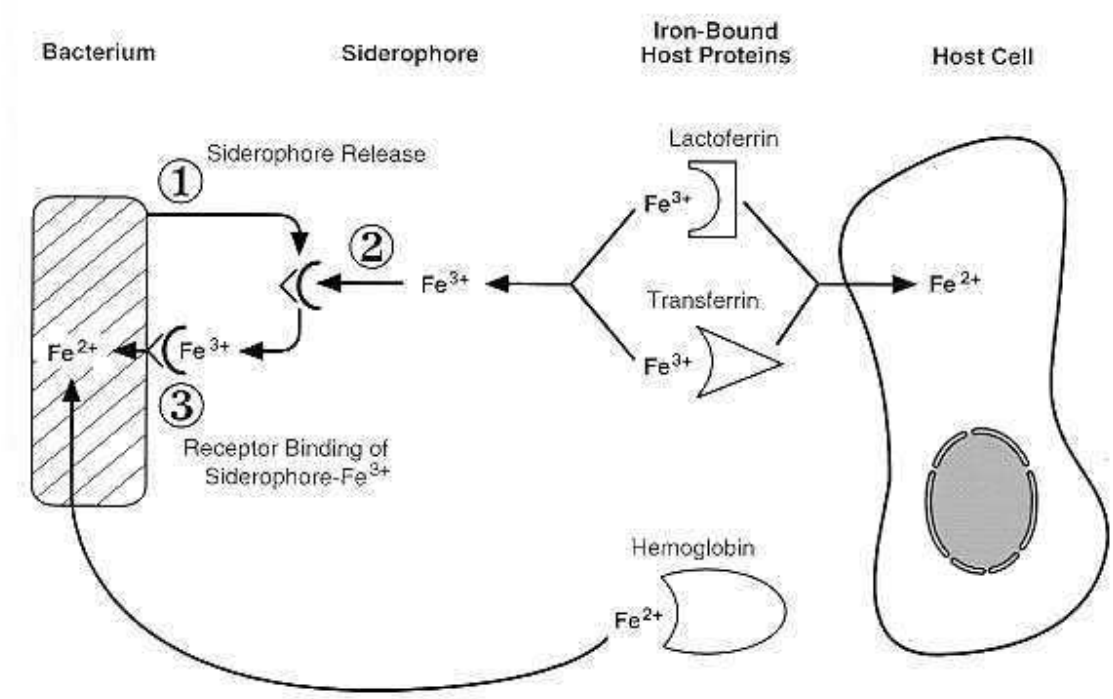


FIGURE 7-6 Competition between host cells and bacterial pathogens for iron, illustrating the importance of siderophores. Since free iron is scarce in tissue fluids and blood, bacterial siderophores compete effectively for Fe^{3+} bound to lactoferrin and transferrin.

Epilogue

Many factors determine the outcome of the bacterium-host relationship. The host must live in an environment filled with a diverse population of microorganisms. Because of the magnitude of the infectious-disease problem, we strive to understand the natural immune mechanisms of the host so that future improvements in resistance to bacterial infections may be possible. Similarly, massive research efforts are being expended to identify and characterize the virulence factors of pathogenic bacteria and hence allow us to interrupt

the pathogenic mechanisms of virulent bacteria. The availability of an array of antibiotics and vaccines has provided the medical profession with powerful tools to control or cure many infections. Unfortunately, these drugs and vaccines have eliminated no bacterial disease from the human or animal populations, and bacterial infections and drug resistance remain a serious medical problem.

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