Bacterial Virulence Factors

Many factors determine bacterial virulence, or ability to cause infection and disease.

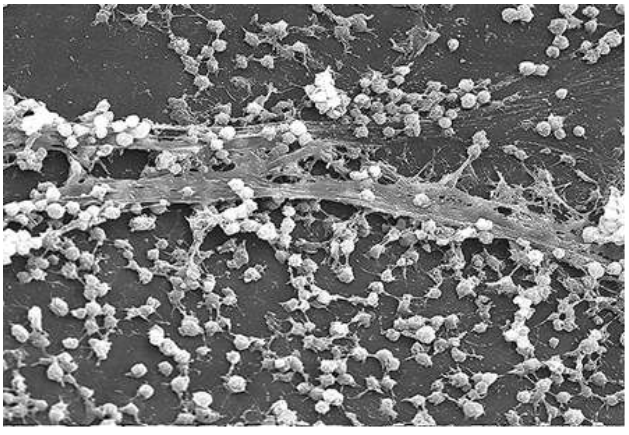
Virulence Factors for Adhesion

The first two steps in pathogenesis are exposure and adhesion. Adhesin is a protein or glycoprotein found on pathogen surface that attaches to receptors on the host cell. Many bacteria have pili, hair like appendages that extend from bacterial surface and help mediate adherence of the bacteria to host cell surfaces. For example, some *E coli* strains have type 1 pili, which adhere to epithelial cell receptors containing D-mannose.

Streptococcus pyogenes also have hair-like appendages, termed fimbriae that extend from the cell surface. **Lipoteichoic acid, protein F, and M protein** are found on the fimbriae. The lipoteichoic acid and protein F cause adherence to buccal epithelial cells; this adherence is mediated by fibronectin, which acts as the host cell receptor molecule. M protein acts as an antiphagocytic molecule and is a major virulence factor.

The Role of Bacterial Biofilms

Biofilm growth can also act as an adhesion factor. A biofilm is a community of bacteria that produce a glycocalyx, known as extrapolymeric substance (EPS), which allows the biofilm to attach to a surface. A single species or more than one species of bacteria is involved to form a biofilm. The EPS allows the bacteria to adhere to the host cells and makes it harder to physically remove the pathogen. The EPS also provides protection against the immune system and antibiotic treatments, preventing antibiotics from reaching the bacterial cells within the biofilm.



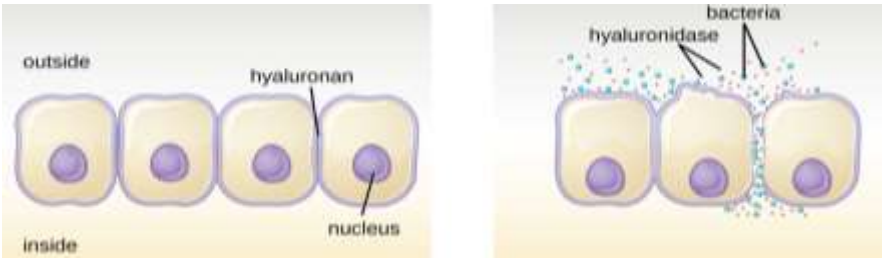
Bacterial Exoenzymes and Toxins as Virulence Factors

After exposure and adhesion, the next step in pathogenesis is invasion, which can involve enzymes and toxins.

1. Exoenzymes

Some pathogens produce extracellular enzymes, or exoenzymes, which have a wide variety of targets. Each of these exoenzymes functions in a particular tissue to facilitate invasion or support its own growth and defend against the immune system. For example,

A. Hyaluronidase S, an enzyme produced by *S. aureus*, degrades hylauronan, which acts as an intercellular cement between adjacent cells in connective tissue. This allows the pathogen to pass through the tissue layers and spread elsewhere in the body.



B. *S. aureus's* DNAse degrades extracellular DNA to escape and spread through tissue. Bacterial and host cells die at the infection site, lysing and releasing their contents. Masses of extracellular DNA can trap bacteria and stop their spread. *S. aureus* and other pathogens use this strategy to degrade and escape extracellular DNA webs produced by immune system phagocytes to trap the bacteria.

C. Many pathogens produce phospholipases to degrade the phospholipids of cell membranes and cause lysis of target cells. *B. anthracis* produces phospholipase C. When *B. anthracis* is ingested by phagocytic cells, phospholipase C degrades the phagosome membrane before it can fuse with the lysosome, allowing the pathogen to escape into the cytoplasm and multiply.

D. Bacterial pathogens also produce protein-digesting enzymes, or proteases. For example collagenase digests collagen, the main protein in connective tissue. Similar to hyaluronidase, collagenase allows the pathogen to penetrate and spread through the host tissue by digesting this connective tissue protein.

2. Toxins

In addition to exoenzymes, certain pathogens are able to produce toxins, biological poisons that assist in their ability to invade and cause damage to tissues. The ability of a pathogen to produce toxins to cause damage to host cells is called toxigenicity.

Toxins can be categorized as endotoxins or exotoxins.

A. The lipopolysaccharide (LPS) found on the outer membrane of gram-negative bacteria is called endotoxin. Lipopolysaccharide is composed of lipid A, a core glycolipid, and an O-specific polysaccharide side chain.

During infection and disease, gram-negative bacterial pathogens release endotoxin either when the cell dies, resulting in the breakdown of the membrane, or when the bacterium undergoes binary fission. The lipid A component is responsible for the toxic properties and triggers the immune system's inflammatory response.

If the concentration is low, the inflammatory response may provide the host an effective defense against infection; on the other hand, high concentrations in the blood can cause an increased inflammatory response, leading to a severe drop in blood pressure, multi-organ failure, and death.

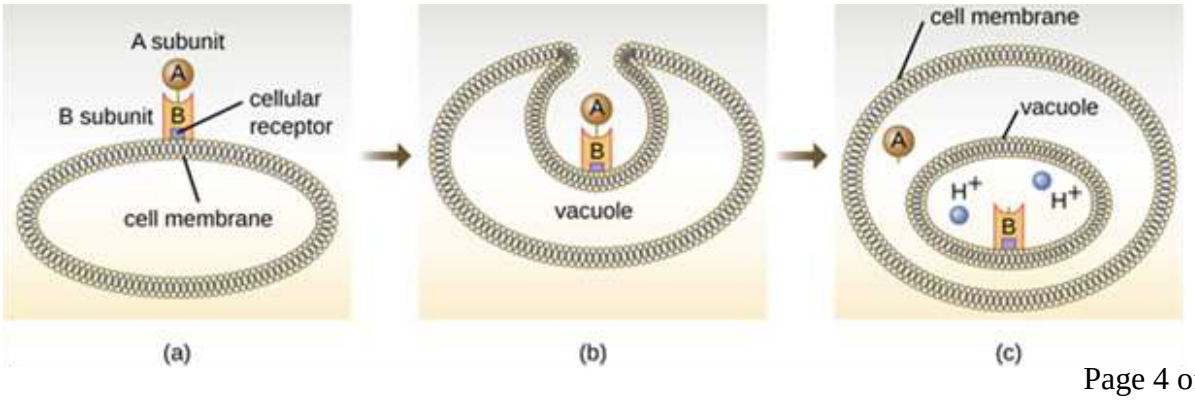
B. Exotoxins are protein molecules produced by many pathogenic bacteria. Although some gram-negative pathogens produce exotoxins, the majority are produced by gram-positive pathogens. Unlike endotoxin, which causes a systemic inflammatory response when released, exotoxins are more specific in their action and each exotoxin targets specific receptors on specific cells and damages those cells. Exotoxins differ from endotoxins in other ways summarized in Table.

Table: Characteristics of Exotoxins and Endotoxins (Lipopolysaccharides).

Exotoxins	Endotoxins
Excreted by living cell; high concentrations in liquid medium.	Integral part of the cell wall of gram-negative bacteria. Released on bacterial death and in part during growth.
Produced by both gram-positive and gram-negative bacteria.	Found only in gram-negative bacteria.
Polypeptides with a molecular weight of 10,000–900,000.	Lipopolysaccharide complexes. molecular weights between 3000 and 5000
Consist of A and B subunits. The B mediates adherence A subunit provides the toxic activity.	Lipid A portion probably responsible for toxicity.
Relatively unstable; toxicity often destroyed by heating at temperatures above 60°C.	Relatively stable; withstand heating at temperatures above 60°C for hours without loss of toxicity.
Highly antigenic; stimulate formation of high-titer antitoxin. Antitoxin neutralizes toxin.	Weakly immunogenic; antibodies are antitoxic and protective. Relationship between antibody titers and protection from disease is less clear.
Converted to antigenic, nontoxic toxoids by formalin, acid. Toxoids are used to immunize (tetanus toxoid).	Not converted to toxoids.
Highly toxic; fatal to animals in microgram quantities or less.	Moderately toxic; fatal for animals in tens to hundreds of micrograms.
Usually bind to specific receptors on cells.	Specific receptors not found on cells.
Usually do not produce fever in the host.	Usually produce fever in the host by release of interleukin-1 and other mediators.
Frequently controlled by plasmids.	Synthesis directed by chromosomal genes.

The exotoxins can be grouped into three categories based on their target:

1. Intracellular targeting toxins have two components (A-B exotoxin): A for activity and B for binding. The B component attaches the toxin to cell surface receptors and gives it cellular specificity. Endocytosis brings the A-B toxin into the host cell and traps it in a vacuole. As the vacuole acidifies, A and B subunits separate. The A subunit enters the cell cytoplasm and disrupts the targeted internal function.



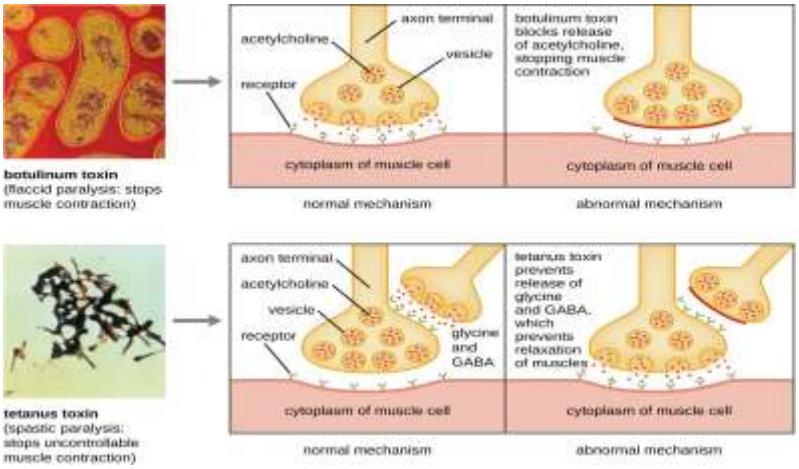
Four unique examples of A-B toxins are the diphtheria, cholera, botulinum, and tetanus toxins.

a. The diphtheria toxin is produced by the gram-positive *Corynebacterium diphtheriae*, the causative agent of nasopharyngeal. After the A subunit separates and gains access to the cytoplasm, it inhibits protein synthesis in the host cell, finally killing the cell.

b. Cholera toxin is an enterotoxin produced by the gram-negative *Vibrio cholerae*. The B subunits bind to receptors on the small intestinal epithelial cell. After entering the cytoplasm, the A subunit activates the enzyme adenyl cyclase, causing increased levels of cyclic adenosine monophosphate (cAMP) and secretion of fluids and electrolytes out of cell, causing diarrhea.

c. *Clostridium botulinum* produces the neurotoxin botulinum toxin (known as botox). It is the strongly toxic known to date. Toxins have light A and heavy protein chain B subunits. The B subunit binds to neurons to allow botulinum toxin to enter the neurons at the neuromuscular junction. The A subunit inhibits release of the neurotransmitter acetylcholine from neurons, results in the inhibition of muscle contractions, leading to muscle relaxation. This could stop breathing and cause death. Because of its action, low concentrations of botox are used for cosmetic and medical procedures.

d. Another neurotoxin is tetanus toxin, which is produced by the *Clostridium tetani*. This toxin also has a light A subunit and heavy protein chain B subunit. Tetanus toxin inhibits the release of glycine and gamma-aminobutyric acid (GABA) from the interneuron, resulting in permanent muscle contraction. The first symptom is typically stiffness of the jaw (lockjaw). Violent muscle contractions in other parts of the body follow, typically ending with respiratory failure and death.

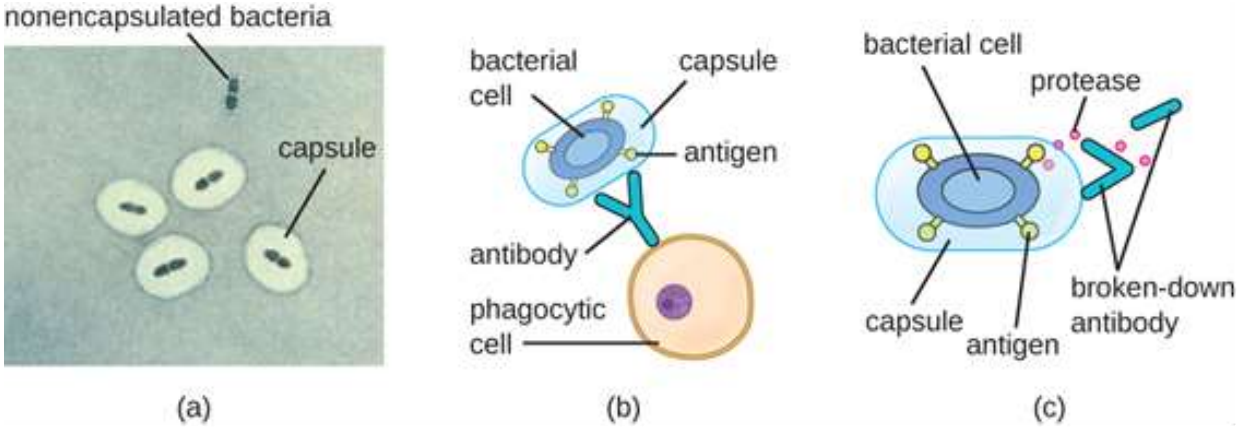


2. Membrane-disrupting toxins affect cell membrane function either by forming pores or by disrupting the phospholipid bilayer in host cell membranes. Two types of membrane-disrupting exotoxins are hemolysins and leukocidins, which form pores in cell membranes, causing leakage of the cytoplasmic contents and cell lysis. Bacterial phospholipases are membrane-disrupting toxins that degrade the phospholipid bilayer of cell membranes rather than forming pores.
3. The third class of exotoxins is the superantigens. These are exotoxins that trigger stimulation of immune cells to secrete cytokines (chemical messengers). The excessive production of cytokines, often called a cytokine storm, produces a strong immune and inflammatory response that can cause life-threatening high fevers, low blood pressure, multi-organ failure, shock, and death.

Virulence Factors for Survival in the Host and Immune Evasion

Evading the immune system is also important to invasiveness. Bacteria use a variety of virulence factors to evade phagocytosis. For example, many bacteria produce capsules, which are used in adhesion but also aid in immune evasion by preventing ingestion by phagocytes. The composition of the capsule prevents immune cells from being able to adhere and then phagocytose the cell. In addition, the capsule makes the bacterial cell much larger, making it harder for immune cells to engulf the pathogen.

Some pathogens can also produce proteases to protect themselves against phagocytosis. The immune system produces antibodies that bind to surface molecules found on bacteria (e.g., capsules, fimbriae, flagella). This binding initiates phagocytosis and proteases combat antibody and clearance by attacking and digesting the antibody molecules.



Regulation of Bacterial Virulence Factors

Environmental signals often control the expression of the virulence genes. Common signals include temperature, iron availability, osmolality, growth phase, pH, and specific ions (eg, Ca^{2+}) or nutrient factors. A few examples are presented in the following.

1. The gene for diphtheria toxin from *Corynebacterium diphtheriae* is carried on bacteriophages. Toxin is produced only by strains lysogenized by the phages. Toxin production is greatly enhanced when *C. diphtheriae* is grown in a medium with low iron.
2. Expression of virulence genes of *Bordetella pertussis* is enhanced when the bacteria are grown at 37 °C and suppressed when they are grown at lower temperatures or in the presence of high concentrations of magnesium sulfate.
3. The virulence factors of *Vibrio cholerae* are regulated on multiple levels and by many environmental factors. Expression of the cholera toxin is higher at pH 6.0 than at pH 8.5 and higher also at 30 °C than at 37 °C.
4. Motility of bacteria enables them to spread and multiply in their environmental or in patients. *Yersinia enterocolitica* and *Listeria monocytogenes* motile when grown at 25 °C but not when grown at 37 °C.