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BACTERIAL VIRULENCE FACTORS

Many factors determine bacterial virulence or the ability tocause infection and disease.

Adherence Factors

When bacteria enter the body of the host, they must adhereto cells of a tissue surface. If they did not adhere, they would be swept away by mucus and other fluids that bathe the tissue surface. **Adherence**, which is only one step in the infectious process, is followed by development of **microcolonies** and subsequent steps in the **pathogenesis of infection**.

The interactions between bacteria and tissue cell surfaces in the adhesion process are complex. Several factorsplay important roles, including **surface hydrophobicity andnet surface charge, binding molecules on bacteria (ligands),and host cell receptor interactions.**

Bacteria and host cells commonly have net negative surface charges and therefore repulsive electrostatic forces. These forces are overcome by **hydrophobic** and other more specific interactions between bacteria and host cells. In general, the more hydrophobic the bacterial cell surface, the greater the adherence to the host cell. Different strains of bacteria within a species may vary widely in their hydrophobic surface properties and ability to adhere to host cells.

Bacteria also have **specific surface molecules** that interact with host cells. Many bacteria have **pili**, thick rod like appendages or **fimbriae**, **shorter "hair like" structures that**

extend from the bacterial cell 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 **epithelialcell receptors**; adherence can be blocked in vitro by addition of d-mannose to the medium. ***E. coli***organisms that cause urinary tract infections commonly do not have d-mannose-mediated adherence but have P-pili, which attach to a portion of the P blood group antigen; the minimal recognition structure is the disaccharide α-d-galactopyranosyl-(1-4)-β-d-galactopyranoside(GAL–GAL binding adhesion). The***E. coli***that cause diarrheal diseases have **pilus**(**fimbriae**)-mediated adherence to intestinal epithelial cells.The type of pili and specific molecular mechanisms of adherence appear to be different depending on the form of the*E. coli* that induce the diarrhea.

Other specific ligand-receptor mechanisms have evolved to promote bacterial adherence to host cells, illustrating the diverse mechanisms used by bacteria. Group A streptococci(***Streptococcus pyogenes***) also have hairlike 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 of the streptococci 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.

Antibodies that act against the specific bacterial ligands that promote adherence (eg, pili and lipoteichoic acid) can block adherence to host cells and protect the host from infection.

After adherence occurs, conformational changes in thehost cell ensue that can lead to cytoskeletal changes allowingorganism uptake by the cell. Sometimes changes in theadhesin molecule after attachment may trigger activation of virulence genes that promote invasion or that result in other pathogenic changes as described below.

Invasion of Host Cells and Tissues

For many disease-causing bacteria, **invasion of the host's epitheliumis central to the infectious process**. Some bacteria(eg, ***Salmonella*** species) invade tissues through the junctions between epithelial cells. Other bacteria (eg, ***Yersinia*** species, *Neisseria gonorrhoeae, Chlamydia trachomatis*) invade specific type sof the host's epithelial cells and may subsequently enterthe tissue. **When inside the host cell**, bacteria may remainenclosed in a vacuole composed of the host cell membrane, or the vacuole membrane may be dissolved and bacteria maybe dispersed in the cytoplasm. Some bacteria (eg, ***Shigella*** species) multiply within host cells, but other bacteria do not.

Invasion is the term commonly used to describe the entry of bacteria into host cells, implying an active role for the organisms and a passive role for the host cells. In many

infections, the bacteria produce **virulence factors** that influence the host cells, causing them to engulf (ingest) the bacteria. The host cells play a very active role in the process.

Toxin production and other virulence properties are generally independent of the ability of bacteria to invade cellsand tissues. For example, *Corynebacterium diphtheriae*is able to invade theepithelium of the nasopharynx and cause symptomatic sore throat even when the *C.diphtheriae* strains are non toxigenic.

In vitro studies with cells in tissue culture have helped characterize the mechanisms of invasion for some pathogens . ***Shigella*** species adhere to host cells in vitro. Commonly, HeLa cells are used; these undifferentiated unpolarized cells were derived from a cervical carcinoma. The adherence causes actin polymerization in the nearby portion of the HeLa cell, which induces the formation of pseudo pods by the HeLa cells and engulfment of the bacteria. Adherence and invasion are mediated at least in part by products of genes located on a large plasmid common to many shigellae. There are multiple proteins, including the **invasion plasmid antigens** (IpA-D), that contribute to the process. Inside the HeLa cells, the **shigella** either are released or escape from the phagocytic vesicle, where they multiply in the cytoplasm. Actin polymerization propels the shigellae within a HeLa cell and from one cellinto another. In vivo the shigellae adhere to integrins on the surface of M cells in Peyer's patches and not to the polarized absorptive cells of the mucosa. M cells normally sample antigensand present them to macrophages in the submucosa. The shigellae are phagocytosed by the M cells and pass through the M cells into the underlying collection of

macrophages.Shigellae inside the M cells and macrophages can cause these cells to die by activating the normal cell death process(apoptosis). The **shigellae** spread to adjacent mucosal cells in amanner similar to the in vitro model by actin polymerization that propels the bacteria.

From studies using cells in vitro, it appears that the adherence-invasion process with ***Y.enterocolitica*** is similar to that of *Shigella*. Yersiniae adhere to the host cell membrane and cause it to extrude protoplasmic projections. The bacteria are then engulfed by the host cell with vacuole formation. Invasion is enhanced when the bacteria are grown at 22 °Crather than at 37°C. When **yersiniae** have entered the cell, the vacuolar membrane dissolves and the bacteria are released into the cytoplasm. In vivo, the **yersiniae** are thought to adhere to and invade the M cells of Peyer's patches rather than the polarized absorptive mucosal cells, much like shigellae.

Listeria monocytogenes from the environment is ingestedin food. Presumably, the bacteria adhere to and invade the intestinal mucosa, reach the bloodstream, and disseminate. The pathogenesis of this process has been studied in vitro. ***L.monocytogenes*** adheres to and readily invades macrophages and cultured undifferentiated intestinal cells. The **listeriae** induce engulfment by the host cells. Proteins, called **internalins**, have a primary role in this process. The engulfment process, movement within a cell and movement between cells, requires actin polymerization to propel the bacteria, aswith shigellae.

Neisseria gonorrhoeaeuses pili as primary adhesins and **opacity associated proteins (Opa)** as secondary adhesins to hostcells. Certain Opa proteins mediate adherence to polymorphonuclearcells. Some **gonococci** survive after phagocytosis by these cells. Pili and Opa together enhance the invasion ofcells cultured in vitro. In uterine (fallopian) tube organ cultures, the **gonococci** adhere to the microvilli of non ciliated cells and appear to induce engulfment by these cells. The **gonococci** multiply intracellularly and migrate to the subepithelial space by an unknown mechanism.

Toxins

Toxins produced by bacteria are generally classified into two groups: exotoxins and endotoxins. Exotoxins are proteins that are most often excreted from the cell. However some exotoxins accumulate inside the cell and are either injected directly into the host or are released by cell lysis. Endotoxins are lipid molecules that are components of the bacterial cell membrane. The primary features of the two groups are listed in Table 3.

A. Exotoxins

Many gram-positive and gram-negative bacteria produce exotoxins of considerable medical importance. Some of the setoxins have had major roles in world history. For example, **tetanus** caused by the toxin of ***Clostridium tetani*** killed as many as50,000

soldiers of the Axis powers in World War II; the Allied forces, however, immunized military personnel against tetanus, and very few died of that disease.

Vaccines have been developed for some of the exotoxin-mediated diseases and continue to be important in the prevention of disease. The sevaccines - called **toxoids**-are made from exotoxins, which are modified so that they are no longer toxic. **Many exotoxins consist of A and B subunits. The B subunit generally mediates adherence of the toxin complex to a host cell and aids entrance of the exotoxin into the host cell. The A subunit provides the toxic activity.**

Table 3: 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; may not need to be released to have biologic activity
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; lipid A portion probably responsible for toxicity
Relatively unstable; toxicity often destroyed rapidly 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 than with exotoxins
Converted to antigenic, nontoxic toxoids by formalin, acid, heat,and so on; toxoids are used to immunize (eg, tetanus toxoid)	Not converted to toxoids
Highly toxic; fatal to animals in	Moderately toxic; fatal for animals in tens to

microgram quantities or less	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 extrachromosomal genes (eg, plasmids)	Synthesis directed by chromosomal genes

Many factors regulate toxin production; when the availability of **inorganic iron** is the factor limiting the growth rate, then maximal toxin production occurs. **The toxin molecule is secreted as a single polypeptide molecule**(molecular weight [MW], 62,000). This native toxin is enzymatically degraded into two fragments, A and B, linked together by a disulfide bond. Fragment B (MW, 40,700) binds to specific host cell receptors and facilitates the entry of fragment A (MW, 21,150) into the cytoplasm. Fragment A inhibits peptide chain elongation factor EF-2 by catalyzing a reaction that attaches an adenosine diphosphate-ribosyl group to EF-2,yielding an inactive adenosine diphosphate-ribose-EF-2complex. Arrest of protein synthesis disrupts normal cellular physiologic functions. Diphtheria toxin is very potent.

Clostridium tetaniis an anaerobic gram-positive rod that causes **tetanus**. *C.tetani* from the environment contaminates wounds, and the spores germinate in the anaerobic environment of the devitalized tissue. Infection often is minor and not clinically apparent. The vegetative forms of ***C.tetani*** produce the toxin **tetanospasmin** (MW, 150,000)that is cleaved by a bacterial protease into two peptides (MW,50,000 and 100,000) linked by a disulfide bond. The toxininitially binds to receptors on the presynaptic membranes of motor neurons. It then migrates by the retrograde axonal transport system to the cell bodies of these neurons to the spinal cord and brainstem. The toxin diffuses to terminals of

inhibitory cells, including both glycinergic interneurons and γ-amino butyric acid (GABA)-secreting neurons from the brainstem. The toxin degrades synaptobrevin, a protein required for docking of neurotransmitter vesicles on the presynaptic membrane. Release of the inhibitory glycine and GABA is blocked, and the motor neurons are not inhibited.Spastic paralysis results. Extremely small amounts of toxincan be lethal for humans. Tetanus is totally preventable in immunologically normal people by immunization with tetanus toxoid.

Some ***S.aureus*** strains growing on mucous membranes or in wounds, elaborate **toxic shock syndrome toxin-1 (TSST-1)**,which causes **toxic shock syndrome**. **TSST-1** is a super antigen and stimulatesT-cells to produce large amounts of interleukin-2 (IL-2) and tumor necrosis factor (TNF). The major clinical manifestations of the disease appear to be secondary to the effects of the cytokines. Many of the systemic effects ofTSST-1 are similar to those of toxicity caused by lipopolysaccharide (LPS).

Some strains of group Aβ-hemolytic **streptococci** produce **pyrogenic exotoxin A** that is similar to or the same as streptococcal erythrogenic toxin, which results in scarlet fever. Rapidly progressive soft tissue infection by streptococcithat produce the pyrogenic exotoxin A has many clinical manifestations similar to those of staphylococcal toxic shock syndrome. The pyrogenic exotoxin A also is a super antigenthat acts in a manner similar to TSST-1.

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