

Microbiology

Virulence & Pathogenicity

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4th lecture

Role of Virulence Factors in Bacterial Invasiveness



Normal Flora

- ❑ The term "normal microbial flora or microbiota" means the population of microorganisms that are associated with a host, established at a particular anatomical location and don't harm their host
- ❑ Microorganisms Important part of innate immunity
 - ▣ Compete for space and nutrients with harmful organisms (The mechanism of bacterial interference may involve competition for receptors or binding sites on host cells, competition for nutrients, mutual inhibition by metabolic or toxic products, mutual inhibition by antibiotic materials or bacteriocins)
 - ▣ first line of defense against microbial pathogens, assist in digestion, play a role in toxin-degradation and contribute to maturation of the immune system.

- Members of the resident flora in the intestinal tract synthesize vitamin K and aid in the absorption of nutrients.
- On mucous membranes and skin, the resident flora may prevent colonization by pathogens and possible disease through "bacterial interference."
- When displaced from normal site to other areas → may cause disease
 - ▣ Example: *Staphylococcus aureus* in nose and throat can enter a wound and cause serious infections

- ❑ **The microbial communities on human skin and in mucosal areas such as the mouth, esophagus, stomach, colon, and vagina**

- **Skin:** *Staphylococcus epidermidis* & *Staphylococcus aureus* (in small numbers)

- **Nasopharynx :** Any amount of the following: Diphtheroids, nonpathogenic *Neisseria* species

- **Gastrointestinal tract and rectum :**

Various Enterobacteriaceae except *Salmonella*, *Shigella*, *Yersinia*, *Vibrio*, and *Campylobacter* species

- **Genitalia**

Any amount of the following: *Corynebacterium* species, *Lactobacillus* species

GLOSSARY

- ❑ **Adherence (adhesion, attachment):** The process by which bacteria stick to the surfaces of host cells. Once bacteria have entered the body, adherence is a major initial step in the infection process.
- ❑ **Infection:** Multiplication of an infectious agent within the body. Multiplication of the bacteria that are part of the normal flora of the gastrointestinal tract, skin, etc, is generally not considered an infection; on the other hand, multiplication of pathogenic bacteria (eg, *Salmonella* species)—even if the person is asymptomatic—is deemed an infection.
- ❑ **Invasion:** The process whereby bacteria, animal parasites, fungi, and viruses enter host cells or tissues and spread in the body.
- ❑ **Nonpathogen:** A microorganism that does not cause disease; may be part of the normal flora.

- ❑ **Opportunistic pathogen:** An agent capable of causing disease only when the host's resistance is impaired (ie, when the patient is "immunocompromised").
- ❑ **Pathogen:** A microorganism capable of causing disease.
- ❑ **Pathogenicity:** The ability of an infectious agent to cause disease.
- ❑ **Toxigenicity:** The ability of a microorganism to produce a toxin that contributes to the development of disease.
- ❑ **Virulence:** The quantitative ability of an agent to cause disease. Virulent agents cause disease when introduced into the host in small numbers. Virulence involves adherence, invasion, and toxigenicity

Virulence is a measure of **pathogenicity** and is measured by the number of organisms required to cause disease.

- The **50% lethal dose** (LD_{50}) is the number of organisms needed to kill half the hosts, and the **50% infectious dose** (ID_{50}) is the number needed to cause infection in half the hosts.
- Organisms with a lower LD_{50} (or ID_{50}) are said to be more virulent than those with a higher LD_{50} (or ID_{50}) because fewer organisms are needed to cause death or disease.
- The **infectious dose** of an organism required to cause disease varies greatly among the pathogenic bacteria.

For example, *Shigella* and *Salmonella* both cause diarrhea by infecting the GI tract, but the infectious dose of *Shigella* is less than 100 organisms, whereas the infectious dose of *Salmonella* is on the order of 100,000 organisms.

The infectious dose of bacteria depends primarily on their virulence factors (e.g., pili, exotoxins or endotoxins)

The final outcome of host-parasite relationships

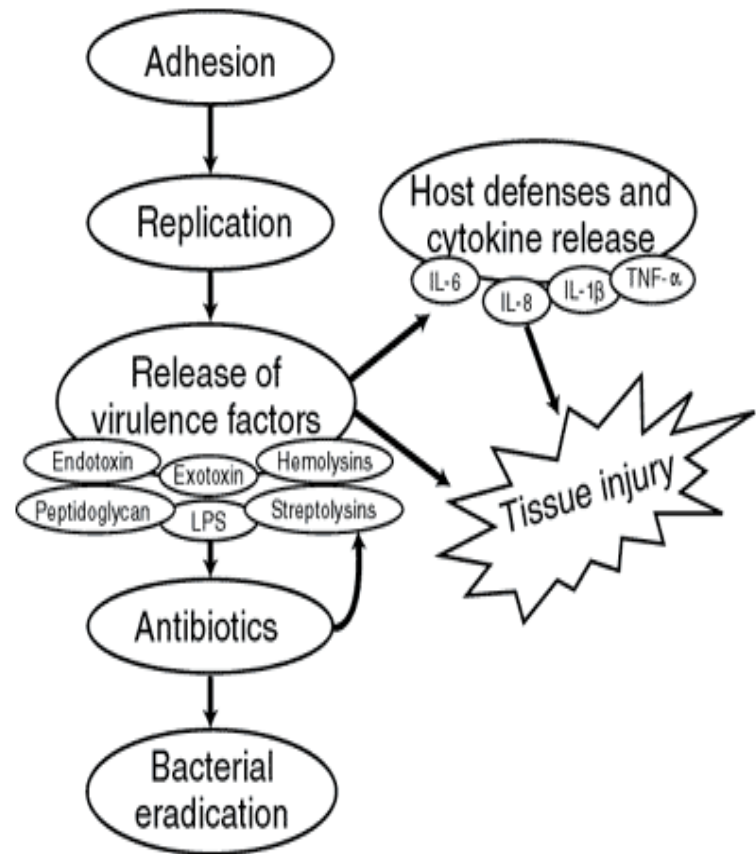
- ▣ **Dependent on three factors:**
 - **The actual number of parasites in the body**
 - **How virulent the parasite is**
- ▣ **How effective the host's resistance is to infection**
 - Disease often incurred if parasite is particularly virulent, even if the host has high resistance
 - Disease also incurred if virulence isn't particularly intense, but the host's resistance is weakened
- ▣ Age
- ▣ Illness
- ▣ Immunosuppressive drugs

Methods by which pathogens cause disease:

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- Adhesion.
- Colonization.
- Invasion .
- Evasion of host defenses.
- Toxin releas .



Adhesion

- ❖ which is only one step in the infectious process, is followed by development of microcolonies and subsequent steps in the pathogenesis of infection.
- ❖ Specific adherence involves complementary chemical interactions between the host cell or tissue surface and the bacterial surface. In the language of medical microbiologist, a bacterial "adhesin" attaches covalently to a host "receptor" so that the bacterium "docks" itself on the host surface. The adhesins of bacterial cells are
 - Filamentous hemagglutinin of *Bordetella pertussis* (binds to cilia of RT)
 - Fimbriae, pili
 - Glycocalyx (dental plaque)
 - Lectin
 - Capsule
 - S layer
 - Slime layer
 - Teichoic and lipoteichoic acids

1. surface hydrophobicity and net surface charge, binding molecules on bacteria (ligands), and host cell receptor interactions.

2. Pili

hair-like appendages 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 epithelial cell receptors. The *E. coli* that cause diarrheal diseases have pilus-mediated adherence to intestinal epithelial cells

3. fimbriae

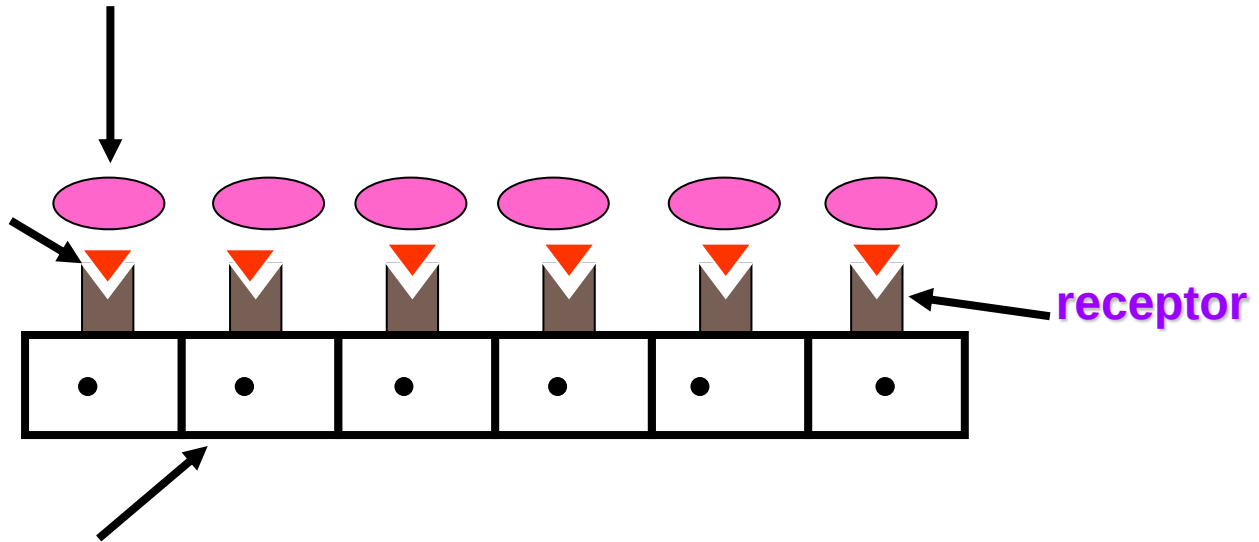
Group A streptococci (*Streptococcus pyogenes*) also have hair-like appendages, termed fimbriae, that extend from the cell surface. Lipoteichoic acid, protein F, and M protein are found on the fimbriae. The lipoteichoic acid and protein F cause adherence of the streptococci to buccal epithelial cells; 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.

Adhesion

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BACTERIUM

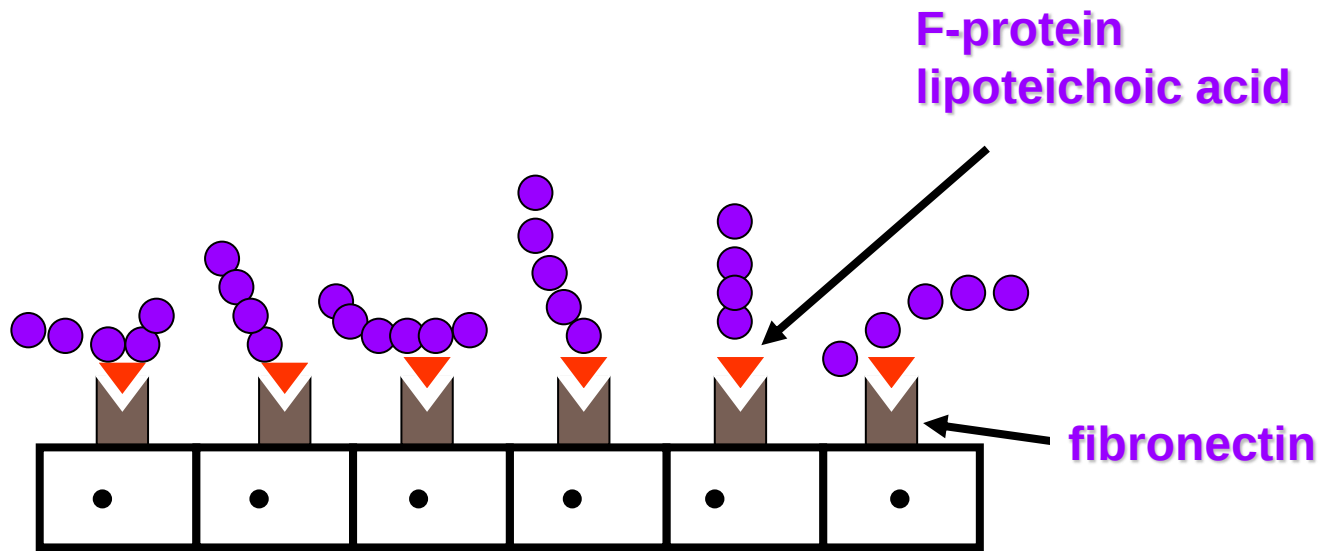
adhesin



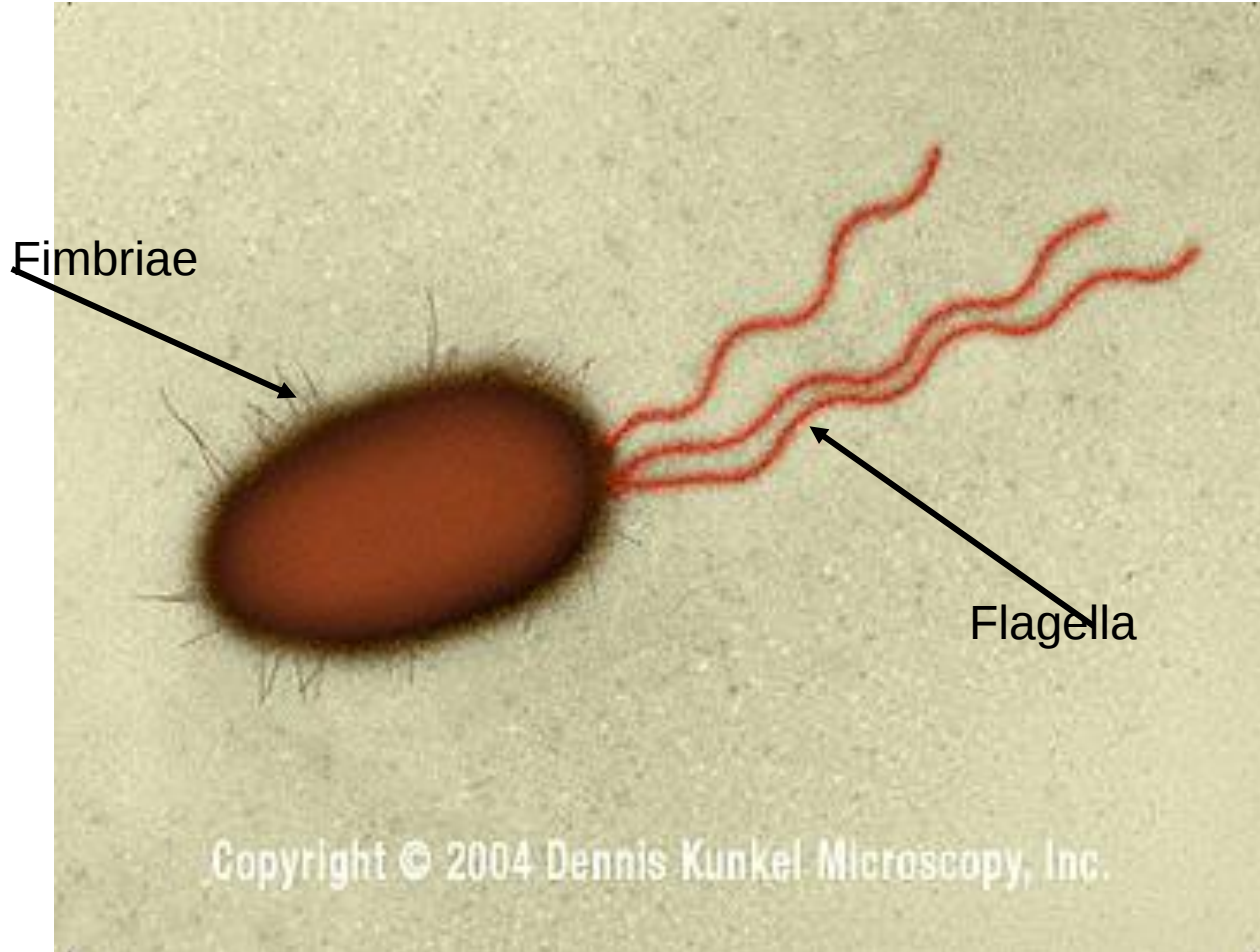
EPITHELIUM

S. pyogenes

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E. coli



colonization

- **Attachment and colonization (multiplication) by the pathogen**
 - **Pathogen must be able to adhere to and colonize host cells and tissue;**
 - **this is mediated by special molecules or structures called adhesins**

Invasion of Host Cells & Tissues

- "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.
 - **Gaining of access to deeper tissues**
 - **Penetration into the circulatory system**
- **Growth and multiplication of the pathogen - pathogen must find an appropriate environment**
 - pH
 - Oxygen tension
 - Nutrient availability (including specialized growth factors)
 - Intracellular growth
 - *Mycobacterium tuberculosis* in alveolar macrophages
 - Extracellular growth
 - Pyogenic infections – pus formation in extracellular spaces (pyogenic streptococci – *S. pyogenes*)

Portals of Entry

- Each infectious organism has its own portal of entry and portal of exit from the host
- Entry:
 - ▣ Respiratory tract via nose and throat
 - *M. tuberculosis* can survive in dried sputum for weeks
 - ▣ Gastrointestinal tract via mouth
 - *S. typhi* multiply only in cells of intestinal mucosa
 - ▣ Skin and mucus membranes
 - ▣ Genitourinary system (STD)
 - ▣ Blood (insects, transfusions, needles)
- Exit: Usually the same as the portals of entry

Dissemination by Evasion

- Pathogens spread throughout the host tissue and sometimes even gain entry into lymphatic capillaries and then eventually into the circulation
 - ▣ Localized infection = limited
 - ▣ Systemic dissemination = multiple organs
- Enhanced by microbial products and enzymes (virulence factors)

- **Capsules and fimbriae** – antiphagocytic, requires antibodies for opsonization
- **Leukocidins** - extracellular enzymes that kill phagocytic leukocytes by causing degranulation of lysosomes
- **Hemolysins** - extracellular enzymes that kill erythrocytes by forming a pore (iron released)
 - **Streptolysin O** – inactivated by oxygen, beta hemolysis when incubated anaerobically (*S. pyogenes*)
 - **Streptolysin S** – not oxygen sensitive, beta hemolysis under aerobic conditions
 - If phagocytosed, acts as a leukocidin and kills macrophages
- **Coagulase** – clots fibrinogen in plasma, protecting pathogen from phagocytosis and other host defenses
- **Collagenase** – Degrades collagen in connective tissue promoting the spread of the pathogen
- **Deoxyribonuclease** – reduces viscosity of exudates, improving pathogen mobility

- **Hydrogen peroxide and ammonia** – damage to respiratory and urogenital systems
- **Hyaluronidase** – degrades hyaluronic acid in intercellular ground substances holding cells together – increases mobility
- **IgA protease** – Degrades antibody (IgA isotype) into Fab and Fc fragments
- **Protein A** – binds IgG at Fc, prevents C' activation
- **Streptokinase (fibrinolysin, staphylokinase)**– binds plasminogen and induces production of plasmin, and digestion of fibrin clots – increasing mobility of pathogen from clotted areas

Toxins

- **Toxigenicity** : the capacity of an organism to produce a toxin
- **Intoxications**: diseases caused as a result of toxin produced inside the body
- **Toxin** : a specific substance, often a metabolic product of the organism, that damages the host in some specified manner
- **Toxemia**: symptoms caused by toxins if they enter the blood of the host
- **Toxoid** : Inactivated toxin used in vaccine
- **Antitoxin** : Antibodies against a specific toxin

Characteristics of Exotoxins

1. Protein Water soluble / Heat Liabile Excreted by living cell
2. Exotoxins are produced by several gram-positive and gram-negative bacteria, in contrast to endotoxins, which are present only in gram-negative bacteria.
3. toxicity often destroyed rapidly by heating at temperatures above 60°C
4. Have specific disease associations and specific effects in the host (e.g. cholera toxin, diphtheria toxin)
5. Highly antigenic (Induce production of neutralizing antibodies (antitoxins))
6. controlled by extrachromosomal genes (eg, plasmids & bacteriophages)

7. Usually do not produce fever in the host

8. Many exotoxins have an **A–B subunit** structure; the **A** subunit possesses the **toxic activity** (the A subunit has the enzyme activity that causes damage to the host), and the **B** subunit is responsible for **binding the exotoxin to specific receptors** on the membrane of the **human cell**. **GM1 for cholera toxin** (A-B) toxin. Enterotoxin. Stimulates cAMP to cause severe diarrhea

Type of Exotoxins

1. Cytotoxins

do general tissue damage (kill cells)

2. Neurotoxins

interfere with normal nerve impulses (**damage nervous tissue**)

3. Enterotoxins

effect cells lining the G.I. Tract (**damage the small intestine**)

4. Cytotoxins cAMP-producing increase cAMP levels

5. Superantigens – trigger massive T cell stimulation and cytokine production (TNF-alpha)

Mechanism of Action of Exotoxins

- Inhibition of protein synthesis
- Impairment of nerve synapse function
- Membrane transport disrupted (e.g. electrolyte imbalance resulting in a flow of water into the intestine)
- Damage to plasma membrane
- Increased cAMP levels (cyclic adenosine monophosphate)
- Cytokine production induced by superantigens

Two Types of Membrane Disrupting Exotoxins

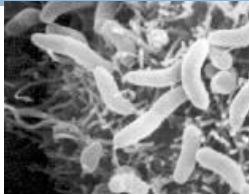
- 1: A channel forming (pore-forming) type of exotoxin that inserts itself into the normal host cell membrane and makes an open channel (pore)
 - ▣ Exotoxin attaches to the cholesterol portion of the host cell plasma membrane
 - ▣ Examples: Leukocidins (pneumococci, streptococci, and staphylococci) and Hemolysins (streptococci)
- 2: A phospholipid-hydrolyzing phospholipase exotoxin destroys membrane integrity.
 - ▣ The exotoxins 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.
 - ▣ Example: gas gangrene caused by *Clostridium perfringens* α -toxin

Exotoxin Transport Mechanisms

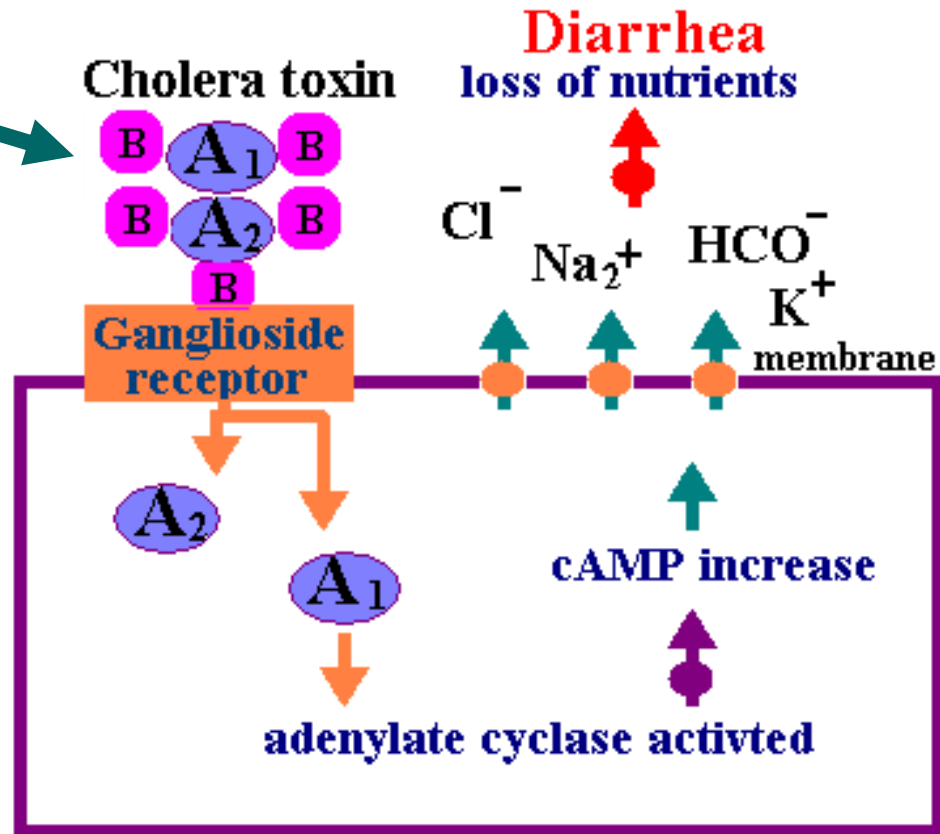
- A close look at the AB Model
 - ▣ Exotoxins lack an independent cell entry function and must bind to specific receptors on host cell membranes to be transported across the membrane
 - ▣ The “A” subunit of the toxin exerts its toxic effect (the A subunit has the enzyme activity that causes damage to the host)
 - ▣ The “B” subunit binds to the host receptor (e.g. ganglioside receptors are used for different exotoxins – GM1 for cholera toxin; GT1 for tetanus toxin; GD1 for botulinum toxin)

Toxins: cause hyperactivation

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Vibrio cholerae



Properties of Neurotoxins

- Can be ingested as a preformed toxin or made in the body
- Affect the nervous system directly and the small intestine indirectly
- Examples:
 - ▣ Staphylococcal enterotoxin B (SEB)
 - ▣ *Bacillus cereus* emetic toxin (vomiting)

▣ Botulinum toxin

- Flaccid paralysis caused by inhibition of acetylcholine activity at neuromuscular junction
- Ingestion of preformed toxin
- Infant botulism: spores germinate in the gut (dust, honey)

▣ Tetanus toxin

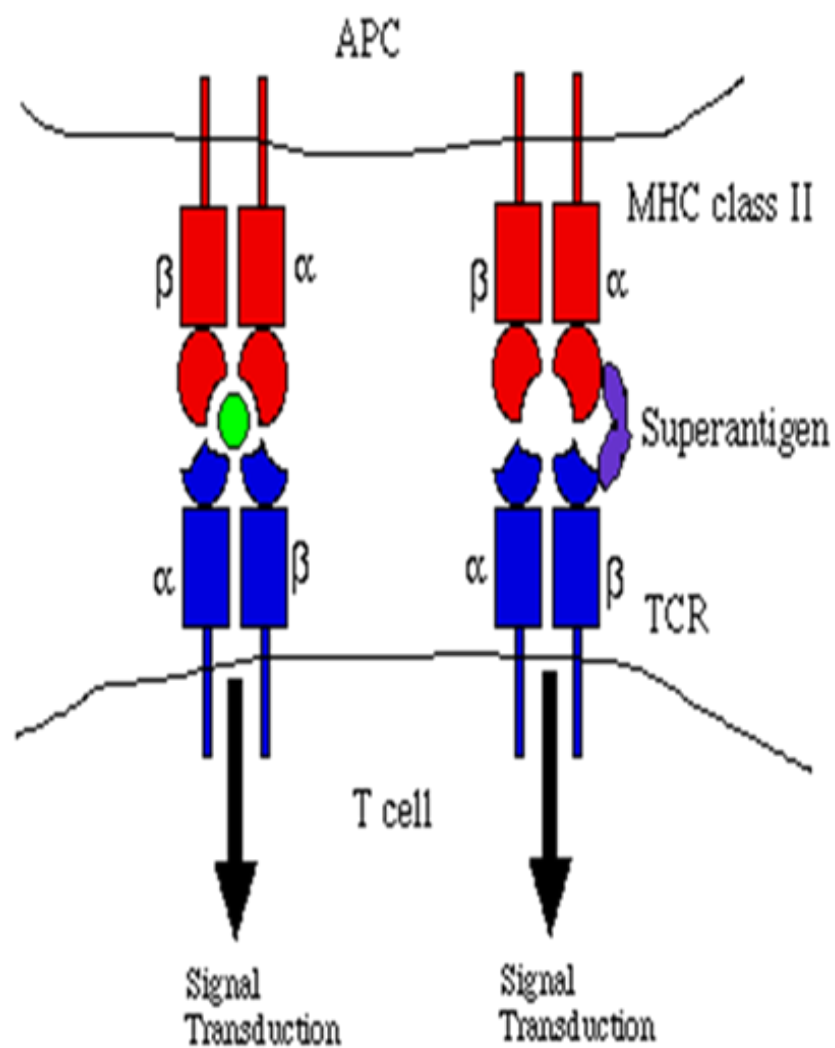
- Spores germinate in necrotic tissue
- Spastic paralysis caused by blocking inhibitory nerve impulses that cause constant stimulation of somatic motor neurons

Properties of Enterotoxins

- Directly affect intestinal mucosa
 - ▣ Vomiting, dysentery and diarrhea
- Elicit massive fluid secretion into intestine
- Water and electrolytes move across intestinal cells into the lumen of the gut
- Examples:
 - ▣ Cholera toxin
 - ▣ Escherichia coli
 - ▣ Staphylococcal food poisoning
 - ▣ Shigella dysentery
 - ▣ Enterohemorrhagic and enteroinvasive E. coli

Properties of Superantigens

- Pyrogenic (fever-inducing) toxins
- Bind to MHC class II on macrophages and the variable region on subsets of T cell receptors of CD4+ TH1 cells (inflammataory T cells)
- Induce macrophages to produce TNF-alpha and Interleukin-1 (IL-1) = endogenous pyrogen)
- Cause an intense immune response due to release of cytokines from host cells, causes Fever, nausea, vomiting, diarrhea, shock, death
- Examples of superantigens:
 - ▣ Staphylococcal enterotoxins
 - ▣ Exfoliatin toxin (Staphylococcus, scalded skin syndrome)
 - ▣ Pyrogenic toxism of staph and strep
 - ▣ Toxic shock syndrome toxin (TSST-1) - Staphylococcus



Endotoxins (LPS)

1. Part of the cell wall of gram-negative bacteria. Released on bacterial death and in part during growth
2. Found only in gram-negative bacteria
3. Lipopolysaccharide complexes. Lipid A portion probably responsible for toxicity
4. stable; withstand heating at temperatures above 60°C for hours without loss of toxicity
5. Weakly immunogenic; does not stimulate a strong adaptive immune response
6. Usually produce fever in the host by release of interleukin-1 and other mediators
7. Synthesis directed by chromosomal genes

8. The endotoxins of gram-negative bacteria are the best-established causes of septic shock, but surface molecules of gram-positive bacteria can also cause septic shock.

Two features of septic shock are interesting:

1. Septic shock is different from toxic shock. In septic shock, the bacteria are in the bloodstream, whereas in toxic shock, it is the toxin that is circulating in the blood.
2. Septic shock can cause the death of a patient even though antibiotics have killed the bacteria in the patient's blood (i.e., the blood cultures have become negative).

This occurs because septic shock is mediated by cytokines, such as TNF and IL-1, which continue to act even though the bacteria that induced the cytokines are no longer present.

The following can be observed clinically

Fever

leukopenia

Hypoglycemia

hypotension

shock resulting in impaired perfusion of essential organs (eg, brain, heart, kidney);

intravascular coagulation

death from massive organ dysfunction.

Evasion of host defenses

Some pathogenic bacteria are inherently able to resist the bactericidal components of host tissues. For example, the capsule of some bacteria protects the organisms against cell lysis by cationic proteins in sera or in phagocytes. The outer membrane of Gram-negative bacteria is not easily penetrated by hydrophobic compounds such as bile salts which are harmful to the bacteria. Pathogenic mycobacteria have a waxy cell wall that resists attack or digestion by most tissue bactericides. And intact lipopolysaccharides (LPS) of Gram-negative pathogens may protect the cells from complement-mediated lysis or the action of lysozyme.

MICROBIAL STRATEGIES TO AVOID PHAGOCYTIC KILLING:



1-Avoiding Contact with Phagocytes

- ❖ Certain internal tissues (e.g. the lumen of glands) and surface tissues (e.g. the skin) are not reached by phagocytes.

2-Inhibition of Phagocytic Engulfment

- ❖ Classical examples of antiphagocytic substances on the bacterial surface include:
 - ❑ -Polysaccharide capsules.
 - ❑ -M protein and fimbriae .
 - ❑ -Surface slime (polysaccharide) .
 - ❑ -O antigen associated with LPS.

3-Products of Bacteria that Kill or Damage Phagocytes

- ❖ One obvious strategy in defense against phagocytosis is direct attack by substances which produced by pathogens that cause damage to phagocytes have been referred to as "aggressins". Most of these are actually extracellular enzymes or toxins that kill phagocytes. Phagocytes may be killed by a pathogen before or after ingestion.

4-Survival Inside of Phagocytes

Some bacteria survive inside of phagocytic cells, in either neutrophils or macrophages. (intracellular parasites). Most intracellular bacteria have special (genetically-encoded) mechanisms which interfere with the bactericidal activities of the host cell. These include:

- **a. Inhibition of phagosome-lysosome fusion.** The bacteria survive inside of phagosomes because they prevent the discharge of lysosomal contents into the phagosome environment. Specifically phagolysosome formation is inhibited in the phagocyte. This is the strategy employed by *M. tuberculosis*, and the *Chlamydiae*.
□
- b. Survival inside the phagolysosome.** With some intracellular bacteria, phagosome-lysosome fusion occurs but the bacteria are resistant to inhibition and killing by the lysosomal constituents.
- **c. Escape from the phagosome.** Early escape from the phagosome vacuole is essential for growth and virulence of some intracellular pathogens. This is a strategy employed by some bacteria which produce a phospholipase enzyme that lyses the phagosome membrane within thirty seconds after ingestion.

5. Evading Complement

- Antibodies that are bound to bacterial surfaces will activate complement by the **classical pathway** and bacterial polysaccharides activate complement by the **alternative pathway**. Bacteria in serum and other tissues, especially Gram-negative bacteria, need protection from the antimicrobial effects of complement before and during an immunological response. One role of **capsules** in bacterial virulence is to protect the bacteria from complement activation and the ensuing inflammatory response.