Pharmaceutical

Microbiology

Dr. Mohammed Hussien Taleb

Chapter 2

Part 1

Pathogenesis of Bacterial Infection

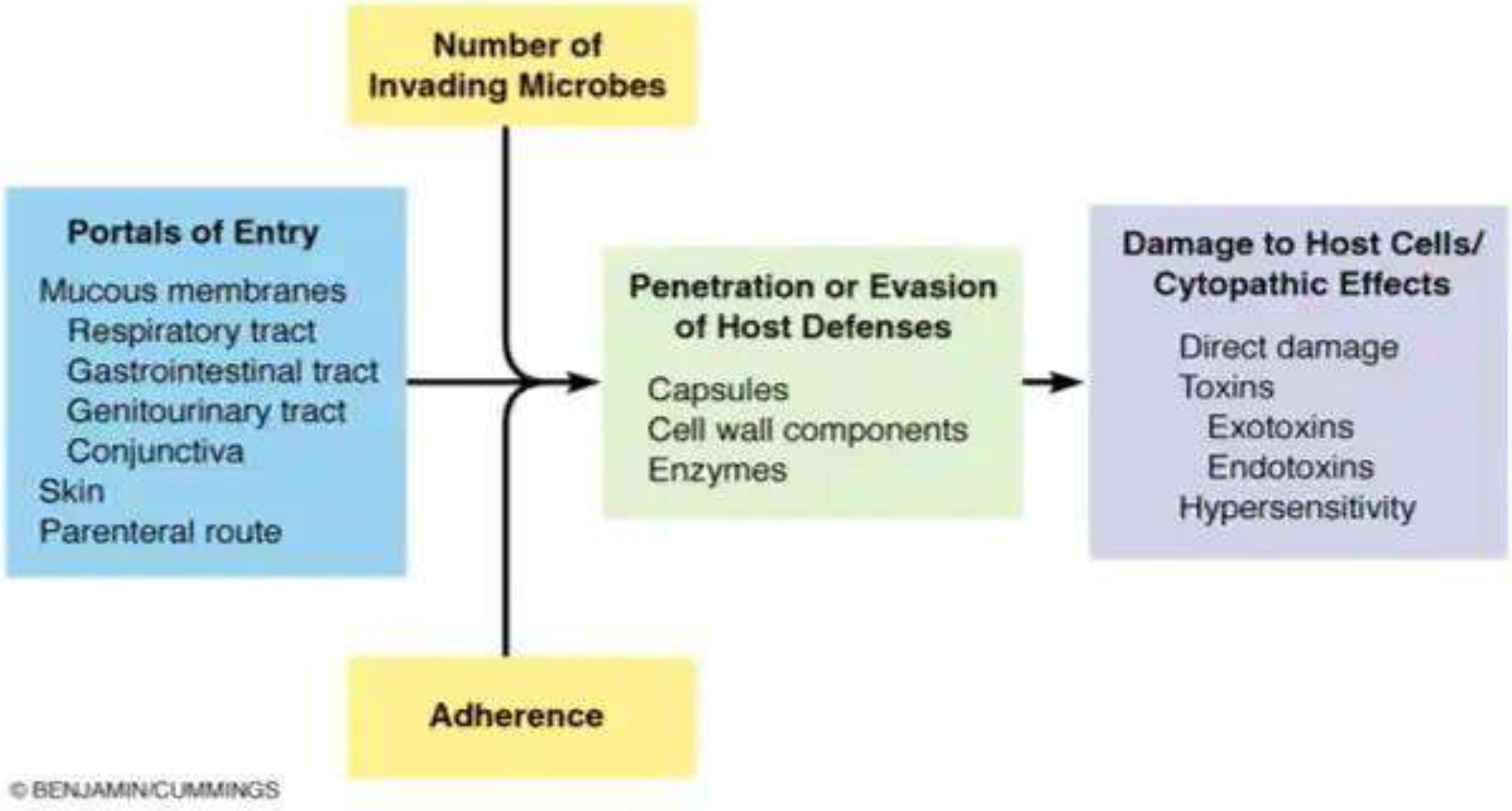
Types of microorganism

Kingdom	Pathogenic Microorganisms	Type of Cells
Animal	Helminths	Eukaryotic
Plant	None	Eukaryotic
Protist	Protozoa	Eukaryotic
	Fungi	Eukaryotic
Prokaryote	Bacteria	Prokaryotic
	Viruses	Noncellular

Steps of bacterial pathogenesis

1. Transmission from the source of infection into the portal of entry.
2. Evasion of primary host defense.
3. Adherence to mucous membrane.
4. Colonization by growth of the bacteria at the site of adherence.
5. Disease symptoms caused by bacterial toxin or invasion.
6. Host immune response during steps 3,4,5
7. Progression or resolution of the disease.

Bacterial Mechanisms of Pathogenicity: How Microorganisms Cause Disease



- **Step in the pathogenesis of infections disease:**

1. ***Entry:*** of the pathogen into the body by: (Penetration, inhalation, ingestion and introduction of the pathogens directly into the blood. [shades needles]
2. ***Attachment:*** of the pathogen to some tissues within the body.
3. ***Multiplication:*** with local or system
4. ***Invasive / spread*** of the pathogens
5. ***Evasion of a host defenses***.
6. ***Damage to host tissue (s).*** extensive or death.

Important Microbiological definitions

- **Types of pathogen**
- **Obligate pathogens** are always associated with disease (e.g. Treponema pallidum and HIV).
- **Conditional pathogens** may cause disease if certain conditions are met.
- For example, Bacteroides fragilis is a normal commensal of the gut but if it invades the peritoneal cavity, it will cause severe infection.
- **Opportunistic pathogens** usually cause infection when the host defences are compromised.
- For example, Pneumocystis jiroveci usually causes lung infection only in a host who has **severely compromised T-cell immunity**.

- **Adherence (adhesion, attachment):**

The process by which bacteria stick to the surfaces of host cells. After bacteria have entered the body, adherence is a major initial step in the infection process. The terms adherence , adhesion , and attachment are often used interchangeably.

- **Carrier:**

A person or animal with asymptomatic infection that can be transmitted to another susceptible person or animal.

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, and so on 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.
- **Microbiota:** Microbial flora harbored by normal, healthy individuals.
- **Nonpathogen:** A microorganism that does not cause disease; may be part of the normal microbiota.

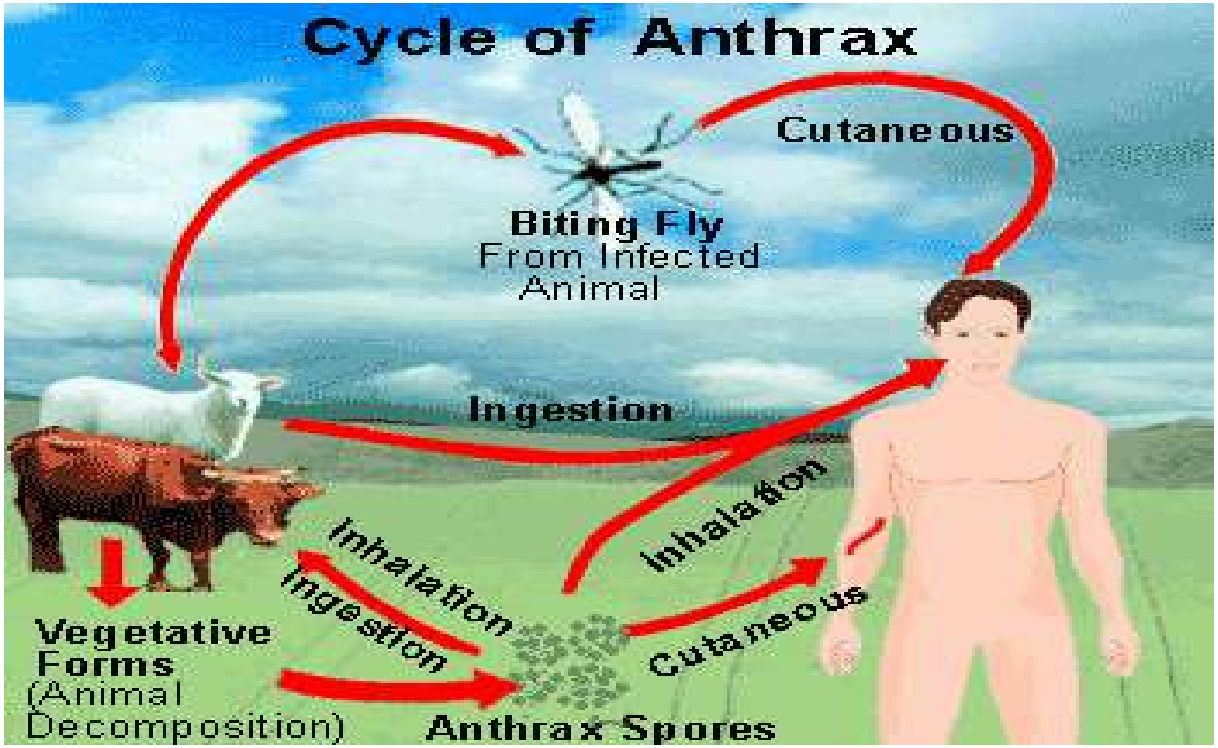
- **Pathogen:** A microorganism capable of causing disease.
- **Pathogenicity:** The ability of an infectious agent to cause disease.
- **Opportunistic pathogen**: An **agent capable of causing disease only when the host's resistance is impaired (ie, when the patient is "immunocompromised").**

- **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, persistence, invasion, and toxigenicity** .

Transmission Of Infection

- Some **bacteria that commonly cause disease in humans exist primarily in animals and incidentally infect humans.**
- For example, **Salmonella and Campylobacter species typically infect animals and are transmitted in food products to humans.**
- For example, Y. pestis (plague) has a well-established life cycle in rodents and rodent fleas, and transmission by the fleas to humans is inadvertent.

- Bacillus anthracis (anthrax) lives in the environment, occasionally infects animals, and is transmitted to humans by products such as raw hair from infected animals



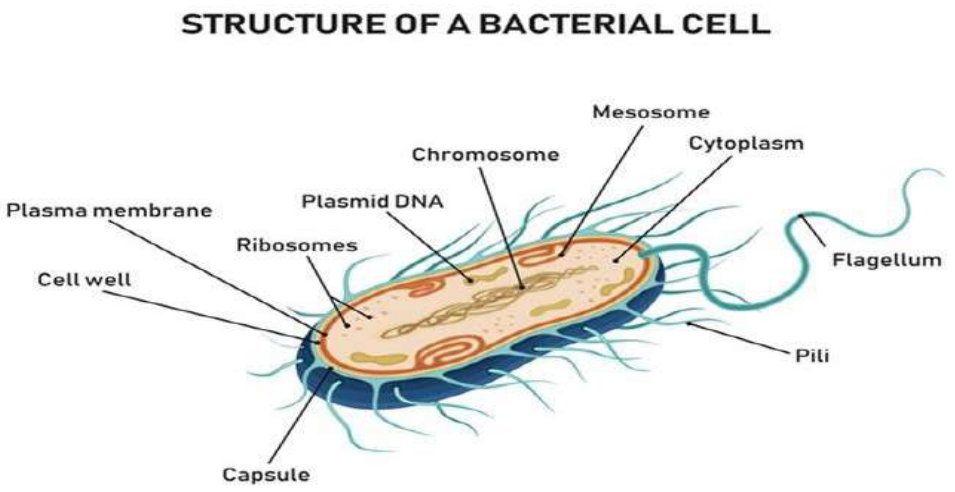
Bacterial Virulence Factors

1-Adherence Factors

- When **bacteria enter the body of the host, they must adhere to 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 factors play important roles, including
- **surface hydrophobicity**
- **net surface charge,**
- **binding molecules on bacteria (ligands),**
- **host cell receptor interactions.**

- **Bacteria** also **have specific surface molecules that interact with host cells.** Many bacteria have pili, (thick rodlike appendages) or fimbriae, (shorter "hairlike" 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 epithelial cell receptors**



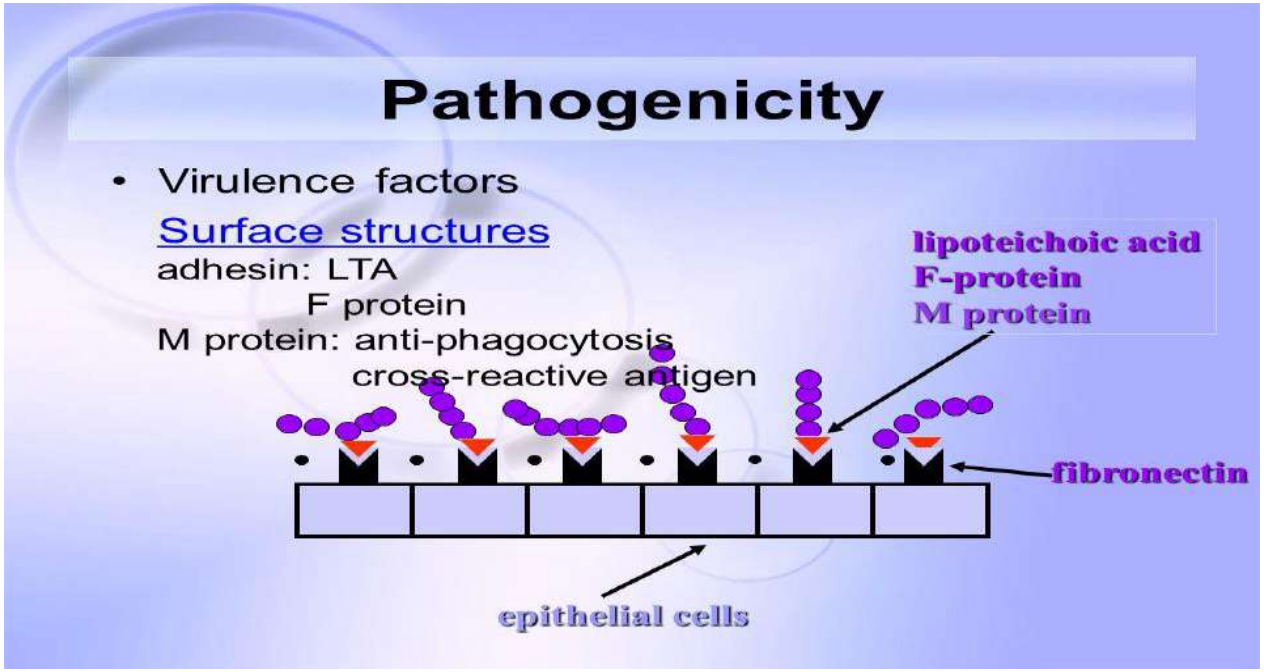
- Bacteria and host cells commonly have net negative surface charges and therefore repulsive electrostatic forces. These forces are overcome by hydrophobic and other more specific interactions between bacteria and host cells.

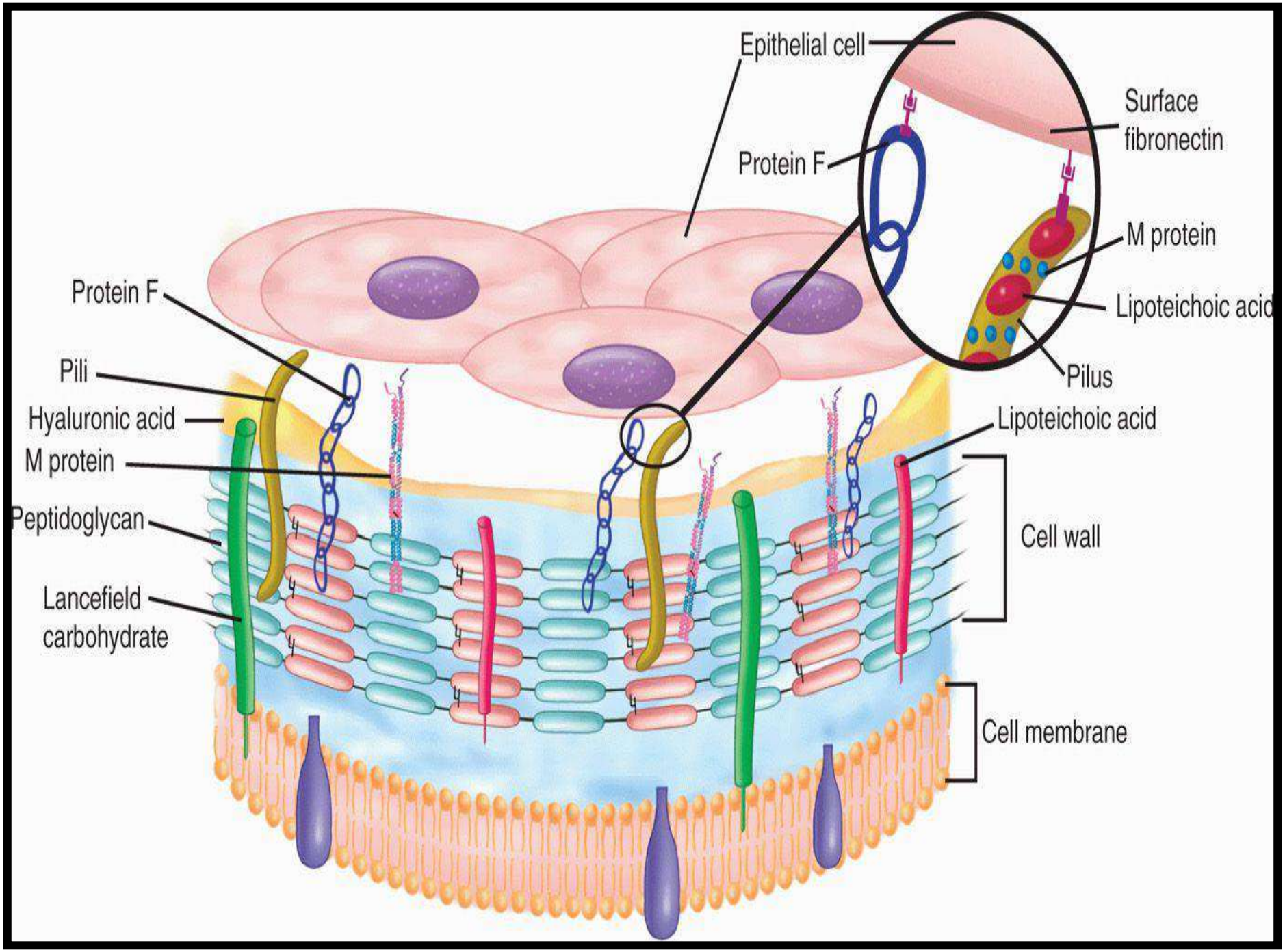
- In general, **the more hydrophobic the bacterial cell surface, the greater the adherence to the host cell.**

- Different **strains of bacteria within a species may vary widely in their hydrophobic surface properties and ability to adhere to host cells.**

- 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.




Epithelial cell
Surface fibronectin
Protein F
M protein
Lipoteichoic acid
Pilus
Protein F
Pili
Hyaluronic acid
M protein
Peptidoglycan
Lancefield carbohydrate
Lipoteichoic acid
Cell wall
Cell membrane

- 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 the host cell ensue that can lead to cytoskeletal changes allowing organism uptake by the cell.
- Sometimes changes in the adhesin molecule after attachment may trigger activation of virulence genes that promote invasion or that result in other pathogenic changes

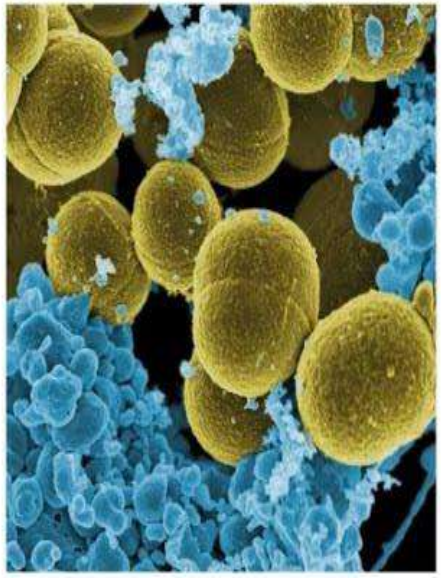
2-Invasion

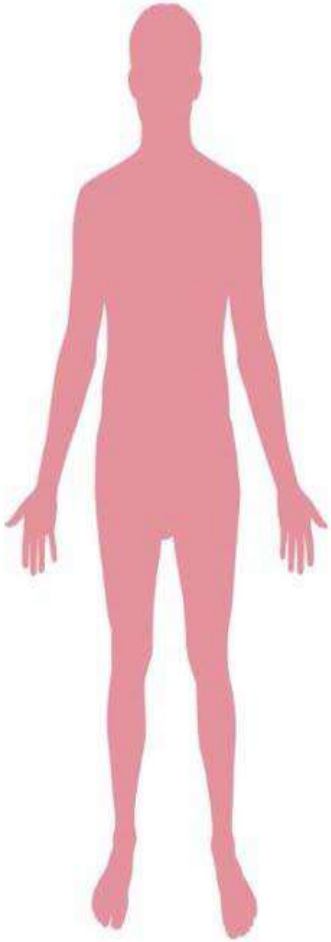
is the term commonly used to describe **the entry of bacteria into host cells, implying an active role for the organisms** and **a passive role for the host cells.**

In many infections, the bacteria produce virulence factors that influence the host cells, causing them to engulf (ingest) the bacteria.

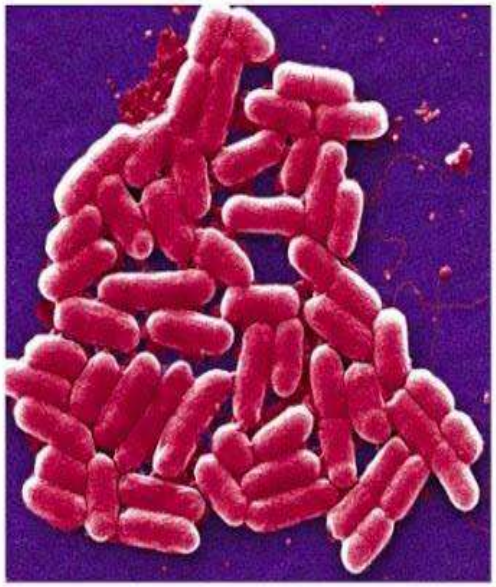
incubation period	onset of illness	period of invasion	convalescent period
(length varies)	(and prodromal phase)	(length varies)	(recovery phase)

some bacteria are more contagious during the **incubation period**





some bacteria are more infectious during the **invasive period**



- 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 cells and tissues.**

- For example, **C. diphtheriae is able to invade the epithelium of the nasopharynx and cause symptomatic sore throat even when the C. diphtheriae strains are nontoxigenic.**

- **Invasion of Host Cells and Tissues For many disease-causing bacteria, invasion of the host's epithelium is central to the infectious process.**
- Some bacteria (eg, Salmonella species) invade tissues through the junctions between epithelial cells.
- Other bacteria (eg, Yersinia species, N gonorrhoeae, Chlamydia trachomatis) invade specific types of the host's epithelial cells and may subsequently enter the tissue.

- When **inside the host cell, bacteria may remain enclosed in a vacuole composed of the host cell** membrane, or the vacuole membrane may be dissolved and bacteria may be dispersed in the cytoplasm.
- Some bacteria (eg, Shigella species) multiply within host cells, but other bacteria do not.

Chapter 2

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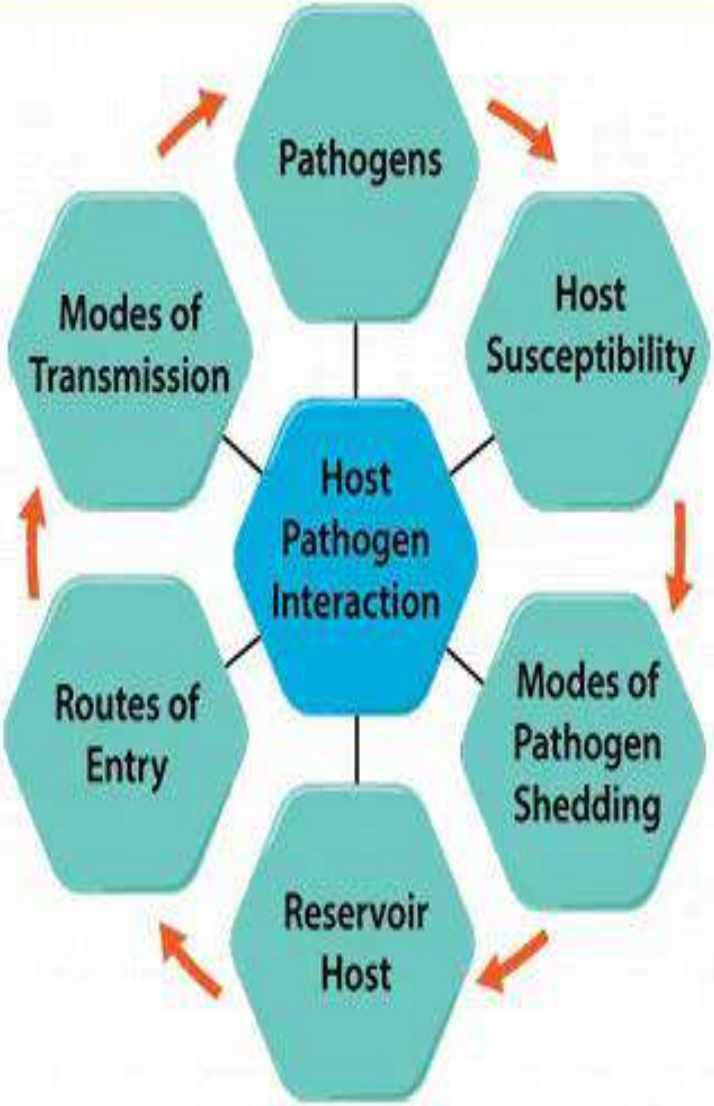
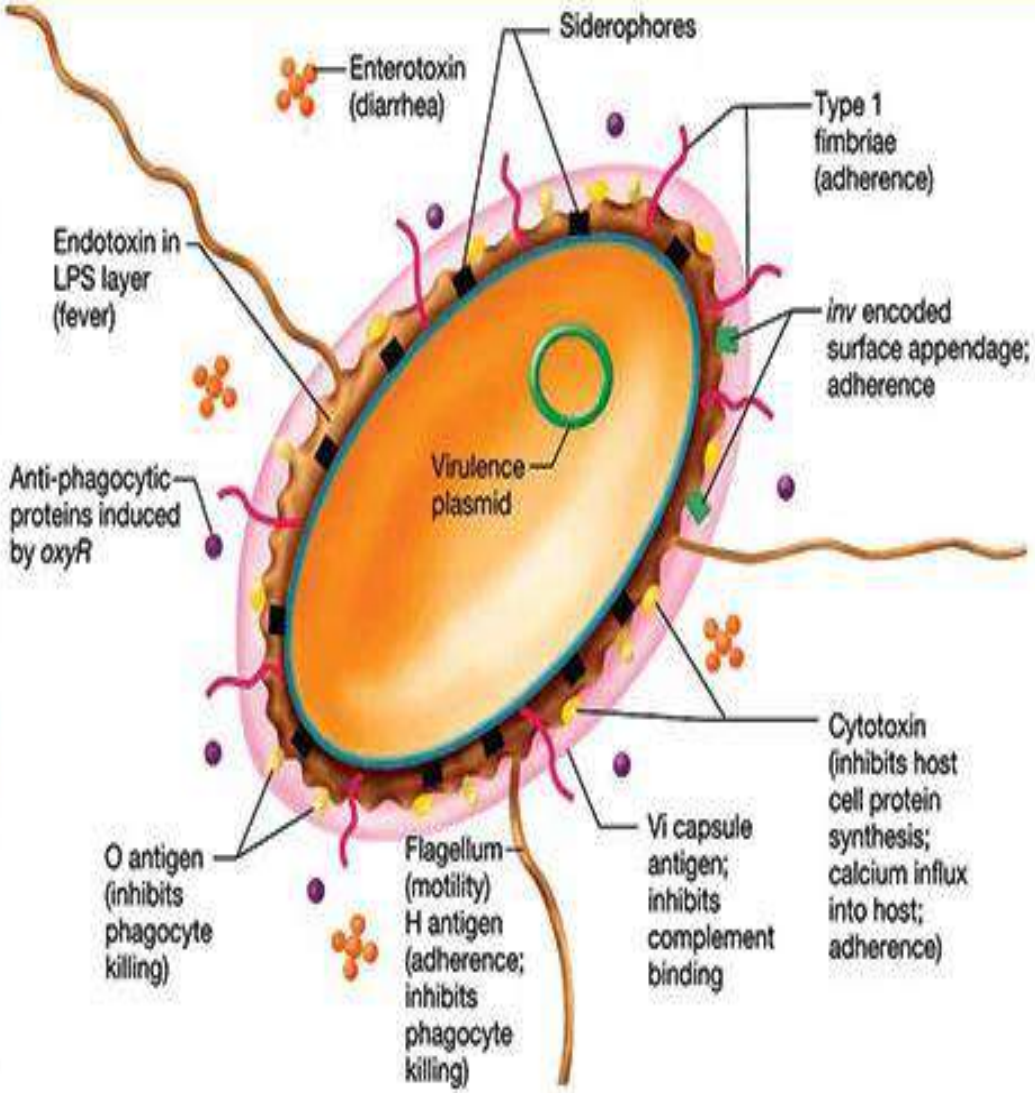
Factors affecting bacterial pathogenicity



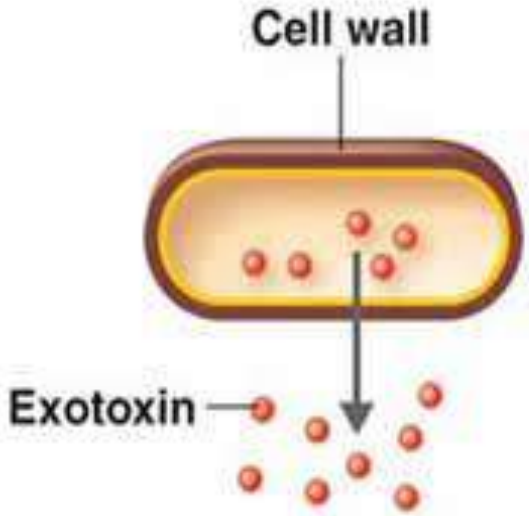


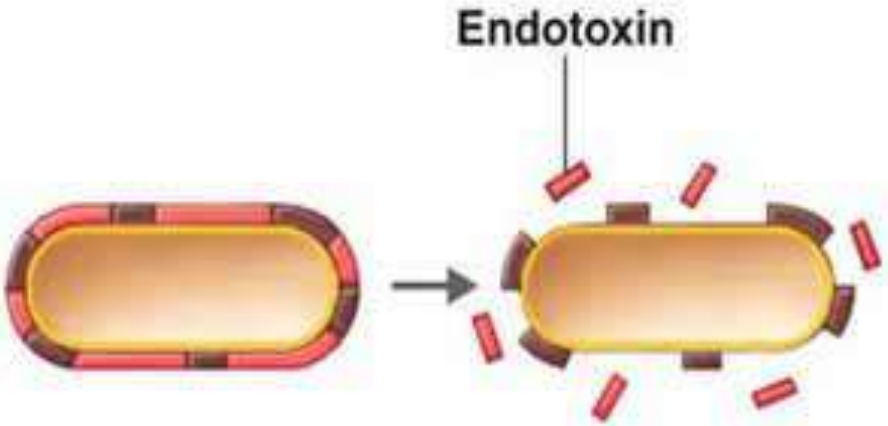
3-Toxins

- 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.

ENDOTOXINS	EXOTOXINS
1. Integral part of cell wall	1. Released from the cell before or after lysis
2. Endotoxin is LPS; lipid A is toxic	2. Protein
3. Heat stable	3. Heat labile
4. Antigenic; questionable immunogenicity	4. Antigenic and immunogenic
5. Toxoids not be produced	5. Toxoids can be produced
6. Many effects on host	6. Specific in effect on host
7. Produced only by gram-negative organisms	7. Produced by gram-positive & gram-negative organisms

Differences Between Exotoxins and Endotoxins





(a) Exotoxins are proteins produced inside pathogenic bacteria, most commonly gram-positive bacteria, as part of their growth and metabolism. The exotoxins are then secreted or released into the surrounding medium following lysis.

(b) Endotoxins are the lipid portions of lipopolysaccharides (LPSs) that are part of the outer membrane of the cell wall of gram-negative bacteria (lipid A; see Figure 4.13c). The endotoxins are liberated when the bacteria die and the cell wall breaks apart.

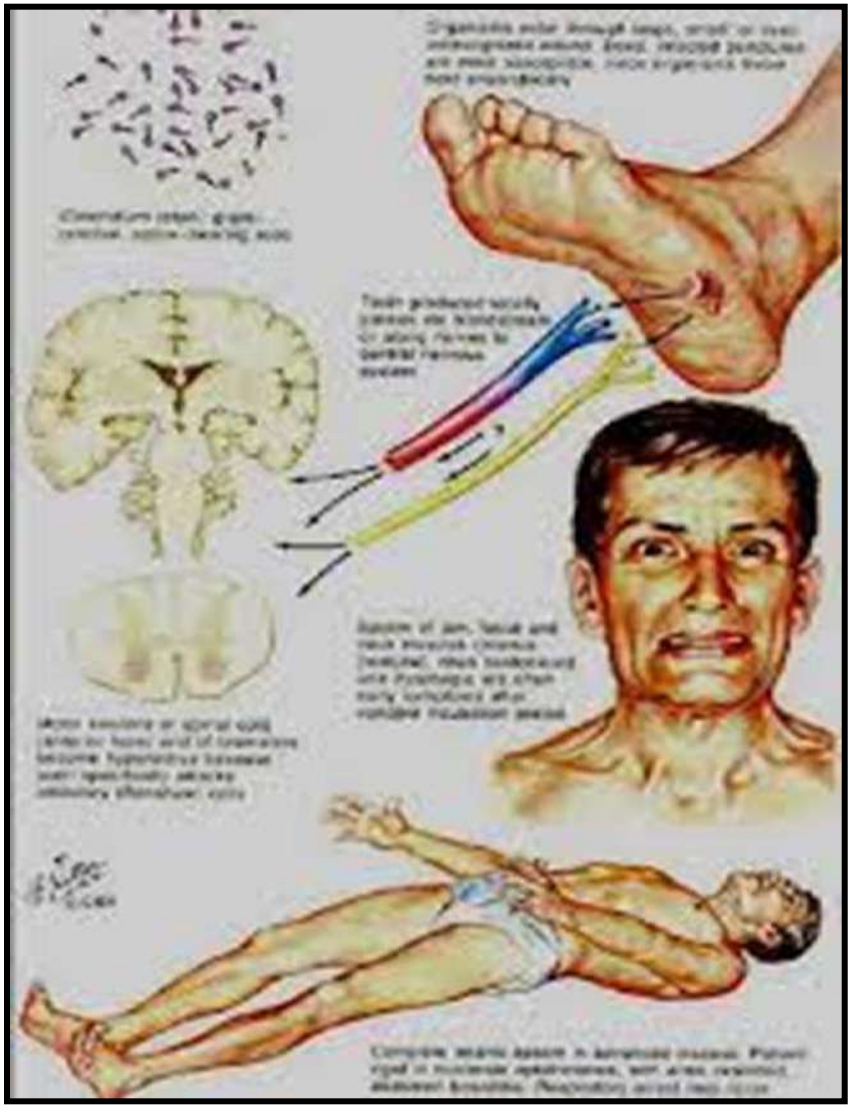
Differences B/W exotoxin and endotoxin

	Exotoxin	Endotoxin
Species	Some species of both Gram-positive and Gram-negative bacteria	Most Gram-negative bacteria and *Listeria*
Protein Location	Proteins secreted from cell	Part of cell (lipopolysaccharide) that fragments off
Gene Location	Genes for exotoxin are in plasmid or bacteriophage	Genes for endotoxin are on bacterial chromosome
Toxicity	High toxicity	Low toxicity
Antigenicity	Highly antigenic (host forms antibodies called antitoxins)	Poorly antigenic
Vaccine	Vaccine available (formed from toxoids)	No vaccine available
Heat Stability	Heat labile	Heat stable
Example	Think cholera, tetanus, botulism	Think meningococcemia, sepsis

A. Exotoxins

- **Many** gram-positive and gram-negative bacteria produce exotoxins of considerable medical importance
- Some of these toxins had major roles in world history.
- For example, tetanus caused by the toxin of *C. tetani* killed as many as 50,000 soldiers of the Axis powers in World War II;
- However, the Allied forces, immunized military personnel against tetanus, and very few died of that disease.

Tetanus exotoxin

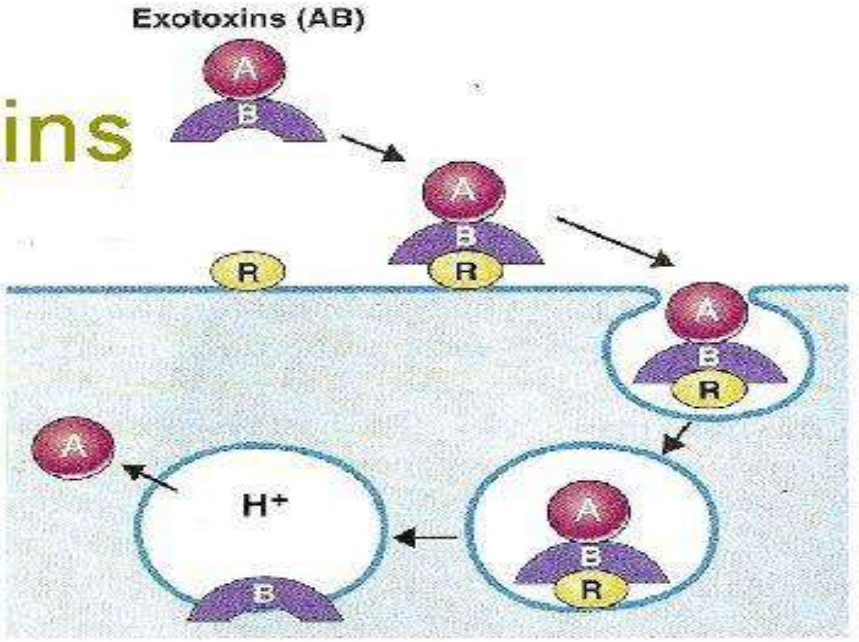


- Many exotoxins consist of A and B subunits. The A subunit provides the toxic activity.
- The B subunit generally **mediates**
- **1- Adherence of the toxin complex to a host cell**
- **2- Aids entrance of the exotoxin into the host cell.**
- Examples of some pathogenetic mechanisms associated with exotoxins are given below.

Bacterial exotoxin

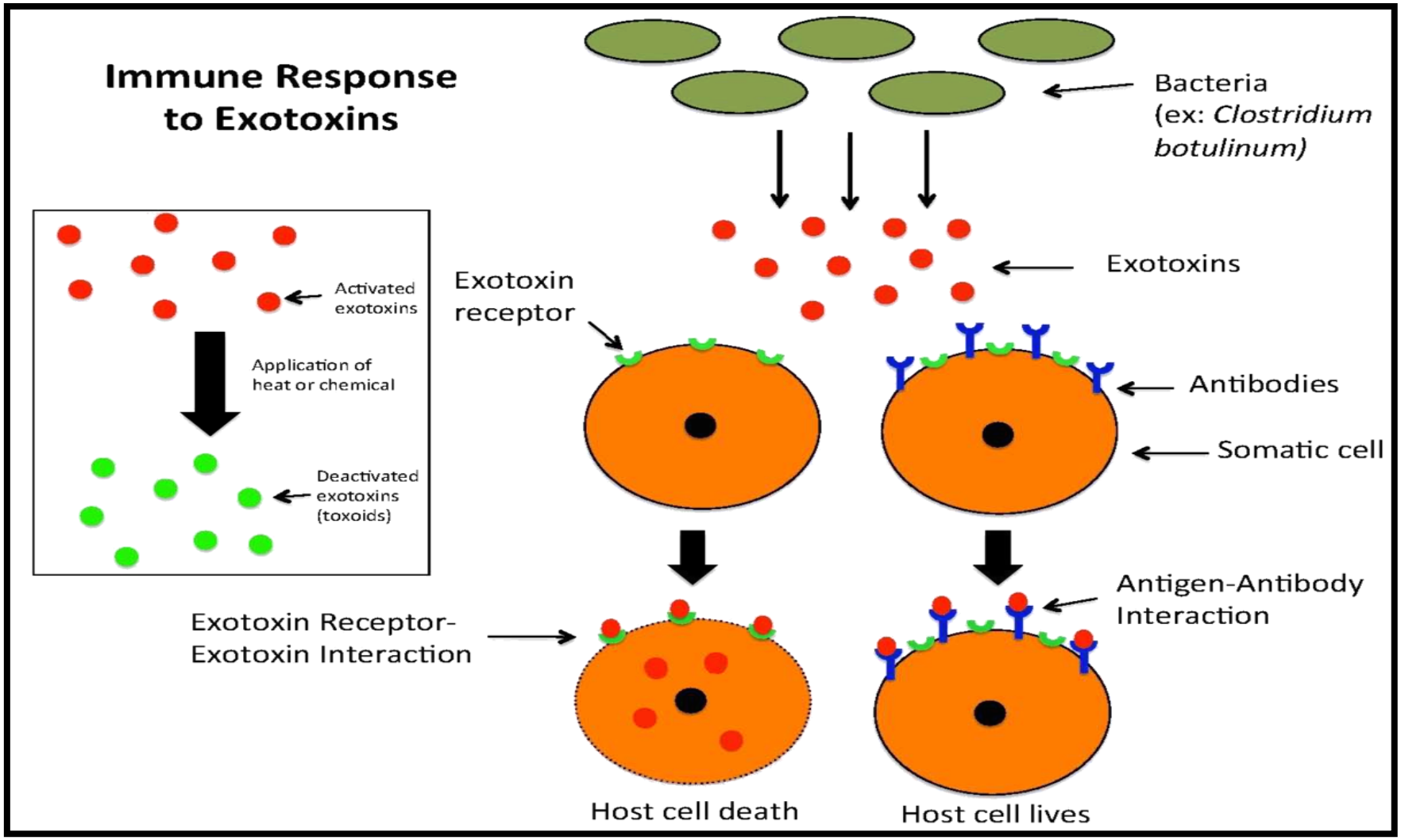
Bacterial Exotoxins

- Exotoxins
  - Initial location outside cells
  - Transported into host cells
  - Alter host cell physiology and metabolism
  - Typical A – B toxins



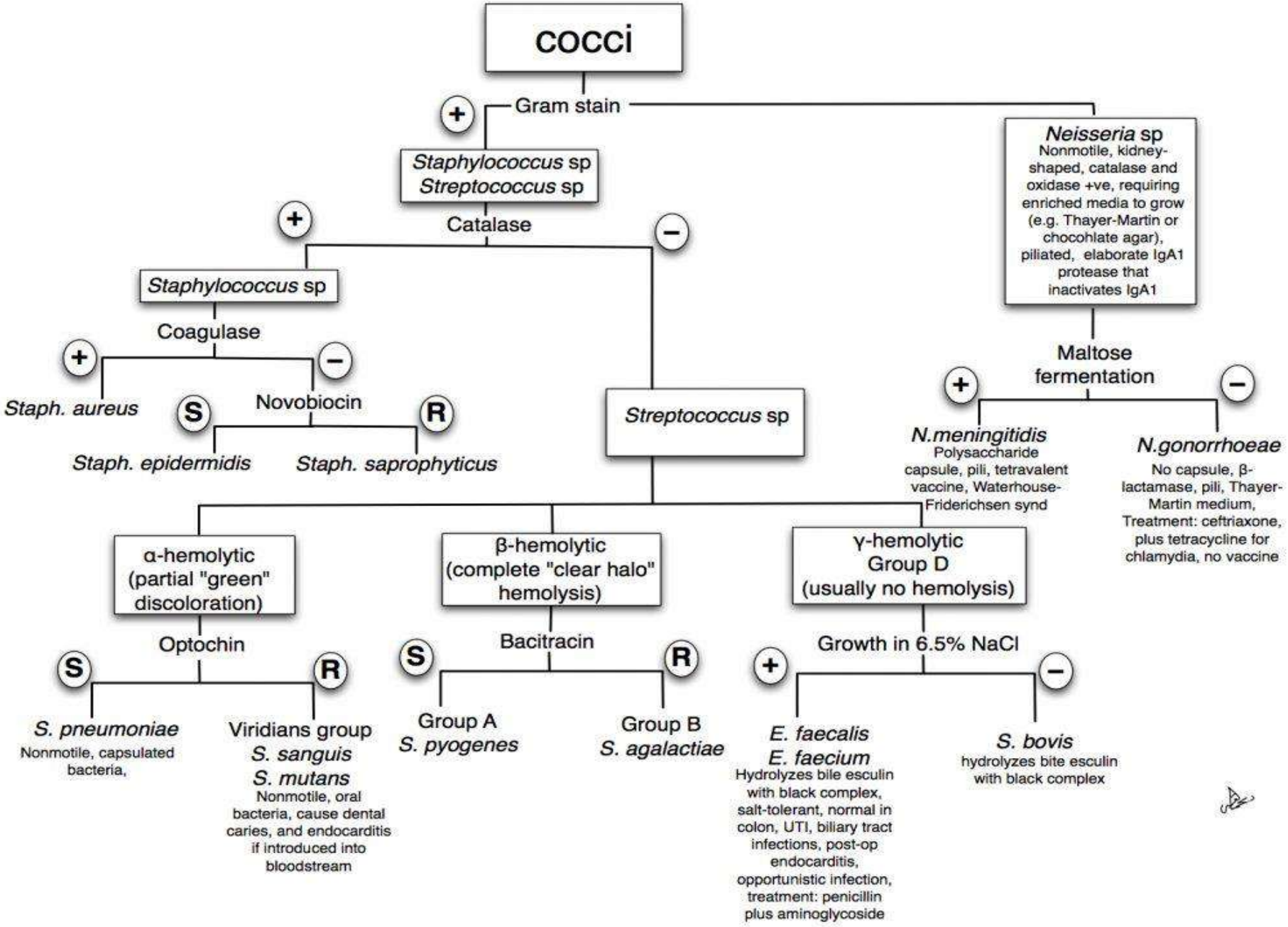
AB toxin enters cells via:
1) Receptor mediated endocytosis
2) Fusion of vesicle with lysosome
3) Acid environment of lysosome reduces disulfide bonds and releases A into cell
4) A has various cellular activities

Exotoxin host cell interaction



1- Some **strains of group A β-hemolytic streptococci produce pyrogenic exotoxin A** that is similar to or the same as **streptococcal erythrogenic toxin**, which results in **scarlet fever.**

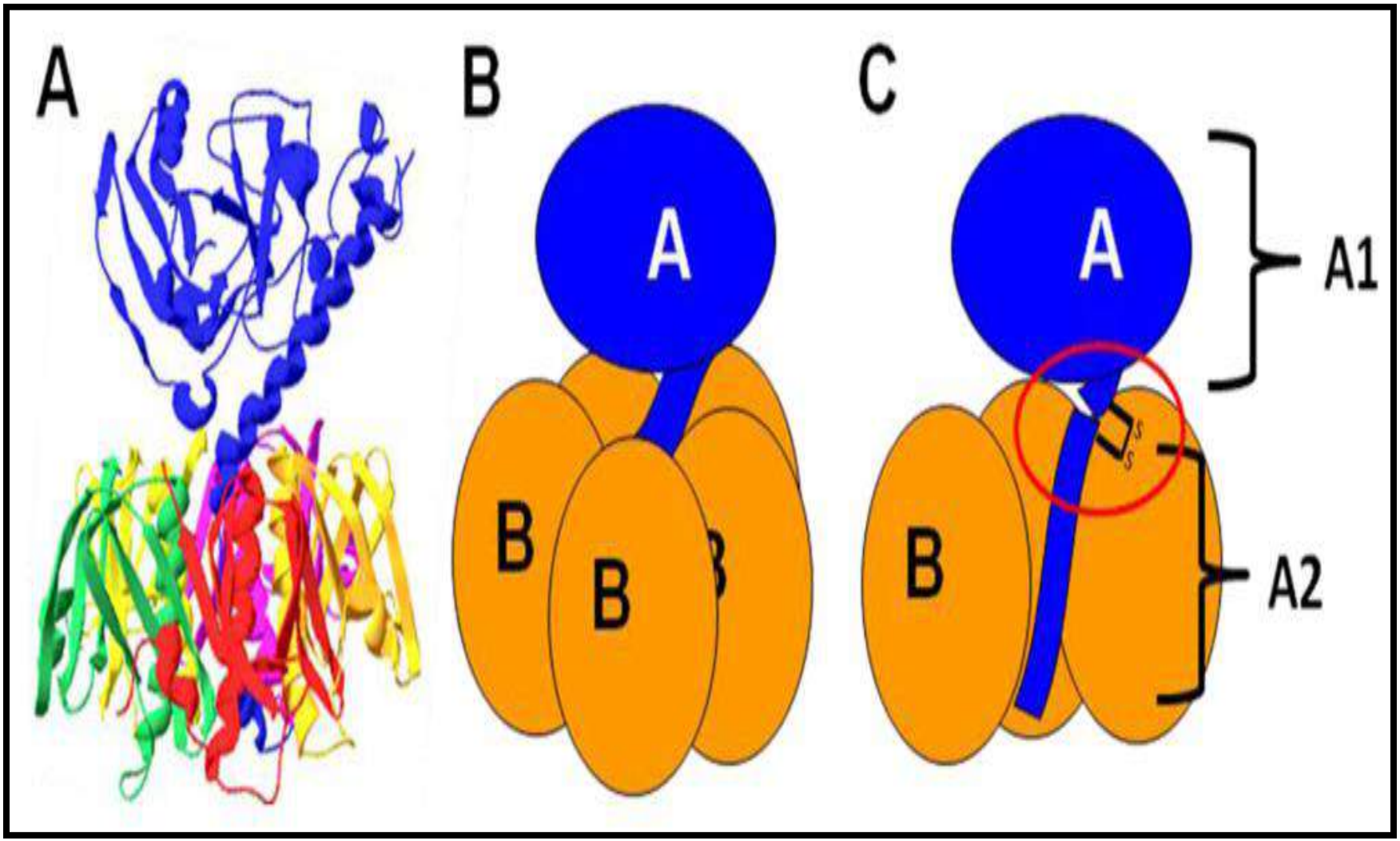
- **Rapidly progressive soft tissue infection by streptococci that produce the pyrogenic exotoxin A has many clinical manifestations similar to those of staphylococcal toxic shock syndrome.**
- **The pyrogenic exotoxin A also is a super antigen that acts in a manner similar to TSST-1.**


cocci
Gram stain
+
Staphylococcus sp
Streptococcus sp
Catalase
+
−
Staphylococcus sp
Coagulase
+
−
Staph. aureus
S
Novobiocin
R
Staph. epidermidis
Staph. saprophyticus
Streptococcus sp
Neisseria sp
Nonmotile, kidney-shaped, catalase and oxidase +ve, requiring enriched media to grow (e.g. Thayer-Martin or chocohlate agar), piliated, elaborate IgA1 protease that inactivates IgA1
Maltose fermentation
+
−
N.meningitidis
Polysaccharide capsule, pili, tetravalent vaccine, Waterhouse-Friderichsen synd
N.gonorrhoeae
No capsule, β-lactamase, pili, Thayer-Martin medium, Treatment: ceftriaxone, plus tetracycline for chlamydia, no vaccine
α-hemolytic (partial "green" discoloration)
β-hemolytic (complete "clear halo" hemolysis)
γ-hemolytic Group D (usually no hemolysis)
Optochin
S
R
S. pneumoniae
Nonmotile, capsulated bacteria,
Viridians group
S. sanguis
S. mutans
Nonmotile, oral bacteria, cause dental caries, and endocarditis if introduced into bloodstream
Bacitracin
S
R
Group A
S. pyogenes
Group B
S. agalactiae
Growth in 6.5% NaCl
+
−
E. faecalis
E. faecium
Hydrolyzes bile esculin with black complex, salt-tolerant, normal in colon, UTI, biliary tract infections, post-op endocarditis, opportunistic infection, treatment: penicillin plus aminoglycoside
S. bovis
hydrolyzes bite esculin with black complex

- 2. Exotoxins Associated with Diarrheal Diseases and Food Poisoning
- Exotoxins associated with diarrheal diseases are frequently called enterotoxins.
- V. cholerae has produced epidemic diarrheal disease (cholera) in many parts of the world.
- After entering host via contaminated food or drink, V. cholerae penetrates the intestinal mucosa and attaches to microvilli of the brush border of gut epithelial cells.
- V. cholerae, usually of the serotype O1 (and O139), can produce an enterotoxin with a MW of 84,000.

- The toxin consists of two subunits—
- Subunit B. has five identical peptides and rapidly binds the toxin to cell membrane ganglioside molecules.
- Subunit A, which is split into two peptides, A1 and A2, linked by a disulfide bond, and
- Subunit A enters the cell membrane and causes a large increase in adenylate cyclase activity and in the concentration of cAMP.

V cholera Exotoxin

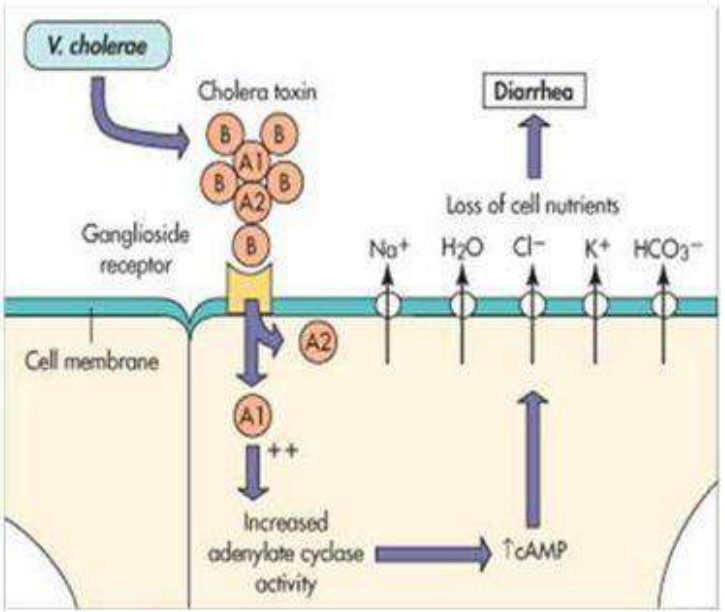


- The net effect is rapid secretion of electrolytes into the small bowel lumen, with impairment of sodium and chloride absorption and loss of bicarbonate.
- Life-threatening massive diarrhea (eg, 20–30 L/day) can occur, and acidosis develops.

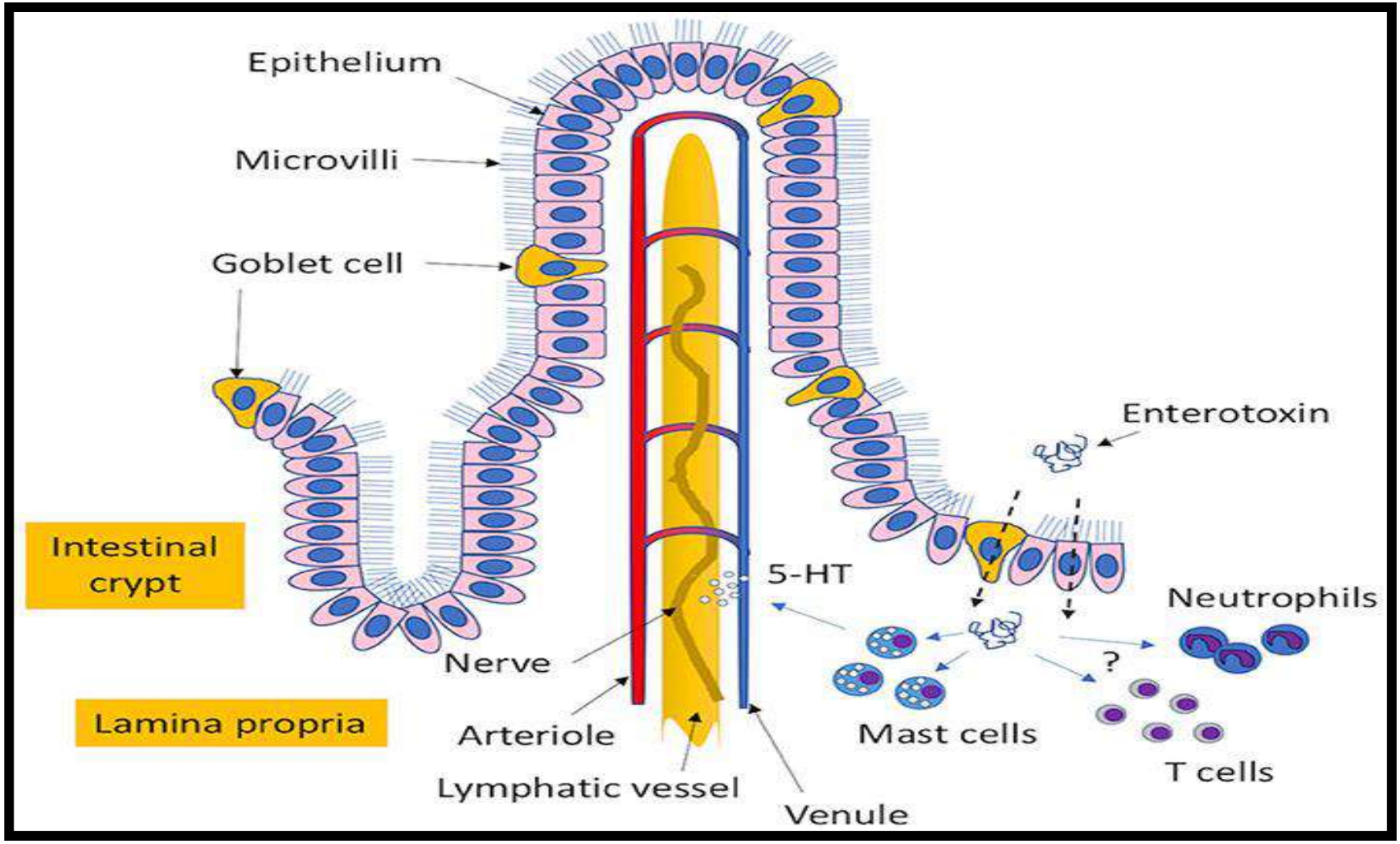
- The deleterious effects of cholera are due to fluid loss and acid–base imbalance;
- Treatment, therefore, is by electrolyte and fluid replacement.

Examples of exotoxins: Cholera toxin

- Vibrio cholera – diarrhoea
- Toxin A – increased cAMP – which controls the efflux of H_2O ions from cells
- Increased secretion of water and ions into the intestine ➡ Diarrhoea

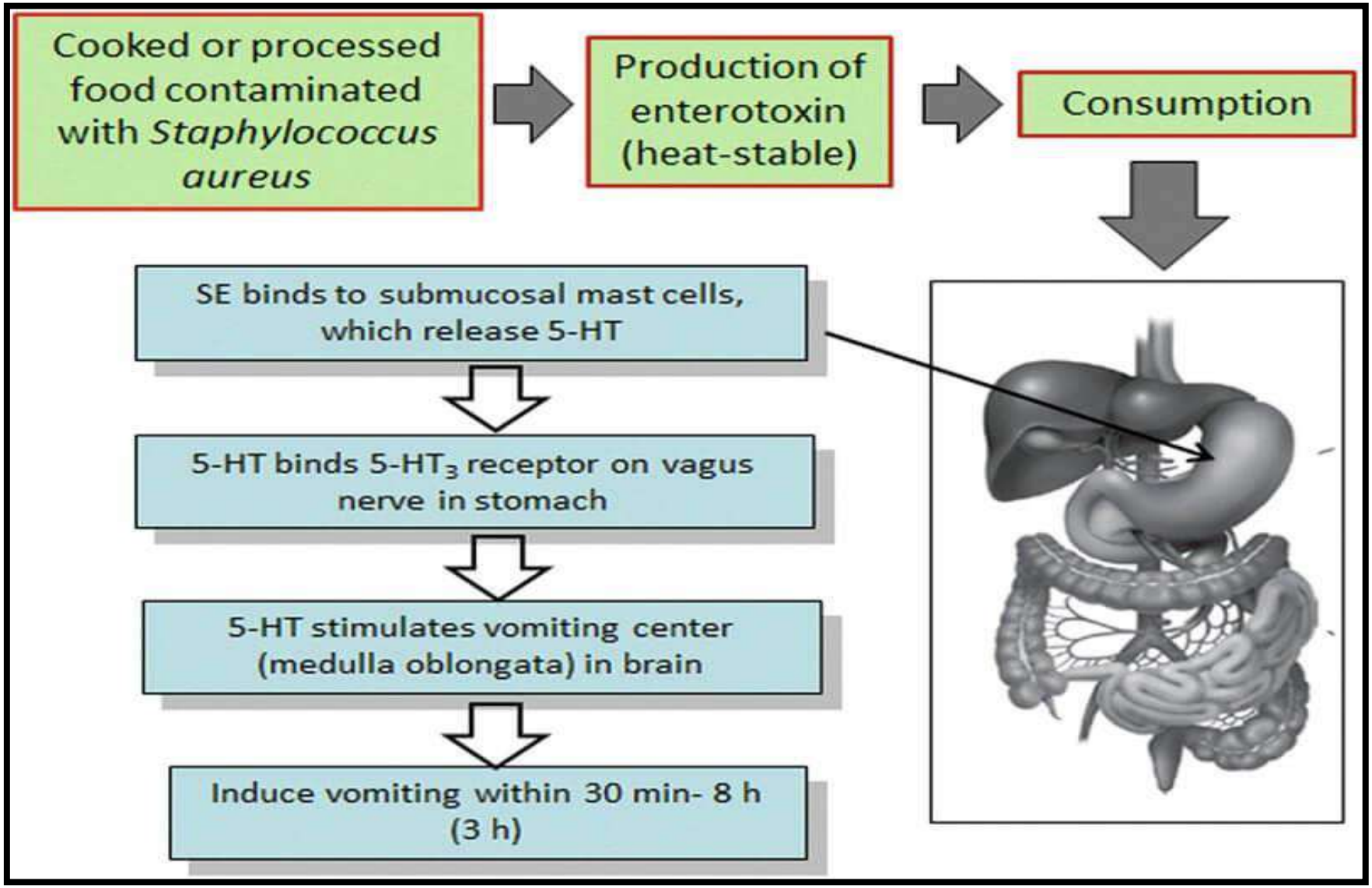


Cholera exotoxin



- 3-Some strains of *S. aureus* produce enterotoxins while growing in meat, dairy products, or other foods. In typical cases, the food has been recently prepared but not properly refrigerated.
- There are at least 7 distinct types of the staphylococcal enterotoxin.
- After the preformed toxin is ingested, it is absorbed in the gut, where it stimulates vagus nerve receptors.

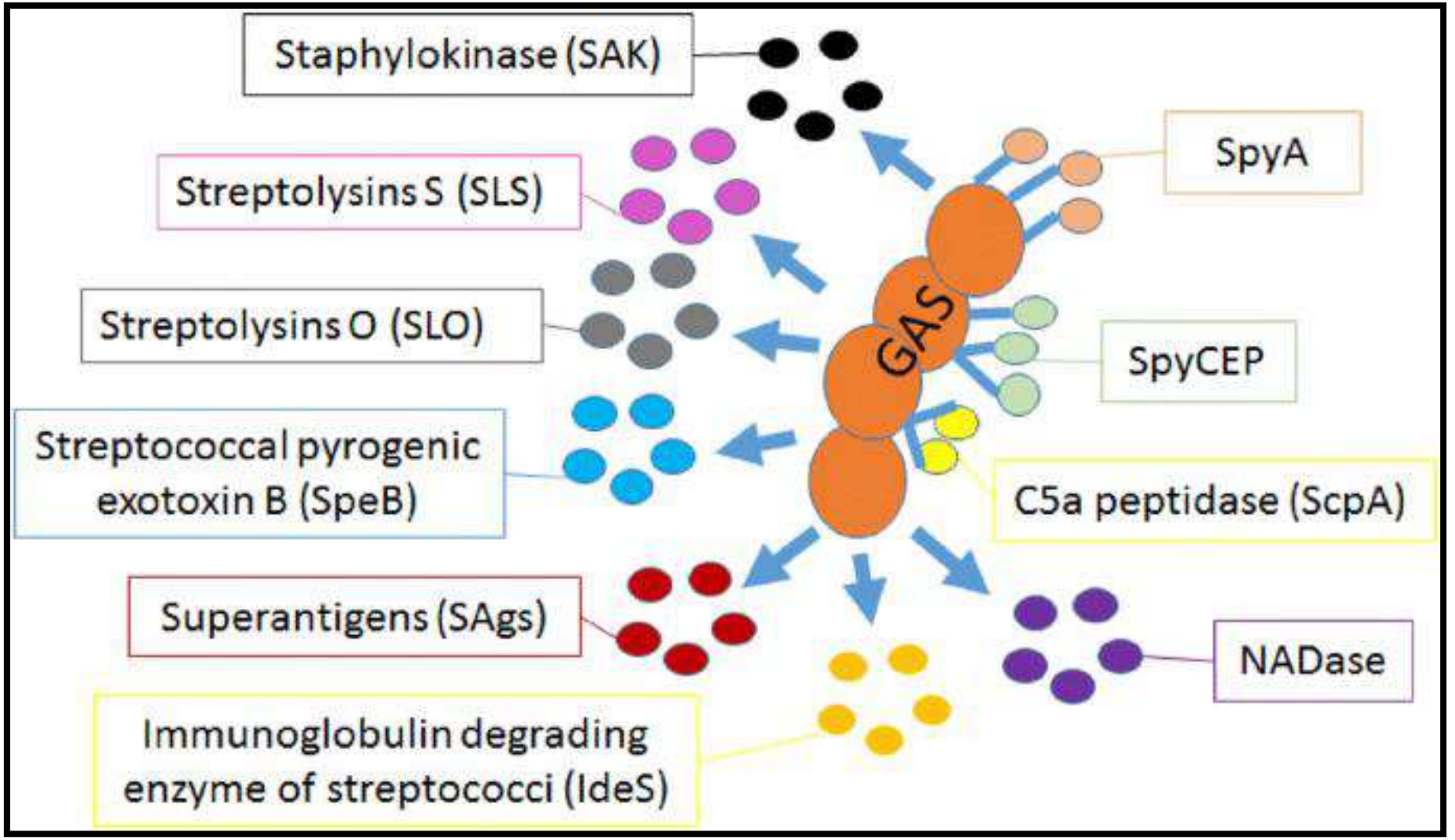
S.aureus entertoxin mechanism of action



- The stimulus is transmitted to the vomiting center in the central nervous system.
- Vomiting, often projectile, results within hours.
- Diarrhea is less frequent. Staphylococcal food poisoning is the most common form of food poisoning. *S aureus* enterotoxins are super antigens.

- **Gram-positive bacteria** have considerably **more cell wall–associated peptidoglycan than do gram-negative bacteria**.
- **Peptidoglycan released during infection** may yield many of the same biologic activities as LPS, although peptidoglycan is invariably much less potent than LPS

Hemolysin

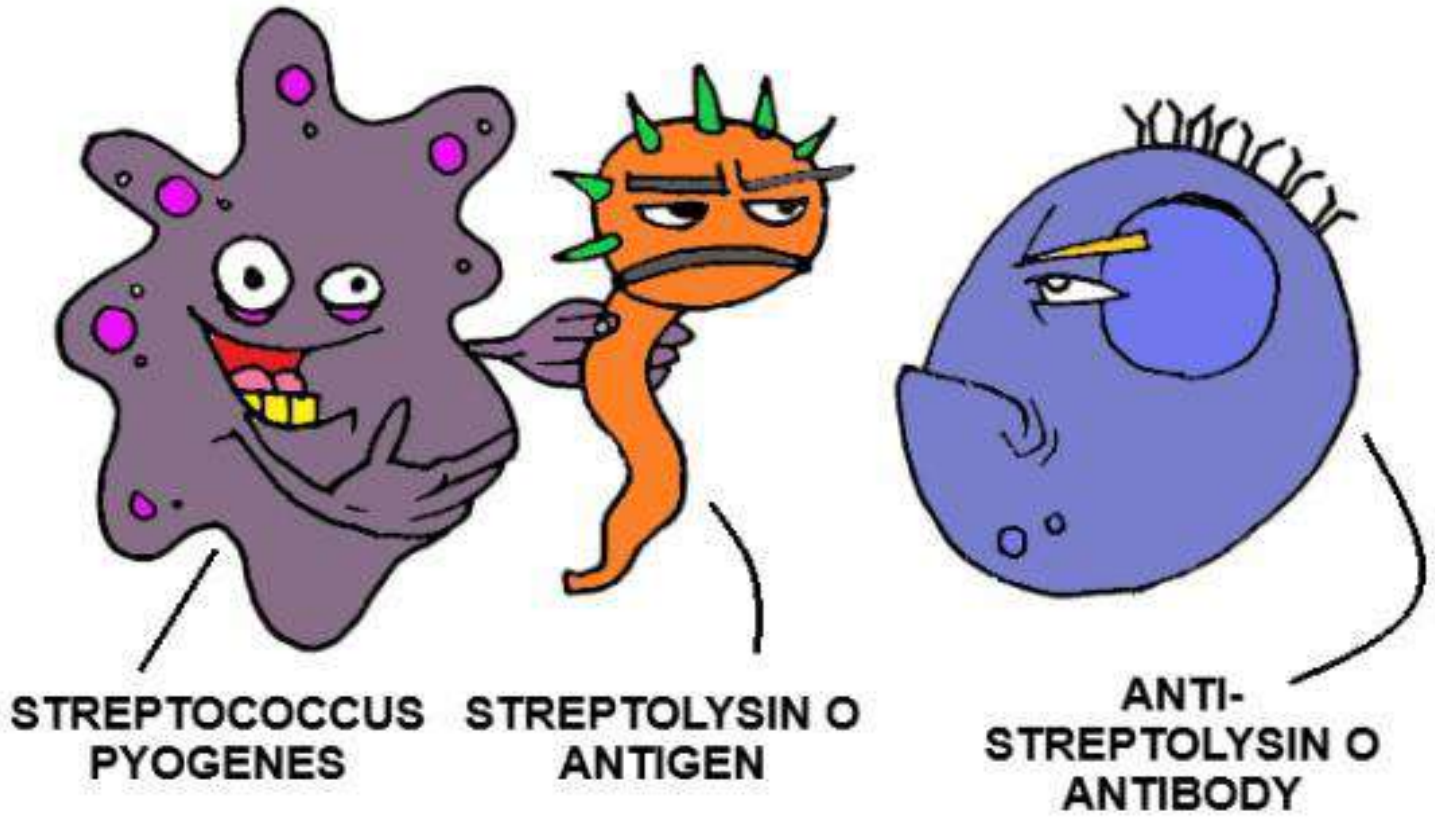

Staphylokinase (SAK)
Streptolysins S (SLS)
Streptolysins O (SLO)
Streptococcal pyrogenic exotoxin B (SpeB)
Superantigens (SAgs)
Immunoglobulin degrading enzyme of streptococci (IdeS)
GAS
SpyA
SpyCEP
C5a peptidase (ScpA)
NADase

- **Elaborates two hemolysins (streptolysins)** that not only lyse the membranes of erythrocytes but also damage a variety of other cell types.
- **Streptolysin O** is a protein .it combines quantitatively with **antistreptolysin O (ASO),** an antibody that appears in humans after infection with any streptococci that produce streptolysin O.

- **Streptolysin O**, for example, is **produced by group A streptococci and is lethal for mice and hemolytic for RBCs from many animals.**
- **Streptolysin O is oxygen labile and can therefore be oxidized and inactivated, but it is reactivated by reducing agents.**

- The **same streptococci also produce oxygen-stable, serum-inducibl streptolysin S, which is not antigenic.**
- This antibody blocks hemolysis by streptolysin O. This phenomenon forms **the basis of a quantitative test for the antibody.**
- An ASO serum titer in excess of 160–200 units is considered abnormally high and suggests either **recent** infection with *S pyogenes* or **persistently** high antibody levels caused by an exaggerated immune response to an earlier exposure in a hypersensitive person

Streptolysin O

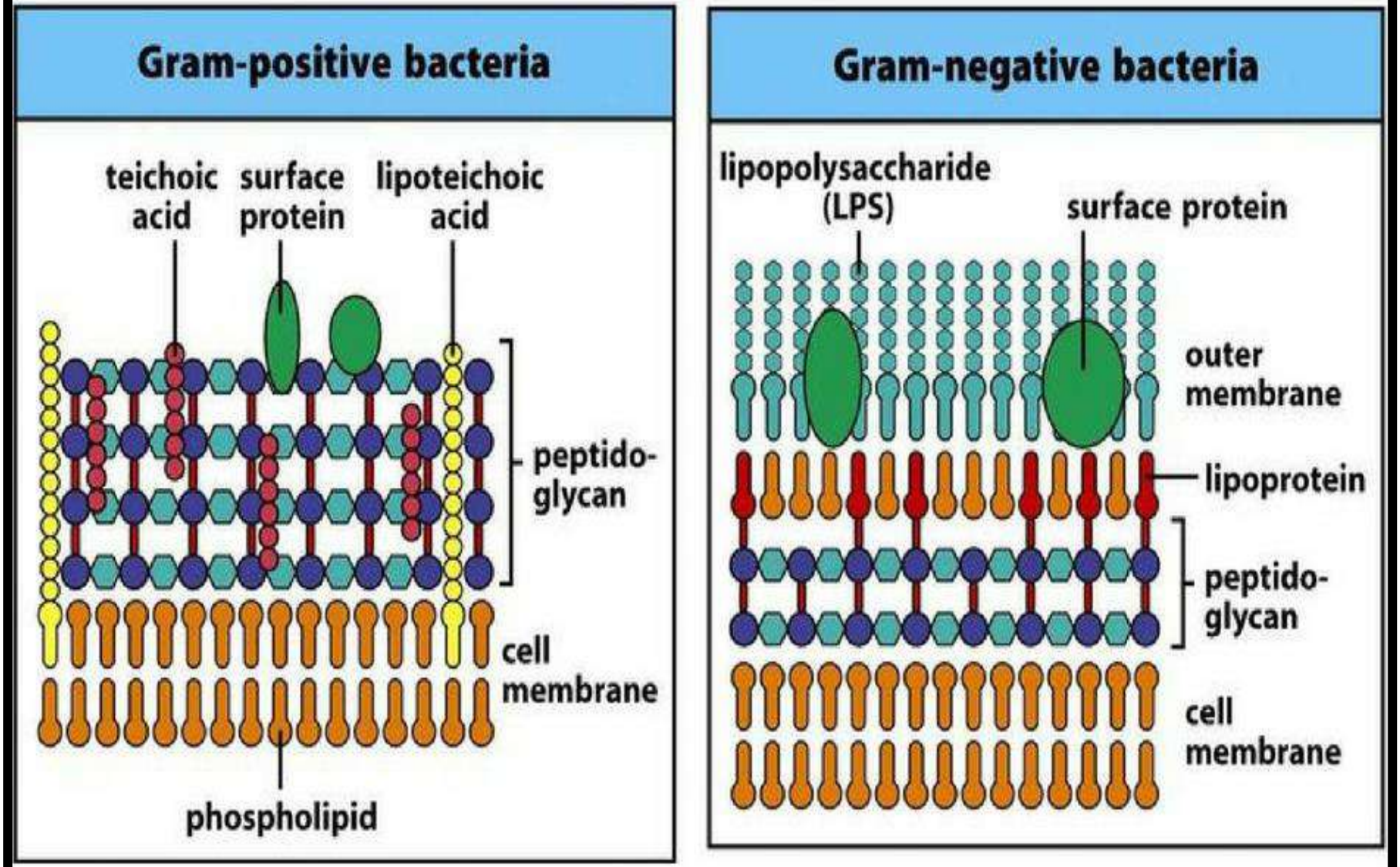


- **Streptolysin S** is the agent responsible for the hemolytic zones around streptococcal colonies growing on the surface of blood agar plates.
- It is not antigenic, but it may be inhibited by a nonspecific inhibitor **that is frequently present in the sera of humans and animals and is independent of past experience with streptococci.**
- Most isolates of *S. pyogenes* produce both of these hemolysins. About 10% may produce only one.

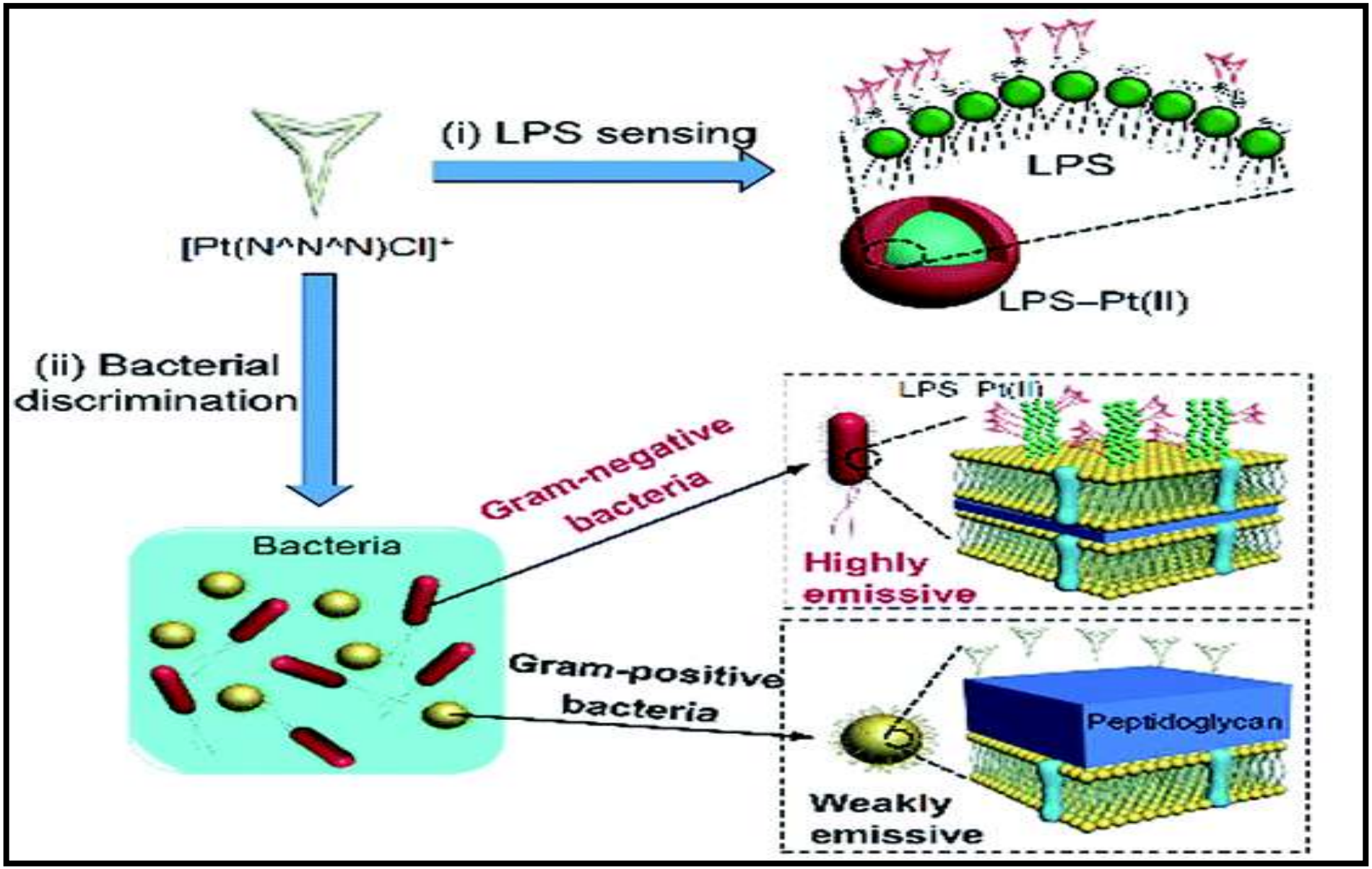
B. Endotoxin

- The LPS (endotoxin) of gram-negative bacteria are bacterial cell wall components that are often liberated when the bacteria lyse.
- The substances are heat-stable, have MWs between 3000 and 5000 (lipooligosaccharides, LOS) and several million (lipopolysaccharides) and can be extracted (eg, with phenol-water). They have three main regions.
- Hypotension occurs early in gram-negative bacteremia or after injection of LPS.

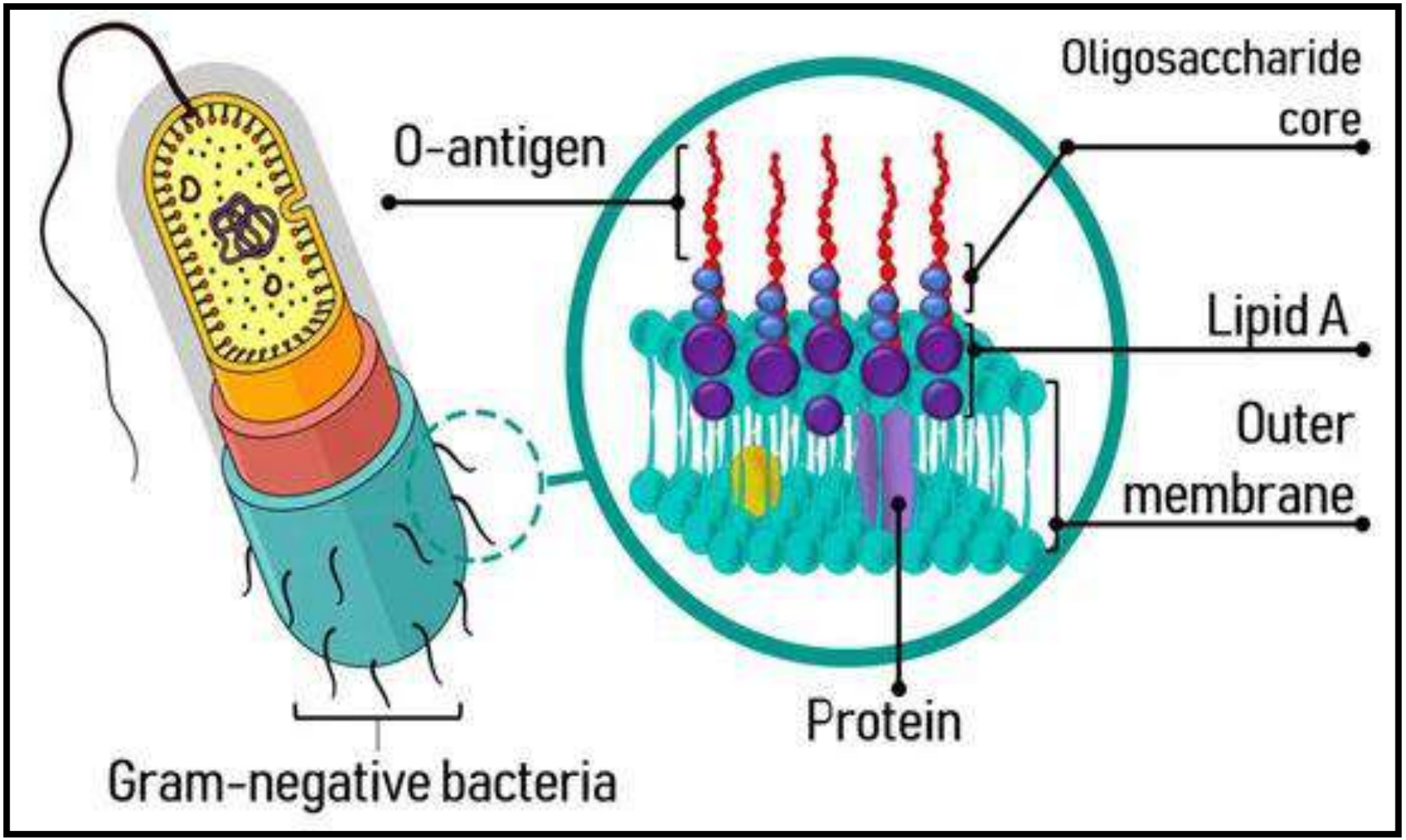
Gram positive bacteria V/S Gram negative baceteria



Endotoxin


(i) LPS sensing
LPS
LPS–Pt(II)
[Pt(N^N^N)Cl]+
(ii) Bacterial discrimination
Bacteria
Gram-negative bacteria
LPS Pt(II)
Highly emissive
Gram-positive bacteria
Peptidoglycan
Weakly emissive

Parts of endotoxin



4-Enzymes

Many species of bacteria produce enzymes **that are not intrinsically toxic** but **do play important roles in the infectious process.** Some of these enzymes are discussed below.

- **Many bacteria produce tissue-degrading enzymes.**

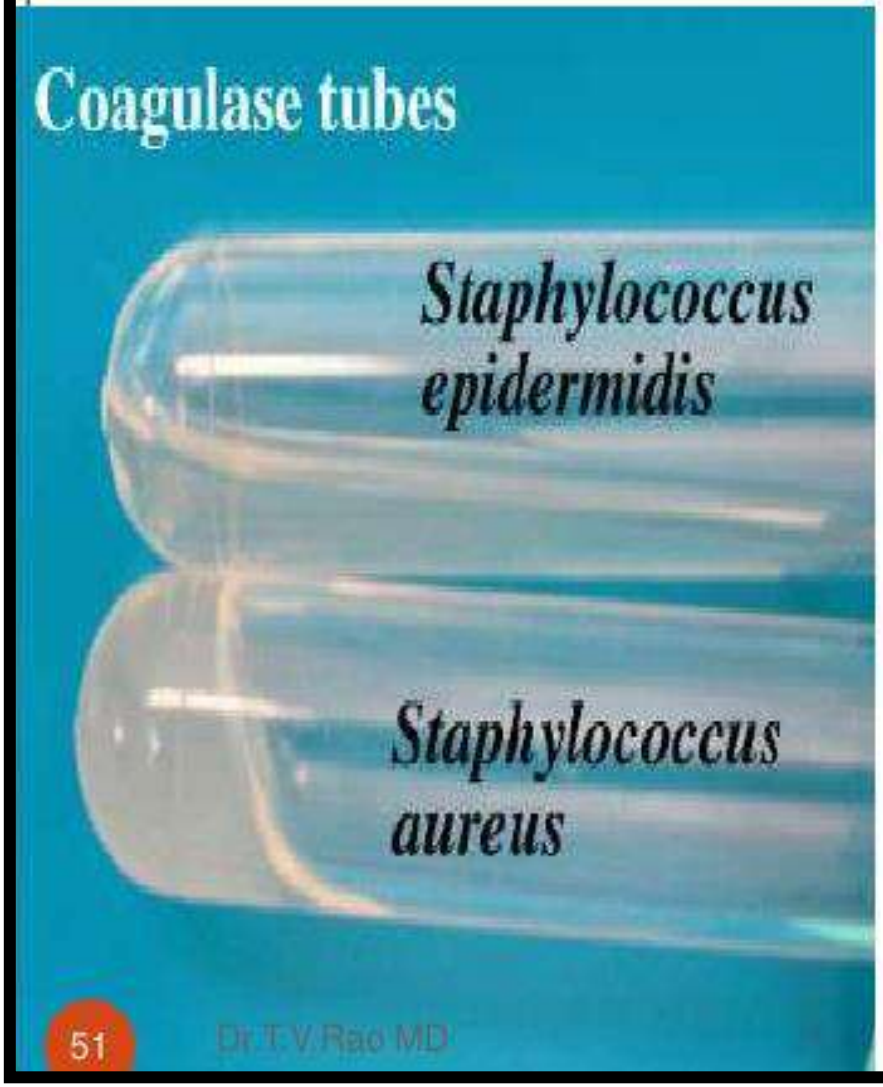
Some Extra Cellular Bacterial Proteins That Act As Invasins:

Invasin	Bacteria Involved	Activity
Hyaluronidase	Streptococci, staphylococci and clostridia	Degrades hyaluronic of connective tissue
Collagenase	*Clostridium*species	Dissolves collagen framework of muscles
Neuraminidase	*Vibrio cholerae*and *Shigella dysenteriae*	Degrades neuraminic acid of intestinal mucosa
Coagulase	Staphylococcus aureus	Converts fibrinogen to fibrin which causes clotting

- The best-characterized are **enzymes from** ***C. perfringens*** ,and, to a lesser extent, **anaerobic bacteria S aureus , and group A streptococci** .
- In addition to lecithinase, *C. perfringens* produces the proteolytic enzyme collagenase, which **degrades collagen the major protein of fibrous connective tissue, and promotes spread of infection in tissue.**
- **The roles of tissue-degrading enzymes in the pathogenesis of infections appear obvious.**

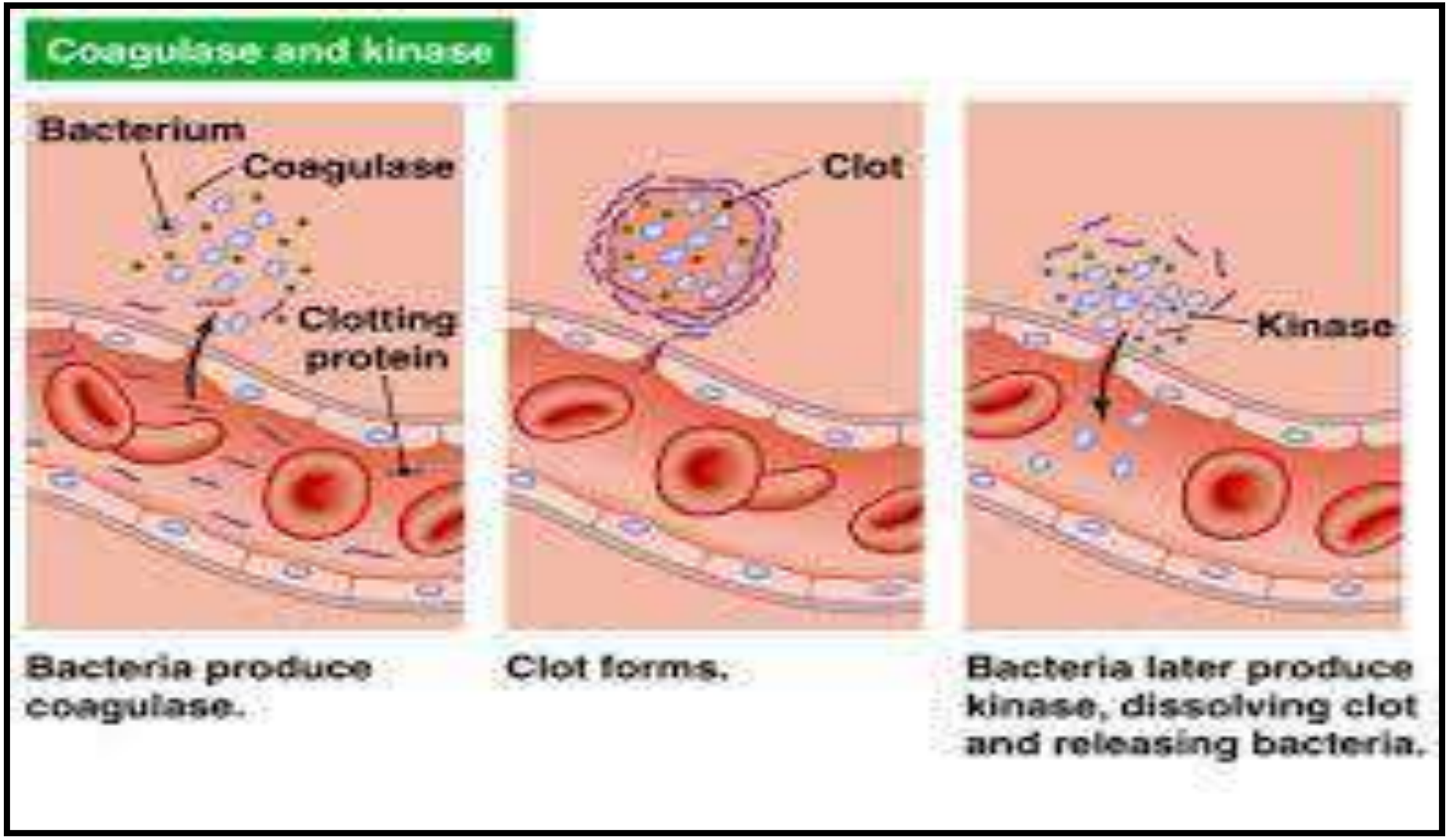
- # 1- Coagulase
- S. aureus produces coagulase, which works in conjunction with blood factors to coagulate plasma.
- Coagulase contributes to the formation of fibrin walls around staphylococcal lesions, which helps them persist in tissues.
- Coagulase also causes deposition of fibrin on the surfaces of individual staphylococci, which may help protect them from phagocytosis or from destruction within phagocytic cells.

Coagulase test



- Coagulase is an enzyme that clots blood plasma by catalyzing the conversion of a soluble protein (fibrinogen) to an insoluble protein (fibrin). This test is performed on Gram-positive, catalase positive species to identify the coagulase positive *Staphylococcus aureus*. Coagulase is a virulence factor of *S. aureus*. The formation of clot around an infection caused by this bacteria likely protects it from phagocytosis.

Coagulase and Kinases



2- Hyaluronidases

- Are **enzymes that hydrolyze hyaluronic acid, a constituent of the ground substance of connective tissue.**
- They are **produced by many bacteria (eg, staphylococci, streptococci, and anaerobes) and aid in their spread through tissues.**

- Hyaluronidase splits hyaluronic acid, an important component of the ground substance of connective tissue.
- Thus, hyaluronidase aids in spreading infecting microorganisms (spreading factor).
- Hyaluronidases are antigenic and specific for each bacterial or tissue source.
- After infection with hyaluronidase-producing organisms, specific antibodies are found in the serum.

3-Streptokinase

- Many hemolytic streptococci produce streptokinase (fibrinolysin), a substance that activates a proteolytic enzyme of plasma.
- This enzyme is then able to dissolve coagulated plasma and probably aids in the rapid spread of streptococci through tissues.
- Streptokinase has been used in treatment of acute myocardial infarction to dissolve fibrin clots .

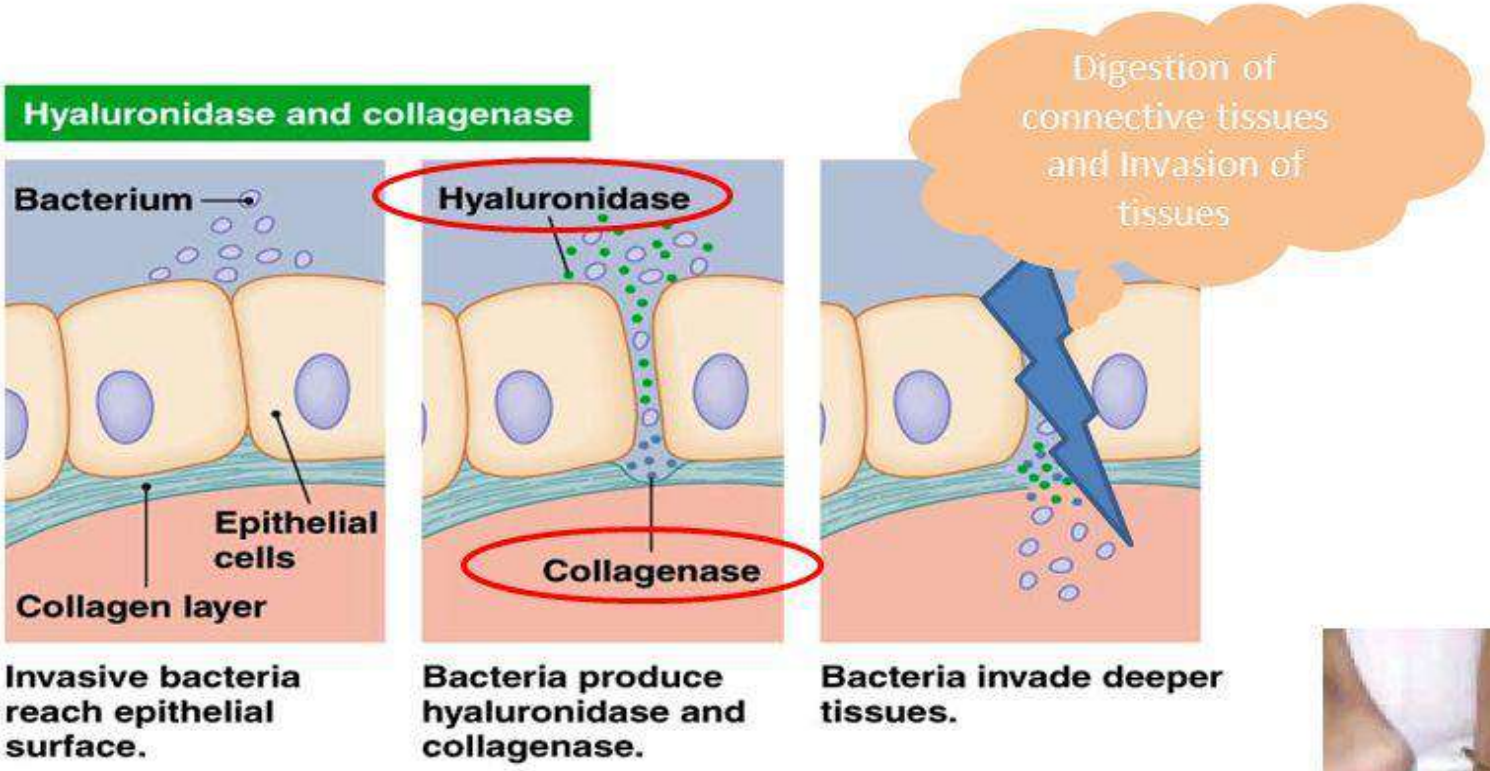
Streptokinase (Fibrinolysin)

- Streptokinase is produced by many strains of group **A β-hemolytic streptococci.** It transforms the plasminogen of human plasma into plasmin, an active proteolytic enzyme that digests fibrin and other proteins, allowing the bacteria to escape from blood clots.
- This process of digestion may be interfered with by nonspecific serum inhibitors and by a specific antibody, antistreptokinase. Streptokinase has been given intravenously for treatment of pulmonary emboli, coronary artery, and venous thromboses.

4. DNases

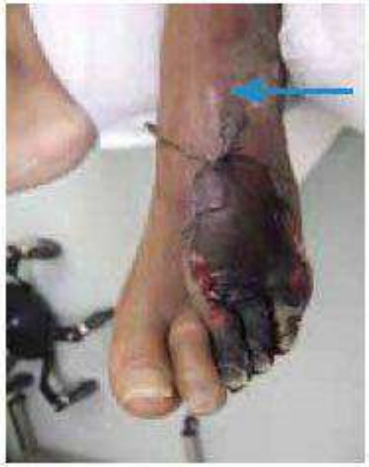
- Streptococcal deoxyribonucleases A, B, C, and D degrade DNA (DNases) and similar to streptokinase facilitate the spread of streptococci in tissue by liquefying pus.
- Mixtures of streptokinase and DNases are used in "enzymatic debridement."
- They help to liquefy exudates and facilitate removal of pus and necrotic tissue; antimicrobial drugs thus gain better access, and infected surfaces recover more quickly. An antibody to DNAse develops after streptococcal infections (normal limit, 100 units), especially after skin infections.

(b) Hyaluronidase and collagenase



Hyaluronidase: is present in *Staphylococcus aureus* (Skin infections) *and Streptococcus pyogenes (Sore throat)*

Collagenase: is present in Clostridium perfringens (gas gangrene)



End of chapter 2