Bacterial Pathogenesis

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MICROORGANISM AND HOST

- **Saprophytism** – Living on dead or decaying organic matter.
- **Parasitism** – Living on or within another living organism - there are different types of host-parasite relationships.
  - **Commensalism** – parasite lives on/in the host without causing any disease.
  - **Symbioism** – mutally beneficial relationship
  - **Opportunistic pathogen** – The organism is generally harmless, but can cause disease when it gains access to other sites or tissues.
  - **Obligate pathogen** – parasite always causes disease .
- **Infectivity** – It is the capacity of the organism to penetrate the tissues of host, to survive the host defenses, and to multiply and disseminate in host.
- **Pathogenicity** – It is the capacity of the microbial species to produce disease.

SOURCES OF INFECTION

Sources of infection are animal and inanimate in nature.

Animal sources

- Normal flora
- Animals in incubation period of disease
- Animals with overt disease.
- Convalescent carrier animals – In these animals shedding of the pathogen occurs for varying periods after clinical recovery. The period may vary from weeks to months.
- Contact carrier or subclinical infections – They acquire pathogenic organisms from other animals suffering with infectious disease without contracting the disease themselves. Such animals are called as contact or subclinical carriers. The carrier state may be temporary for a few days or lasting for months.

Inanimate sources (fomites)

Contaminated utensils, feed and water troughs, vehicles, etc.

TRANSMISSION

Disease can be transmitted by direct or indirect contact.

Direct contact

- contact with discharges or aerosols from the animal.
- Coitus.
- Vertical transmission from mother to offspring.

Indirect contact

- Organisms excreted by the infected animal are carried in/on various vehicles like feed, water, litter, clothing, footwear, farm house products and by-products, equipments, personnel, logistics, air or dust. Such contaminated objects are called as fomites.
- Contaminated instruments may also spread the infection.

Routes of Entry

- **Inhalation**
- **Ingestion**
- **Inoculation through the skin or mucous membrane**
- **Coitus or artificial insemination.**
- **Transplacental / *in ovo***
- **Hospital acquired infections - nosocomial infections.**
- **Physician induced infections - iatrogenic infections.**

Opportunistic infections

- compromised people/animal
  - normal flora
    - Skin
      - *Staphylococcus aureus*
      - *S. epidermidis*
      - *Propionibacterium acnes*
    - Intestine
      - *Bacteroides*
        - high numbers
      - *Enterobacteriaceae*
        - low number
  - environment
    - Dermatophytes
  - nosocomial

PATHOGENICITY

PATHOGENICITY: **The ability to cause disease**

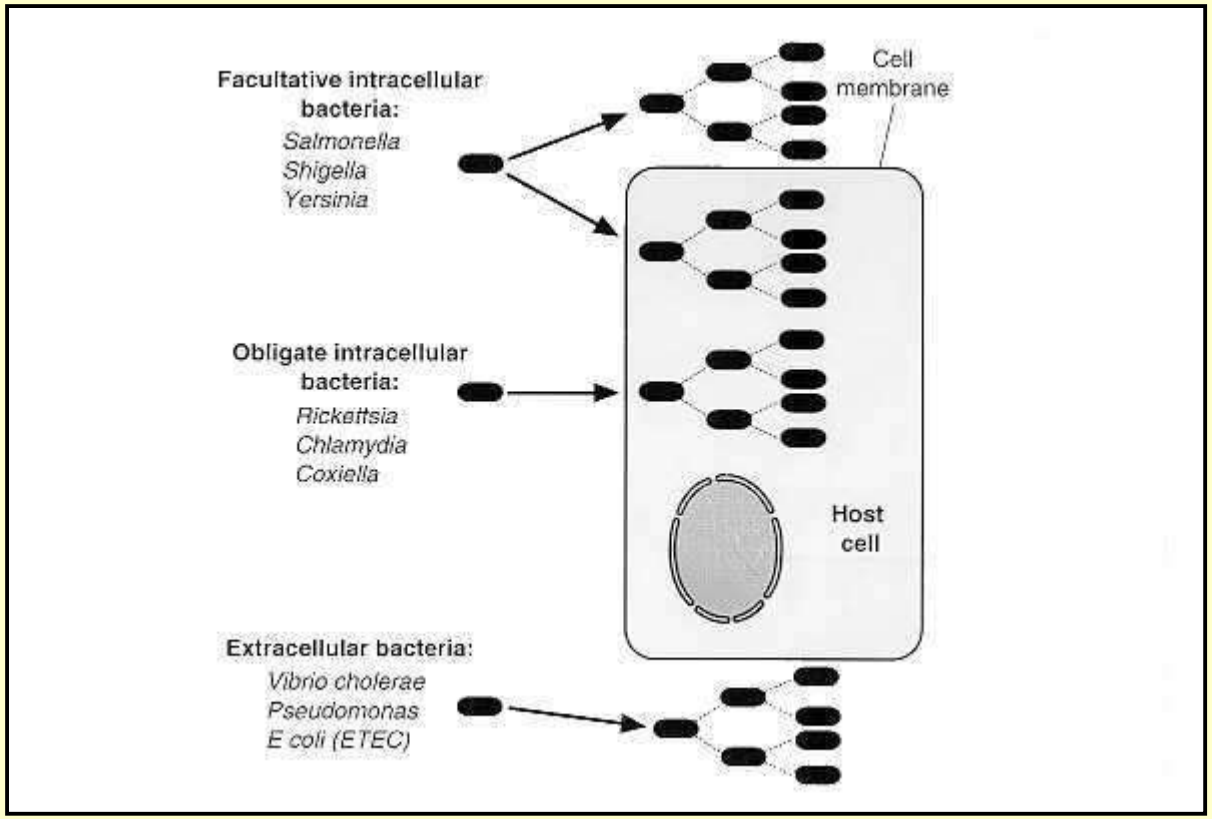
- **virulence factors**
- **number of initial organisms**
- **immune status**

The "objective" of bacteria is to multiply rather than to cause disease; it is in the best interest of the bacteria not to damage or kill the host.

A disease ensues when the balance between bacterial pathogenicity and host resistance is upset.

Pathogenic bacteria can be grouped into three categories on the basis of their invasive properties for eukaryotic cells:

- **Extracellular**
- **Obligate Intracellular**
- **Facultative Intracellular .**



TERMINOLOGIES

Bacteraemia

- Bacteraemia is the presence of bacteria in blood.
- The pathogenic organisms may gain entry in to a blood capillary or venule actively or passively from the initial site of entry.
- Once in blood stream the organism can cause localized infection or spread to various parts of the body, e.g. Leptospires reach the kidneys following bacteraemia.
- Organisms can directly gain access to the blood by first infecting the lymphoid system.

Septicaemia

- Septicaemia is the presence of actively multiplying bacteria in the blood.
- One of most severe septicaemia is anthrax in which the number of bacteria in blood may often exceed the erythrocytes in blood.
- Septicaemic infections often start as localized infections that later become generalized, e.g, streptococcal pharyngitis, bubonic plague.

Toxaemia

- Toxemia is the presence of toxins in blood.

- Bacteria cause disease by 2 basic mechanisms:

  1-Direct damage of host cells

  2-Indirectly by stimulating exaggerated host inflammatory/immune response

VIRULENCE

VIRULENCE: is the measure of the pathogenicity of an organism to cause disease; usually used to describe the difference in disease causing capability between two different strains of the same species.

Virulence can be expressed as LD_{50} (lethal dose for 50% of the inoculated hosts) or ID_{50} (infectious dose for 50% of the inoculated hosts).

Virulence Factors

VIRULENCE FACTORS: The factors produced by a microorganism and induce pathology in a host are called virulence factors. These factors help pathogen to

(1) invade the host,

(2) cause disease, and

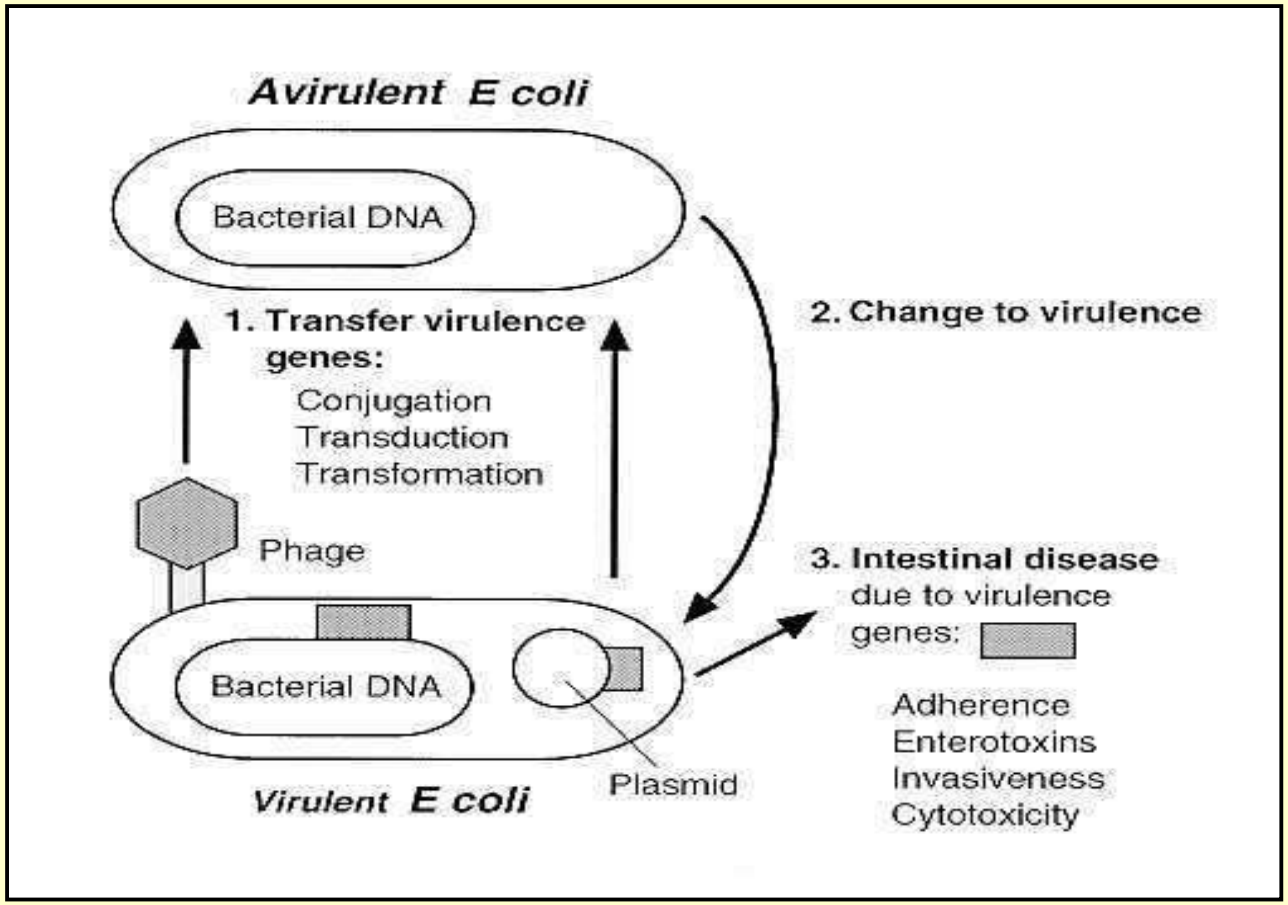
(3) evade host defenses.

Virulence factors are classified into two categories –

1. **Virulence factors that promote bacterial colonization of the host:**
    - **Adherence Factors**
    - **Invasion and/or Spreading Factors**
    - **Compete for iron and other nutrients;**
    - **Evasion of host immune responses**
2. **Virulence factors that damage the host.**
    - **Exotoxins**
    - **Endotoxins**

PATHOGENIC ATTRIBUTES
OR
VIRULENCE FACTORS
OF BACTERIA

Genetic Basis for Virulence



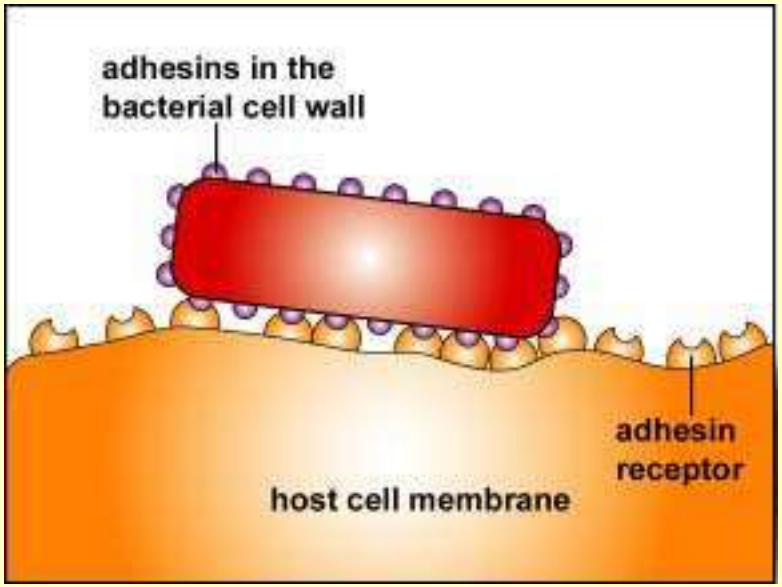
Virulence factors in bacteria may be encoded on chromosomal DNA, bacteriophage DNA, plasmids, or transposons. For example, the heat-labile enterotoxin (LTI) of *E coli* is plasmid encoded, the heat-labile toxin (LTII) is encoded on the chromosome, and diphtheria toxin of *C.diphtheriae* is coded by phage. Virulence factors are acquired by bacteria by vertical or horizontal gene transfer

Bacterial Virulence Factors that Promote Colonization in the Host

Factors for contact with host cells

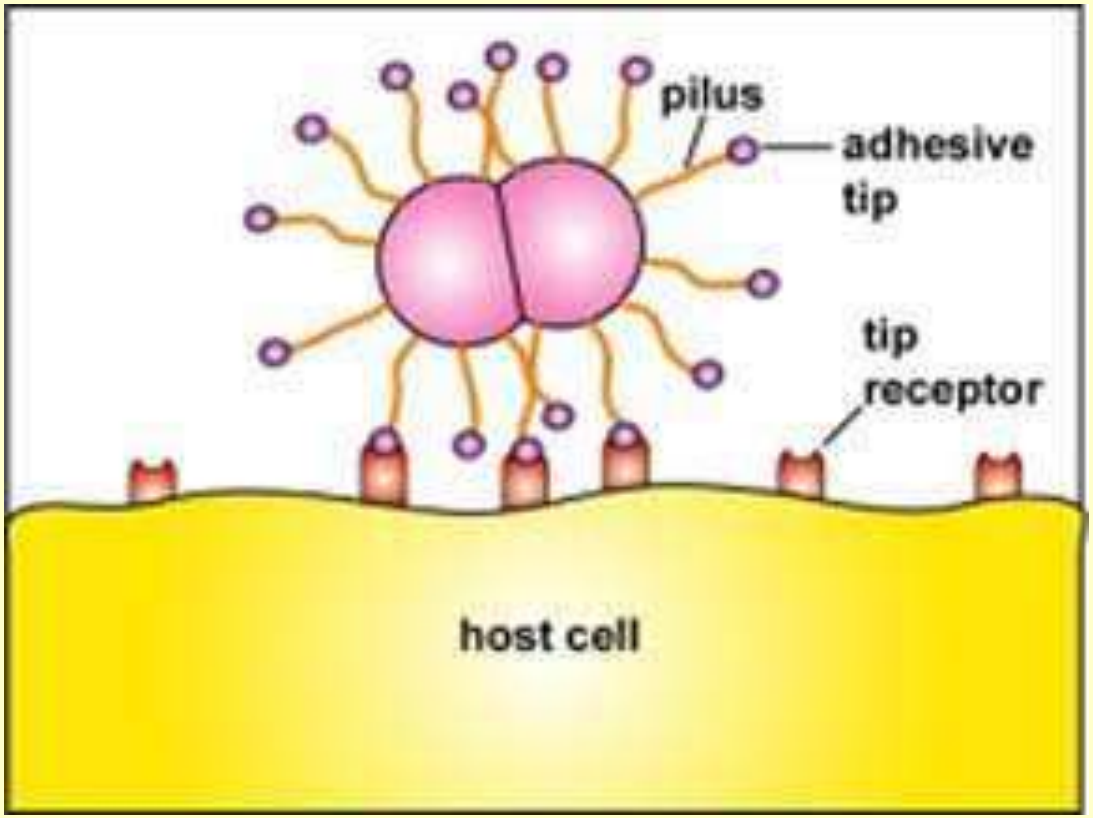
1. **Bacterial motility -** e.g., non-motile mutants of *Vibrio cholerae* are less virulent than the motile wild types.
2. **Bacterial enzymes -** e.g., *Streptococcus pyogenes* produces **streptokinase** that facilitate spread of the bacterium by liquefying the fibrin clot

Factors for adherence with host cells - adhesins

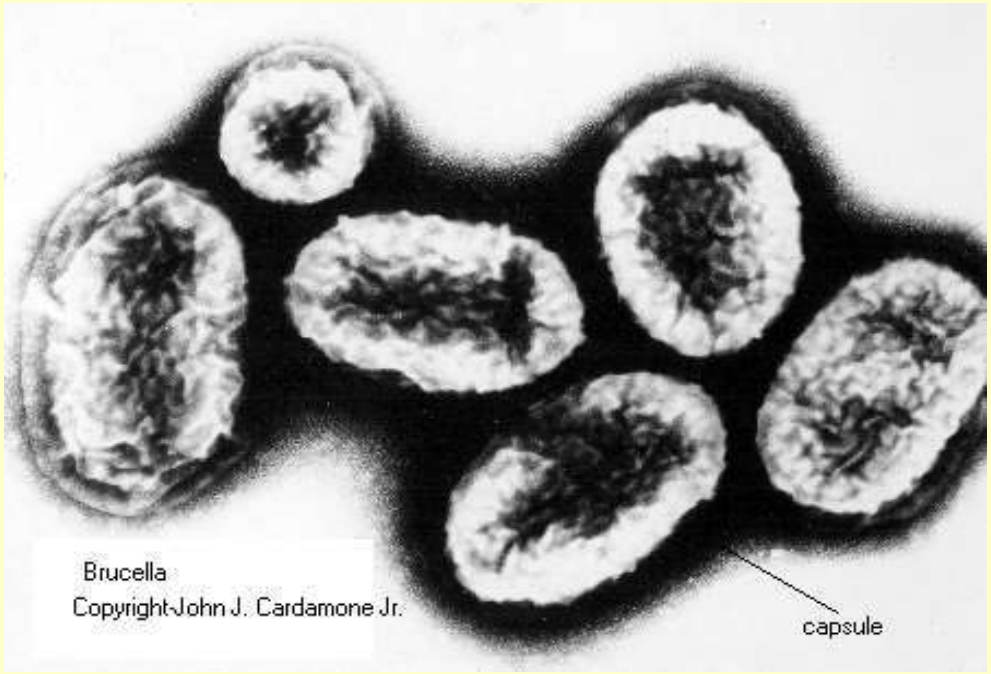


Adhesins are proteins found on the cell wall of various bacteria that bind to specific receptor molecules on the surface of host cells and enable the bacterium to adhere intimately to that cell in order to colonize and resist physical removal, e.g. common fimbriae, capsule, biofilm, liptotechoic acid, Fibronectin binding protein (FBP), etc.

Adherence factors - Pili (fimbriae)

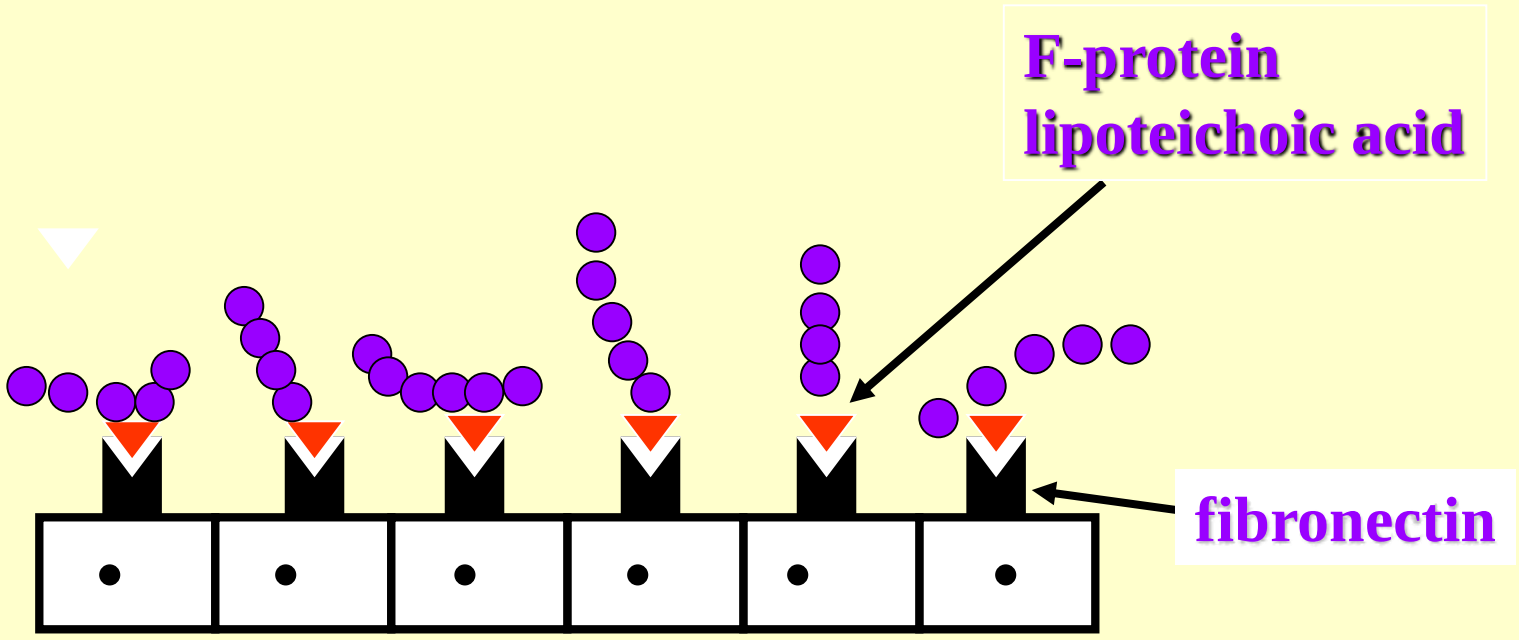


Adherence factors - capsules (biofilms)



Adherence factors – FBP

(eg. *S.pyogenes)*



Spreading Factors

"Spreading Factors" are a family of bacterial enzymes that affect the physical properties of tissue matrices and intercellular spaces, thereby promoting the spread of the pathogen.

- **Hyaluronidase** - depolymerize hyaluronic acid, the interstitial cement substance of connective' tissue; produced by streptococci, staphylococci, and clostridia.
- **Collagenase** - breaks down collagen; produced by *Clostridium histolyticum* and *Clostridium perfringens*.
- **Neuraminidase -** degrades neuraminic acid (also called sialic acid) present on epithelial cells of the mucosa; produced by *Vibrio cholerae*, *Shigella dysenteriae*, *P.multocida,* and *M.haemolytica*
- **Streptokinase** and **Staphylokinase** - convert inactive plasminogen to plasmin which digests fibrin.
- **Edema Factor** of *B.anthracis* - adenylate cyclase activity promote bacterial invasion.

Invasins

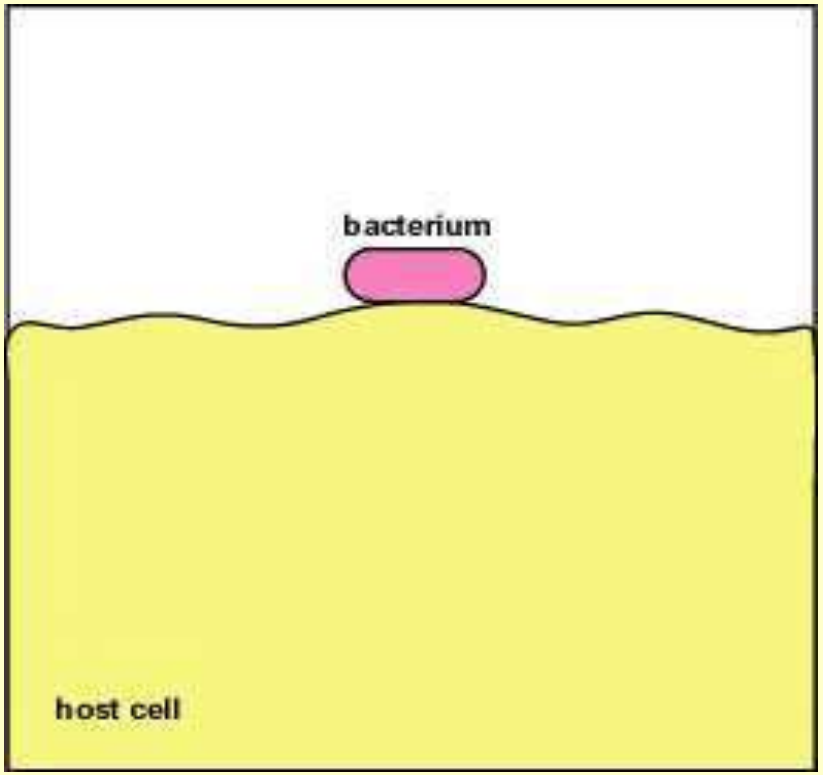
- Some bacteria have mechanisms by virtue of which they initiate phagocytosis in non-phagocytic cells for invasion by:
  - binding to some receptor on cell, eg. *Yersinia pestis*
  - injecting invasins, such as Type III secretion system in bacterial cytoplasm, eg. *Salmonella*

In either case changes in host cell cytoskeleton cause the bacteria to be ingested

- Some pathogens can utilize actin fibres intracellularly to move through host cells (transcytosis), eg. *Listeria monocytogenes*

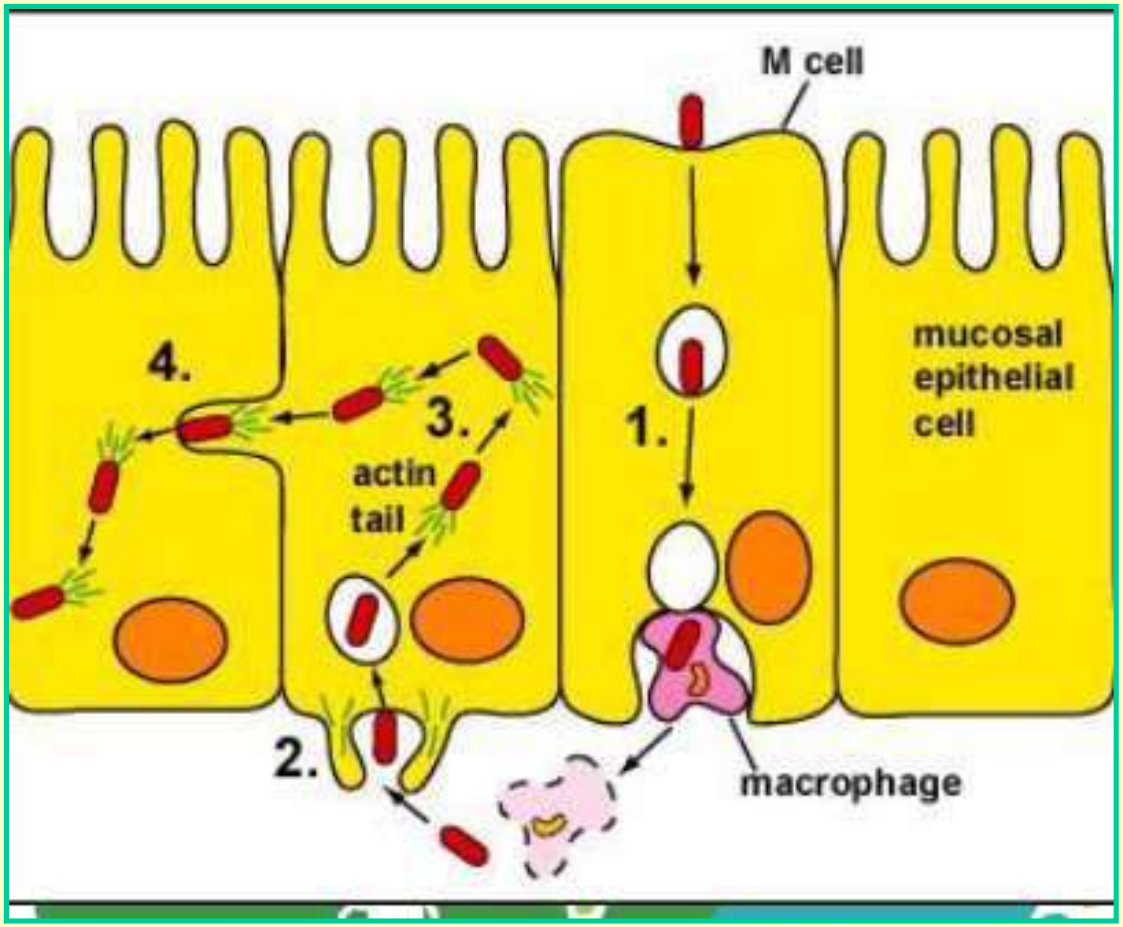
- Invasins may also mediate uptake of bacteria into professional phagocytic cells in a way that bypass normal phagosome formation

The Type III Secretion system in Bacteria



The bacteria having the type III secretion system on contact with cells, delivers proteins into the cells **which cause polymerization and depolymerization of actin filaments** resulting in cytoskeletal rearrangement. Thus the invasins is able to trick the non-phagocytic cell into behaving like a phagocyte and engulf the bacterium into phagosome like vacuole. The bacteria then cause the vacuole membrane to rupture and escape into the cytoplasm

Transcytosis



The Ability to Compete for Nutrients and Iron

Bacteria compete for nutrients by **synthesizing specific transport systems or cell wall components** capable of binding limiting substrates and transporting them into the cell.

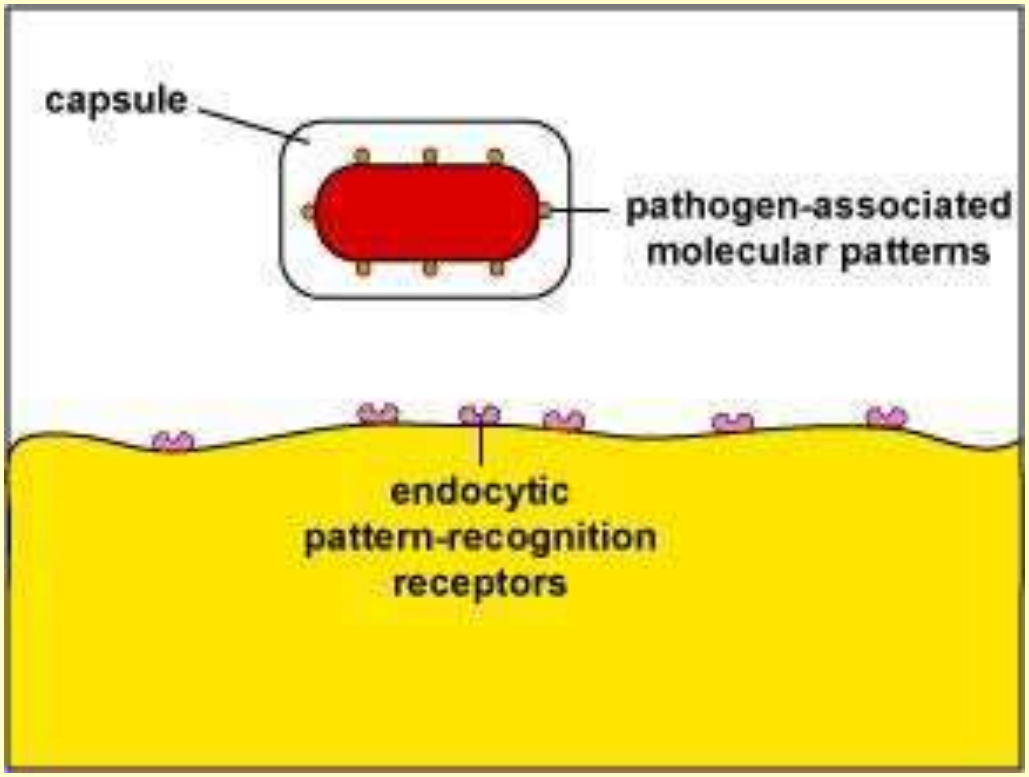
- **Siderophores--**low MW compounds that chelate iron with very high affinity, eg. *E.coli*
- **Direct binding** of host transferrin, lactoferrin, ferritin, or heme by bacterial surface receptors, eg. *Yersinia* species
- **Exotoxins** that lyse host cells (can be used to obtain other nutrients as well), eg. haemolysins

Bacterial Virulence Factors that helps in Evasion of Immune Response

Evasion of Innate Immune Responses

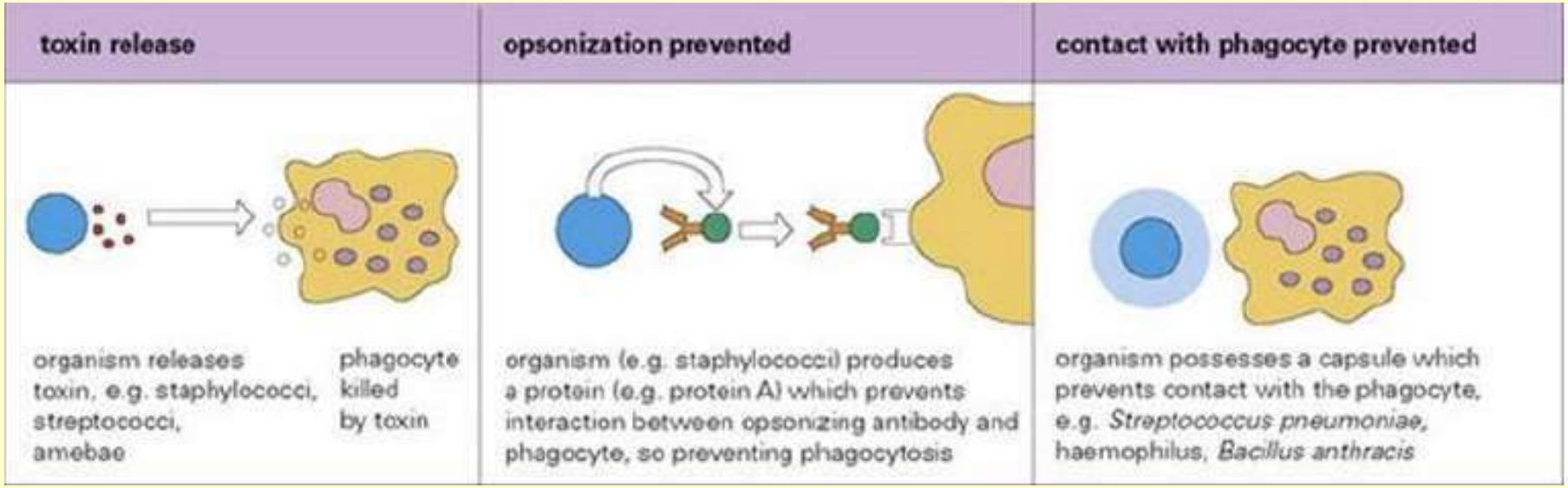
- **Invade or remain confined in regions inaccessible to phagocytes.** e.g. the lumen of glands and the skin are not patrolled by phagocytes.
- **Avoid provoking an inflammatory response**.
- **Hide the antigenic surface of the bacterial cell.** eg, S.*aureus* produces coagulase which clot fibrin on the bacterial surface
- **Inhibit chemotaxis of phagocytes,** e.g. Streptococcal streptolysin, fractions of *Mycobacterium tuberculosis* and *Clostridium ø* toxin suppresses neutrophil chemotaxis
- **Inhibit ingestion by phagocytes,** e.g. capsule inhibit recognition and engulfment by phagocytes
- **Resistance to complement mediated lysis (serum resistance),** e.g. capsule, LPS, S-layers, etc.

Capsules Blocking the Attachment of Bacteria to Phagocytes



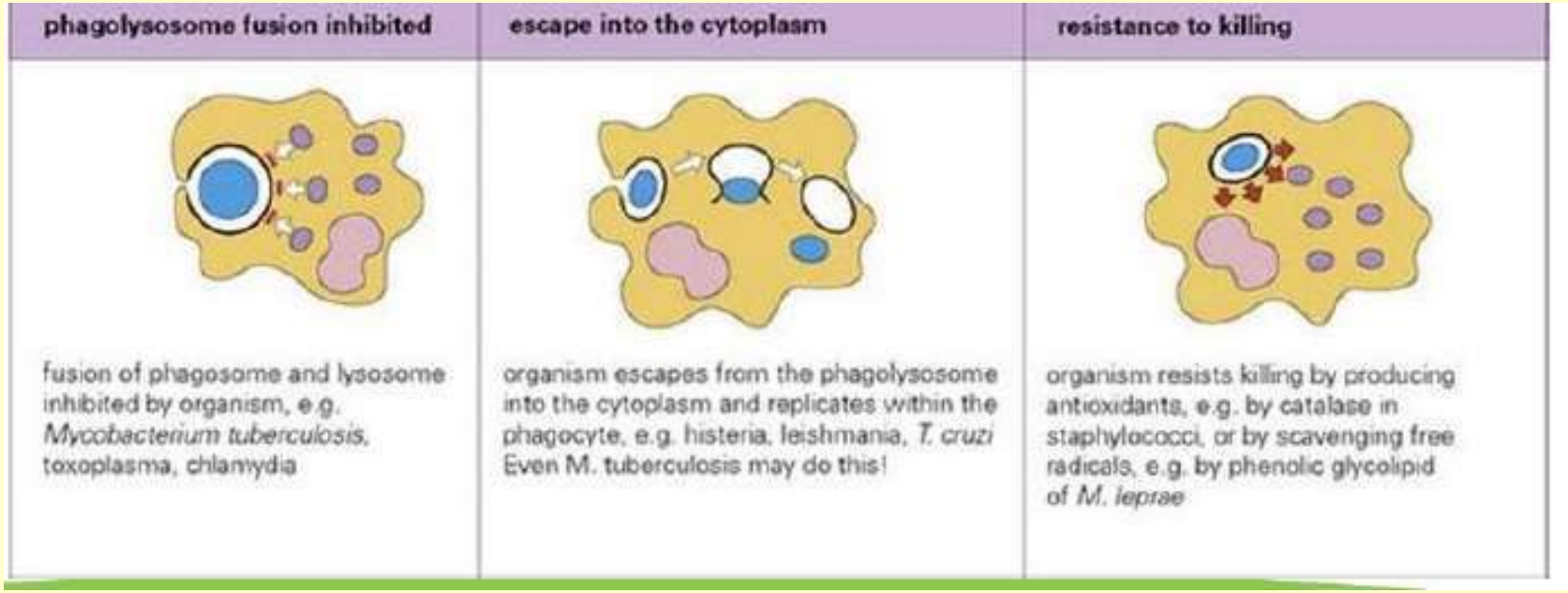
Resistance to opsonization/phagocytosis

ii. **LPS O polysaccharide**
iii. **S-layer**
iv. **Extracellular products:** enzymes that inactivate C5a chemoattractant (*S. pyogenes),* toxins that kill phagocytes (leukotoxins) *(Mannheimia haemolytica),*inhibit migration, or reduce oxidative burst.



Strategies for surviving phagocytosis:

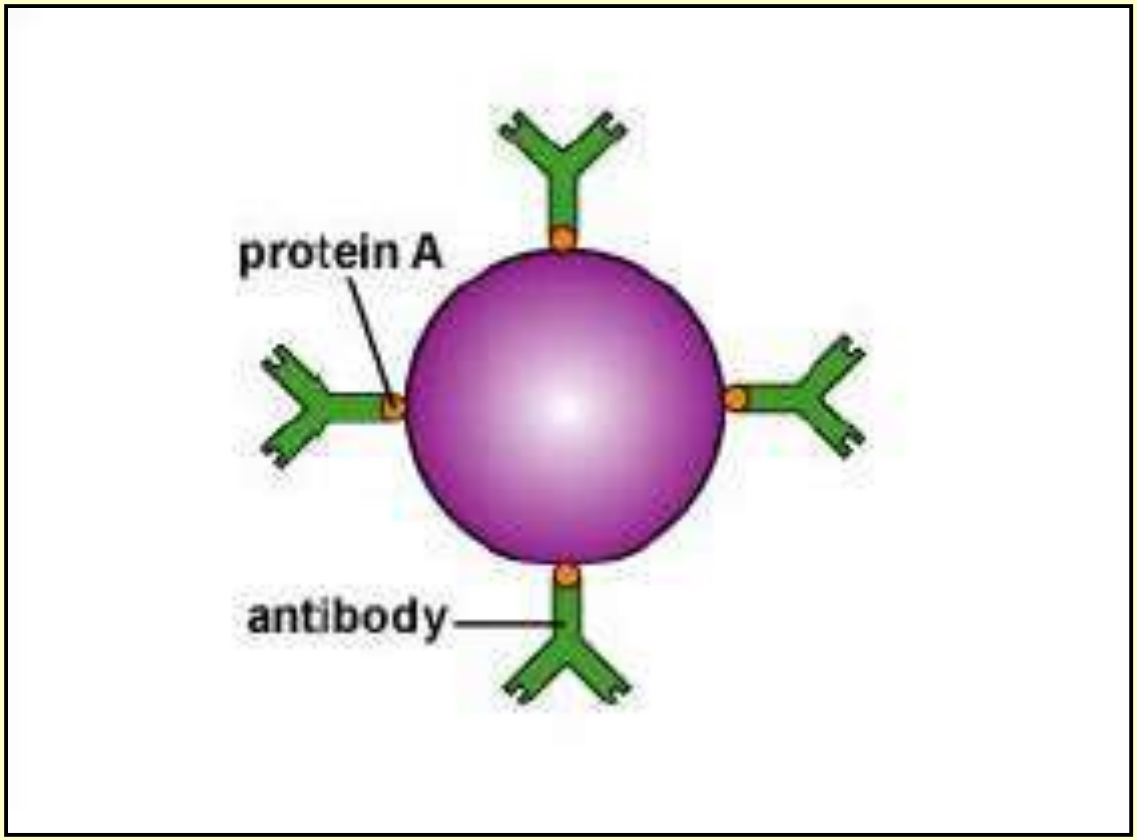
I. Escape from phagosome before fusion with lysosome (example: *Listeria monocytogenes*, mediated by listeriolysin*)*
II. Prevent phagosome-lysosome fusion – eg, *Salmonella*, *Mycobacterium* , *Legionella* and *Chlamydia*
III. Express factors that allow survival in phagolysosome



Evasion of Adaptive Immune Defenses

- **Antigen masking -** Some bacteria are able to coat themselves with host proteins such as fibrin, fibronectin, lactoferrin, or transferrin and in this way avoid antibodies.
- **Antigenic switching or phase variation -** one way certain bacteria can evade antibodies is by changing the adhesive tips of their pili or vary other surface proteins so that antibodies already made will no longer "fit."
- *Staphylococcus aureus* produces **protein A** while *Streptococcus pyogenes* produces **protein G**. which non-specifically binds IgG with very high affinity
- **Immunoglobulin proteases -** Bacteria such as *Haemophilus influenzae* , *Streptococcus pneumoniae* , *Helicobacter pylori* , *Shigella flexneri* , *Neisseria meningitidis* , *Neisseria gonorrhoeae* and Enteropathogenic *E. coli* produce enzymes that degrade the antibodies found in body secretion **(**IgA).

Staphylococcus aureus Resisting Opsonization via Protein A



The Fc portion of the antibody IgG, the portion that would normally binds to Fc receptors on phagocytes, instead binds to protein A on *Staphylococcus aureus*. In this way the bacterium becomes coated with a protective coat of antibodies that do not allow for opsonization.

Bacterial Virulence Factors that damage the Host

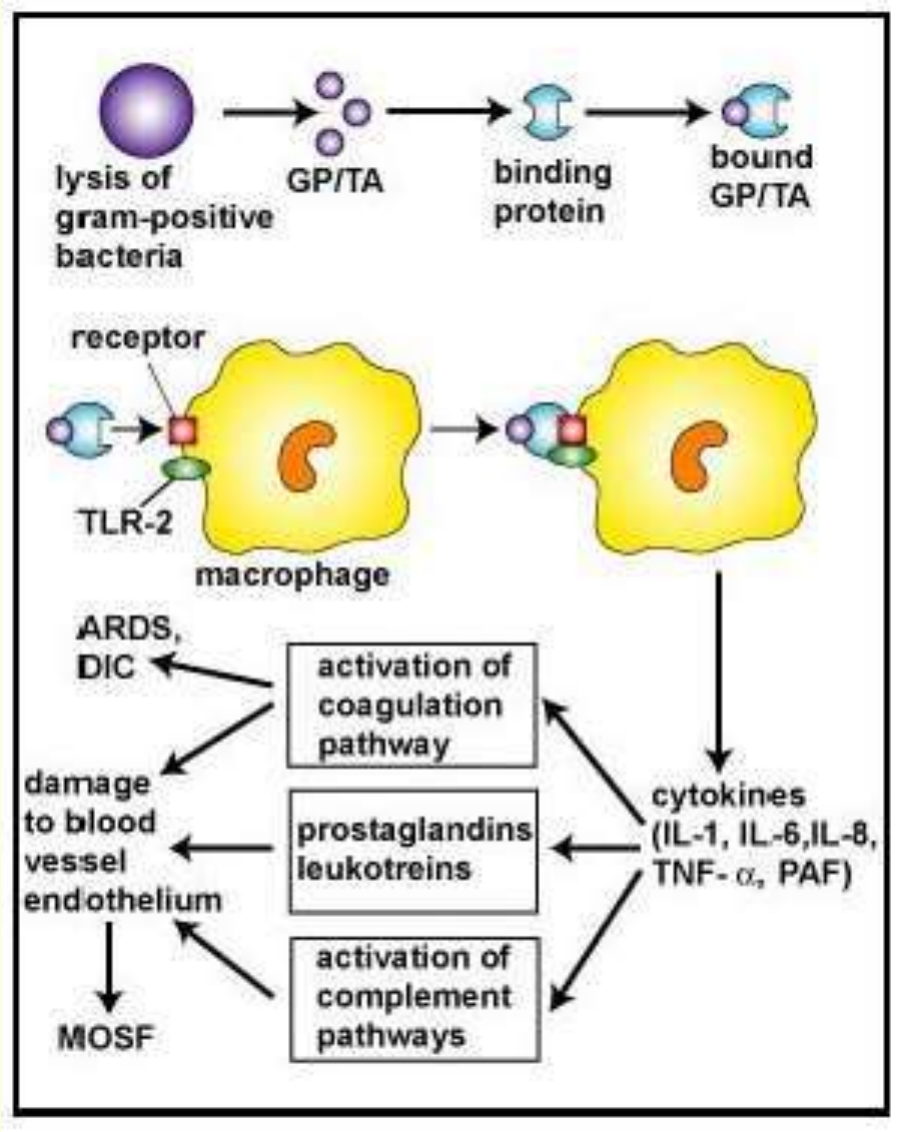
Virulence factors that damage the host include:

- The cell wall components that bind to host cells causing them to synthesize and secrete pro-inflammatory cytokines and chemokines.
- Toxins.
- Induce autoimmune responses.

Bacterial Cell Wall Components that Promote Synthesis and Secretion of Inflammatory Cytokines and Chemokines.

- **LPS of Gram-negative bacteria, and teichoic acids and glycopeptides of Gram-positive bacteria induces cytokine production and secretion**
- These cytokines, such as **TNF-alpha, IL-1, interleukin-6 IL-6, IL-8**, and **platelet-activating factor (PAF)** promote inflammation and lead to **activation of the complement pathways and the coagulation pathway**.
- **At moderate levels**, inflammation, products of the complement pathways, and products of the coagulation pathway are **essential for body defense**. However, these **when excessive produced in excessive amounts cause exaggerated inflammatory response which leads to MOSF.**
- In some bacteria, **lipoproteins in the outer membrane** may also play a role in leading to **excessive cytokine production.**

Harmful Effects of Glycopeptides and Teichoic Acid Released During Gram-Positive Infections



BACTERIAL TOXINS

BACTERIAL TOXINS

Toxins are of two types:

- **Exotoxin**
- **Endotoxin**

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ENDOTOXINS

- Endotoxins are the **t**he lipopolysaccharides component of the outer membrane of cell wall of the Gram negative bacterial cell
- The endotoxins are released into the medium only following the death or the lysis of cells which occurs during late growth stages of culture.
- The toxic effects of endotoxins are observed only after they are released in to the medium.
- All endotoxins exhibit similar pharmacological effects. They cause pyrexia, blood changes and shock.

- LPS is bound by LPS binding proteins (LBP) in plasma, which then binds CD14. This complex binds Toll-like receptor 4 (TLR4) on macrophages and monocytes.
- Macrophages and monocytes release cytokines (IL-1, IL-6, IL-8, TNF alpha, Platelet Activating Factor), which subsequently trigger prostaglandin and leukotriene release
- The complement and coagulation cascades are activated.
- Endotoxic shock occurs when bacterial products reach high enough levels in the blood to trigger exaggerated cytokine release, complement activation,, and coagulation cascade activation in many parts of the body.
- Circulatory system collapse followed by multiple organ system failure occurs.

Harmful Effects of LPS-Endotoxin

a. fever production

b. inflammation

c. tissue destruction

d. respiratory distress

e. capillary damage (leading to petechial rash , capillary leakage, and hypovolemia)

f. intravascular coagulation

g. hypotension

h. decreased cardiac output

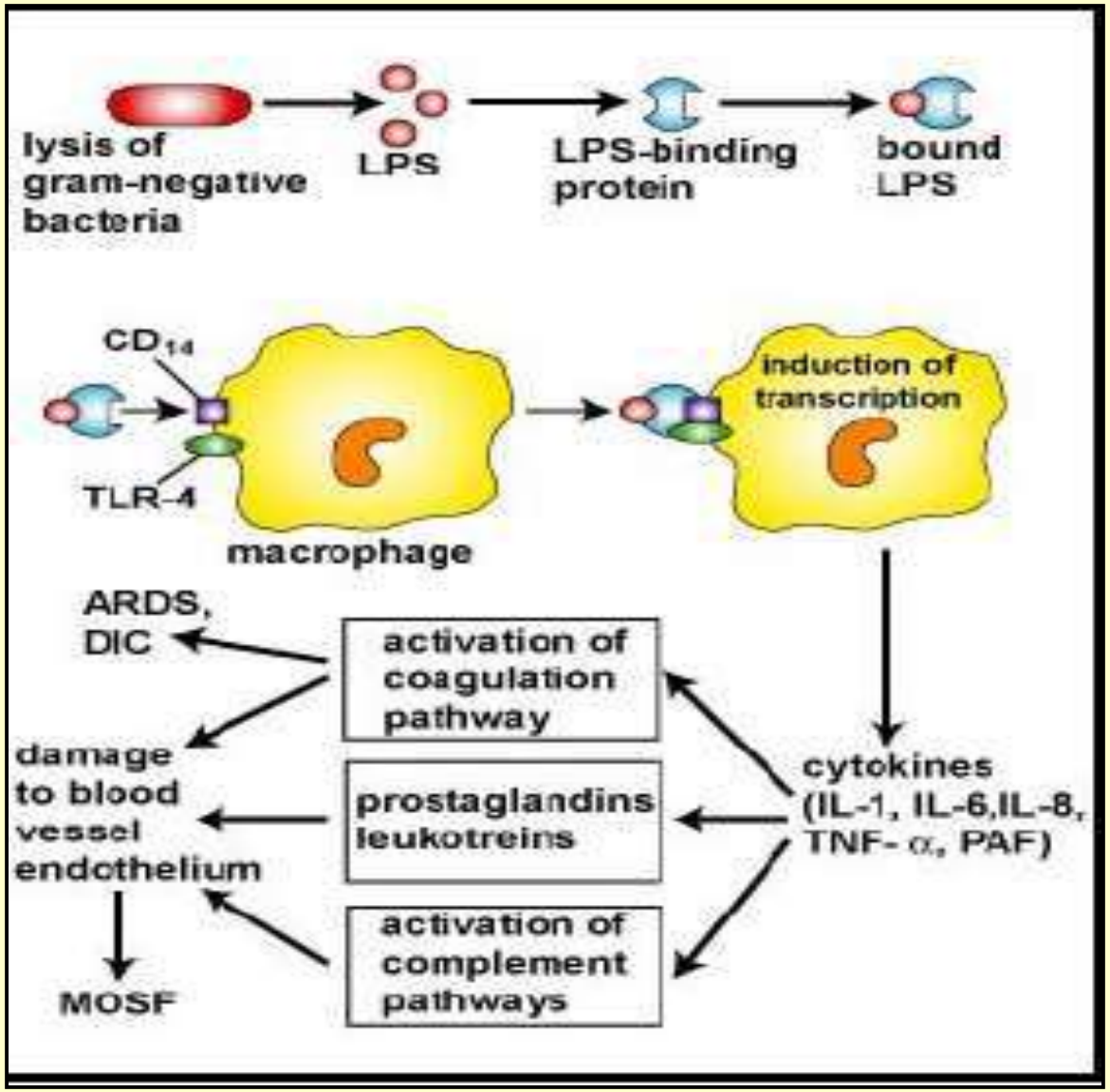
i. irreversible shock

j. wasting of the body

k. diarrhea (from endotoxin in intestines)

l. allow bacteria to cross the blood-brain barrier

Harmful Effects of LPS-Endotoxin



Exotoxins

- produced by bacteria (both Gram-positive and Gram-negative)
- released into the surrounding environment
- proteins in nature
- usually enzymes
- heat stable (high mol. wt.) or heat labile (low mol. wt.)
- functions for the bacteria are usually unknown
- site of action is more localized and is confined to particular cell types
- exotoxins are excellent antigens - elicit specific antibodies called antitoxins

Exotoxins v/s Endotoxins

	Comparison of Properties	
Property	**Exotoxin**	**Endotoxin**
Source	Certain species of gram-positive and gram-negative bacteria	Cell wall of gram-negative bacteria
Secreted from cell	Yes	No
Chemistry	Polypeptide	Lipopolysaccharide
Location of genes	Plasmid or bacteriophage	Bacterial chromosome
Toxicity	High (fatal dose on the order of 1 µg)	Low (fatal dose on the order of hundreds of micrograms)
Clinical effects	Various effects (see text)	Fever, shock
Mode of action	Various modes (see text)	Includes TNF and interleukin-1
Antigenicity	Induces high-titer antibodies called antitoxins	Poorly antigenic
Vaccines	Toxoids used as vaccines	No toxoids formed and no vaccine available
Heat stability	Destroyed rapidly at 60°C (except staphylococcal enterotoxin)	Stable at 100°C for 1 hour
Typical diseases	Tetanus, botulism, diphtheria	Meningococcemia, sepsis by gram-negative rods

TNF = tumor necrosis factor.

Types of Exotoxins

On the basis of mode of action:

- super antigens, e.g. Toxic shock syndrome toxin-1 produced by some strains of *Staphylococcus aureus*
- toxins that act on the extracellular matrix of connective tissue, e.g. *Clostridium perfringens* collagenase
- A-B toxins, e.g. botulinum toxin
- exotoxins that damage host cell membranes, e.g. botulinum toxin

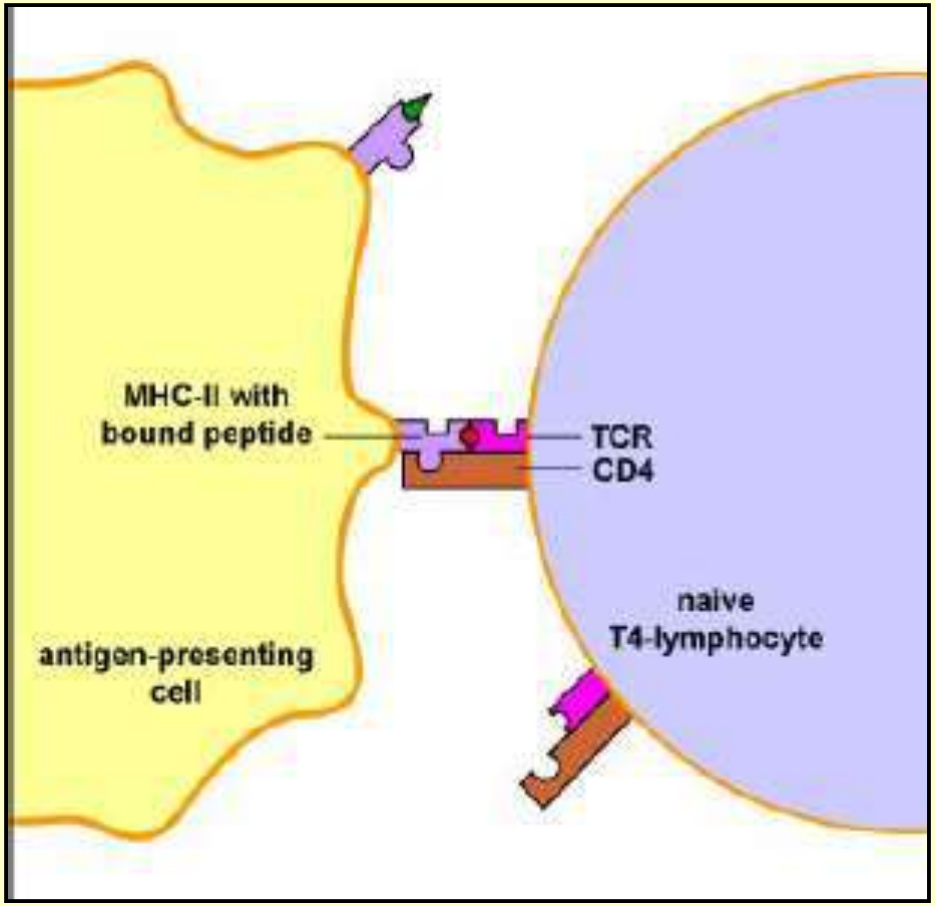
On the basis of site of action:

- cytotoxins, e.g.diphtheria toxin and erythrogenic toxins
- neurotoxins, e.g.botulinum toxin and tetanus toxin.
- enterotoxins, e.g. cholera toxin and staphylococcal enterotoxin

Super antigens

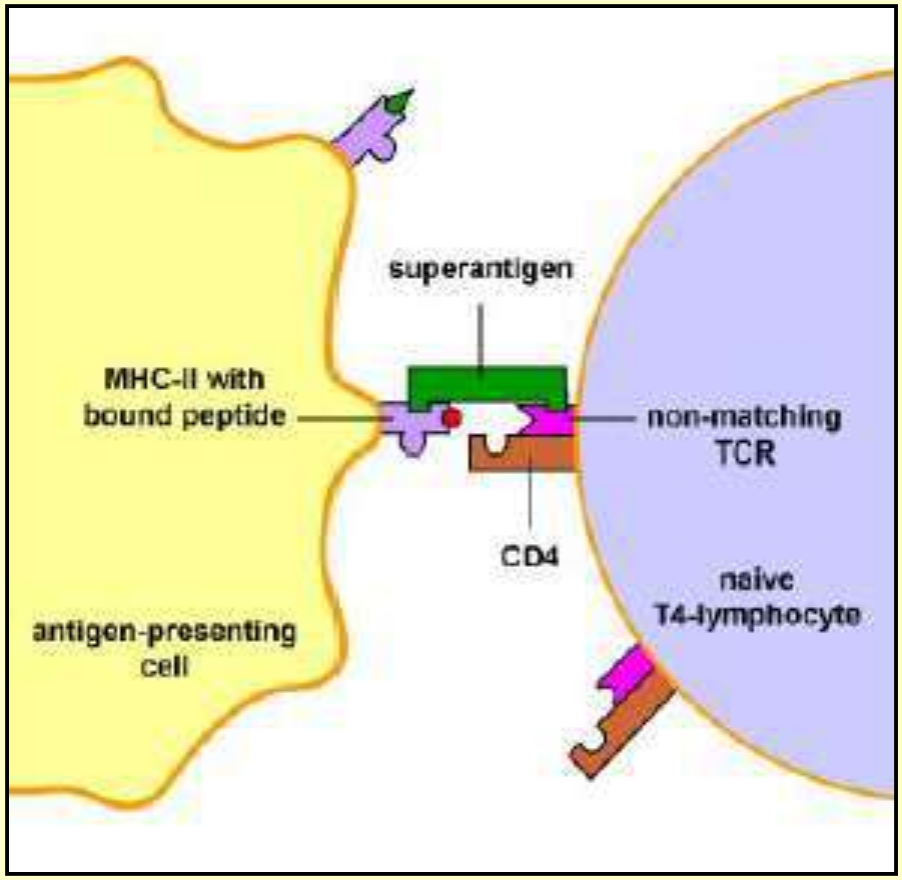
- bind directly to the MHC-II molecules on macrophages without being processed and cross link non-specifically with many TCRs on multiple T-cells.
- cross linking causes stimulation of up to 1 in 5 T-cells in the body (normal antigens cause stimulation of 1 in 10,000).
- results in the secretion of excessive amounts of interleukin-2 (IL-2)
- high levels of IL-2 in the blood lead to symptoms such as fever, nausea, vomiting, diarrhea, and malaise.
- stimulation of IL-2 secretion can also lead to production of other cytokines such as TNF-alpha, IL-1, IL-8, and PAF, which can lead to SIRS (Systemic Inflammatory Response Syndrome)
- e.g., Toxic shock syndrome toxin-1 (TSST-1), produced by some strains of *Staphylococcus aureus*

Binding of T4-Lymphocytes to Conventional Antigens



Conventional antigens are only recognized, after processing and presentation with MHC-II molecules by antigen presenting cells, by specific T4-lymphocytes having a TCR with a shape that corresponds to a peptide of that antigen

Binding of Super antigens



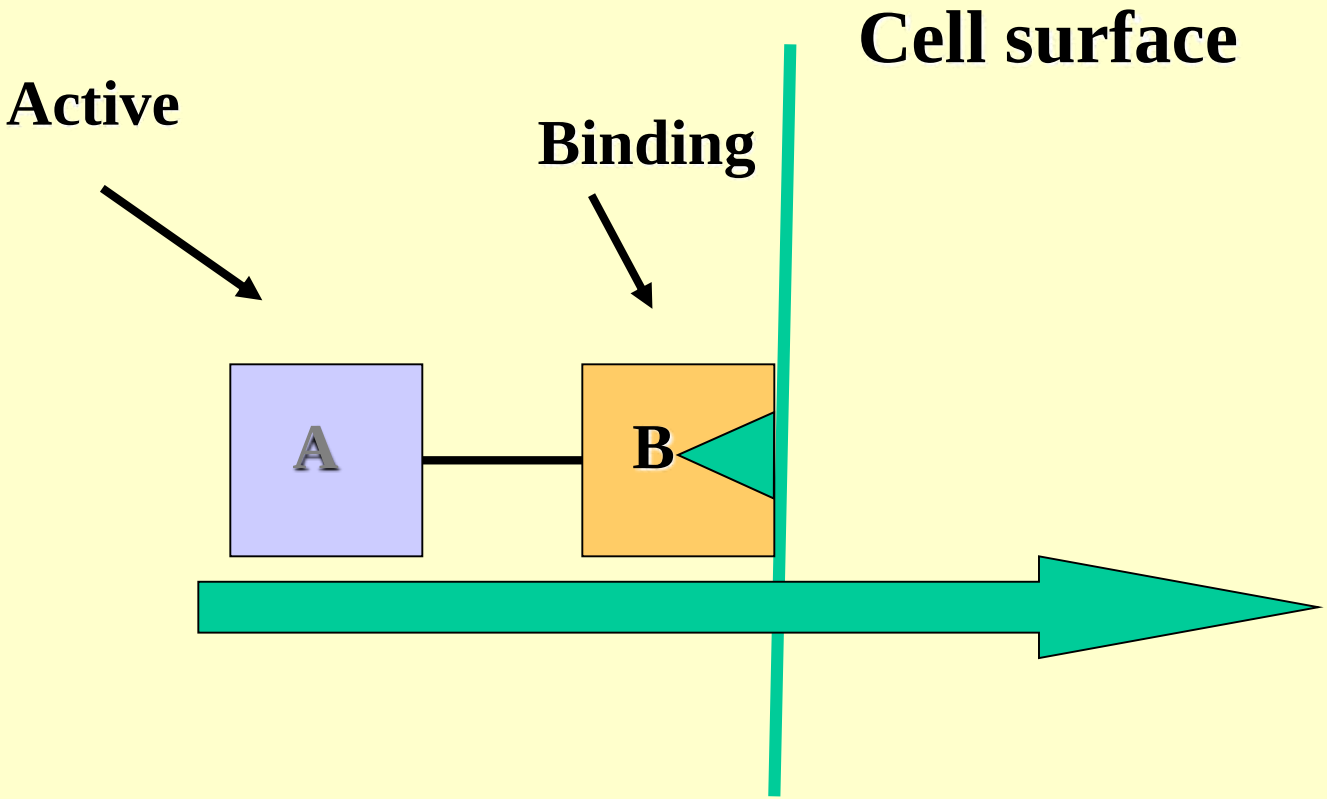
Super antigens bind directly to the outside of MHC-II molecules and the TCRs and activate many T4-lymphocytes. A specific TCR is not required for activation.

Toxins that act on the extracellular matrix of connective tissue

- **break down of host macromolecules , such as collagen, hyaluronic acid, proteins including immunoglobulins, etc.**
- **play an important role in disease development by providing nutrients and/or helping in dissemination deeper in body tissues**
- **cause extensive tissue damage**
- **Examples:**
  - **Hyaluronidase –aid in the spread of bacteria by degrading extracellular matrix.**
  - **Collagenase –aids in dissemination**
  - **DNase–reduces viscosity of debris from dead cells**
  - **Proteases – tissue damage**

A-B toxins

- consist of two parts: A (active) - enzymatic component, and B – binding component
- determines the host cell specificity of the toxin .



A-B toxins

B component binds the exotoxin to a receptor molecule on the host cell

↓

After binding the exotoxin is translocated across the host cell membrane (some A-B toxins enter by endocytosis)

(some passes directly through the host cell's membrane)

↓

A-component of the toxin separates from the B-component

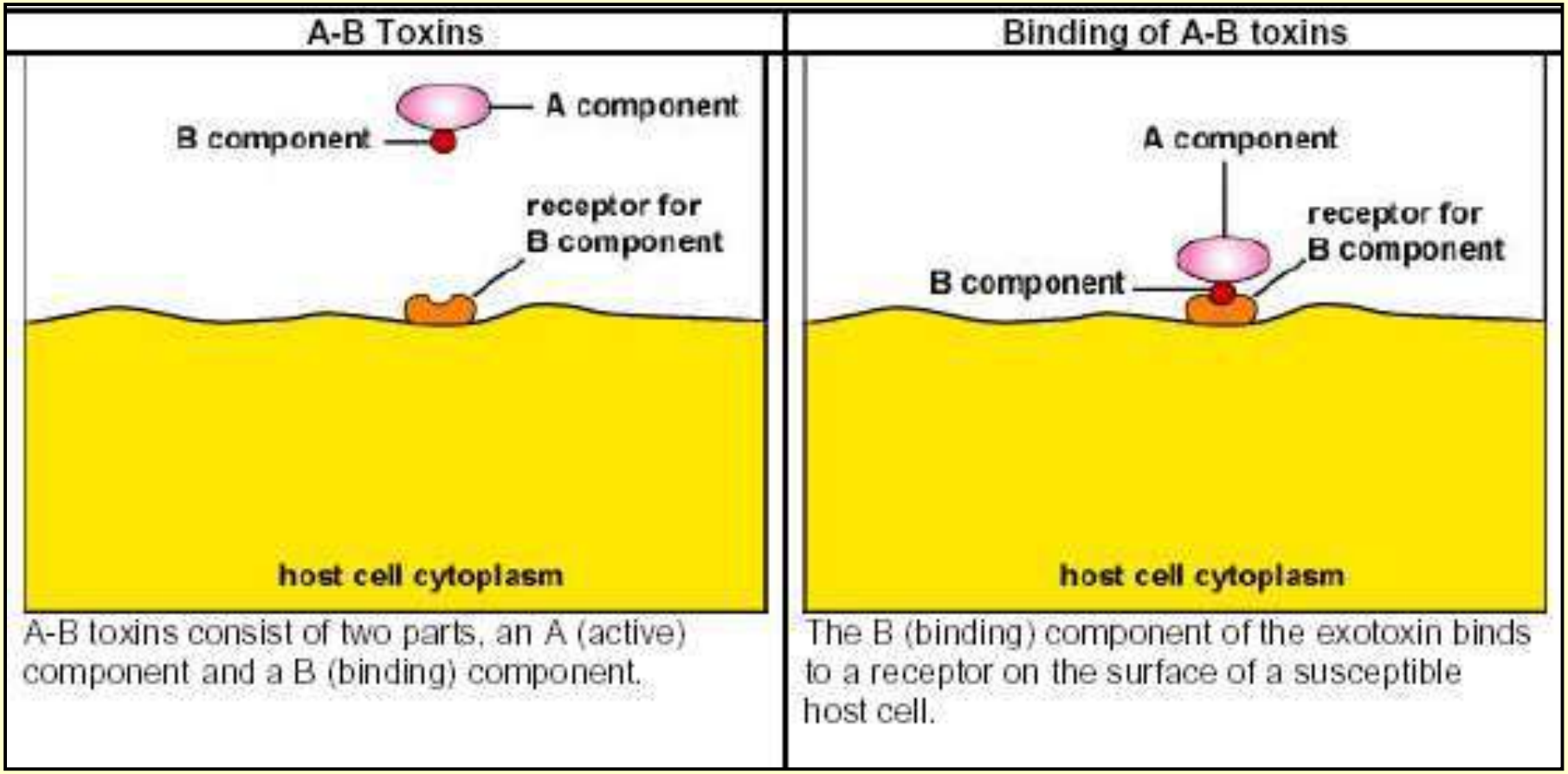
↓

A-component is released or enter the cytoplasm of host cell

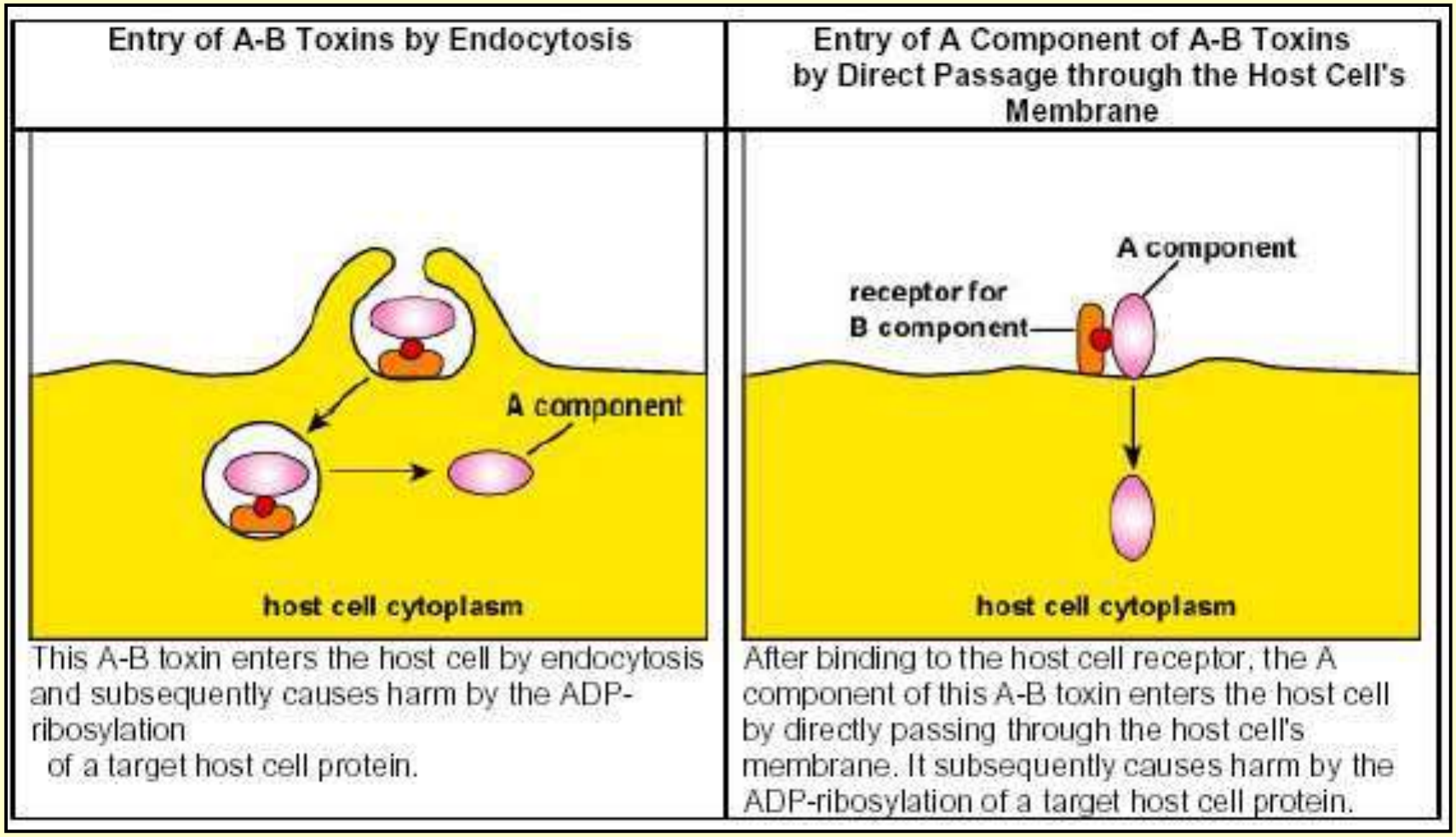
↓

A component then catalyze an enzymatic reaction

A-B toxins


A-B Toxins
A component
B component
receptor for
B component
host cell cytoplasm
A-B toxins consist of two parts, an A (active) component and a B (binding) component.
Binding of A-B toxins
A component
receptor for
B component
B component
host cell cytoplasm
The B (binding) component of the exotoxin binds to a receptor on the surface of a susceptible host cell.

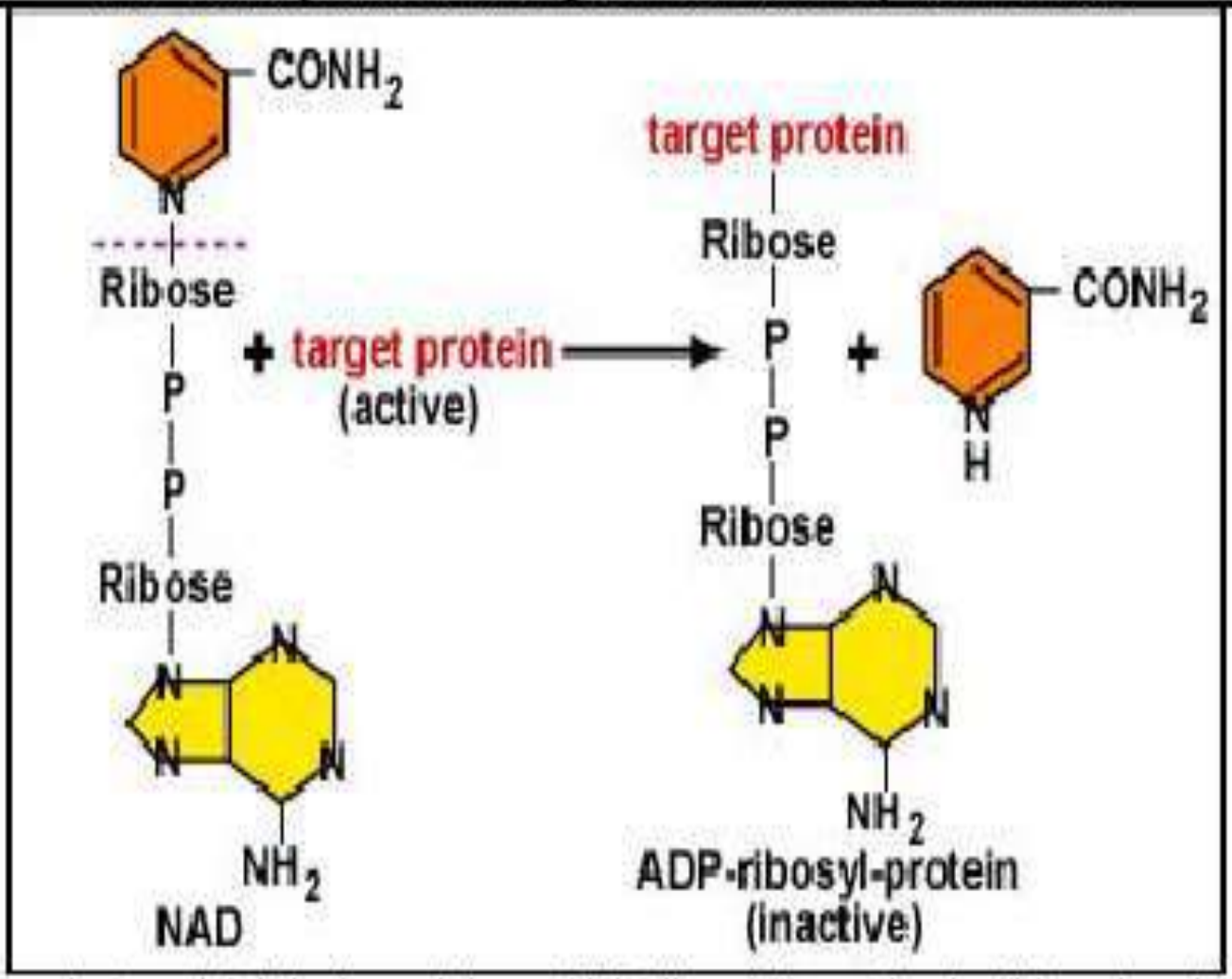
A-B toxins


Entry of A-B Toxins by Endocytosis
A component
host cell cytoplasm
This A-B toxin enters the host cell by endocytosis and subsequently causes harm by the ADP-ribosylation of a target host cell protein.
Entry of A Component of A-B Toxins by Direct Passage through the Host Cell's Membrane
A component
receptor for B component
host cell cytoplasm
After binding to the host cell receptor, the A component of this A-B toxin enters the host cell by directly passing through the host cell's membrane. It subsequently causes harm by the ADP-ribosylation of a target host cell protein.

A-B toxins - Types

- Those with ADP-ribosylating activity e.g. cholera toxin, *E. coli* heat labile toxin, *Pseudomonas aeruginosa* and diphtheria toxins.
- Those with a lytic activity on 28S rRNA e.g. shiga and shiga-like (vero)toxins.
- Those with a partially characterized site of action e.g. botulinum toxin, tetanus toxin and anthrax lethal toxin.

ADP- Ribosylation of a Target Host Protein by an A-B Toxin



The A component of most A-B toxins catalyzes ADP-ribosylation of host cell target proteins. The ADP-ribosyl group is removed from the coenzyme NAD (see dashed line) and is covalently attached to a host cell target protein. This causes the inactivation of that target protein.

A-B toxins - Examples

a) **Diphtheria toxin:** ADP-ribosylation of host EF-2; host cells are killed due to the blocking of translation of mRNA into polypeptides.

b) **Cholera toxin:** ADP-ribosylation of a cAMP regulatory protein, which causes loss of ion regulation, water loss, diarrhea.

c) **Shiga toxin:** cleaves host rRNA, which blocks translation and kills the host cell.

d) ***Clostridium botulinum***: large subunit targets neurons, small subunit cleave snare proteins inhibiting neurotransmitter release from neurons-causes paralysis

ADP-ribosylating A-B toxins

- Cholera toxin and *E. coli* labile toxin

- ADP-ribosylation of regulator protein Gs
- adenylate cyclase activation
- cyclic AMP ⇧
- block intestinal epithelial cells from taking in sodium from the lumen of the intestines
- active ion and water secretion
- diarrhea

A-B toxins with lytic activity on 28S rRNA

- **Shiga toxin - Shigella and EHEC**

- lyses rRNA in ribosome

↓

- inhibits protein synthesis

↓

- death of epithelial cells

↓

- poor water absorption

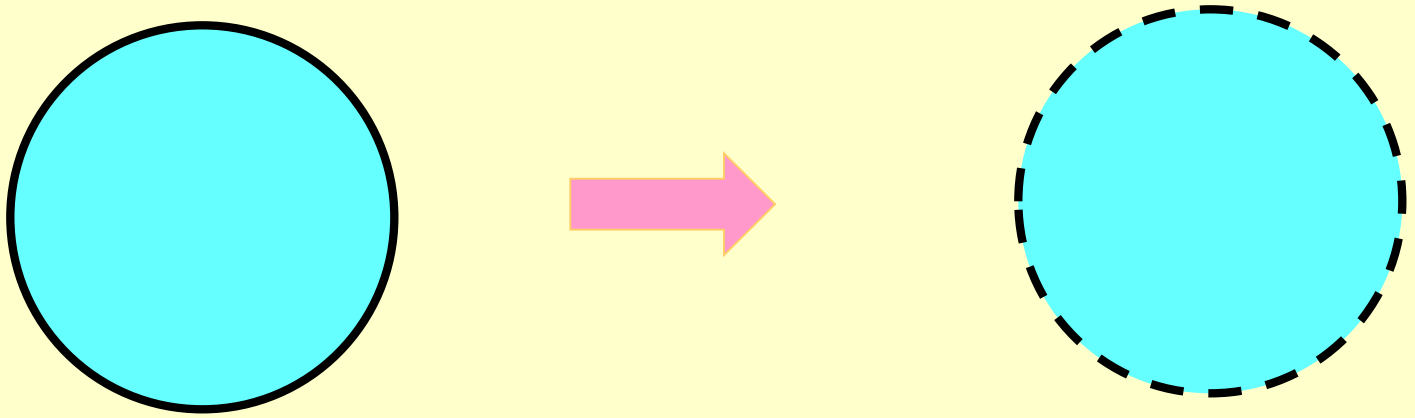
↓

- diarrhea

Membrane damaging exotoxins

- Cause damage or disruption of plasma membranes, which leads to osmotic lysisand cell death.

- Three types of membrane disrupting toxins:
  a)Enzymes that hydrolyze phospholipids: phospholipase, sphingomyelinase
  b)Toxins with detergent-like surfactant activity that disrupt membrane by lipid solubilization
  c)Pore forming toxins (the most common): proteins that insert in the host membrane and form a hydrophilic pore



Membrane damaging exotoxins

Examples:

- ***C. perfringens* phospholipase or alpha toxin (lecithinase)**
- **Leukocidin - *Staphylococcus aureus* and *Streptococcus pyogenes***
- **Elastase - *Pseudomonas aeruginosa***
- **Haemolysins**

Virulence Factors that Induce Autoimmune/Hypersensitivity Responses

- **Producing cross-reacting antibodies or auto reactive cytotoxic T-lymphocytes made in response to bacterial antigens that accidentally cross-react with epitopes on host cells** destroying the host cells to which they have bound and/or activate the classical complement pathway that stimulates the **inflammatory response** resulting in more tissue damage, e.g. **rheumatic fever** triggered by some strains of *Streptococcus pyogenes*
- **Stimulating the production of immune complexes** that **activate the complement pathway** resulting in **inflammatory response, which** destroys tissues, e.g. **acute glomerulonephritis** following infection by *Streptococcus pyogenes*.