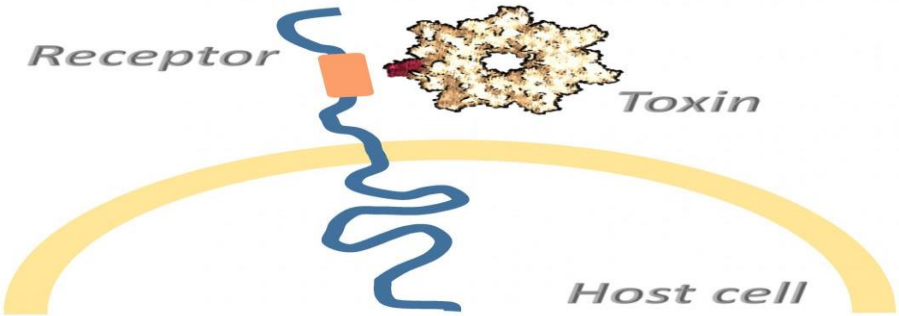
Cihan University - Erbil
College of Science
Department of Biology

Bacterial Toxins



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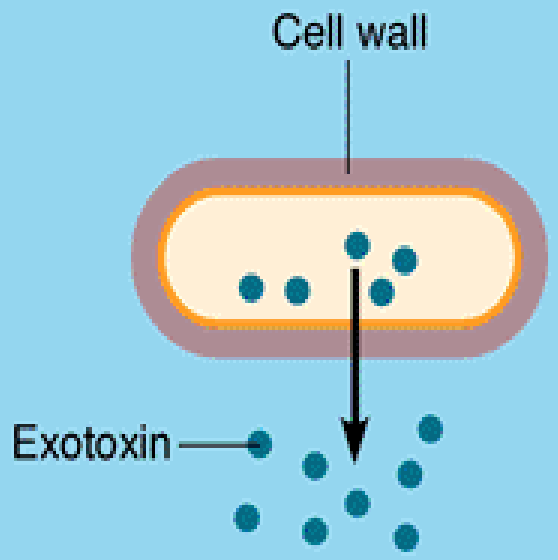
Two types of bacterial toxins

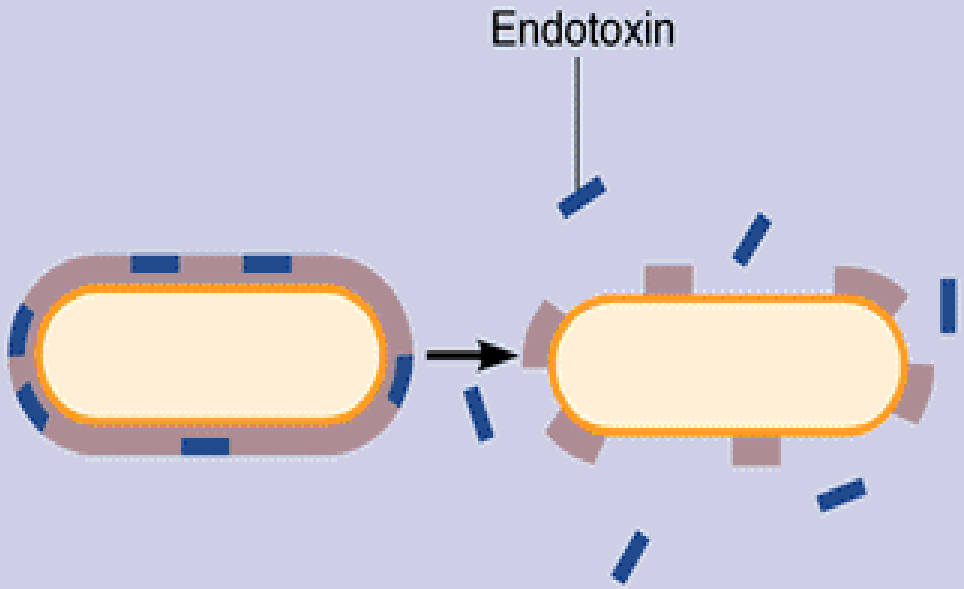
1. **Lipopolysaccharides**: are associated with the cell walls of Gram –ve bacteria.
   - ✓ The lipopolysaccharide (LPS) component of the Gram-ve bacterial outer membrane bears the name **endotoxin** because of its association with the cell wall of bacteria.

2. **Proteins**: may be released into the extracellular environment of pathogenic bacteria.

   ✓Most of the protein toxins are thought of as **exotoxins**, since they are "released" from the bacteria and act on host cells at a distance.



(a) **Exotoxins** are produced inside mostly gram-positive bacteria as part of their growth and metabolism. They are then released into the surrounding medium.



(b) **Endotoxins** are part of the outer portion of the cell wall (lipid A; see Figure 4.12c) of gram-negative bacteria. They are liberated when the bacteria die and the cell wall breaks apart.

1. BACTERIAL PROTEIN TOXINS

❖The protein toxins aresoluble proteins secreted by living bacteriaduring **exponential growth**.

❖The production of protein toxins is specific to a particular bacterial species

- (e.g. only ***Clostridium tetani*** produces **tetanus** toxin.
  - only **Corynebacterium diphtheriae** produces the **diphtheria toxin**.

❖Usually, **virulent strains** of the bacterium produce the toxin (or range of toxins) while non-virulent strains do not.

1. BACTERIAL PROTEIN TOXINS

- Toxin is the **major** determinant of virulence.

- **Both** Gram-positive and Gram-negative bacteria produce soluble toxins.

- Bacterial protein toxins are the most **potent** poisons known and may show activity at very high dilutions.

1. BACTERIAL PROTEIN TOXINS

- The protein toxins **resemble enzymes**.
- Like enzymes bacterial exotoxins are:

  - ✓proteins
  - ✓denatured by heat, acid, proteolytic enzymes
  - ✓have a high biological activity (most act catalytically)
  - ✓exhibit specificity of action

1. BACTERIAL PROTEIN TOXINS

❖Bacterial protein toxins are **highly specific** in the substrate utilized and in their mode of action.

❖Usually the site of damage caused by the toxin indicates the location of the substrate for that toxin.

1. BACTERIAL PROTEIN TOXINS

- Terms such as
- "**enterotoxin**"
- "**neurotoxin**"
- "**leukocidin**”
- "**hemolysin**"

are used to indicate the target site of some well-defined protein toxins.

1. BACTERIAL PROTEIN TOXINS

❖Certain protein toxins have very **specific cytotoxic** activity (i.e., they attack specific cells, for example, **tetanus** or **botulinum** toxins)

❖Some (as produced by staphylococci, streptococci, clostridia, etc.) have fairly **broad cytotoxic activity** and cause nonspecific death of tissues (necrosis).

1. BACTERIAL PROTEIN TOXINS

❖ Toxins that are **phospholipases** may be relatively nonspecific in their cytotoxicity.

❖ This is also true of pore-forming "**hemolysins**" and "**leukocidins**".

❖ A few protein toxins cause death of the host and are known as "**lethal toxins**",

- (e.g. anthrax toxin).

1. BACTERIAL PROTEIN TOXINS

❖Protein toxins are strongly antigenic.

❖**In vivo**, specific antibody (antitoxin) neutralizes the toxicity of these bacterial proteins.

❖**In vitro**, specific antitoxin may not fully inhibit their enzymatic activity.

1. BACTERIAL PROTEIN TOXINS

❖Protein toxins are inherently unstable:

In time they lose their toxic properties but retain their antigenic ones.

❖**Toxoids:** are detoxified toxins which retain their antigenicity and their immunizing capacity

1. BACTERIAL PROTEIN TOXINS

❖ **The formation of toxoids can be accelerated by:**

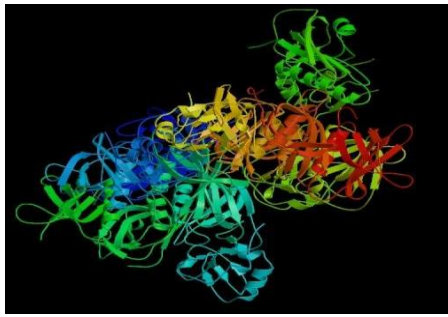
✓treating toxins with a variety of reagents including **formalin**, **iodine**, **pepsin**, etc.

✓The mixture is maintained at 37^{o} at pH range 6 to 9 for several weeks.

✓Toxoids can be use for artificial immunization against diseases caused by pathogens .

✓E.g immunizing against diphtheria and tetanus that are part of the DPT vaccine.

1. BACTERIAL PROTEIN TOXINS



A + B SubunitArrangement of Protein Toxins

❖**Many protein toxins, consist of two components:**

1. **Subunit A:** responsible for the enzymatic activity of the toxin.

2. **Subunit B**: concerned with binding to a
   - specific receptor on the host cell membrane and transferring the enzyme across the membrane.

1. BACTERIAL PROTEIN TOXINS

- The enzymatic component is not active until it is released from the native toxin.

- Isolated A subunits are enzymatically active and but lack binding and cell entry capability.

- Isolated B subunits may bind to target cells (and even block the binding of the native A+B toxin), but they are nontoxic

2. ENDOTOXINS

❖**Endotoxins** are:

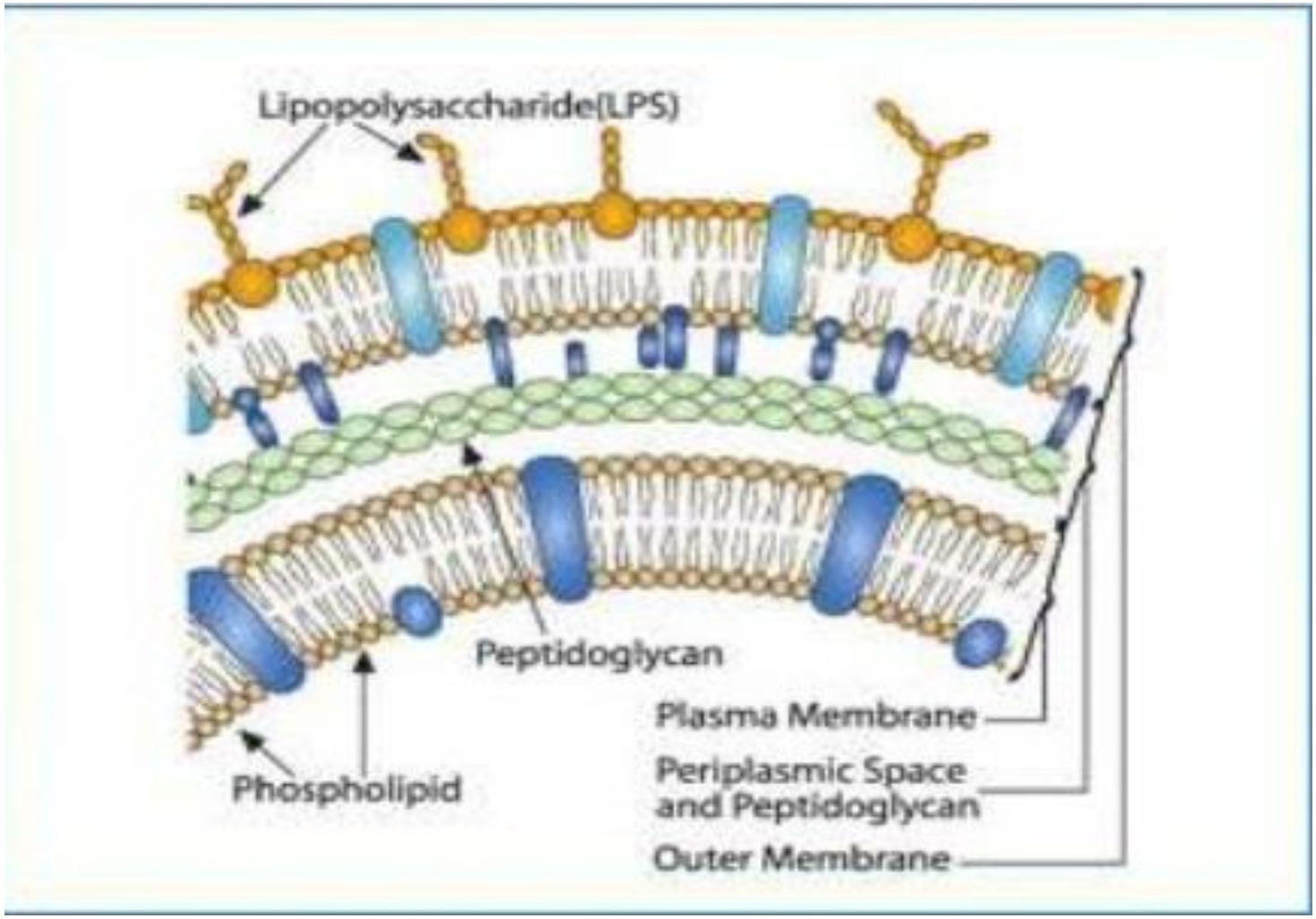
❖Part of the outer cell wall of bacteria.

❖Invariably associated with Gram-ve bacteria as constituents of the outer membrane of the cell wall.

❖**Lipopolysaccharide** (LPS) participates:

In a number of outer membrane functions essential for **bacterial growth** and **survival** .

2. ENDOTOXINS



2. ENDOTOXINS

The lipopolysaccharide complex associated with the outer envelope of **Gram-ve** bacteria such as

E. Coli

Salmonella

Shigella

Pseudomonas

and other leading pathogens.

2. ENDOTOXINS

❖The biological activity of endotoxin is associated with the **lipopolysaccharide** (**LPS**).

❖Toxicity is associated with the lipid component (**Lipid A**) and polysaccharide

❖Immunogenicity antigenicity is associated with the components.

2. ENDOTOXINS

- Most part of endotoxins remain associated with the cell wall until disintegration of the bacteria.

  - ✓In vivo, this results from **autolysis**, **external lysis**, and **phagocytic** digestion of bacterial cells.

  - ✓Small amounts of endotoxin may be released in a **soluble** form, especially by young cultures.

2. ENDOTOXINS

- Compared to the classic exotoxins of bacteria, endotoxins are less potent and less specific in their action, since they do not act enzymatically.

- Endotoxins are heat stable (boiling for 30 min does not destabilize endotoxin).
  - ✓but certain powerful oxidizing agents such as , **superoxide, peroxide** and **hypochlorite** degrade them.

- Endotoxins, although strongly antigenic, cannot be converted to toxoids.

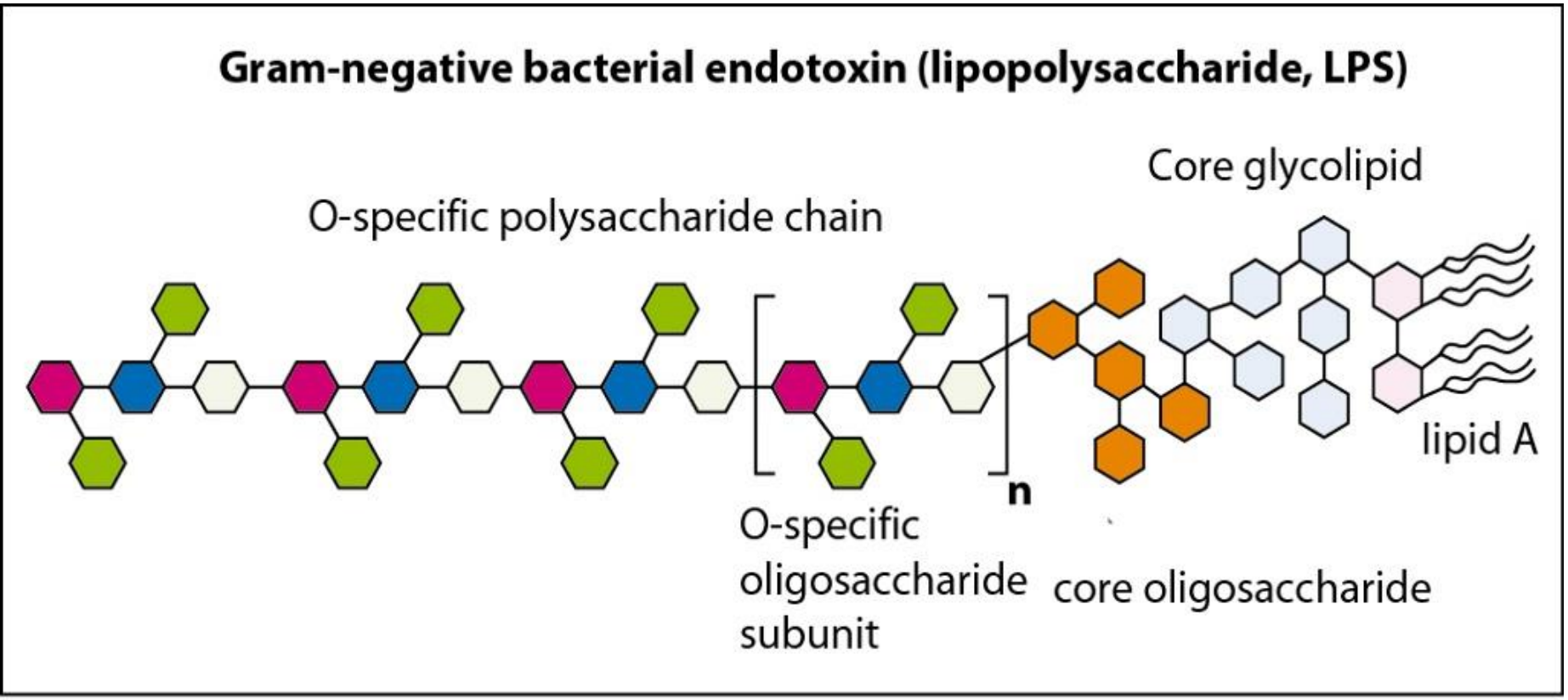
2. ENDOTOXINS

❖In a basic ground plan common to all endotoxins, LPS consists of three components:

(1)Lipid A

(2)Core polysaccharide

(3) O polysaccharide



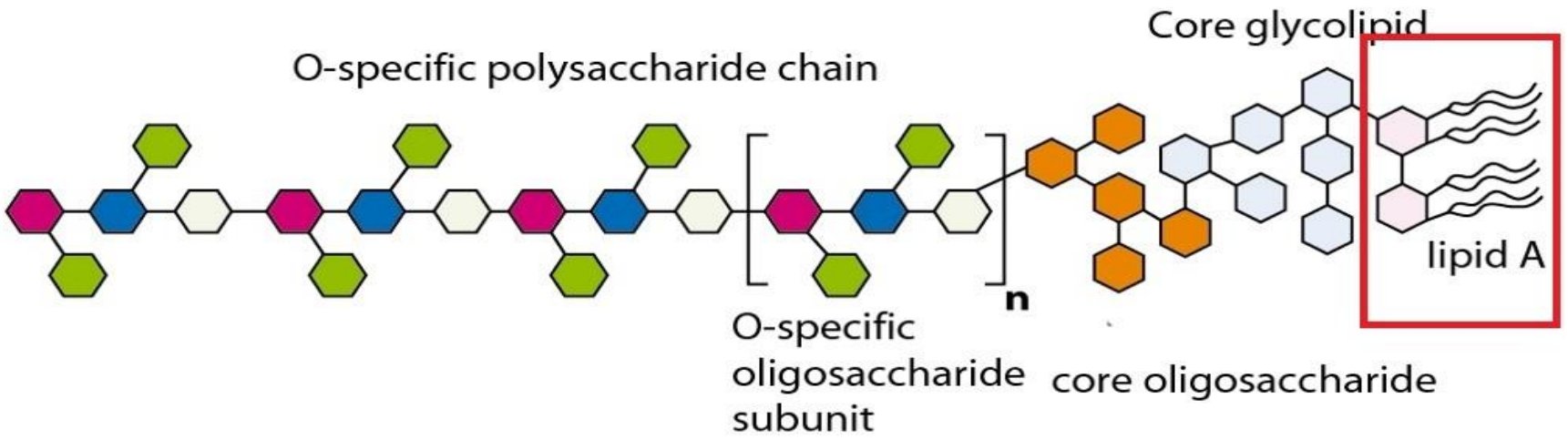
2. ENDOTOXINS

1. Lipid A:

- ❖ Lipid component of LPS .
- ❖ Contains the hydrophobic, membrane-anchoring region of LPS.

- ❖ Lipid Aconsists of **a phosphorylated N-acetylglucosamine** (NAG) dimer with 6 or 7 fatty acids (FA) attached.

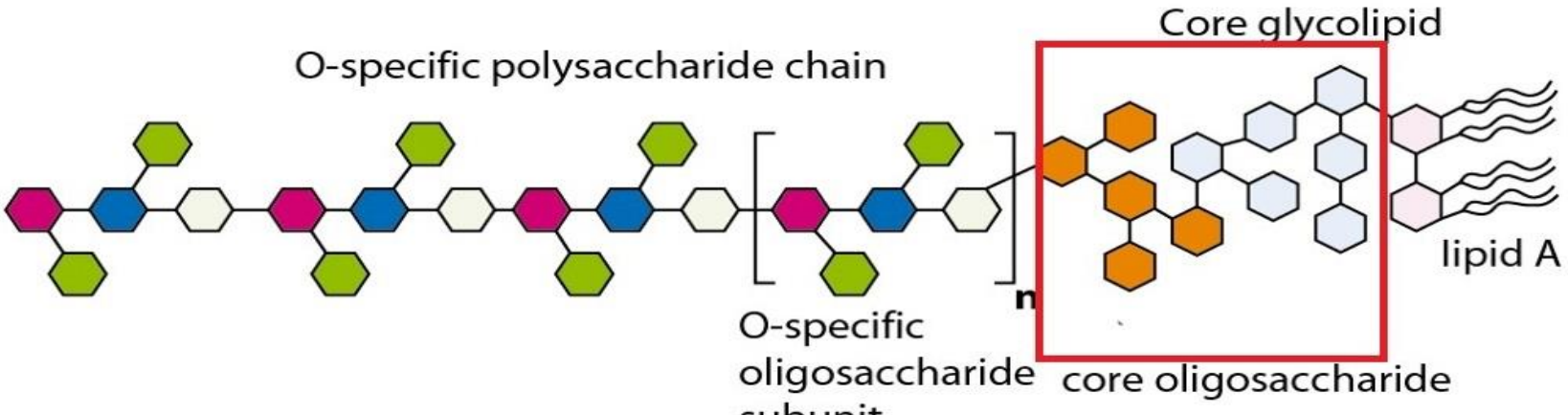
- ❖ The structure of Lipid A is highly conserved among Gram-ve bacteria.
  - ✓ Among ***Enterobacteriaceae*** Lipid A is virtually constant



2. ENDOTOXINS

2. Core (R) polysaccharide:

- ❖Is attached to the 6 position of one NAG.
- ❖The R antigen consists of a short chain of sugars.
- ❖Two unusual sugars – present
  - ❖Heptose and
  - ❖2-keto-3-deoxyoctonoic acid (KDO), in the core polysaccharide.
  - ❖KDO is unique and invariably present in LPS and so has been an indicator in assays for LPS (endotoxin).

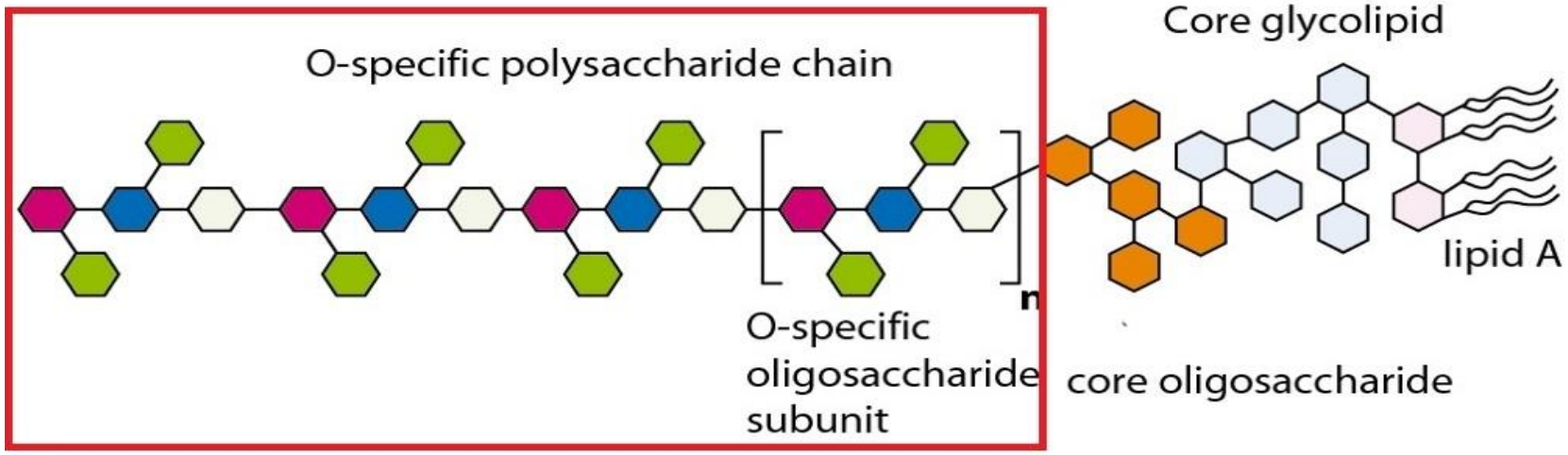


2. ENDOTOXINS

3. O polysaccharide (also referred to as the **O antigen** or **O side chain**):

- ❖Is attached to the core polysaccharide.
- ❖Consists of repeating oligosaccharide subunits made up of 3-5 sugars.

- ❖The individual chains vary in length ranging up to 40 repeat units.
- ❖O polysaccharide is much longer than the core polysaccharide and it maintains the hydrophilic domain of the LPS molecule.



2. ENDOTOXINS

❖A major **antigenic** determinant (antibody-combining site) of the Gram-negative cell wall resides in the **O polysaccharide**.

❖Great variation occurs in the composition of the sugars in the O side chain between species and even strains of Gram-negative bacteria.

2. ENDOTOXINS

❖ Regardless of the bacterial source

✓ all endotoxins produce the same range of biological effects in the animal host.

✓ Injection of living or killed Gram-ve cells, or purified LPS, into experimental animals wide spectrum of nonspecific **pathophysiological reactions related to inflammation** such as

- ✓ fever
- ✓ changes in white blood cell counts
- ✓ disseminated intravascular coagulation
- ✓ tumor necrosis
- ✓ hypotension
- ✓ shock

2. ENDOTOXINS

The role of Lipid A

- Physiological **activities** of endotoxins- mediated mainly by the Lipid A component of LPS.

- Lipid A is the **toxic** component of LPS.

Lipid A → **surfaces of macrophages** → **mediate the pathophysiological response**

2. ENDOTOXINS

The role of the O polysaccharide

❖Although nontoxic, the polysaccharide side chain (O antigen) of LPS may act as a determinant of virulence in Gram-ve bacteria.

❖O polysaccharide is responsible for the property of "**smoothness**" of bacterial cells, which may contribute to their **resistance** to phagocytic engulfment.

❖O polysaccharide is **hydrophilic** and may allow diffusion or delivery of the **toxic lipid** in the hydrophilic (in vivo) environment.

CHARACTERISTICS OF BACTERIAL ENDOTOXINS AND EXOTOXINS		
PROPERTY	ENDOTOXIN	EXOTOXIN
Chemical nature	Lipopolysaccharide (mw = 10 kDa)	Protein (mw = 50-1000 kDa)
Relationship to cell	Part of outer membrane	Extracellular, diffusible
Denatured by boiling	No	Usually
Antigenic	Yes	Yes
Form toxoid	No	Yes
Potency	Relatively low (>100ug)	Relatively high (1 ug)
Specificity	Low degree	High degree
Enzymatic activity	No	Usually
Pyrogenicity	Yes	Occasionally