BACTERIAL TOXINS

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TOXIGENESIS

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.

1. BACTERIAL PROTEIN TOXINS

❖The protein toxins are soluble proteins secreted by living bacteria during 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.

❖ Toxin is the major determinant of virulence.

❖Both Gram-positive and Gram-negative bacteria produce soluble protein 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

❖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.

- ❖Terms such as "enterotoxin", "neurotoxin", "leukocidin" or "hemolysin" are used to indicate the target site of some well-defined protein toxins.

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).

❖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).

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.

- 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 (first discovered by Ehrlich)

BACTERIAL PROTEIN TOXINS

❖The formation of toxoids can be accelerated by:

- ✓ treating toxins with a variety of reagents including formalin, iodine, pepsin, ascorbic acid, ketones, 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 where the primary determinant of bacterial virulence is toxin production.
  - ✓E.g immunizing against diphtheria and tetanus that are part of the DPT vaccine.

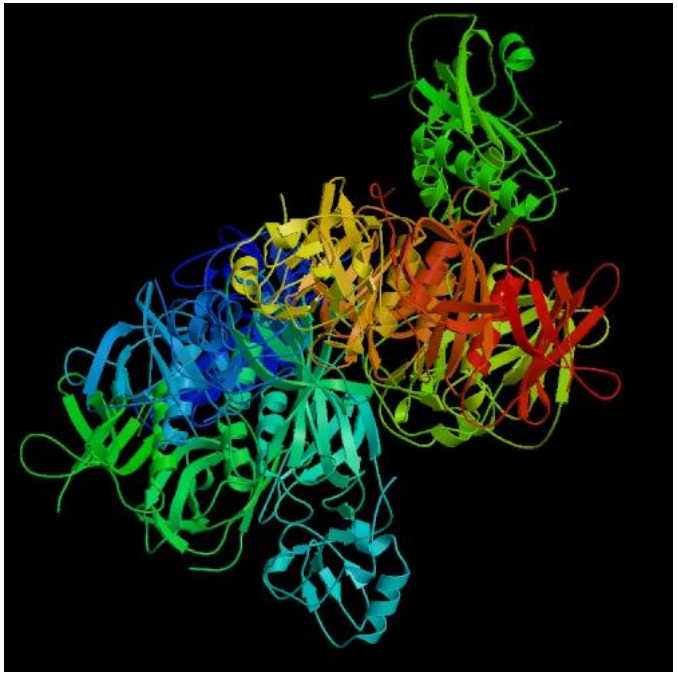
1. BACTERIAL PROTEIN TOXINS

A + B Subunit Arrangement 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.

- ❖ 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.



Tertiary structure of the pertussis toxin produced by *Bordetella pertussis*. Pertussis toxin is a member of the A-B bacterial toxin superfamily. It is a hexameric protein comprising five distinct subunits,

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.

❖NB: **Endotoxin:**

- ❖Occasionally used to refer to any "cell-associated" bacterial toxin.
- ❖But should be reserved for the lipopolysaccharide complex associated with the outer envelope of Gram-ve bacteria such as *E. coli*, *Salmonella*, *Shigella*, *Pseudomonas*, *Neisseria*, *Haemophilus*, and other leading pathogens.

❖Lipopolysaccharide (LPS) participates:

- ❖ in a number of outer membrane functions essential for bacterial growth and survival, especially within the context of a host-parasite interaction.

2. ENDOTOXINS

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

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

❖Immunogenicity (antigenicity) is associated with the polysaccharide components.

❖The cell wall antigens (**O antigens**) of Gram-negative bacteria are components of LPS.

❖LPS activates complement by the alternative (properdin) pathway and may be a part of the pathology of most Gram-negative bacterial infections.

2. ENDOTOXINS

NB 2: 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.

❖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.

CHARACTERISTICS OF BACTERIAL ENDOTOXINS AND EXOTOXINS		
PROPERTY	ENDOTOXIN	EXOTOXIN
Chemical nature	Lipopolysaccharide (mw = 10kDa)	Protein (mw = 50-1000kDa)
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

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2. ENDOTOXINS

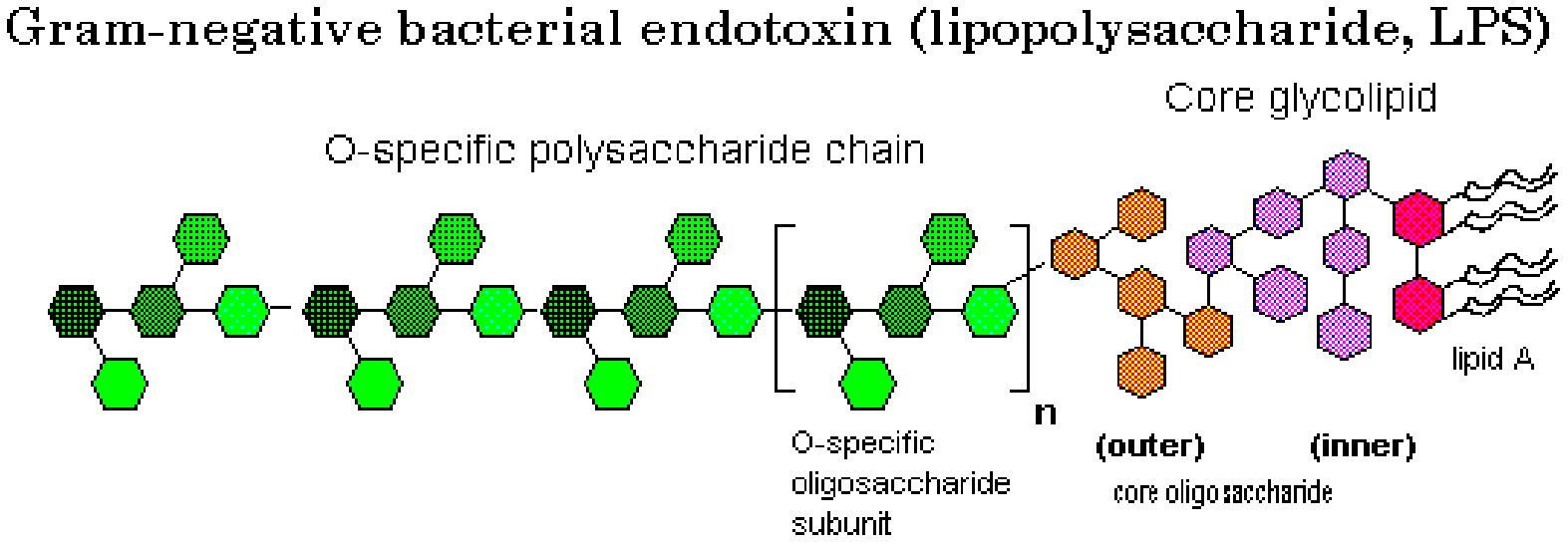
Lipopolysaccharides:

❖ Are complex amphiphilic molecules with a mw of about 10kDa, that vary widely in chemical composition both between and among bacterial species.

❖ 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: heat stable



2. ENDOTOXINS

1. Lipid A :

- Lipid component of LPS.
- Contains the hydrophobic, membrane-anchoring region of LPS.
- Lipid A consists of a phosphorylated N-acetylglucosamine (NAG) dimer with 6 or 7 fatty acids (FA) attached.
- 6 FA are found. All FA in Lipid A are saturated.
- Some FA are attached directly to the NAG dimer and others are esterified to the 3-hydroxy fatty acids that are characteristically present.
- 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.
- ❖For example: KDO - Hep - Hep - Glu - Gal - Glu - GluNAc.
- ❖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

- Core polysaccharide is common to all members of a bacterial genus with minor variations (e.g. *Salmonella*).
  - but it is structurally distinct in other genera of Gram-negative bacteria.
  - *Salmonella*, *Shigella* and *Escherichia* have similar but not identical cores.

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 unique group of sugars, called **dideoxyhexoses**, occurs in the O polysaccharide.

❖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

LPS and virulence of Gram-negative bacteria

❖Endotoxins are toxic to most mammals.

❖They are strong antigens but they seldom elicit immune responses which give full protection to the animal against secondary challenge with the endotoxin.

❖Cannot be toxoided.

❖Endotoxins released from multiplying or disintegrating bacteria significantly contribute to the symptoms of Gram-ve bacteremia and septicemia ⟶ important pathogenic factors in Gram-ve infections.

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.
- ❖Its biological activity depends on
  - ✓a peculiar conformation determined by the glucosamine disaccharide,
  - ✓PO_4 groups,
  - ✓Acyl chains, and
  - ✓KDO-containing inner core.
- ❖Lipid A is known to react at the surfaces of macrophages → release cytokines that mediate the pathophysiological response to endotoxin.

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.

2. ENDOTOXINS

❖Long side chains of LPS afforded by the O polysaccharide may prevent host complement from depositing on the bacterial cell surface which would bring about bacterial cell lysis.

❖O polysaccharide may supply a bacterium with its specific ligands (adhesins) for colonization which is essential for expression of virulence.

❖O-polysaccharide is antigenic, and the usual basis for antigenic variation in Gram-ve bacteria rests in differences in their O polysaccharides.

Pathogenicity Islands

Pathogenicity Islands (PAI):

- ❖Are a distinct class of genomic islands which are acquired by horizontal gene transfer.

- ❖Incorporated in the genome of pathogenic bacteria
  - ✓usually absent from non-pathogenic organisms of the same or closely related species.
- ❖They occupy relatively large genomic regions ranging from 10-200 kb
  - ✓encode genes which contribute to virulence of the pathogen.

- ❖Typical examples:
  - ✓adhesins, toxins, iron uptake systems, invasins, etc.

Pathogenicity Islands

❖One species of bacteria may have more than one pathogenicity island.

✓For example, in *Salmonella*, five pathogenicity islands have been identified.

❖Found mainly in Gram-ve bacteria, but have been shown in a few Gram-positives.

❖Found in pathogens that undergo gene transfer by plasmid, phage, or a conjugative transposon and are typically transferred through mechanisms of horizontal gene transfer (HGT).

Pathogenicity Islands

❖May be located on the bacterial chromosome or may be a part of a plasmid.

❖Are rich in Guanine + Cytosine content.

❖They are flanked by direct repeats i.e., the sequence of bases at two ends are the same.

❖Are associated with tRNA genes, which target sites for the integration of DNA.

❖Have characteristics of transposons in that they carry functional genes

- ✓ e.g. integrase, transposase, or part of insertion sequences
- ✓may move from one tRNA locus to another on the chromosome or plasmid.

Pathogenicity Islands

❖Play a vital role in the virulence of bacterial pathogens of humans , animals and plants.

❖The availability of a large number of genome sequences of pathogenic bacteria and their nonpathogenic relatives

⟹ identification of novel pathogen-specific genomic islands.

❖PAI apparently -acquired during the speciation of pathogens from their nonpathogenic or environmental ancestors.

- ❖The acquisition of PAI is an ancient evolutionary event that led to the appearance of bacterial pathogens on a timescale of millions of years

- ❖May also represent a mechanism that contributes to the appearance of new pathogens.

Pathogenicity Islands

❖Knowledge about PAI, their structure, their mobility, and the pathogenicity factors they encode is helpful:

a) In gaining a better understanding of bacterial evolution and interactions of pathogens with eucaryotic host cells

b) Also may have important practical implications such as providing delivery systems for vaccination and tools for the development of new strategies for therapy of bacterial infections.

Pathogenicity Islands

- PAIs represent distinct genetic elements encoding virulence factors of pathogenic bacteria
  - Belong to a more general class of **genomic islands**
  - Common genetic elements sharing a set of unifying features.
  - Genomic islands have been acquired by horizontal gene transfer.
  - In recent years many different genomic islands have been discovered in a variety of pathogenic as well as non-pathogenic bacteria.
  - Because they promote genetic variability, genomic islands play an important role in microbial evolution