جامعة الانبار

كليه العلوم

قسم علوم الحياة

Bacterial toxins

Assisstant .prof .Dr.Muthanna Hamid Hassan

Lecture 9

Pore-forming toxins

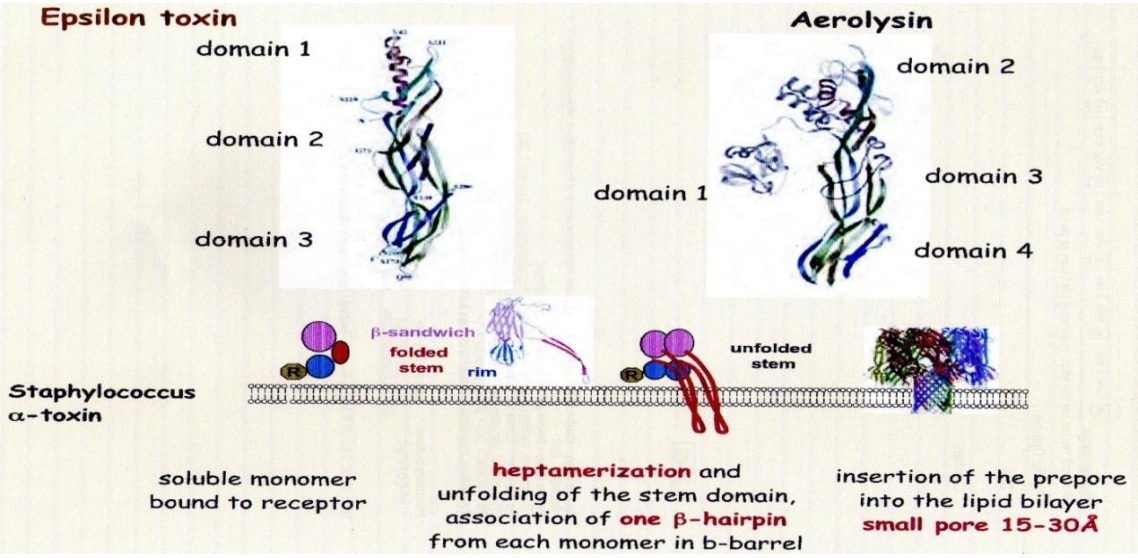
السموم التي تكون ثقوب بالاغلفه

Pore-forming toxins Lec. 9

Staphylococcal alpha toxin (alpha-hemolysin)

The best characterized and most potent membrane-damaging toxin of *S. aureus* is alpha toxin. It is expressed as a monomer that binds to the membrane of susceptible cells. Subunits then oligomerize to form heptameric rings with a central pore through which cellular contents leak.

In humans, platelets and monocytes are particularly sensitive to alpha toxin. Susceptible cells have a specific receptor for alpha toxin which allows the toxin to bind causing small pores through which monovalent cations can pass. The mode of action of alpha hemolysin is likely by osmotic lysis



Staphylococcal alph-toxin (haemolysin) is known to be produced *in vivo.* It is important in killing neutrophils. It probably has the narrowest substrate specificity among the phospholipases, and is a hot-cold haemolysin: lysis of erythrocytes occurs only on cooling after incubation at 37.The most likely explanation of this phenomenon

is that, when cooled below their phase-transition temperature, the remaining phospholipids undergo quasi-crystalline formation, thereby generating intramembranous stresses incompatible with structural integrity.

EPSILON TOXIN (ETX)

ETX is an example of an aerolysinlike, pore-forming toxin. *C. perfringens* ETX and *C. septicum* alpha-toxin are structurally related to aerolysin although ETX shows no significant sequence homology with aerolysin at the amino acid level. ETX and *C. septicum* alpha-toxin form heptameric pores, like aerolysin, and are very potent cytolysins. ETX is considered the major virulence factor of *C. perfringens* types B and D . This toxin causes blood pressure elevation, increased contractility of smooth muscle, vascular permeability increase, as well as brain and lung edema in multiple animal species, while in goats ETX also causes colitis . ETX is the third most potent clostridial toxin after botulinum toxin and tetanus toxin, with a mouse lethal dose of 100 ng/kg . ETX is secreted as aprototoxin (32,981 Da) , which is converted into a fully active toxin (~1000 times more toxic than the prototoxin) when activated by proteases such as trypsin, chymotrypsin, and a metalloproteinase named lambda toxin that is produced by *C. perfringens* .

ETX is also active on a few cell lines .The cytotoxicity is associated with pore formation that causes a rapid loss of intracellular K+, an increase of Cl- and Na+, with an increase of Ca++ occurring later. ETX induces a rapid and dramatic increase in permeability and it is thought that pore formation in the cell membrane is likely responsible for the permeability change of cell monolayers. Actin cytoskeleton and organization of tight and adherens junctions are not altered upon ETX treatment.

ß-toxin

It is a sphingomyelinase which damages membranes rich in this lipid. The classical test for ß-toxin is lysis of sheep erythrocytes. The majority of human isolates of *S. aureus* do not express ß-toxin. A lysogenic bacteriophage is known to encode the toxin.

Leukocidin

It is a multicomponent protein toxin produced as separate components which act together to damage membranes. Leukocidin forms a hetero-oligomeric transmembrane pore composed of four LukF and four LukS subunits, thereby forming an octameric pore in the affected membrane. Leukocidin is hemolytic, but less so than alpha hemolysin.

Only 2% of all of *S. aureus* isolates express leukocidin, but nearly 90% of the strains isolated from severe dermonecrotic lesions express this toxin, which suggests that it is an important factor in necrotizing skin infections.

Cholesterol-binding cytolysins (CBCs)

These proteins, more commonly known as 'SH-activated cytolysins', are made by some 23 taxonomically different species of Gram-positive bacteria, not all of which are pathogens. They are lethal, cardiotoxic, antigenically related, and their lytic and lethal activities are blocked by cholesterol. Recent work requires that we abandon certain perceptions about these toxins which are enshrined in the older nomenclature.

For example, *purified* toxins are not O2-1abile, and are not activated by sulihydryl compounds, and do not depend on a cysteine residue for activity. The cholesterol is irreversibly inhibit the lytic and lethal properties of these toxins. Interaction with cholesterol is thought to be the key primary event in their interaction with susceptible membranes, which leads to the impairment of the latter; cholesterol plays no further part in the subsequent damage process. However, the role of cholesterol has been interpreted in terms of mediating the oligomerisation process which leads to membrane damage.

Four examples of CBCs from pathogenic species include

1- **Streptolysin O (SLO**): It is lethal almost certainly due to its cardiotoxicity. The, SLO-induced increases in proinflammatory cytokines IL-I,and tumor necrosis factor a (TNF-α) , accumulation of poly morpho nuclear leucocytes (PMNs) in lung and soft tissue in cases of streptococcal toxic shock syndrome .

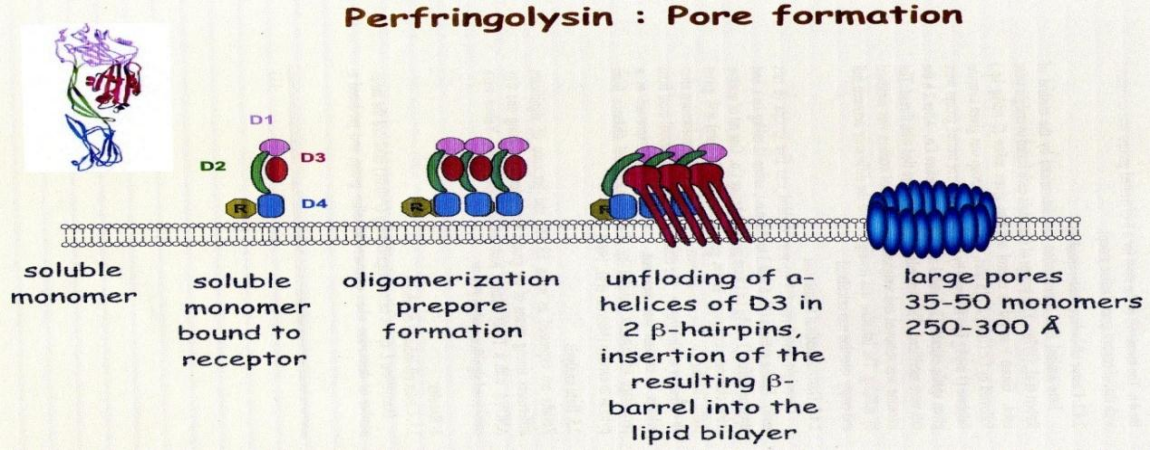
2- ***Listeriolysin 0 (LLO)****:* Is the most important virulence determinant of *Listeria monocytogenes.* It plays an important part in mediating the escape of *L. monocytogenes* from intraphagocytic vacuoles. LLO will rapidly kill cells by rupturing the cytoplasmic membrane.

3 - ***Pneumolysin (PLY) :*** This protein is produced by the pathogen *Streptococcus pneumonia* (pneumococcus. It possesses haemolytic activity. In experimental meningitis in guinea pigs, in contrast to the mouse lung model, PLY was not responsible for the potent inflammatory response, but did cause an increase in protein content of cerebrospinal fluid (CSF) .Attempts to develop protective antipneumococcal vaccines have been based on the type-specific capsular polysaccharides. Unfortunately, there are at least 90 known types and current vaccine preparations comprise a blend of polysaccharides from some 23 types.

4- **Perfringolysin O OR THETA TOXIN (PFO)** : PFO is a 54 kDa cytolytic toxin that binds to cholesterol-containing eukaryotic membranes. This toxin forms a large oligomeric prepore complex on the membrane surface prior to insertion into the cell membrane. Structurally, PFO is comprised of 4 domains. The Cterminal domain (domain 4) binds cholesterol and then a conformational change in domain three exposes a 2 β–hairpin that spontaneously inserts into the lipid bilayer. It has been proposed that the high affinity of PFO for cholesterol concentrates the toxin on the target membrane, promoting oligomerization and membrane insertion .The proposed model of PFO pore formation includes the binding of water-soluble PFO monomers to cholesterol of a lipid bilayer mediated by domain 4,then oligomer (a prepore complex) is formed. The oligomers consist of 35 to 50 monomers, forming on the

membrane surface large arcs and rings that, after insertion of their domain three loops, lead to large pores between 250 Å and 300 Å in diameter .

PFO participates with CPA in the production of local lesions of gangrene/malignant edema in humans and animals . Experimental evidence suggests that PFO plays only a minor role in the pathogenesis of gas gangrene by slightly contributing to the inhibition of PMN migration to the site of infection.



Detergent-like toxins

delta toxin

It is a very small peptide toxin produced by most strains of *S. aureus*. It is also produced by *S. epidermidis*. The role of delta toxin in disease is unknown. Staphylococcal delta-toxin acts in a manner similar to that of the cholesterol-binding cytolysins, with an important difference: the binding is nonspecific with no requirement for cholesterol. It initially forms small pores and then islands of membrane or large micelles; this gives rise to its perceived detergent-like properties. There is a family of closely related δ-toxins which inhibit the growth of gonococci. In this case δ-toxin(s) could have important ecological significance in the mixed culture that is characteristic of the real microbial world. Of great interest is the synergy that δ-toxin displays. Sublytic amounts of δ-toxin cause release of cell constituents without lysis.

Bacillus cereus TOXINS

Bacillus cereus has been recognized as an agent of food poisoning since 1955. There are only a few outbreaks a year reported by CDC. It is not a reportable disease, and usually goes undiagnosed. Key virulent factors of *B. cereus* include the ability to form endospores and produce enterotoxins and emetic toxins. There are two types of clinical syndromes associated with each toxin, namely, **rapid-onset emetic syndrome**"short-incubation" and **slow-onset diarrheal syndrome**"long-incubation", it resembles food poisoning caused by Clostridium perfringens.

The diarrheal type is caused by three enterotoxins, namely, haemolysin BL (HBL), a non-hemolytic enterotoxin (NHE), and cytotoxin K (CytK).

-CytK is a single component protein that has not been shown to be involved in food poisoning. All three enterotoxins are cytotoxic and cell membrane active toxins that will make holes or channels in membranes. HBL and NHE proteins exhibit a conformation known as 'beta-barrel' that can insert into cellular membranes, thus creating pores or channels on the cell membranes of target cells. The effect is loss of cellular membrane potential and eventually cell death. Similarly, CytK is also a pore-forming protein, but is more related to other hemolysins. These enterotoxins can also activate the adenylyl cyclase pathway, leading to intestinal fluid secretion.

- Nhe is composed of NheA, NheB and NheC. The three genes encoding the Nhe components constitute an operon. The nhe genes have been cloned separately, and expressed in either *Bacillus subtilis or Escherichia coli.* Separate expression showed that all three components are required for biological activity.

- The hemolytic enterotoxin, HBL, is encoded by the hblCDA operon. The three protein components, L1, L2 and B, constitute a hemolysin. B is for binding; L1 and L2 are lytic components. This toxin also has dermonecrotic and vascular permeability activities, and it causes fluid accumulation in rabbit ileal loops.

The other clinical illness *B. cereus* triggers is the emetic type. It is characterized by nausea and vomiting and abdominal cramps and has an incubation period of 1 to 6

hours. It resembles Staphylococcus aureus food poisoning in its symptoms and incubation period. The emetic syndrome is caused by a heat-stabile toxin called **cereulide** that is found only in emetic strains and is not part of the standard pathogenesis of *B. cereus*. Cereulide is a ring-shaped structure contains three repeats of four amino acids. Cereulide is believed to either function as a potassium ion channel that alters the cell membrane permeability of nerve cells or activate 5-HT_3 (serotonin) receptors, leading to increased afferent vagus nerve stimulation.

Reference

- *Microbial toxins : current research and future trends*. Proft, Thomas. Norfolk: Caister Academic Press. 2009.
- Lectures by Prof. Dr. Mai Talib Falih - University of Baghdad / college of Science .2011

- علم السموم البكتريا ، الاستاذ الدكتور رحاب العزاوي2010

السموم الميكروبية رشا حمد الميهي وجمهوريه مصر العربية 2014