جامعة الانبار

كليه العلوم

قسم علوم الحياة

Bacterial toxins

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Lecture 10

Endotoxins

السموم الداخليه

Toxins acting on the cell surface: Immune system (Superantigens): enterotoxins and toxic shock syndrome toxin of *S. aureus* lec 10

S. aureus secretes two types of toxins with superantigen activity, **enterotoxins**, of which there are six antigenic types (named **SE-A, B, C, D, E and G**), and **toxic shock syndrome toxin** (**TSST-1**).

Enterotoxins cause diarrhea and vomiting when ingested and are responsible for staphylococcal food poisoning. TSST-1 is expressed systemically and is the cause of toxic shock syndrome (TSS). When expressed systemically, enterotoxins can also cause toxic shock syndrome. In fact, enterotoxins B and C cause 50% of non-menstrual cases of TSS. TSST-1 is weakly related to enterotoxins, but it does not have emetic activity. TSST-1 is responsible for 75% of TSS, including all menstrual cases. TSS can occur as a sequel to any staphylococcal infection if an enterotoxin or TSST-1 is released systemically, and the host lacks appropriate neutralizing antibodies. Superantigens stimulate T cells non-specifically without normal antigenic recognition Up to one in five T cells may be activated, whereas only 1 in 10,000 are stimulated during a usual antigen presentation.Cytokines are released in large amounts, causing the symptoms of TSS.

Staphylococcal exfoliatins (ETs)

Staphylococcal exfoliatin (epidermolysin) is important in staphylococcal'scalded skin syndrome' (SSSS), a disease of newborn babies. The disease is characterised by a region of erythema which usually begins around the mouth and, in 1-2 days, extends over the whole body. The most striking feature of the disease, however, is that the epidermis, although apparently healthy, can be displaced and wrinkled like the skin of a ripe peach by the slightest pressure. Soon large areas of epidermis become lifted by a layer of serous fluid and peel at the slightest touch. Large areas of the body rapidly become denuded in this way and the symptoms resemble those of massive scalding.

The toxin causes cleavage of desmosomes (specialised cell membrane thickenings through which cells are attached to each other) in the stratum granulosum. ETs are serine proteases with high substrate specificity, which selectively recognize and hydrolyze desmosomal proteins in the skin. There are two antigenically distinct forms of the toxin, ETA and ETB. The toxins have esterase and protease activity and apparently target a protein which is involved in maintaining the integrity of the epidermis. . Staphylococcal exfoliative toxin B has been shown to specifically cleave desmoglein 1, a cadherin that is found in desmosomes in the epidermis.

In SSSS, blistering affects only the superficial skin and not the mucosa or deeper skin layers. This phenomenon is elegantly explained by the selectivity of desmoglein cleavage and the differential expression of particular desmogleins in different layers of the skin and mucosa. The ETs selectively hydrolyze Dsg-1, whereas Dsg-3 remains unaffected.

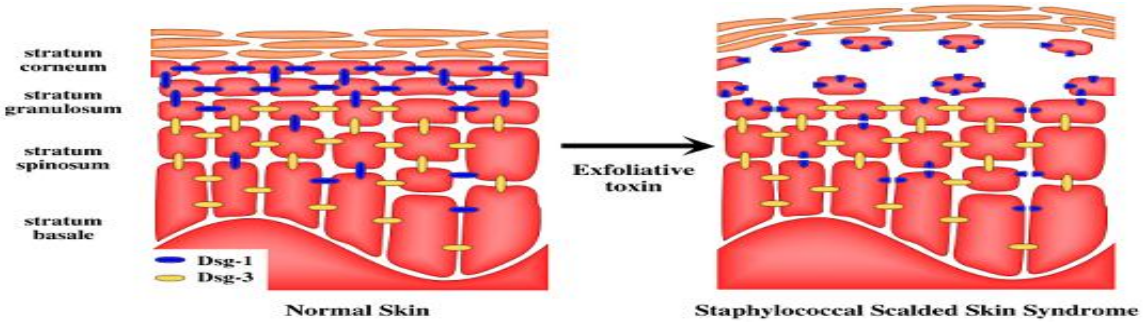

Fig: Differential distribution of desmoglein isoforms in the epidermis explains the exfoliative-toxin-induced splitting at the stratum granulosum. Schematic representation of the desmoglein distribution in (A) healthy skin and (B) skin exposed to exfoliative toxin. In all strata, except the stratum granulosum, the exfoliative-toxin-mediated hydrolysis of desmoglein 1 (Dsg-1) is compensated by desmoglein 3 (Dsg-3). Dsg-3 is absent in the stratum granulosum, which explains the cell detachment and the splitting of the epidermal layers upon the hydrolysis of Dsg-1.

(V) Cell-associated toxins

There are two chemically distinct types of toxin considered: lipopolysaccharide (endotoxin; LPS) and protein. The Gram-negative bacteria produce endotoxin. However, the examination of cell-bound proteins of *Yersinia pestis* from organisms grown *in vivo* led to the discovery of a toxin lethal for mice and guinea-pigs.

Protein toxins of *Yersinia pestis*

Plague is one of the most deadly diseases of man'the black death' and has, over several thousands of years, claimed millions of lives. The causative organism of plague, *Yersinia pestis,* is primarily a parasite of rodents in which it is endemic in many areas of the world. Only when man comes into close proximity with infected rodents do outbreaks of human plague occur. The disease is spread from rat to rat and from rat to man by fleas .

The principal features of human plague can be reproduced in guinea pigs and mice. The symptoms of plague - high fever and vascular damage – are characteristic of intoxication with endotoxin. However, it is extremely unlikely that endotoxin alone is the main toxin involved in plague. It is much more likely to act in conjunction with one or more other potentially toxic fractions from Y. *pestis.*

*Plague murine toxin is a protein which, although highly lethal for mice and rats, is relatively nontoxic for guinea-pigs, rabbits, dogs and monkeys.

*A completely separate guinea-pig toxin complex exists comprising at least two cell wall/ membrane protein components, one of which will kill mice, although both are needed to kill guinea-pigs. However, the nature of the toxin or toxins of Y. *pestis* and their role in the human disease syndrome are still far from clear.

Endotoxins

Endotoxins are part of the outer membrane of the cell wall of Gram-negative bacteria .Endotoxin is invariably associated with Gram-negative bacteria whether the organisms are pathogenic or not. Although the term "endotoxin" is occasionally used to refer to any cell-associated bacterial toxin, in bacteriology it is properly reserved to refer to the **lipopolysaccharide** complex associated with the outer membrane of Gram-negative pathogens such as *Escherichia coli*, *Salmonella*, *Shigella*, *Pseudomonas*, *Neisseria*, *Haemophilus influenzae*, *Bordetella pertussis* and *Vibrio cholerae*. The biological activity of endotoxin is associated with the lipopolysaccharide (LPS). **Toxicity** is associated with the lipid component (**Lipid A**) and **immunogenicity** is associated with the **polysaccharide** components.

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 minutes does not destabilize endotoxin), but certain powerful oxidizing agents such as superoxide, peroxide and hypochlorite, have been reported to neutralize them. Endotoxins, although antigenic, cannot be converted to toxoids.

LPS can be extracted from whole cells by treatment with 45% phenol at 90^{o}. Mild hydrolysis of LPS yields Lipid A plus polysaccharide.

LPS and virulence of Gram-negative Bacteria

- The O polysaccharide and virulence

Virulence, and the property of "smoothness", is associated with an intact **O polysaccharide**, The involvement of the polysaccharide chain in virulence is shown by the fact that small changes in the sugar sequences in the side chains of LPS result in major changes in virulence. How are the polysaccharide side chains involved in the expression of virulence? There are a number of possibilities:

1. O-specific antigens could allow organisms to **adhere** specifically to certain tissues, especially epithelial tissues.

2. Smooth antigens probably allow **resistance to phagocytes**, since rough mutants are more readily engulfed and destroyed by phagocytes.

3. The hydrophilic O polysaccharides could act as water-solubilizing **carriers for toxic Lipid A**. It is known that the exact structure of the polysaccharide can greatly influence water binding capacity at the cell surface.

4. The O antigens could provide **protection from damaging reactions with antibody and complement**.

5. The O-polysaccharide or **O antigen** is the basis of **antigenic variation** among many important Gram-negative pathogens including *E. coli, Salmonella* and *Vibrio cholerae*.

- Lipid A and virulence

The physiological activities of LPS are mediated mainly by the **Lipid A** component of LPS. Lipid A is a powerful biological response modifier that can stimulate the mammalian immune system .

- The injection of living or killed Gram-negative cells or purified LPS into experimental animals causes a wide spectrum of nonspecific pathophysiological reactions, such as fever, changes in white blood cell counts, disseminated intravascular coagulation, hypotension, shock and death. Injection of fairly small doses of endotoxin results in death in most mammals.

In monocytes and macrophages, three types of events are triggered during their interaction with LPS:

1. **Production of cytokines**, including IL-1("endogenous pyrogen"), IL-6, IL-8, tumor necrosis factor (TNF) and platelet-activating factor. These, in turn, stimulate

production of prostaglandins and leukotrienes. These are powerful mediators of inflammation and septic shock that accompanies endotoxin toxemia

2. **Activation of the complement cascade**. C3a and C5a cause histamine release and affect neutrophil chemotaxis and accumulation. The result is inflammation.

3. **Activation of the coagulation cascade**. Initial activation of Hageman factor (blood-clotting Factor XII) can activate several humoral systems resulting in : a. coagulation, thrombosis , b. activation of the complement alternative pathway (which leads to inflammation) , c. plasmin activation which leads to fibrinolysis and hemorrhaging , d. kinin activation releases bradykinins and other vasoactive peptides which causes hypotension .The net effect is to induce inflammation, intravascular coagulation, hemorrhage and shock. LPS also acts as a **B cell mitogen**, stimulating the polyclonal differentiation and multiplication of B-cells and the secretion of immunoglobulins, especially IgG and IgM.

Delta endotoxins (**δ-endotoxins**)

also called **Cry** and **(Cyt** toxins) are pore-forming toxins produced by Bacillus thuringiensis species of bacteria. They are useful for their insecticidal action. During spore formation the bacteria produce crystals of this protein. When an insect ingests these proteins, they are activated by proteolytic cleavage. The N-terminus is cleaved in all of the proteins and a C-terminal extension is cleaved in some members. Once activated, the endotoxin binds to the gut epithelium and causes cell lysis by the formation of cation-selective channels, which leads to death. The activated region of the delta toxin is composed of three distinct structural domains: an N-terminal helical bundle domain involved in membrane insertion and pore formation; a beta-sheet central domain involved in receptor binding; and a C-terminal beta-sandwich domain that interacts with the N-terminal dom ain to form a channel

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