جامعة الانبار

كليه العلوم

قسم علوم الحياة

Bacterial toxins

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Lecture 7

Toxins that act on 28S rRNA

السموم المؤثرة على 28S rRNA

ii) Toxins that act on 28S rRNA Lec7

Shiga toxin (chromosomally encoded) is a potent cytotoxin, Stx is produced by the enteric pathogens *Shigella dysenteriae* serotype I and a collective group of *E. coli* strains called Stx-producing *E. coli* (STEC) which includes serotype O157:H7 and other enterohemorrhagic *E. coli* (EHEC). Stx from *S. dysenteriae* serotype I is involved in the pathogenesis of shigellosis, whilst shiga-like toxins (phage encoded) are primarily produced by enterohemorraghic *E. coli*. They share a common mode of action.

Shiga toxins are a family of related toxins Stx, Stx1 and Stx2. The toxin requires highly specific receptors on the cells' surface in order to attach and enter the cell; species such as cattle, swine, and deer which do not carry these receptors may harbor toxigenic bacteria without any ill effect, shedding them in their feces, from where they may be spread to humans.The syndromes associated with shiga toxin include dysentery, hemorrhagic colitis, and hemolytic uremic syndrome. The onset of symptoms is generally within a few hours, with higher doses leading to more rapid onset. There is no antidote for the toxin. Supportive care requires maintenance of fluid and electrolyte levels, and monitoring and support of kidney function.Immunoassays are available for rapid diagnosis of the toxin.

The toxicity of Shiga toxin for the mouse (LD50) is <20 micrograms/kg by intravenous or intraperitoneal administration. Initially, Stx was called Verotoxin due to its cytotoxicity against Vero cells in culture. Upon the discovery that Verotoxin could be neutralized by an antitoxin against purified Stx from *Shigella*, the name Stx came into use and is the more common name to date .

Table. The toxin has been given several trivial names depending on the bacterium that produces it and the gene that encodes it.

Source Organism	Gene Designation	Toxin Name	Older Names
Shigella dysenteriae, type I	stx	Shiga toxin (Stx)	Shiga toxin
Escherichia coli	stx1	Shiga toxin 1 (Stx1)	Shiga-like toxin I, Verotoxin 1
	stx2	Shiga toxin 2 (Stx2)	Shiga-like toxin II, Verotoxin 2

Characteristics of Shiga Toxin Enterotoxic, neurotoxic and cytotoxic. Encoded by chromosomal genes. Two domain (A-5B) structure

*Enterotoxic effect:

Shiga toxin adheres to small intestine receptors .Blocks absorption (uptake) of electrolytes, glucose, and amino acids from the intestinal lumen

*Cytotoxic Effect:

-B subunit of Shiga toxin binds host cell glycolipid

-A domain is internalized via receptor-mediated endocytosis (coated pits) .Causes irreversible inactivation of the 60S ribosomal subunit, thereby causing: Inhibition of protein synthesis , cell death and microvasculature damage to the intestine . Hemorrhage (blood & fecal leukocytes in stool)

*Neurotoxic Effect: Fever, abdominal cramping are considered signs of neurotoxicity.

Structure

The toxin has two subunits molecular weight of 68,000 da /designated A(32,000 molecular weight) and B(7,700 molecular weight) —and is one of the AB_5 toxins.

Five copies of the B-subunit protein associate to form a pentamer with five-fold symmetry. This structure turns out to resemble that of the B-subunit of cholera toxin, which was a bit of a surprise because the amino acid sequences are very different.

The B subunit is binds to specific glycolipids on the host cell, specifically globotriaosylceramide (Gb3). Following this. A subunit is proteolytically nicked to yield a ca. 28-kDa peptide (A_1) and a 4-kDa peptide (A_2); these peptides remain linked by a disulfide bond. The A_1 peptide contains the enzymatic activity, and the A_2 peptide serves to bind the A subunit to a pentamer of five identical subunits.The A1 component then binds to the ribosome, disrupting protein synthesis. Stx-2 has been found to be approximately 400 times more toxic (as quantified by LD50 in mice) than Stx-1.Gb3 is, for unknown reasons, present in greater amounts in renal epithelial tissues, to which the renal toxicity of Shiga toxin may be attributed. Gb3 is also found in CNS neurons and endothelium, which may lead to neurotoxicity.

Mechanism of Action of Shiga Toxin

The toxin acts on the lining of the blood vessels, the vascular endothelium. The B subunits of the toxin bind to a component of the cell membrane known as Gb3 and the complex enters the cell. When the protein is inside the cell, the A subunit interacts with the ribosomes to inactivate them. The A subunit of Shiga toxin is an N-glycosidase that modifies the RNA component of the ribosome to inactivate it and so bring a halt to protein synthesis leading to the death of the cell(induces apoptosis). The vascular endothelium has to continually renew itself, so this killing of cells leads to a breakdown of the lining and to hemorrhage.

The first response is commonly a bloody diarrhea.The toxin is effective against small blood vessels, such as found in the digestive tract, the kidney, and lungs, but not against large vessels such as the arteries or major veins. A specific target for the toxin appears to the vascular endothelium of the glomerulus. This is the filtering structure

that is a key to the function of the kidney. Destroying these structures leads to kidney failure and the development of the often deadly and frequently hemolytic uremic syndrome. Food poisoning with Shiga toxin often also has effects on the lungs and the nervous system.

The A subunit has N-glycosidase activity that cleaves an adenosine residue from 28S ribosomal RNA of the 60S ribosomal subunit. As a result, it inhibits protein synthesis, causing cell death by apoptosis. The five B subunits form a structure that binds the globotriaosylceramide (Gb3) receptor on the surface of eukaryotic cells. Gb3 is expressed by Paneth cells in the intestinal mucosa and by kidney epithelial cells .

Stx is constituted by a pentamer of B subunits bound to a catalytic A subunit. The B subunits bind to globotriaosylceramide (Gb3) expressed by some eukaryotic cells **(1)** Stx is internalized by endocytosis **(2)** Subsequently, Stx undergoes retrograde transport to the trans-Golgi network (TGN) **(3)** and then to the endoplasmic reticulum (ER) **(4)** In the ER, Stx encounters its target, the ribosome, inactivating it **(4)** As a consquence, Stx inhibits protein synthesis, causing cell death by apoptosis.

Stx in intestinal disease.

There is a variety of data showing the involvement of Stx in diarrhea and enterocolitis, beginning with early demonstrations that purified Stx can cause fluid accumulation and histological damage when injected into ligated intestinal loops.

*One possible mechanism for fluid secretion in response to Stx involves the selective killing of absorptive villus tip intestinal epithelial cells by Stx. In rabbit ileum, the Gb_3 receptor is present in much higher concentrations in villus cells than in the secretory crypt cells, and so the death of absorptive cells and preservation of secretory crypt cells could shift the usual balance of intestinal absorption and secretion toward net secretion. The available evidence therefore suggests that unlike LT or CT, Stx does not

increase active secretion of Cl^- ions. Intravenous administration of purified Stx1 or Stx2 to rabbits can produce nonbloody diarrhea, suggesting other potential mechanisms of diarrhea besides binding of toxin to villus tip cells.

Infections with the two strains resulted in equivalent diarrheal stool volumes, but in animals receiving the Stx-positive strain, the stools were consistently more bloody and there was greater destruction of capillary vessels within the connective tissue of the colonic mucosa.

Shiga-like toxin, also known as **verotoxin**,

A toxin generated by *Escherichia coli*. It is named for its similarity to the AB5-type Shiga toxin produced by the bacteria *Shigella dysenteriae*. Verotoxin (VT) was first reported by Konowalchuk *et al*. in 1977. Although VT-producing *E. coli* strains belong to several different serotypes, 0157:H7 is the dominant serotype isolated from patients suffering from food poisoning. There are two main groups, Stx1 and Stx2 , also known as **SLT-I and SLT-II (**VT1 **&** VT**2)** .

Although Stx1 and Stx2 share enzymatic activity and structural features, they are immunogically distinct. Stx1 is nearly identical to Stx from *Shigella* **(** cross-neutralizable with shiga toxin) , with a difference in only a single amino acid in the catalytic A subunit. Conversely, Stx2 shares only 55% amino acid sequence similarity to Stx1. Stx2 is more potent than Stx1 in humans, and it is commonly associated with hemorrhagic colitis and HUS. The two subgroups of Stx can be found in different combinations in *EHEC* isolates. Epidemiological studies suggest that *EHEC* strains encoding Stx2 are more likely to cause severe disease than isolates that harbor only Stx1 or a combination of Stx1 and Stx2 .

Vero toxin is a small protein that acts to cleave the host cell's rRNA (ribosomal RNA) and hence disrupt protein biosynthesis by ribosomes. The enzymatic A-subunit inhibits protein synthesis in the cell, by selectively attacking the ribosome by removing the adenine residue at position 4324 of the 28S subunit.

Verotoxins: Structure and Functions

Verotoxins are composed of two subunits which are noncovalently linked, a single 31 kDa enzymatic A subunit and a pentamer of 7 kDa receptor binding *B* subunits. VTI B subunit and VT1 holotoxin have been crystallized. The A subunit is activated by proteolytic cleavage resulting in 2 fragments, A1, and A2.The A1, fragment inactivates the 60s ribosome leading to the inhibition of protein synthesis while the A2, fragment remains associated with the B subunit. The B subunit of the toxin binds to the eukaryotic cell surface receptor glycolipid globotriaosylcerarnide, Gb3 .

There are two variants of VT2: VT2e and VT2c, which share some homology to shiga toxin, however they are not cross-neutralizable with shiga toxin antiserum. All verotoxins bind to Gb3. but VT2e(Stx2e) binds preferentially to globotetraosylceramide, Gb4.

Stx and Verotoxins in HUS.

Stx produced in the intestine is assumed to translocate to the bloodstream, although toxin has never been detected in the blood of HUS patients. In polarized intestinal epithelial cells in vitro, Stx moves across the epithelial cell monolayer without obvious cellular disruption, probably through a transcellular, rather than paracellular, pathway.

Damage of the intestinal epithelium by Stx, bacterial lipopolysaccharide (LPS), or other inflammatory mediators could also aid translocation of the toxin to the bloodstream. It is not known how VT travels from the intestinal lumen to gain access to the bloodstream. however, the most likely hypothesis is that VT could translocate nonspecifically across the epithelial cells via transcytotic vesicles . VT could then travel through the blood and bind to Gb3 in the kidney.This is thought to be the initial event leading to the pathological effects of HUS.

It has been demonstrated that there are high levels of Gb3 in cultured human renal microvascular endothelid cells and that these cells are sensitive to the cytotoxic effects of VT .As well it has been shown that Gb3 is present in human glomenili .

Stx is cytotoxic to human renal endothelial cells in vitro. The typical human renal histopathology includes swollen glomerular endothelial cells and deposition of platelets and fibrin within the glomeruli. Stx is believed to damage the glomerular endothelial cells, leading to narrowing of capillary lumina and occlusion of the glomerular microvasculature with platelets and fibrin. The decreased glomerular filtration rate is presumably responsible for the acute renal failure that is typical of HUS. Traversal of the occluded microvasculature could also injure erythrocytes to produce the fragmented cells that are characteristic of HUS.

(iii) Partially characterized site of action

Botulinum neurotoxins, tetanospasmin and the lethal toxin of *B. anthracis* appear to be A-B type exotoxins. Botulinum toxin acts by causing inhibition of release of acetylcholine at the neuromuscular junction. Tetanus toxin is taken up at neuromuscular junctions and transported in axons to synapses. It then acts by inactivating inhibitory neurons. The exotoxins of tetanus and botulism appear to have B components, but the mode of action of their A subunits are not known. The B component of lethal toxin of *B. anthracis* is the protective antigen; interestingly, this also serves as the B subunit for edema toxin.

Bacillus anthracis

Anthrax is a disease caused by *Bacillus anthracis*, a spore-forming, Gram positive, rod-shaped bacterium. The lethality of the disease owes itself to the bacterium's two principal virulence factors: (i) the polyglutamic acid capsule, which is anti-phagocytic, and (ii) the tripartite protein toxin, called anthrax toxin. Anthrax toxin is a mixture of three protein components:

(i) protective antigen (PA), (ii) edema factor (EF),and (iii) lethal factor (LF).

Anthrax toxin is an *A*+*B* toxin

Interestingly, each individual anthrax toxin protein is, in fact, nontoxic. Toxic symptoms are not observed when these proteins are injected individually into laboratory animals. However, the co-injection of PA and EF causes edema, and the co-injection of PA and LF is lethal. The former combination is called edema toxin, and the latter combination is called lethal toxin. Thus the manifestation of physiological symptoms requires, in either case, the presence of the PA component.

The PA requirement observed in animal-model experiments demonstrates a common paradigm for bacterial toxins, called the *A* + *B* paradigm. The *A* component(s) are enzymatically active, and the *B* component is the cell binding component. Anthrax toxin, in fact, is of the form A_2B, where the two enzymes, EF and LF, are the *A* components and PA is the *B* component. Thus PA acts as a Trojan Horse, which carries EF and LF through the plasma membrane into the cytosol, where they may then catalyze reactions that disrupt normal cellular physiology.

Anthrax toxin assembly and translocation

Anthrax toxin protein components must assemble into holotoxin complexes to function. In order for LF and EF to function inside a target cell, they must localize to the cell and enter its cytoplasm. Through a series of steps, PA can translocate EF and LF into the cell . This process starts when the 83-kDa form of PA, called PA83, binds to an anthrax toxin receptor. There are two known homologous receptors, which bind to PA83, called tumor endothelium marker-8 (TEM8) and capillary morphogenesis protein 2 (CMG2). Then a 20 kDa fragment (PA20) is cleaved off of PA83's amino terminus by membrane endoproteases from the furin family. When PA20 dissociates, the remaining receptor-bound portion of PA, called PA63, may assemble into either a heptameric or octameric ring-shaped oligomer. This ring-shaped oligomer is often referred to as the pre-pore (or pre-channel) form of PA, since later in the pathway it will become a translocase pore (or channel). The surface of the pre-pore oligomer,

which was exposed upon release of the PA20 moiety, can then bind to LF and EF.. The heptameric and octameric forms of the PA oligomer may then bind with up to three or four molecules of EF and/or LF, respectively. The cell then endocytoses these assembled complexes and carries them to an acidic compartment in the cell.

Enzyme function of LF and EF

Once in the cytosol, the EF and LF then carry out their respective damage-inducing processes.

1-EF acts as a Ca^{2+} and calmodulin dependent adenylate cyclase that greatly increases the level of cAMP in the cell. This increase in cAMP upsets water homeostasis, severely throws the intracellular signaling pathways off balance, and impairs macrophage function, allowing the bacteria to further evade the immune system.

2-LF also helps the bacteria evade the immune system through killing macrophages. Once in these cells, LF acts as a Zn^{2+}-dependent endoprotease that snips off the N-terminus of mitogen-activated protein kinase kinases (MAPKK). This inhibits these kinases by not allowing them to efficiently bind to their substrates, which leads to altered signaling pathways and ultimately to apoptosis.

Thus, the synergistic effect of these three proteins leads to cellular death through a cascade of events that allow the proteins to enter the cell and disrupt cellular function.

Reference

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