جامعة الانبار

كليه العلوم

قسم علوم الحياة

Bacterial toxins

Assisstant .prof .Dr.Muthanna Hamid Hassan

Lecture 1

Introduction of bacterial toxins

Bacterial Toxins Lec 1

Introduction

A toxin is a poisonous substance produced within living cells or organisms. toxins can be small molecules, peptides, or proteins that are capable of causing disease on contact with or absorption by body tissues interacting with biological macromolecules such as enzymes or cellular receptors.

Toxins were the first bacterial virulence factors to be identified and were also the first link between bacteria and cell biology.

Bacterial Toxigenesis

Toxigenesis, or the ability to produce toxins, is an underlying mechanism by which many bacterial pathogens produce disease. At a chemical level, there are two main types of bacterial toxins, **lipopolysaccharides**, which are associated with the cell wall of Gram-negative bacteria, and **proteins**, which are released from bacterial cells and may act at tissue sites removed from the site of bacterial growth. The **cell-associated** toxins are referred to as **endotoxins** and the **extracellular** diffusible toxins are referred to as **exotoxins**.

Endotoxins are cell-associated substances that are structural components of bacteria. Most endotoxins are located in the cell envelope. In the context of this article, endotoxin refers specifically to the lipopolysaccharide (LPS) or lipooligosaccharide (LOS) located in the outer membrane of Gram-negative bacteria. Although structural components of cells, soluble endotoxins may be released from growing bacteria or from cells that are lysed as a result of effective host defense

mechanisms or by the activities of certain antibiotics. Endotoxins generally act in the vicinity of bacterial growth or presence.

Exotoxins are usually secreted by bacteria and act at a site removed from bacterial growth. However, in some cases, exotoxins are only released by lysis of the bacterial cell. Exotoxins are usually proteins, minimally polypeptides, that act enzymatically or through direct action with host cells and stimulate a variety of host responses. Most exotoxins act at tissue sites remote from the original point of bacterial invasion or growth. However, some bacterial exotoxins act at the site of pathogen colonization and may play a role in invasion.

BACTERIAL PROTEIN TOXINS

Exotoxins are usually secreted by living bacteria during exponential growth. The production of the toxin is generally specific to a particular bacterial species that produces the disease associated with the toxin (e.g. only *Clostridium tetani* produces tetanus toxin; only *Corynebacterium diphtheriae* produces the diphtheria toxin). Usually, virulent strains of the bacterium produce the toxin while nonvirulent strains do not, and the toxin is the major determinant of virulence (e.g. tetanus and diphtheria). At one time, it was thought that exotoxin production was limited mainly to Gram-positive bacteria, but clearly both Gram-positive and Gram-negative bacteria produce soluble protein toxins.

- Bacterial protein toxins are the most powerful human poisons known and retain high activity at very high dilutions. The lethality of the most potent bacterial exotoxins is compared to the lethality of strychnine, snake venom, and endotoxin.

- Usually the site of damage caused by an exotoxin indicates the location for activity of that toxin. Terms such as **enterotoxin**, **neurotoxin**, **leukocidin** or **hemolysin** are descriptive terms that indicate the target site of some well-defined protein toxins.

- A few bacterial toxins that obviously bring about the death of an animal are known simply as **lethal toxins**, and even though the tissues affected and the target site or substrate may be known, the precise mechanism by which death occurs is not clear (e.g. anthrax LF).

- Some bacterial toxins are utilized as **invasins** because they act locally to promote bacterial invasion. Examples are extracellular enzymes that degrade tissue matrices or fibrin, allowing the bacteria to spread. This includes collagenase, hyaluronidase and streptokinase. Other toxins, also considered invasins, degrade membrane components, such as phospholipases and lecithinases.

- The pore-forming toxins that insert a pore into eucaryotic membranes are considered as invasins, as well, but they will be reviewed here.

- Some protein toxins have very **specific cytotoxic activity** (i.e., they attack specific types of cells). For example, tetanus and botulinum toxins attack only neurons. But some toxins (as produced by staphylococci, streptococci, clostridia, etc.) have fairly **broad cytotoxic activity** and cause nonspecific death of various types of cells or damage to tissues, eventually resulting in

necrosis. Toxins that are phospholipases act in this way. This is also true of pore-forming hemolysins and leukocidins.

- Bacterial protein toxins are strongly **antigenic**. *In vivo*, specific antibody neutralizes the toxicity of these bacterial exotoxins (**antitoxin**). However, *in vitro,* specific antitoxin may not fully inhibit their activity. This suggests that the antigenic determinant of the toxin may be distinct from the active portion of the protein molecule. The degree of neutralization of the active site may depend on the distance from the antigenic site on the molecule. However, since the toxin is fully neutralized *in vivo*, this suggests that other host factors must play a role in toxin neutralization in nature.

- Protein exotoxins are inherently **unstable**. In time they lose their toxic properties but retain their antigenic ones. This was first discovered by Ehrlich who coined the term "toxoid" for this product.

Toxoids are detoxified toxins which retain their antigenicity and their immunizing capacity. 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 degrees at pH range 6 to 9 for several weeks.

The resulting toxoids can be used for **artificial immunization** against diseases caused by pathogens where the primary determinant of bacterial virulence is toxin production. Toxoids are effective immunizing agents against diphtheria and tetanus that are part of the DPT (DTP) vaccine.

Nomenclature of toxins

1– Named for host cell attacked:

- **Neurotoxins , Enterotoxins**
- **Cytotoxins (Nephrotoxin ,Hepatotoxin , Cardiotoxin)**

2– Named for producer or disease: cholera, Shiga

3– Named for activity: lecithinase, adenylate cyclase

4-Letter designation: exotoxin A

Toxins with Enzymatic Activity

As proteins, many bacterial toxins **resemble enzymes** in a number of ways. Like enzymes, they are **denatured by heat**, acid and proteolytic enzymes, they **act catalytically**, and they exhibit **specificity of action**. The **substrate** (in the host) may be a component of tissue cells, organs or body fluid.

A plus B Subunit Arrangement

Many protein toxins, notably those that act intracellularly (with regard to host cells), consist of two components:

- one component (**subunit A**) is responsible for the **enzymatic activity** of the toxin
- the other component (**subunit B**) is 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 (**A+B)** toxin.
- Isolated A subunits are enzymatically active but lack binding and cell entry capability.
- Isolated B subunits may bind to target cells (and even block the binding of the native toxin), but they are nontoxic.

There are a variety of ways that toxin subunits may be synthesized and arranged:

- **A + B** indicates that the toxin is synthesized and secreted as two separate protein subunits that interact at the target cell surface;

- **A-B** or **A-5B** indicates that the A and B subunits are synthesized separately, but associated by noncovalent bonds during secretion and binding to their target
- **5B** indicates that the binding domain of the protein is composed of 5 identical subunits.
- **A/B** denotes a toxin synthesized as a single polypeptide, divided into A and B domains that may be separated by proteolytic cleavage.

Attachments and mechanism of toxin entry

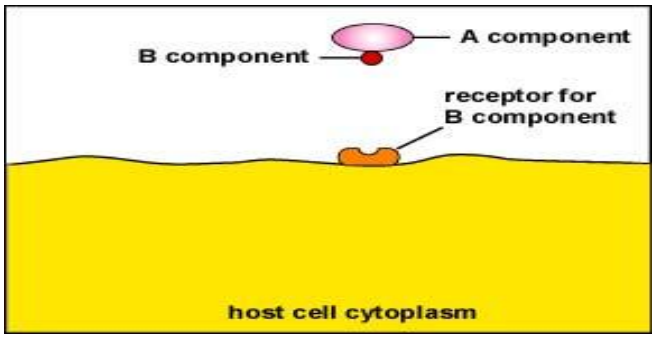
There are at least **two mechanisms of toxin entry into target cells.**

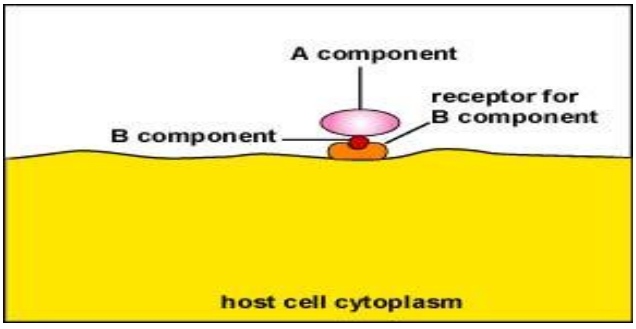
- **direct entry**, the B subunit of the native (A+B) toxin binds to a specific receptor on the target cell and induces the formation of a pore in the membrane through which the A subunit is transferred into the cell cytoplasm.

- an alternative mechanism, the native toxin binds to the target cell and the A+B structure is taken into the cell by the process of **receptor-mediated endocytosis** (**RME**). The toxin is internalized in the cell in a membrane-enclosed vesicle called an **endosome**. H^+ ions enter the endosome lowering the internal pH which causes the A+B subunits to separate. The B subunit affects the release of the A subunit from the endosome so that it will reach its target in the cell cytoplasm. The B subunit remains in the endosome and is recycled to the cell surface.

In both cases above, a large protein molecule must insert into and cross a membrane lipid bilayer, either the cell membrane or the endosome membrane. This activity is reflected in the ability of most A+B or A/B toxins, or their B components, to insert into artificial lipid bilayers, creating ion permeable pathways. If the B subunit contains a hydrophobic region (of amino acids) that insert into the membrane (as in the case of the diphtheria toxin), it may be referred to as the T (translocation) domain of the toxin.

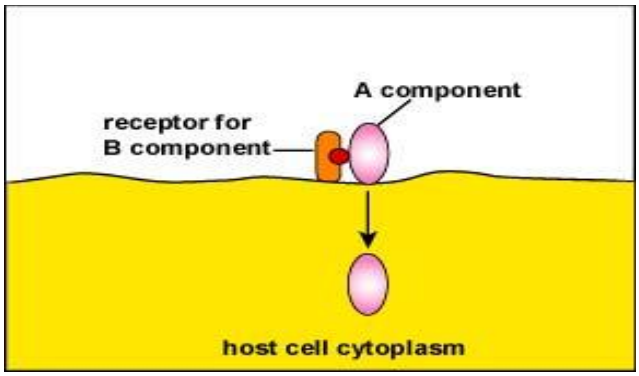
Binding of A-B toxins



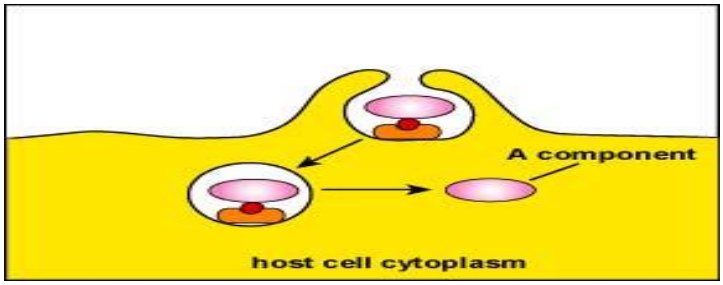


The B (binding) component of the exotoxin binds to a receptor on the surface of a susceptible host cell.

Entry of A Component of A-B Toxins by Direct Passage through the Host Cell's Membrane



Entry of A-B Toxins by Endocytosis



A few bacterial toxins (e.g. diphtheria) are known to utilize both direct entry and RME to enter into host cells, which is not surprising since both mechanisms are variations on a theme.

Bacterial toxins with similar enzymatic mechanisms may enter their target cells by different mechanisms. Thus, the diphtheria toxin and *Pseudomonas* exotoxin A, which have identical mechanisms of enzymatic activity, enter their host cells in slightly different ways.

The **specific receptors** for the B subunit of toxins on target cells or tissues are usually sialogangliosides (glycoproteins) called **G-proteins** on the cell membrane. For example, the cholera toxin utilizes the ganglioside GM1, and tetanus toxin utilizes ganglioside GT1 and/or GD1b as receptors on host cells.

Reference

- *Microbial toxins : current research and future trends*. Proft, Thomas. Norfolk: Caister Academic Press. 2009.
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Lecture 2

ADP-ribosylation toxins

ADP-ribosylation toxins Lec2

ADP-ribosylation is a posttranslational modification of proteins that involves the addition of one or more ADP-ribose moieties. These reactions are involved in cell signaling and the control of many cell processes, including DNA repair and apoptosis.

ADP-ribosylation toxins

ADP-ribosylation is also responsible for the actions of some bacterial toxins, such as cholera toxin, diphtheria toxin, and pertussis toxin. These toxin proteins are ADP-ribosyltransferases that modify target proteins in human cells.

Diphtheria

- In 1884, Loeffler concluded that *C. diphtheriae* produced a soluble toxin, and thereby provided the first description of a bacterial exotoxin. In 1888, Roux and Yersin demonstrated the presence of the toxin in the cell-free culture fluid of *C. diphtheriae* which, when injected into suitable lab animals, caused the systemic manifestation of diphtheria.

- Two years later, von Behring and Kitasato succeeded in immunizing guinea pigs with a heat-attenuated form of the toxin and demonstrated that the sera of immunized animals contained an antitoxin capable of protecting other susceptible animals against the disease. This modified toxin was suitable for immunizing animals to obtain antitoxin, but it was found to cause severe local reactions in humans and could not be used as a vaccine. In 1909, Theobald Smith, in the U.S., demonstrated that diphtheria toxin that had been neutralized by antitoxin forming a **Toxin-Anti-Toxin complex (TAT)** remained immunogenic and eliminated

local reactions seen in the modified toxin. For some years, beginning about 1910, TAT was used for active immunization against diphtheria. TAT had two undesirable characteristics as a vaccine. *First, the toxin used was highly toxic, and the quantity injected could result in a fatal toxemia unless the toxin was fully neutralized by antitoxin. *Second, the antitoxin mixture was horse serum, the components of which tended to be allergenic and to sensitize individuals to the serum.

- In 1913, Schick designed a skin test as a means of determining susceptibility or immunity to diphtheria in humans. Diphtheria toxin will cause an inflammatory reaction when very small amounts are injected intracutaneously. The Schick Test involves injecting a very small dose of the toxin under the skin of the forearm and evaluating the injection site after 48 hours. A positive test (inflammatory reaction) indicates susceptibility (nonimmunity). A negative test (no reaction) indicates immunity (antibody neutralizes toxin) .
- In 1924, Ramon demonstrated the conversion of diphtheria toxin to its nontoxic, but antigenic, equivalent (**toxoid**) by treating with formaldehyde. He provided humanity with one of the safest and surest vaccines of all time, the diphtheria toxoid
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- In 1951, Freeman made the remarkable discovery that pathogenic (toxigenic) strains of *C. diphtheriae* are lysogenic, (i.e., are infected by a temperate Beta phage), while non lysogenized strains are avirulent. Subsequently, it was shown that the gene for toxin production is located on the DNA of the Beta phage

Pathogenicity

The pathogenicity of *Corynebacterium diphtheriae* includes two distinct phenomena:

1. **Invasion** of the local tissues of the throat, which requires colonization and subsequent bacterial proliferation. Little is known about the adherence mechanisms of *C. diphtheriae,* but the bacteria produce several types of pili. The diphtheria toxin, as well, may be involved in colonization of the throat.

2. **Toxigenesis**: bacterial production of the toxin. The diphtheria toxin causes the death eucaryotic cells and tissues by inhibition protein synthesis in the cells. Although the toxin is responsible for the lethal symptoms of the disease, the virulence of *C. diphtheriae* cannot be attributed to toxigenicity alone, since a distinct invasive phase apparently precedes toxigenesis.

Toxigenicity

Two factors have great influence on the ability of *Corynebacterium diphtheriae* to produce the diphtheria toxin: (1) the **presence of a lysogenic prophage** in the bacterial chromosome and (2) **low extracellular concentrations of iron**. The gene for toxin production occurs on the chromosome of the prophage, but a bacterial repressor protein controls the expression of this gene. The repressor is activated by iron, and it is in this way that iron influences toxin production. High yields of toxin are synthesized only by lysogenic bacteria under conditions of iron deficiency

The role of B-phage. Only those strains of *Corynebacterium diphtheriae* that are lysogenized by a specific Beta phage produce diphtheria toxin. A phage lytic cycle is not necessary for toxin production or release. The **phage contains the structural gene for the toxin molecule(tox gene)**. The original proof rested in the demonstration that lysogeny of *C. diphtheriae* by various mutated Beta phages leads to production of nontoxic but antigenically-related material (called **CRM** for **"cross-reacting material**"). CRMs have shorter chain length than the diphtheria toxin molecule but cross react with diphtheria antitoxins due to their antigenic similarities to the toxin.

Even though the tox gene is not part of the bacterial chromosome, the regulation of toxin production is under bacterial control since the DtxR (regulatory) gene is on the bacterial chromosome and toxin production depends upon bacterial iron metabolism.

The role of iron. In artificial culture the most important factor controlling yield of the toxin is the concentration of inorganic iron (Fe^{++} or Fe^{+++}) present in the culture medium. Toxin is synthesized in high yield only after the exogenous supply of iron has become exhausted (This has practical importance for the industrial production of toxin to make toxoid. Under the appropriate conditions of iron starvation, *C. diphtheriae* will synthesize diphtheria toxin as 5% of its total protein).

Presumably, this phenomenon takes place in vivo as well. It is the regulation of toxin production in the bacterium that is partially controlled by iron. The **tox gene** is regulated by a mechanism of negative control wherein a repressor molecule, product of the **DtxR gene**, is activated by iron. The active repressor binds to the tox gene operator and prevents transcription. When iron is removed from the repressor (under growth

conditions of iron limitation), **derepression** occurs, the repressor is inactivated and transcription of the **tox genes** can occur. Iron is referred to as a **corepressor** since it is required for repression of the toxin gene There is no evidence to suggest a key role of the toxin in the life cycle of the organism. Since the organism synthesize up to 5% of its total protein as a toxin that specifically inhibits protein synthesis in eukaryotes and archaea , it possibly the toxin assists colonization of the throat (or skin) by killing epithelial cells or neutrophils and since mass immunization against diphtheria has been practiced, the disease has virtually disappeared, and *C. diphtheriae* is no longer a component of the normal flora of the human throat and pharynx. It may be that the toxin played a key role in the colonization of the throat in nonimmune individuals and, as a consequence of exhaustive immunization, toxigenic strains have become virtually extinct.

Structure of Diphtheria toxin

Diphtheria toxin is a single polypeptide chain of 535 amino acids consisting of two subunits linked by disulfide bridges. Binding to the cell surface of the less stable of these two subunits allows the more stable part of the protein to penetrate the host cell

The diphtheria toxin (DTx) is a two-component bacterial exotoxin synthesized as a single polypeptide chain containing an A (active) domain and a B (binding) domain. Proteolytic nicking of the secreted form of the toxin separates the A chain from the B chain. The B chain contains a hydrophobic T (translocation) region, responsible for insertion into the endosome membrane in order to secure the release of A.

The toxin binds to a specific receptor now known as the heparin-binding epidermal growth factor (HB-EGF receptor) on susceptible cells and enters by receptor-mediated endocytosis. Acidification of the endosome vesicle results in unfolding of the protein and insertion of the T segment into the endosomal membrane. Apparently, as a result of activity on the endosome membrane, the A subunit is cleaved and released from the B subunit as it inserts and passes through the membrane. The specific membrane receptor, (HB-EGF) precursor is a protein on the surface of many types of cells. The occurrence and distribution of the HB-EGF receptor on cells determines the susceptibility of an animal species, and certain cells of an animal species, to the diphtheria toxin. Normally, the HB-EGF precursor releases a peptide hormone that influences normal cell growth and differentiation. One hypothesis is that the HB-EGF receptor itself is the protease that nicks the A fragment and reduces the disulfide bridge between it and the B fragment when the A fragment makes its way through the endosomal membrane into the cytoplasm.

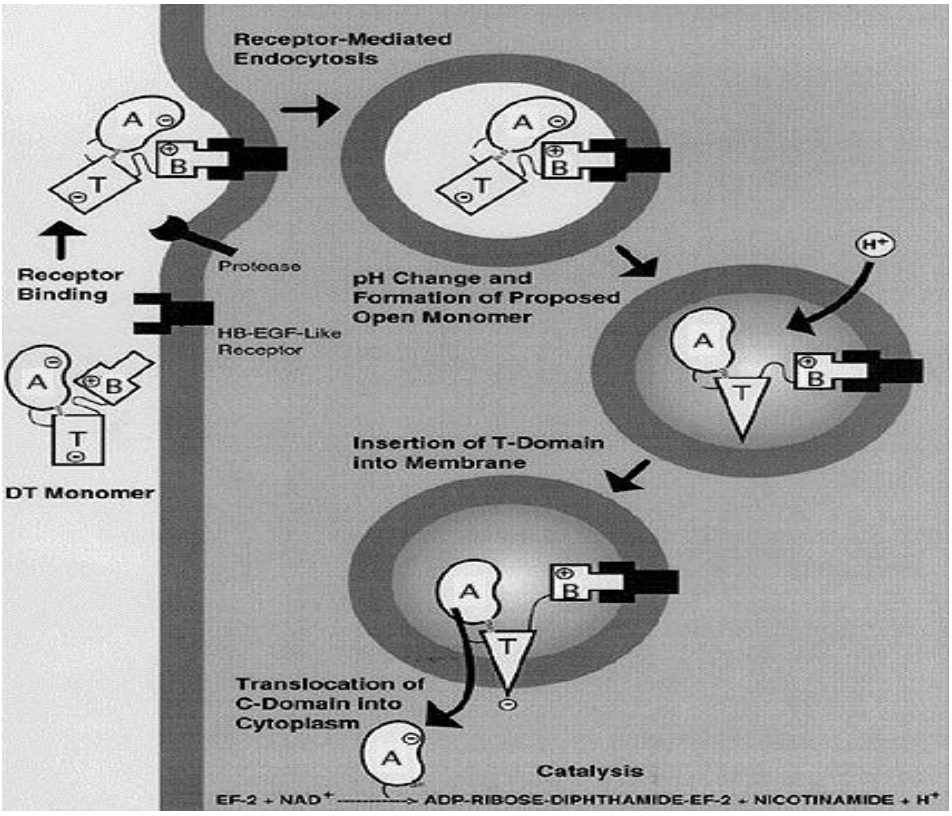

Figure. Uptake and activity of the diphtheria toxin in eucaryotic cells.

Fragment A catalyzes the transfer of ADP-ribose from NAD to the eukaryotic Elongation Factor 2 which inhibits the function of the latter in protein synthesis. Ultimately, inactivation of all of the host cell EF-2 molecules causes death of the cell. Attachment of the ADP ribosyl group occurs at an unusual derivative of histadine called diphthamide.The toxin transfers an ADP-ribose from NAD+ to a diphthamide residue (a modified Histidine amino acid) found within the EF-2 protein. EF-2 is needed for the moving of tRNA from the A-site to the P-site of the ribosome during translation. The ADP-ribosylation is reversible when by giving high doses of nicotinamide (or vitamin B3), one of the reaction's products.

This is NAD+-diphthamide ADP-ribosyltransferase, it acts as a RNA translational inhibitor.

Diphtheria toxin is extraordinarily potent The lethal dose for humans is about 0.1 µg of toxin per kg of bodyweight. A massive release of toxin into the body will likely cause lethal necrosis of the heart and liver.

Pseudomonas aeruginosa toxins

Pseudomonas aeruginosa produces exotoxin A (ETA) and four type III cytotoxins: ExoS, ExoT, ExoU and ExoY. Different clinical isolates of P. aeruginosa can express one or more of these four cytotoxins. The catalytic activity of each type III cytotoxin is activated by a host protein.

ETA is the most potent protein toxin that *P. aeruginosa* secretes, and it inhibits mammalian protein synthesis by **ADP-ribosylation of elongation factor 2 (EF2).**

ExoU is lipase that disrupts membrane function in mammalian cells.

ExoY is an adenylate cyclase that elevates intracellular cyclic AMP (cAMP) to supra-physiological levels, which indirectly disrupts the actin cytoskeleton.

One role of ExoS and ExoT is to disrupt the actin cytoskeleton through two independent enzymatic activities: Rho GTPase-activating protein (GAP) activity and ADP-ribosylation.

Exotoxin A

Exotoxin A has exactly the same mechanism of action as the diphtheria toxin; it causes the ADP ribosylation of eucaryotic elongation factor 2 resulting in inhibition of protein synthesis causing cell death. Exotoxin A is responsible for local tissue damage, bacterial invasion, and (possibly) immunosuppression . Purified exotoxin A is highly lethal for mice which supports its role as a major systemic virulence factor of *P. aeruginosa*. The production of Exotoxin A is regulated by exogenous iron, but the details of the regulatory process are distinctly different in *C. diphtheriae* and *P. aeruginosa*.Exotoxin A appears to mediate both local and systemic disease processes caused by *Pseudomonas aeruginosa*. It has necrotizing activity at the site of bacterial colonization and is thereby thought to contribute to the colonization process. Toxinogenic strains cause a more virulent form of pneumonia than nontoxinogenic strains. In terms of its systemic role in virulence, purified Exotoxin A is highly lethal for animals including primates. The protein is a single polypeptide chain of 613 amino acids. The x-ray crystallographic structure of exotoxin A, determined to 3.0-A resolution, shows the following: an amino-terminal domain, composed primarily of antiparallel beta-structure and comprising approximately half of the molecule; a middle domain composed of alpha-helices; and a carboxyl-terminal domain comprising approximately one-third of the molecule. The carboxyl-terminal domain is the ADP-ribosyltransferase of the toxin.

Reference

- *Microbial toxins : current research and future trends*. Proft, Thomas. Norfolk: Caister Academic Press. 2009.
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Lecture 3

Cholera toxins

Cholera Lec 3

V. cholerae produces **cholera toxin**, the model for enterotoxins, whose action on the mucosal epithelium is responsible for the characteristic diarrhea of the disease cholera. In its extreme manifestation, cholera is one of the most rapidly fatal illnesses known. The watery diarrhea is speckled with flakes of mucus and epithelial cells ("rice-water stool") and contains enormous numbers of vibrios. The loss of potassium ions may result in cardiac complications and circulatory failure. Untreated cholera frequently results in high (50-60%) mortality rates.

Colonization of the Small Intestine

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There are several characteristics of pathogenic *V. cholerae* that are important **determinants of the colonization** process. These include **adhesins**, **neuraminidase**, **motility**, chemotaxis and **toxin** production. If the bacteria are able to survive the gastric secretions and low pH of the stomach, they are well adapted to survival in the small intestine. *V. cholerae* is resistant to bile salts and can penetrate the mucus layer of the small intestine, possibly aided by secretion of neuraminidase and proteases (mucinases).

Specific adherence of *V. cholerae* to the intestinal mucosa is probably mediated by long filamentous fimbriae that form bundles at the poles of the cells. These fimbriae have been termed **Tcp pili** (for **toxin coregulated pili**), because expression of these pili genes is coregulated with expression of the cholera toxin genes. Not much is known about the interaction of Tcp pili with host cells, and the host cell receptor for these fimbriae has not been identified. Tcp pili share amino acid sequence

similarity with N-methylphenylalanine pili of *Pseudomonas* and *Neisseria*.

Two other possible adhesins in *V. cholerae* are a surface protein that agglutinates red blood cells (**hemagglutinin**) and a group of outer membrane proteins which are products of the **acf** (**accessory colonization factor**) genes. acf mutants have been shown to have reduced ability to colonize the intestinal tract. *V. cholerae* produces a protease originally called **mucinase** that degrades different types of protein including fibronectin, lactoferrin and cholera toxin itself. Its role in virulence is not known but it probably is not involved in colonization since mutations in the mucinase gene (designated hap for **hemagglutinin protease**) do not exhibit reduced virulence. It has been suggested that the mucinase might contribute to detachment rather than attachment. Possibly the vibrios would need to detach from cells that are being sloughed off of the mucosa in order to reattach to newly formed mucosal cells.

Cholera Toxin

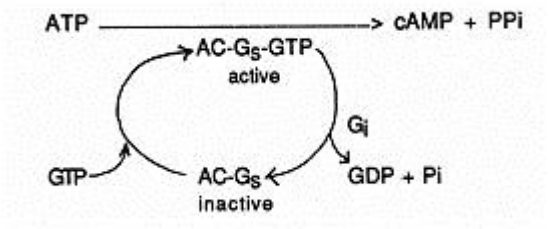
In 1969 Finkelstein and LoSpalluto had purified the toxin and shown it to be a 84 kDa protein. CT is made up of two types of subunits, a 56kDa oligomer composed of several identical "light" subunits responsible for receptor binding and a single "heavy" 28kDa toxic-active subunit; these subunits were later renamed B (for binding) and A (for toxic-active), respectively. In the assembled CT the toxic-active A-subunit (CTA) is embedded in the circular B-subunit homopentamer (CTB pentamer) responsible for toxin binding to cells.

Although being synthesized as a single polypeptide chain, CTA is post-translationally modified through the action of a *V. cholerae* protease that generates two fragments, CTA1 and CTA2, which however still remain

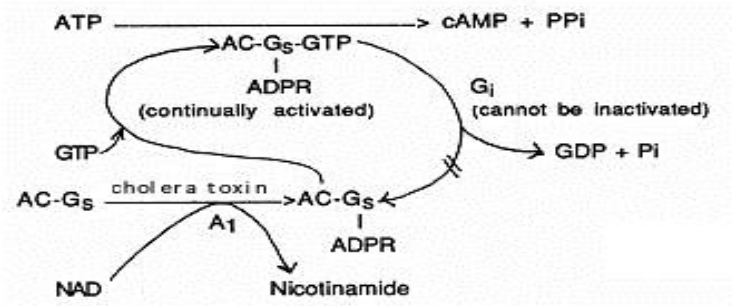
linked by a disulphide bond. The toxic activity of CTA resides in CTA1, whereas CTA2 serves to insert CTA into the CTB pentamer. These many polar bonds together with a tight packing of subunits via hydrophobic interactions could by themselves explain the outstanding stability of pentameric CTB to proteases, bile components and other factors in the intestinal milieu

Cholera toxin **activates the adenylate cyclase enzyme** in cells of the intestinal mucosa leading to increased levels of intracellular cAMP, and the secretion of H_20, Na^+, K^+, Cl^-, and HCO_3^- into the lumen of the small intestine. The effect is dependent on a specific receptor, monosialosyl ganglioside (GM1 ganglioside) present on the surface of intestinal mucosal cells. The bacterium produces an invasin, neuraminidase, during the colonization stage which has the interesting property of degrading gangliosides to the monosialosyl form, which is the specific receptor for the toxin. Once it has entered the cell, the A1 subunit enzymatically transfers ADP ribose from NAD to a protein (called Gs or Ns), that regulates the adenylate cyclase system which is located on the inside of the plasma membrane of mammalian cells.

The process is complex. Adenylate cyclase (AC) is activated normally by a regulatory protein (GS) and GTP; however activation is normally brief because another regulatory protein (Gi), hydrolyzes GTP. The normal situation is described as follows.



The A1 fragment catalyzes the attachment of ADP-Ribose (ADPR) to the regulatory protein forming Gs-ADPR from which GTP cannot be hydrolyzed. Since GTP hydrolysis is the event that inactivates the adenylate cyclase, the enzyme remains continually activated. This situation can be illustrated



Thus, the net effect of the toxin is to cause cAMP to be produced at an abnormally high rate which stimulates mucosal cells to pump large amounts of Cl^- into the intestinal contents. H_2O, Na^+ and other electrolytes follow due to the osmotic and electrical gradients caused by the loss of Cl^-. The lost H_2O and electrolytes in mucosal cells are replaced from the blood. Thus, the toxin-damaged cells become pumps for water and electrolytes causing the diarrhea, loss of electrolytes, and dehydration that are characteristic of cholera.

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Lecture 4

Other *V. cholerae* O1/O139 toxins

(ii) Other *V. cholerae* O1/O139 toxins. Lec4

The second toxin identified in *V. cholerae* O1 strains is **Zot** (for zonula occludens
toxin). The *zot* gene encodes a predicted 44.8-kDa peptide , whose native form has not yet been purified. The *zot* gene is present in most O1 and O139 strains, and if a strain is CT positive, it is almost always *zot* positive. Crude Zot diminishes the resistance of rabbit ileal tissue in Ussing chambers without causing detectable changes in potential difference.

The onset of action of crude Zot is immediate, and the activity is reversible. Electron microscopy has revealed penetration of an electron-dense marker into the zonula occluden of rabbit ileum after Zot treatment, and freeze-fracture analysis of the zonula occludens reveals a marked decrease in the number of junctional strands. Furthermore, Zot has been reported to cause F-actin rearrangement in rat intestinal epithelial cells (IEC-6 cells) in vitro and rabbit ileum in vivo . In an endothelial cell line, Zot treatment increased the proportion of F to G actin . Together, these observations indicate an effect of Zot on the zonula occludens or epithelial tight junctions, possibly through a rearrangement of F actin, and suggest that Zot may contribute to diarrhea in cholera by
altering the permeability of intestinal tissue.

Several signal transduction mechanisms (e.g., calcium, PKC, tyrosine kinase, cyclic AMP) have been shown to regulate tight junctions in intestinal epithelial cells , and current data implicate PKC in the response of intestinal cells to Zot .

Intestinal Diseases Caused by *E. coli*

As a pathogen, *E. coli* is best known for its ability to cause intestinal diseases.

Five classes (virotypes) of *E. coli* that cause diarrheal diseases are now recognized: **enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), and enteroaggregative *E. coli* (EAEC)**

Table-1. Diarrheagenic *E. coli*: virulence determinants and characteristics of disease

Diarrheagenic *E. coli*	virulence determinants	toxin	characteristics of disease
ETEC	fimbrial adhesins e.g. the K-88 fimbrial Ag is found on strains from piglets; K-99 Ag is found on strains from calves and lambs; CFA I, and CFA II, are found on strains from humans non invasive.The infectious dose 10^8 cells	produce LT and/or ST toxin (plasmid-encoded toxins)	watery diarrhea in infants and travelers; no inflammation, no fever
EIEC	nonfimbrial adhesins, possibly outer membrane protein ,invasive (penetrate and multiply within epithelial cells). The infectious dose 10^6 organisms Unlike typical *E. coli*, EIEC are non-motile, do not decarboxylate lysine and do not ferment lactose.Both plasmid and	does not produce shiga toxin	dysentery-like diarrhea (mucous, blood), severe inflammation, fever

	chromosomal genes are involved in conferring pathogenicity.The invasion phenotype, encoded by a high molecular weight plasmid		
EPEC	non fimbrial adhesin (intimin) a plasmid-encoded protein referred to as EPECadherence factor (EAF) enables localized adherence of bacteria to intestinal cells.Moderately invasive (not as invasive as *Shigella* or EIEC).Some types of EPEC are referred to as diffusely adherent *E. coli* (DAEC).The infectious dose 10^6 organisms	does not produce LT or ST; some reports of shiga-like toxin	usually infantile diarrhea; watery diarrhea with blood, some inflammation, no fever; symptoms probably result mainly from invasion rather than toxigenesis
EAEC	adhesins not characterized non invasive.Attach to tissue culture cells in an aggregative manner	produce ST-like toxin(EnteroAggregative ST) (EAST) and a hemolysin	persistent diarrhea in young children without inflammation or fever
EHEC	adhesins not characterized, probably fimbriae , *E. coli* O157:H7 is the prototypic EHEC	does not produce LT or ST but does produce shiga toxin	pediatric diarrhea, copious

	and most often implicated in illness worldwide The bacteria do not invade mucosal cells. The infectious dose10 - 100 cells	[Verocytotoxin-producing*E.coli* (VTEC) Shiga-toxin-producing*E.coli* (STEC)].The toxin is phage encoded and its production is enhanced by iron deficiency.The EHEC plasmid is known to encode the enterohemolysin (*ehx*) as well as a fimbrial antigen potentially involved in colonization.	bloody discharge (hemorrhagic colitis), intense inflammatory response, may be complicated by hemolytic uremia syndrome (HUS).

. A summary of the characteristics of diarrheagenic strains of *E. coli* is given in Table 1.

Reference

- *Microbial toxins : current research and future trends*. Proft, Thomas. Norfolk: Caister Academic Press. 2009.
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Bacterial toxins

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Lecture 5

E. coli heat-labile enterotoxin

Escherichia coli heat-labile enterotoxin (LT) is the causative agent of traveller's diarrhoea, and it is also responsible for the deaths of hundreds of thousands of children per year in developing countries.

The LTs of *E. coli* are oligomeric toxins that are closely related in structure and function to the cholera enterotoxin (CT) expressed by *Vibrio cholerae*. LT and CT share many characteristics including holotoxin structure, protein sequence (ca. 80% identity), primary receptor identity, enzymatic activity, and activity in animal and cell culture assays; some differences are seen in toxin processing and secretion and in helper T-lymphocyte responses.

There are two major serogroups of LT, LT-I and LT-II, which do not cross-react immunologically.

(i) LT-I. LT-I is an oligomeric toxin of ca. 86 kDa composed of one 28-kDa A subunit and five identical 11.5-kDa B subunits .

The B subunits are arranged in a ring or "doughnut" and bind strongly to the ganglioside GM_1 and weakly to GD1b and some intestinal glycoproteins.

The A subunit is responsible for the enzymatic activity of the toxin and is proteolytically cleaved to yield A_1 and A_2 peptides joined by a disulfide bond.

Two closely related variants of LT-I which exhibit partial antigenic cross-reactivity have been described. These variants are called LTp (LTp-I) and LTh (LTh-I) after their initial discovery in strains isolated from pigs or humans, respectively. The genes encoding LT (*elt* or *etx*) reside on plasmids that also may contain genes encoding ST and/or colonization factor antigens (CFAs), After binding to the host cell membranes, the

toxin is endocytosed and translocated through the cell in a process involving trans-Golgi vesicular transport.

The cellular target of LT is adenylate cyclase located on the basolateral membrane of polarized intestinal epithelial cells. The A_1 peptide has an ADP-ribosyltransferase activity and acts by transferring an ADP-ribosyl moiety from NAD to the alpha subunit of the GTP-binding protein, G_S, which stimulates adenylate cyclase activity.

ADP-ribosylation of the $G_{S\alpha}$ subunit results in adenylate cyclase being permanently activated, leading to increased levels of intracellular cyclic AMP (cAMP). cAMP-dependent protein kinase (A kinase) is thereby activated, leading to supranormal phosphorylation of chloride channels located in the apical epithelial cell membranes.

The major chloride channel activated by LT and CT is Cystic fibrosis transmembrane conductance regulator (**CFTR**), the ion channel that is defective in cystic fibrosis. The net result is stimulation of Cl^- secretion from secretory crypt cells and inhibition of NaCl absorption by villus tip cells.

The increased luminal ion content draws water passively through the paracellular pathway, resulting in osmotic diarrhea.

CT and LT have been shown as well to decrease the absorption of fluid and electrolytes from the intestinal lumen. Muller *et al.* have reported that both CT and LT induce cAMP-dependent inhibition of the H^+/peptide cotransporter in the human intestinal cell line Caco-2. Interestingly, since the H^+/peptide cotransporter does not possess sites for phosphorylation by protein kinase A (PKA), the authors propose that the effect is mediated through PKC. This hypothesis would suggest another novel mechanism of CT and LT and requires substantiation in other systems.

In addition to its enterotoxic properties, LT has the ability to serve as a mucosal adjuvant. Mutants of LT which retain adjuvanticity while

eliminating the ADP-ribosyltransferase activity have been constructed. Mice immunized orally or intranasally with ovalbumin or fragment C of tetanus toxin together with the mutant LTs have developed higher levels of serum and local antibodies to these antigens than when the antigens are delivered without LT.

(ii) LT-II. The LT-II serogroup of the LT family shows 55 to 57% identity to LT-I and CT in the A subunit but essentially no homology to LT-I or CT in the B subunits . Two antigenic variants, LT-IIa and LT-IIb, which share 71 and 66% identity in the predicted A and B subunits, respectively, have been described. LT-II increases intracellular cAMP levels by similar mechanisms to those involved with LT-I toxicity, but LT-II uses GD1 as its receptor rather than GM_1 . As noted above, there is no evidence that LT-II is associated with human or animal disease.

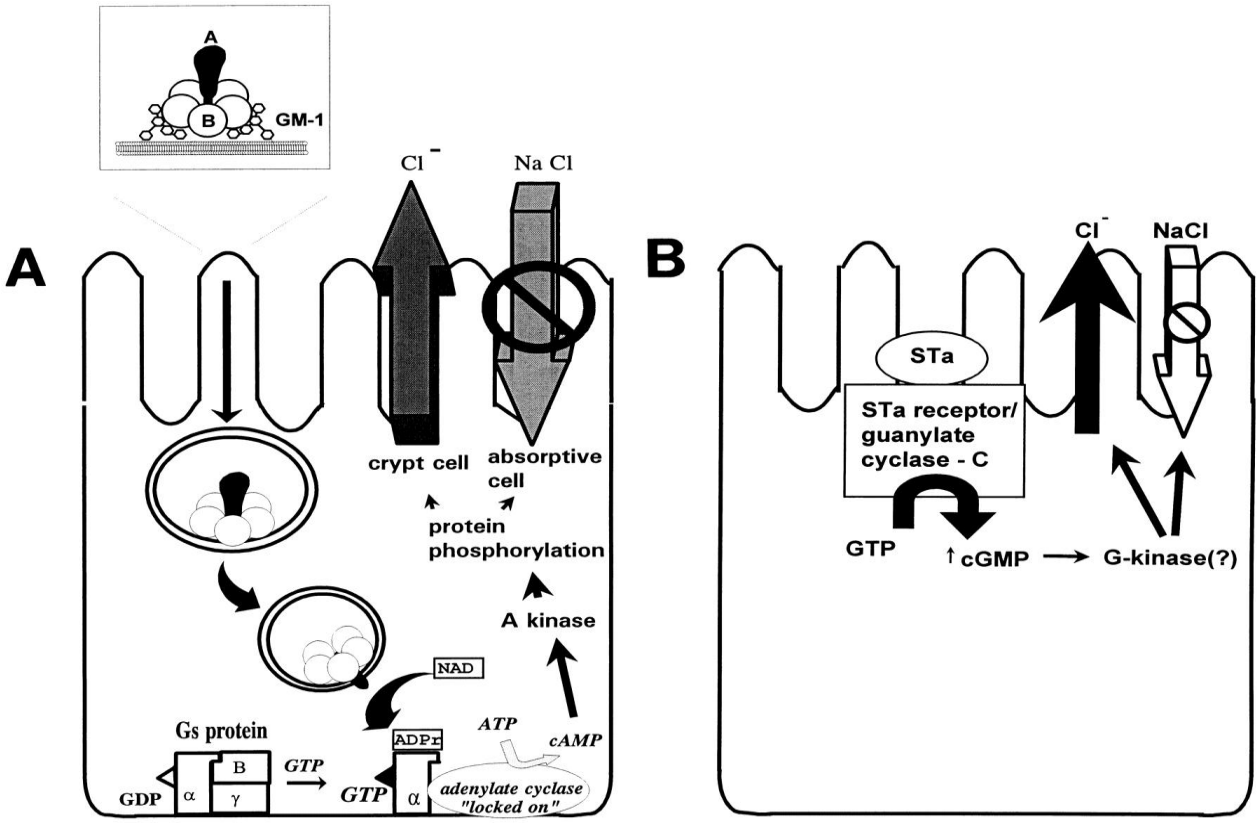

FIG.. Classic mechanisms of action of ETEC toxins

Heat-stable toxins(STs) .

The STs are small, monomeric toxins that contain multiple cysteine residues, whose disulfide bonds account for the heat stability of these toxins. Heat-stable enterotoxins are small peptides that are secreted by enterotoxigenic bacteria. ST peptides are active even after 60 min of heating at 95 °C .

There are two unrelated classes of STs that differ in structure and mechanism of action: the methanol soluble protease resistant and guanyl cyclase C (GC-C) binding STa and the methanol insoluble and protease sensitive STb. STb is a 48 amino acid peptide associated with disease in cattle, but not in humans, and it does not bind to GC-C. STb was shown to increase intracellular levels of Ca2.

STa (also called ST-I) toxins are produced by ETEC and several other gram-negative bacteria including *Yersinia enterocolitica* and *V. cholerae* non-O1.STa has about 50% protein identity to the EAST1(enteroaggregative heat-stable toxin) of EAEC.

(i) STa. There are two variants, designated STp (ST porcine or STIa) and STh (ST human or STIb), after their initial discovery in strains isolated from pigs or humans, respectively. Both variants can be found in human ETEC strains.

STa is initially produced as a 72-amino-acid precursor (pre-pro form) that is cleaved by signal peptidase 1 to a 53-amino-acid peptide. This form is transported to the periplasm, where the disulfide bonds are formed by the chromosomally encoded DsbA protein.

An undefined protease processes the pro-STa to the final 18- or 19-residue mature toxin which is released by diffusion across the outer membrane.

The major receptor for STa is a membrane-spanning enzyme called guanylate cyclase C (GC-C), which belongs to a family of receptor cyclases that includes the atrial natriuretic peptide receptors GC-A and GC-B. Additional receptors for STa may exist, but GC-C is the only receptor identified definitively. GC-C is located in the apical membrane of intestinal epithelial cells, and binding of ligands to the extracellular domain stimulates the intracellular enzymatic activity.

Binding of STa to GC-C stimulates GC activity, leading to increased intracellular cGMP levels . This activity leads ultimately to stimulation of chloride secretion and/or inhibition of sodium chloride absorption, resulting in net intestinal fluid secretion.The intermediate steps involved in this process are controversial, and roles for both cGMP-dependent kinases and cAMP-dependent kinases have been reported.

Ultimately, the CFTR chloride channel is activated, leading to secretion of Cl^- ions into the intestinal lumen. In contrast to the 15- to 60-min lag time needed for LT to translocate to and activate the basolateral adenylate cyclase complex, STa acts much faster due to the apical location of its cyclase receptor.

The secretory response to STa may also involve phosphatidylinositol and diacylglycerol release, activation of PKC, elevation of intracellular calcium levels, and microfilament (F-actin) rearrangement .

(ii) STb. STb is associated primarily with ETEC strains isolated from pigs, although some human ETEC isolates expressing STb have been reported.

STb is initially synthesized as a 71-amino-acid precursor protein, which is processed to a mature 48-amino-acid protein with a molecular weight of 5.1 kDa.The STb protein sequence has no homology to that of STa, although it does contain four cysteine residues which form disulfide bonds.Unlike STa, STb induces histologic damage in the intestinal

epithelium, consisting of loss of villus epithelial cells and partial villus atrophy. suggested recently that the toxin may bind nonspecifically to the plasma membrane prior to endocytosis. Unlike the chloride ion secretion elicited by STa, STb stimulates the secretion of bicarbonate from intestinal cells. STb does not stimulate increases in intracellular cAMP or cGMP concentrations, although it does stimulate increases in intracellular calcium levels from extracellular sources.

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Bacterial toxins

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Lecture 6

Bordetella pertussis

Bordetella pertussis **lec 6**

Whooping cough (**pertussis**) is caused by the bacterium ***Bordetella pertussis***. *B. pertussis* is a very small Gram-negative aerobic coccobacillus that appears singly or in pairs. Its metabolism is respiratory, never fermentative.

Bordetella pertussis colonizes the cilia of the mammalian respiratory epithelium. Generally, it is thought that *B. pertussis* does not invade the tissues, but some recent work has shown the bacterium in alveolar macrophages.

The two most important colonization factors are the filamentous hemagglutinin (FHA) and the pertussis toxin (PTx).

Filamentous hemagglutinin is a large (220 kDa) protein that forms filamentous structures on the cell surface. FHA binds to galactose residues on a sulfated glycolipid called sulfatide which is very common on the surface of ciliated cells. Mutations in the FHA structural gene reduce the ability of the organism to colonize, and antibodies against FHA provide protection against infection. However, it is unlikely that FHA is the only adhesin involved in colonization.

One of the toxins of *B. pertussis*, the pertussis toxin (PTx), is also involved in adherence to the tracheal epithelium. Pertussis toxin is a 105 kDa protein composed of six subunits: S1, S2, S3, (2)S4, and S5. The toxin is both secreted into the extracellular fluid and cell bound. Some components of the cell-bound toxin (S2 and S3) function as adhesins, and appear to bind the bacteria to host cells. S2 and S3 utilize different receptors on host cells.

S2 binds specifically to a glycolipid called lactosylceramide, which is found primarily on the ciliated epithelial cells. S3 binds to a glycoprotein found mainly on phagocytic cells.

Toxins Produced by *B. pertussis*

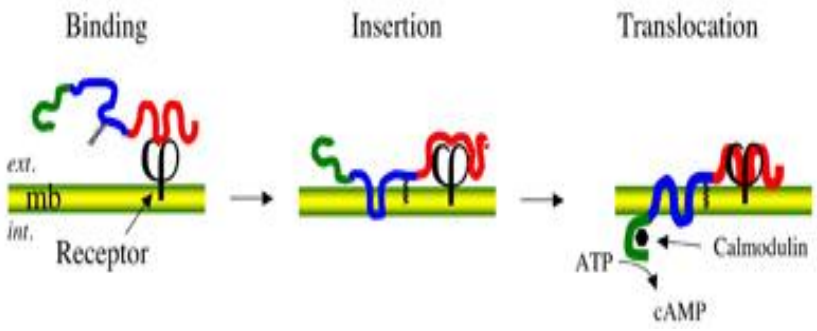
B. pertussis produces a variety of substances with toxic activity in the class of exotoxins and endotoxins.

1-secretes its own **invasive adenylate cyclase** which enters mammalian cells (*Bacillus anthracis* produces a similar enzyme, EF). This toxin acts locally to reduce phagocytic activity and probably helps the organism initiate infection. This toxin is a 45 kDa protein that may be cell-associated or released into the environment.Mutants of *B. pertussis* in the adenylate cyclase gene have reduced virulence in mouse models. The organisms can still colonize but cannot produce the lethal disease.The adenylate cyclase toxin (CyaA) is a single polypeptide with an enzymatic domain (i.e., adenylate cyclase activity) and a binding domain that will attach to host cell surfaces.

An interesting feature of the adenylate cyclase toxin is that it is active only in the presence of a eukaryotic regulatory molecule called calmodulin, which up-regulates the activity of the eukaryotic adenylate cyclase. The adenylate cyclase toxin is only active in the eukaryotic cell since no similar regulatory molecule exists in procaryotes.

CyaA is able to invade eukaryotic cells where it is activated by calmodulin to produce supra physiological levels of cAMP, with a unique mechanism of penetration into eukaryotic cells (see scheme below): after binding through its C-terminal part (red), to a receptor, the αMβ2 integrin, at the surface of target cells, the central region (blue) is inserted

into the plasma membrane and, then, the N-terminal catalytic domain (green) is directly translocated across the membrane into the cytosol of the cells

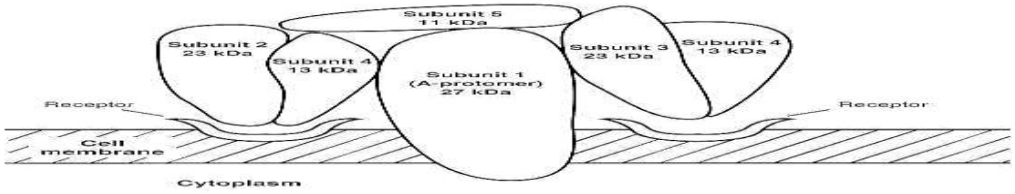


2-produces a highly **lethal toxin** (formerly called dermonecrotic toxin) also called **Heat labile Toxin** which causes inflammation and local necrosis adjacent to sites where *B. pertussis* is located. The lethal toxin is a protein composed of four subunits. It causes necrotic skin lesions when low doses are injected subcutaneosly in mice and is lethal in high doses.

3-produces a substance called the **tracheal cytotoxin** which is toxic for ciliated respiratory epithelium and which will stop the ciliated cells from beating. This substance is not a classic bacterial exotoxin since it is not composed of protein. The tracheal cytotoxin is a peptidoglycan fragment, which appears in the extracellular fluid where the bacteria are actively growing. The toxin kills ciliated cells and causes their extrusion from the mucosa. It also stimulates release of cytokine IL-1, and so causes fever.

4-produces the **pertussis toxin**, **PTx**, a protein that mediates both the colonization and toxemic stages of the disease. PTx is a two component, A+B bacterial exotoxin. The A subunit (S1) is an ADP ribosyl transferase. The B component, composed of five polypeptide

subunits (S2 through S5), binds to specific carbohydrates on cell surfaces. PTx is transported from the site of growth of the Bordetella to various susceptible cells and tissues of the host, the A subunit is inserted through the membrane and released into the cytoplasm in a mechanism of direct entry.



Binding of pertussis toxin to cell membranes

STRUCTURE OF PERTUSSIS TOXIN

Pertussis toxin consists of six polypeptides held together by oncovalent interactions and arranged in the A-B architecture typical of many bacterial toxins.In its native, secreted, form is a 952 residue hexamer comprised of subunits S1-S5, S4 being repeated.

The A subunit gains enzymatic activity and transfers the ADP ribosyl moiety of NAD to the membrane-bound regulatory protein Gi that normally inhibits the eukaryotic adenylate cyclase. The Gi protein is inactivated and cannot perform its normal function to inhibit adenylate cyclase. The conversion of ATP to cyclic AMP cannot be stopped and intracellular levels of cAMP increase. This has the effect to disrupt cellular function, and in the case of phagocytes, to decrease their phagocytic activities such as chemotaxis, engulfment, the oxidative burst, and bacteridcidal killing.

Adenylate cyclase activated by pertussis toxin (The pertussis A subunit transfers the ADP ribosyl moiety of NAD to the membrane-bound

regulatory protein Gi that normally inhibits the eukaryotic adenylate cyclase. The Gi protein is inactivated and cannot perform its normal function to inhibit adenylate cyclase. The conversion of ATP to cyclic AMP cannot be stopped.)

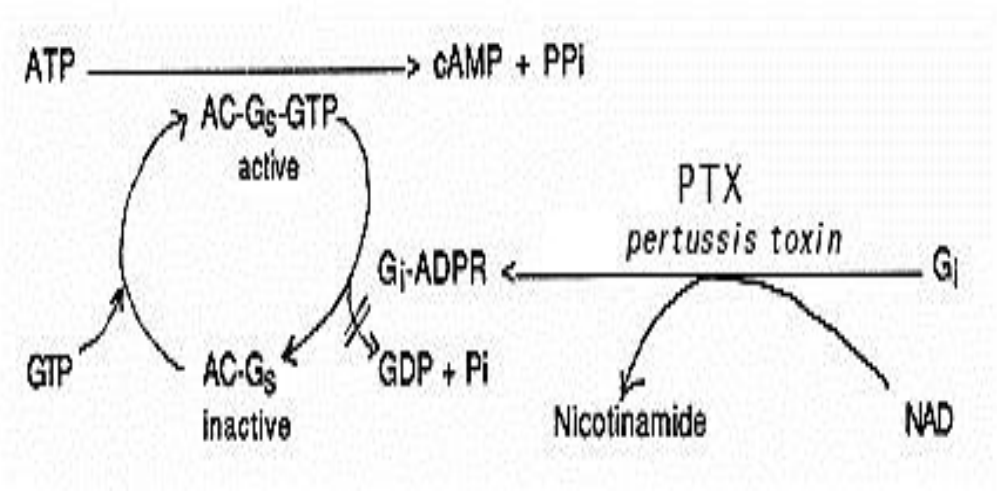


Figure . pertussis toxin (ptx) in their ability to interfere with the regulation of the eukaryotic adenylate cyclase complex.

The Whooping Cough Vaccine

the pertussis vaccine has been given in combination with vaccines against diphtheria and tetanus. The combination is known as the **DTP** vaccine. Recently, infants have been able to receive a vaccine that combines the DTP vaccine with the vaccine against *Haemophilus influenzae* type b meningitis (Hib). This vaccine is called **DTPH**. The diphtheria-tetanus-pertussis vaccine using acellular pertussis is known as **DTaP**.

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Bacterial toxins

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Lecture 7

Tetanus toxin

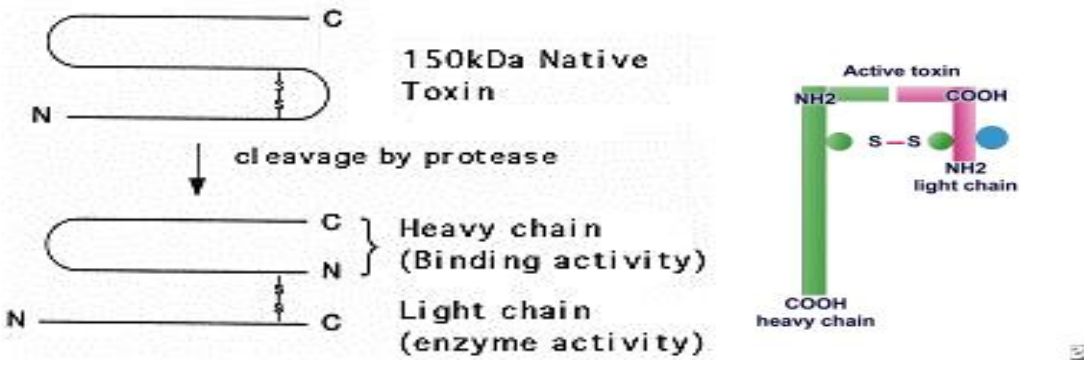
Tetanus toxin Lec7

Tetanus toxin is the neurotoxin produced by the vegetative cell of *Clostridium tetani* in anaerobic conditions, causing tetanus. It is sometimes called spasmogenic toxin, tetanospasmin or abbreviated to TeTx or TeNT.*C. tetani* also produces the exotoxin tetanolysin, the effects of which are as yet unclear.The genes encoding these toxin are located on separate plasmids within the bacterium.

Tetanus toxin spreads through tissue spaces into the lymphatic and vascular systems. It enters the nervous system at the neuromuscular junctions and migrates through nerve trunks and into the central nervous system (CNS) by retrograde axonal transport by using dyneins.

Structure

The protein tetanus toxin has a molecular weight of 150kDa. It is made up of two parts: a 100kDa heavy or B-chain and a 50kDa light or A-chain. The chains are connected by a disulfide bond.



- The B-chain binds to disialogangliosides (GD2 and GD1b) on the neuronal membrane.

ToxinAction

Tetanospasmin initially binds to peripheral nerve terminals. It is

transported within the axon and across synaptic junctions until it reaches the central nervous system. There it becomes rapidly fixed to gangliosides at the presynaptic inhibitory motor nerve endings, and is taken up into the axon by endocytosis. The effect of the toxin is to block the release of inhibitory neurotransmitters (glycine and gamma-amino butyric acid) across the synaptic cleft, which is required to inhibit nervous impulse. If nervous impulses cannot be checked by normal inhibitory mechanisms, it produces the generalized muscular spasms characteristic of tetanus. Tetanospasmin appears to act by selective cleavage of a protein component of synaptic vesicles, synaptobrevin II, and this prevents the release of neurotransmitters by the cells.

The receptor to which tetanospasmin binds has been reported as ganglioside GT and/or GD1b, but its exact identity is still in question. Binding appears to depend on the number and position of sialic acid residues on the ganglioside. Isolated B fragments, but not A fragments, will bind to the ganglioside. The A fragment has toxic (enzymatic) activity after the B fragment secures its entry. Binding appears to be an irreversible event so that recovery depends on sprouting a new axon terminal.

The action of the A-chain stops the affected neurons from releasing the inhibitory neurotransmitters GABA (gamma-aminobutyric acid) and glycine by degrading the protein synaptobrevin. The consequence of this is dangerous overactivity in the muscles from the smallest stimulus—the failure of inhibition of motor reflexes by sensory stimulation. This causes generalized contractions of the agonist and antagonist musculature, termed a tetanic spasm.

The Botulinum Toxins

Neurotoxin production is the unifying feature of the species *C. botulinum*.

Seven types of toxins have been identified and allocated a letter (A-G). Most strains produce one type of neurotoxin but strains producing multiple toxins has been described. Different serotypes have distinct protein structures, modes of action and potencies.

Botulin toxin produced by *Clostridium botulinum* is often believed to be a potential bioweapon as it is so potent that it takes about 75 nanograms to kill a person (LD50 of 1ng/kg, assuming an average person weighs ~75kg); 500 grams of it would be enough to kill half of the entire human population.

Botulinum toxin is secreted under anaerobic conditions (without oxygen) by a bacterium commonly found in soil called *Clostridium botulinum*. In nature the toxin is found in association (complexed) with non-toxic proteins which include haemagglutinin proteins.

Botulinum toxin blocks transmission of messages from nerves to muscles and therefore weakens muscles temporarily.Accidental ingestion of large quantities of botulinum toxin e.g. from improperly canned foods can lead to an acute paralytic illness called botulism.

Structure of botulinum toxin

Botulinum toxins are proteins that have similar molecular structures and weights of around 140-170 kDA. The toxins are produced as a single polypeptide chain, weighing approximately 150 kDA, and are only weakly toxic. The chain is activated by a process of proteolytic cleavage, to form a di-chain molecule (comprised of a heavy chain and a light chain), linked by a disulphide bond.

The light chain (~50 kD - amino acids 1-448) acts as a zinc (Zn^{2+}) endopeptidase similar to tetanus toxin with proteolytic activity located at the N-terminal end (see image below). The heavy chain (~100 kD -

amino acids 449-1280) provides cholinergic specificity and is responsible for binding the toxin to presynaptic receptors; it also promotes light-chain translocation across the endosomal membrane.

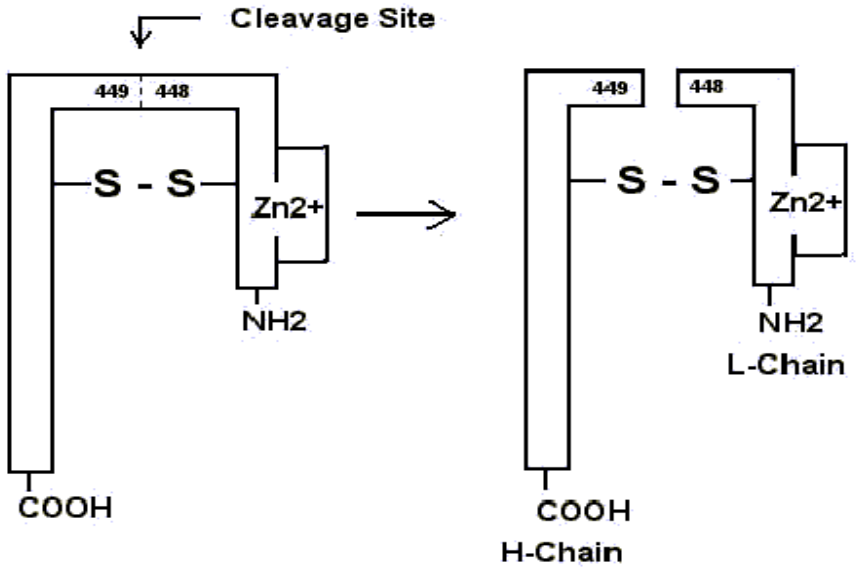


Fig: - Structure of botulinum toxin B ,Proteolytic activity is located at the N-terminal end of the light chain of botulinum toxin type A.

Mode of action of botulinum toxin

The botulinum toxins are very similar in structure and function to the tetanus toxin, but differ dramatically in their clinical effects because they target different cells in the nervous system. Botulinum neurotoxins predominantly affect the peripheral nervous system reflecting a preference of the toxin for stimulatory motor neurons at a neuromuscular junction. The primary symptom is weakness or flaccid paralysis. Tetanus toxin can affect the same system, but the tetanospasmin shows a tropism for inhibitory motor neurons of the central nervous system, and its effects are primarily rigidity and spastic paralysis.

Inhibition of acetylcholine release

The active light chain of botulinum toxin has a specific affinity to cleave certain proteins involved in the mechanism of acetylcholine exocytosis. Botulinum toxin type A cleaves SNAP-25 so that:

- Acetylcholine vesicles cannot fuse with the presynaptic membrane
- Acetylcholine is not released
- Neuromuscular transmission is blocked
- Muscle weakness and paralysis occurs

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Bacterial toxins

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Lecture 8

Toxins that act on 28S rRNA

Toxins that act on 28S rRNA Lec8

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 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.

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 .

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. 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 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 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 enters systemic circulation through absorption by the epithelium, enabling its access to the kidneys. The damage of the GI epithelium caused by *EHEC* likely aids in Stx systemic absorption. Stx is endocytosed by the eukaryotic cell, bypasses the late endocytic pathway and undergo retrograde transport from the trans-Golgi network to the endoplasmic reticulum (ER) where it encounters its target.

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)** .

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).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 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.

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Bacterial toxins

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Lecture 9

Pore-forming toxins

Pore-forming toxins Lec. 9

Staphylococcal alpha toxin (alpha-hemolysin)

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 aerolysin like, pore-forming toxin. 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 .

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.

Cholesterol-binding cytolysins (CBCs)

Three 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- **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.

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. 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).

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.

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

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.

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.

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.

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Bacterial toxins

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Lecture 10

Endotoxins

Endotoxins Lec (10)

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.

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