جامعة الانبار

كليه العلوم

قسم علوم الحياة

Bacterial toxins

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Lecture 2

ADP-ribosylation toxins

ADP-ribos السموم العاملة على مجموعه

سموم الدفثيريا والزوائف الزنجاريه

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 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 .
- 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.

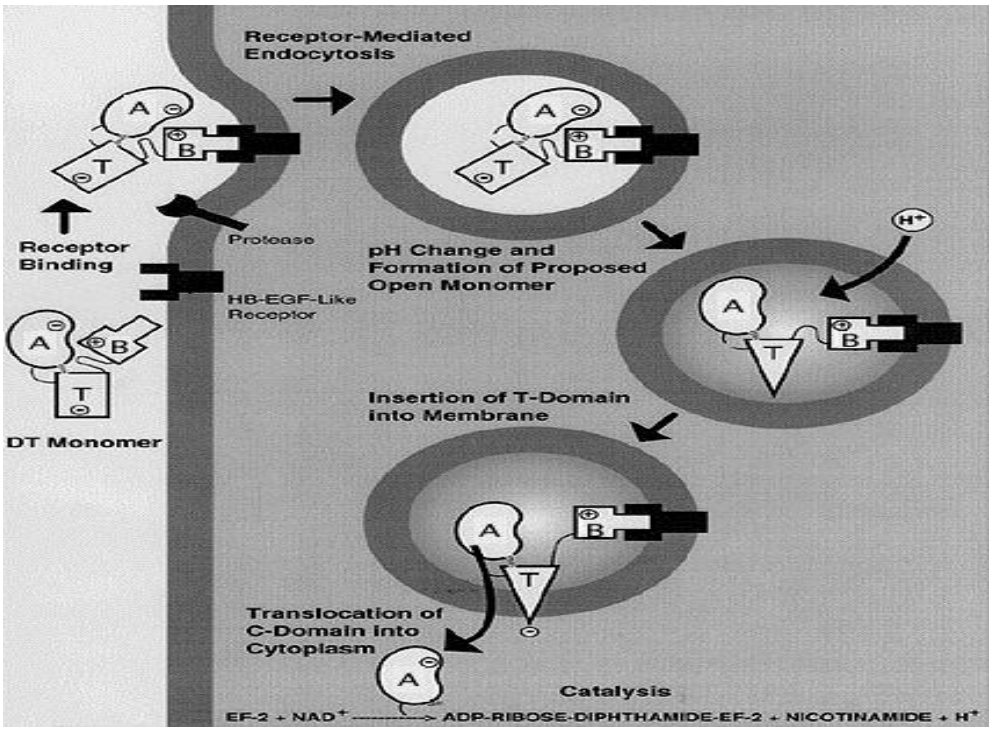


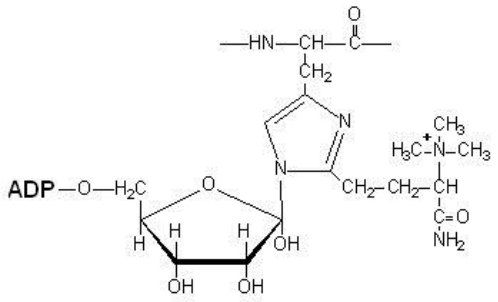
Figure. Uptake and activity of the diphtheria toxin in eucaryotic cells. A represents the A/B toxin's A (C) domain; B is the B (receptor) domain; T is the hydrophobic domain that inserts into the cell membrane.

The membrane translocation of the C domain(A fragment) across the vesicle membrane is facilitated by a cytosolic translocation factor complex which is, in part, composed of Hsp 90 and thioredoxin reductase. Thioredoxin reductase functions to reduce the disulfide bond between the C- and T domains. The function of Hsp 90 is most likely to facilitate the refolding of the C domain(A fragment) to a fully functional ADP ribosyltransferase, so it regains its conformation and its enzymatic activity.

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.



ADP ribosylated diphthamide.

The exotoxin A of Pseudomonas aeruginosa uses a similar mechanism of action. In vitro, the native diphtheria toxin is inactive and can be activated by trypsin in the presence of thiol. The enzymatic activity of fragment A is masked in the intact toxin. 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.

A booster injection should be given about a year later, and it is advisable to administer several booster injections during childhood. Usually, infants in the United States are immunized with a trivalent vaccine containing diphtheria toxoid, pertussis vaccine, and tetanus toxoid (DPT or DTaP vaccine).

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.

So, both ExoS and ExoT inactivate Rho GTPases directly by Rho GAP activity, and indirectly by ADP-ribosyltransferase activity. Why these toxins use both non-covalent (Rho GAP activity) and covalent (ADP-ribosylation) mechanisms to inactive the actin cytoskeleton remains unanswered.

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*. Although it is partially-identical to diphtheria toxin, it is antigenically-distinct. It utilizes a different receptor on host cells than diphtheria toxin, but otherwise it enters cells in the same manner and has the exact enzymatic mechanism. 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. purified Exotoxin A is highly lethal for animals including primates. Indirect evidence involving the role of exotoxin A in disease is seen in the increased chance of survival in patients with *Pseudomonas* septicemia that is correlated with the titer of anti-exotoxin A antibodies in the serum. Also, tox- mutants show a reduced virulence in some models.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. The other two domains are presumably involved in cell receptor binding and membrane translocation.

Reference

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