جامعة الانبار

كليه العلوم

قسم علوم الحياة

Bacterial toxins

Assisstant .prof .Dr.Muthanna Hamid Hassan

Lecture 6

Bordetella pertussis

سموم بكتيريا السعال الديكي

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.

Studies of *B. pertussis* and its adhesins have focused on cultured mammalian cells that lack most of the features of ciliated epithelial cells. However, some generalities have been drawn. 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.

Since the S3 subunit of pertussis toxin is able to bind to the surface of phagocytes, it has been speculated that the bacterium might bind preferentially to phagocytes in order to facilitate its own engulfment. *B. pertussis* produces at least two other types of adhesins, two types of fimbriae and a nonfimbrial surface protein called pertactin, but their role in adherence and pathogenesis is not well established.

Toxins Produced by *B. pertussis*

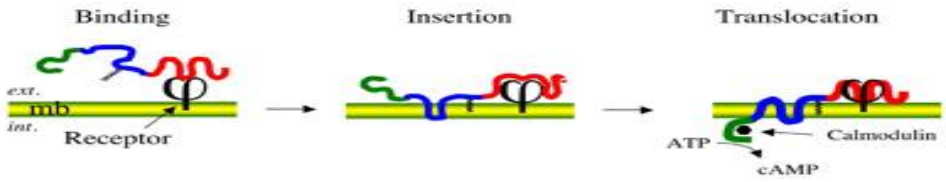
B. pertussis produces a variety of substances with toxic activity in the class of exotoxins and endotoxins.

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.

The adenylate cyclase was originally identified as a hemolysin because it will lyse red blood cells. In fact, it is responsible for hemolytic zones around colonies of *Bordetella pertussis* growing on blood agar. Probably it inserts into the erythrocyte membrane which causes hemolysis.

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,

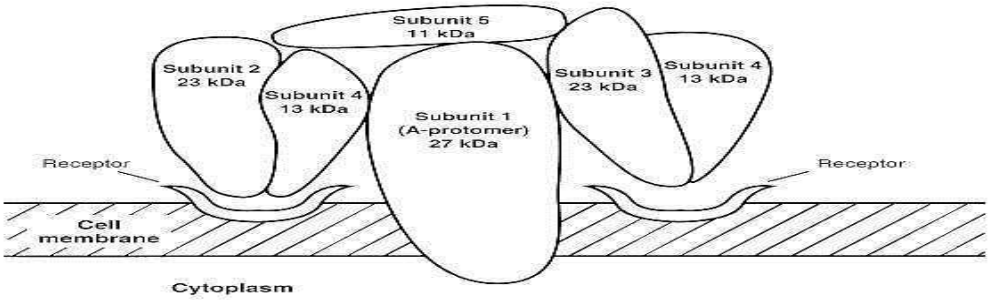
the N-terminal catalytic domain (green) is directly translocated across the membrane into the cytosol of the cells



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. It causes necrotic skin lesions when low doses are injected subcutaneosly in mice and is lethal in high doses. The role of the toxin in whooping cough is not known.

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.Following binding of the B component to host cells, 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. Systemic effects of the toxin include lymphocytosis and alteration of hormonal activities that are regulated by cAMP, such as increased insulin production (resulting in hypoglycemia) and increased sensitivity to histamine (resulting in increased capillary permeability, hypotension and shock). PTx also affects the immune system in experimental animals. B cells and T cells that leave the lymphatics show an inability to return. This alters both AMI and CMI responses and may explain the high freqency of secondary infections that accompany pertussis (the most frequent secondary infections during whooping cough are pneumomia and otitis media).

The pertussis toxin gene has been cloned and sequenced and the subunits expressed in *E. coli*. The toxin can be inactivated and converted to toxoid for use in component vaccines.

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.)

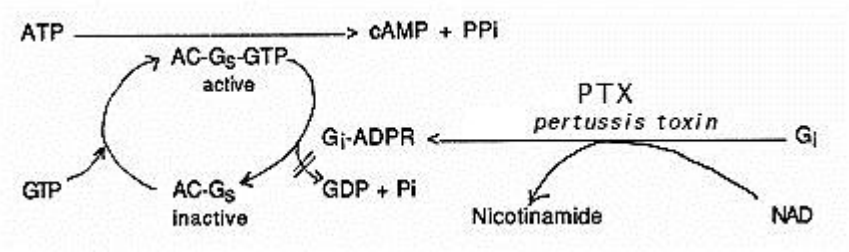


Figure . Comparison between cholera toxin and pertussis toxin (ptx) in their ability to interfere with the regulation of the eukaryotic adenylate cyclase complex.

The Whooping Cough Vaccine

Several new **acellular vaccines** have been developed from purified components of *B. pertussis*. Demonstration of the protective effects of anti-PTx and anti-FHA antibodies in the mouse model, focused vaccine production on combinations of inactivated pertussis toxin (toxoid) and filamentous hemagglutinin. The new vaccine, known as **acellular pertussis** has fewer side effects than the whole cell vaccine and is currently recommended for use under the conditions described below. 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**.

Reference

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