جامعة الانبار

كليه العلوم

قسم علوم الحياة

Bacterial toxins

Assisstant .prof .Dr.Muthanna Hamid Hassan

Lecture 3

Cholera toxins

سموم الكوليرا

Cholera Lec 3

Cholera (frequently called **Asiatic cholera** or **epidemic cholera**) is a severe diarrheal disease caused by the bacterium ***Vibrio cholerae.*** Transmission to humans is by water or food. The natural reservoir of the organism is not known. It was long assumed to be humans, but some evidence suggests that it is the aquatic environment.

Serogrouping of *V. cholerae* is based on the polysaccharides of the somatic (O) antigen. There are more than 200 serogroups of *V. cholerae* but only 2 serogroups – O1 and O139 – cause epidemic disease. There is no proven cross-protection between these 2 serogroups. Serogroup O1 has 2 biotypes: El Tor and classical. Both of these biotypes can be further classified into 2 serotypes: Ogawa and Inaba. Compared with the classical strains, El Tor persists for longer in the environment, causes more asymptomatic cases and is shed more extensively in excreta, even in asymptomatic cases. Although the classical strains are believed to have been responsible for the 6 previous pandemics in modern history, El Tor is responsible for the seventh pandemic, which started in 1961 and continues today.

In 1992, a new variant strain caused extensive epidemics in Bangladesh and India, and subsequently in other parts of south Asia. This strain (*V. cholerae* O139 Bengal) is a genetic derivative of the El Tor biotype in which the O1 biosynthetic genes are replaced by the O139 biosynthetic genes. The spread of the O139 serogroup is restricted to Asia, and over the years the incidence in most parts of Asia has declined except in a few pockets in, for example, China and Thailand. Serogroup O139 is currently responsible for 2–9% of cases in Bangladesh. In 1992 another variant of El Tor emerged;

this has the genetic backbone of the El Tor biotype but produces cholera toxin formerly produced only by classical strains.

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. A healthy person may become hypotensive within an hour of the onset of symptoms and may die within 2-3 hours if no treatment is provided. More commonly, the disease progresses from the first liquid stool to shock in 4-12 hours, with death following in 18 hours to several days. 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 .

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). They withstand propulsive gut motility by their own swimming ability and chemotaxis directed against the gut mucosa.

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. It has been suggested that *V. cholerae* might use these nonfimbrial adhesins to mediate a tighter binding to host cells than is attainable with fimbriae alone.

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

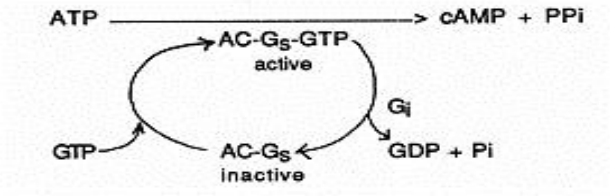
Koch in 1884 proposed that the symptoms caused by *Vibrio cholerae* could be due to a "poison". However, it was not until 1959 when the existence of such a cholera toxin (CT) was conclusively demonstrated. In 1969 Finkelstein and LoSpalluto2 had purified the toxin and shown it to be a 84 kDa protein. 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. The CTB pentamer is held together by approximately 130 hydrogen bonds and 20 salt bridges. These many polar bonds together with a tight packing of subunits via

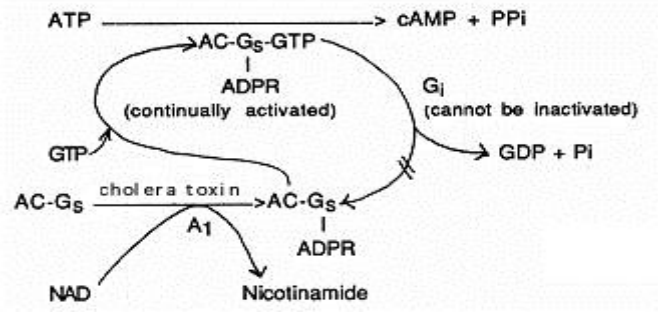
hydrophobic interactions could by themselves explain the outstanding stability of pentameric CTB to proteases, bile components and other factors in the intestinal milieu. It has been suggested that pentamer-pentamer interactions may possibly further add to the stability.

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.

Immunity to Cholera

After natural infection, people also develop toxin-neutralizing antibodies but there is no correlation between antitoxic antibody levels and the incidence of disease in cholera zones. Secretory IgA, as well as IgG and IgM in serum exudate, can be detected in the intestinal mucosa of immune individuals. Although these antibodies presumably have to function in the absence of complement they still bring about protective immunity.

Motility is important in pathogenesis, and antibodies against flagella could immobilize the vibrios. Antibodies against flagella or somatic O antigens could cause clumping and arrested motion of cells. Antitoxic antibodies could react with toxin at the epithelial cell surface and block binding or activity of the the toxin.

The observation that natural infection confers effective and long-lasting immunity against cholera has led to efforts to develop a vaccine which will elicit protective immunity. The first attempts at a vaccine in 1960s were directed at whole cell preparations injected parenterally. At best, 90% protection was achieved and this immunity waned rapidly to the baseline within one year. Purified LPS fractions from different biotypes have also been given as vaccines with variable success. The cholera toxin can be converted to

toxoid in the presence of formalin and glutaraldehyde. The toxoid is a poor antigen, however, and it elicits a very low level of protection.

The CTB-whole cell oral cholera vaccine: The toxicity of CT has precluded its use for human vaccination. Instead, nontoxic CTB has been extensively used without any side effects as a mucosal immunogen in humans. Indeed, recombinantly produced CTB is an important component of an oral cholera vaccine for human use. In addition to CTB, this vaccine also contains inactivated whole-cell cholera vibrios and is being registered (Dukoral®) in more than 60 countries worldwide. The vaccine has proved to be safe and efficiently immunogenic in both adults and children.

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