جامعة الانبار

كليه العلوم

قسم علوم الحياة

Bacterial toxins

Assisstant .prof .Dr.Muthanna Hamid Hassan

Lecture 5

E. coli heat-labile enterotoxin

السموم المعوية الثابتة والحساسة للحرارة

E. coli heat-labile enterotoxin **Lec5**

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 heat-labile enterotoxin (LT) from E. *coli* is a member of a class of medically important bacterial toxins which act intracellularly to catalyze ADP-ribosylation of specific cell proteins. These toxins share an A/B functional organization in which the B fragment mediates specific binding to cell surface receptors while the A fragment contains the enzymatic activity.

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.

LT-I is expressed by *E. coli* strains that are pathogenic for both humans and animals.

LT-II is found primarily in animal *E. coli* isolates and rarely in human isolates, but in neither animals nor humans has it been associated with disease.

(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 (Fig. **4**A).

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.

Although the stimulation of Cl^- as a result of increased intracellular levels of cAMP is **the classical explanation for the mechanism by which LT and CT cause diarrhea**, there is increasing evidence, obtained mostly with CT, that the secretory response to these toxins is considerably more complex.

* **One alternative mechanism** by which these toxins could act involves prostaglandins of the E series (PGE_1 and PGE_2) and platelet-activating factor. Synthesis and release of arachidonic acid metabolites such as prostaglandins and leukotrienes can stimulate electrolyte transport and intestinal motility.

***A second alternative mechanism** involves the enteric nervous system (ENS), which regulates intestinal motility and ion secretion. Serotonin and vasoactive intestinal polypeptide, both of which can stimulate intestinal epithelial cell secretion via the ENS, are released into the human small bowel after treatment with CT.

These alternative secretory mechanisms are supported by a variety of in vitro and in vivo data, and one or more of them could act in concert with the classic mode of action involving cAMP in causing diarrhea due to LT and CT.

The similarity of LT and CT is considered sufficiently high to extrapolate mechanistic similarities between the two toxins, and the validity of these assumptions has proven largely correct, with the exception of the failure of LT to release serotonin. However, observations made to date for secondary effects of CT have not all been demonstrated for LT, nor has the clinical relevance of these secondary secretory effects been substantiated.

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.

(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.

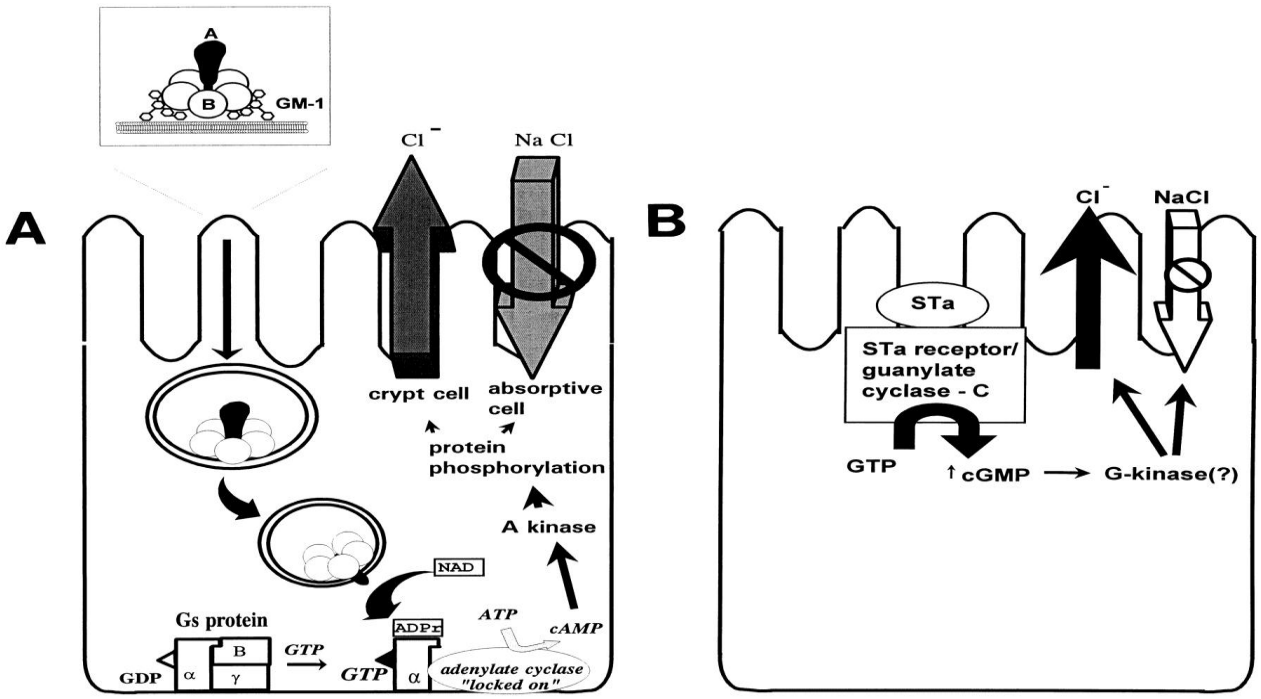

FIG. 4. Classic mechanisms of action of ETEC toxins (see the text for details and additional proposed mechanisms). (A) LT-I. The LT holotoxin, consisting of one A subunit and five B subunits, is internalized by epithelial cells of the small bowel mucosa via endocytosis. The A_1, or catalytic, subunit translocates through the vacuolar membrane and passes through the Golgi apparatus by retrograde transport. In the figure, the A subunit is shown passing through the B subunit ring, but this may not be the case in vivo. A_1 catalyzes the ADP-ribosylation of arginine 201 of the α subunit of G_s-protein (which may be apically located); the ADP-ribosylated G-protein activates adenylate cyclase, which elicits supranormal levels of intracellular cAMP. cAMP is an intracellular messenger which regulates several intestinal epithelial cell membrane transporters and other host cell enzymes, as well as having effects on the cytoskeleton. The activation of the cAMP-dependent A kinase results in phosphorylation of apical membrane transporters (especially the cystic fibrosis transmembrane conductance regulator), resulting in secretion of anions (predominantly Cl^- by a direct effect, and HCO_3^- indirectly) by crypt cells and a decrease in absorption of Na^+ and Cl^- by absorptive cells.

Heat-stable toxins(STs) .

In contrast to the large, oligomeric LTs, 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 guanylyl 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+. Genes for both classes are found predominantly on plasmids, and some ST-encoding genes have been found on transposons.

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. It has recently been reported, that some strains of ETEC may also express EAST1 in addition to STa. STb has been found only in ETEC.

(i) STa. The mature STa is peptide with a molecular mass of ca. 2 kDa. 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. These toxins consist of 18 (STp)or 19 (STh) amino acids including six cysteines that form three intramolecular disulfide linkages. Both peptides share the carboxy-terminal 14 residues which are sufficient for enterotoxicity and referred to as the toxic domain . This toxic domain shows significant homology to the sequence of the mammalian endogenous peptides guanylin , uroguanylin and lymphoguanylin .

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.

A mammalian hormone called guanylin is the endogenous agonist for GC-C. Guanylin is a 15-amino-acid peptide which contains four cysteines and is less potent than STa in

activating GC-C. Guanylin is presumed to play a role in normal gut homeostasis, and GC-C is apparently used opportunistically by STa to cause diarrhea.

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.The receptor for STb is unknown, although it has been 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. STb also stimulates the release of PGE_2 and serotonin, suggesting that the ENS may also be involved in the secretory response to this toxin.

Reference

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