Review

Review of enterotoxigenic *Escherichia coli* enterotoxins

Accepted 15th January, 2016

ABSTRACT

Enterotoxigenic *Escherichia coli* (ETEC) is the most common cause for travellers’ disease and a major cause of diarrhea for infants and newborn animals in developing countries. It produces heat-labile and heat-stable enterotoxins and some other toxins that lead to diarrhea. These enterotoxins are small peptides that are different in their structure and mechanism. Understanding the different structures and mechanisms underlying the process is important to know how ETEC interacts with the hosts and develops diarrhea. Here we give a systematic review on the toxins produced by ETEC and the knowledge here will ultimately help in designing new vaccines and therapy to the disease.

Key words: Enterotoxigenic *Escherichia coli*, ETEC, enterotoxin, LT, ST.

INTRODUCTION

Enterotoxigenic *Escherichia coli* (ETEC) is the major cause of travelers’ diarrhea and a major cause of diarrheal disease in developing countries, especially for children less than 5 years old. According to WHO, ETEC is responsible for about 380,000 deaths each year (WHO, 2006). In addition, ETEC is the most common pathogen of newborn farm animals such as piglets (Nagy and Fekete, 1999; Fairbrother et al., 2005). ETEC infections are linked to acute watery diarrhea, accompanying with headache, fever, and vomiting. Illness develops in 1-3 days after exposure to ETEC and usually lasts 3-4 days. However, in some cases, patients can have diarrhea for a week or more (Yoder et al., 2006).

ETEC was first reported about 55 years ago when De et al. (1956) failed to isolate *Vibrio cholerae* from patients with a classical symptom of cholera. Further study led to the discovery of *E. coli* strains and enterotoxin never found before. Up till now, a big number of ETEC strains, with over 70 O-antigen groups, have been isolated (Sanchez and Holmgren, 2005).

ETEC strains produce various toxins, including a heat-labile toxin (LT) and a heat-stable toxin (ST), and are classified according to their expression of LT, ST, or both (Fleckenstein et al., 2009). LT is a heterohexameric molecule comprising a single A subunit which contains the toxic domains and a pentameric B subunit which recognizes and binds to the receptor. ST are classified as STa/STI and STb/STII. STa and STb are both small cysteine-rich peptides and are unrelated in structure and function. As a matter of fact, they are different molecules with different modes of action. Moreover, ST is associated with more serious diseases and is identified in about 75% of all clinical isolates. Enteroaggregative heat-stable toxin 1, known as EAST1, was initially isolated by Levine et al. (1988) in enteroaggregative *E. coli*. Later, it has been also found in some ETEC strains (Savarino et al., 1993; Paiva de Sousa and Dubreuil, 2001). At nanomolar concentrations or less, these enterotoxins are capable of disrupting the normal homeostasis of the bowel and result in activation of Cl ions secretion and inhibition of Na ions adsorption. The net fluid secretion results in diarrhea (Fleckenstein et al., 2009). Here, we have performed a systematic review and compile aggregate information regarding the enterotoxins.

Heat-labile toxin

LT is a multimeric AB₅ toxin, containing a single A subunit (LTA) and a pentameric B subunit (LTB). It is structurally
and functionally similar to cholera toxin (CT) from *V. cholerae*. LTA contains toxic activity. Its A1 domain, the active toxin domain, connects with a helical molecule A2 domain by a disulfide bridge which anchors to the bridge pertactin (Merritt et al., 1994). LTB is responsible for binding to GM1 gangliosides on the cell surface and then entering into epithelial cells. Heat treatment at 70°C for 10 min releases LTA from the pentameric LTB (Hardy et al., 1988). LT can be further divided into two subtypes: LT-I and LT-II. According to their variation in structure, hosts, and modes of action, LT-I can be subdivided to LT-Ih and LT-Ip and LT-II can be subdivided to LT-IIa and LT-IIb (Merritt et al., 1994).

Sharing approximately 78% nucleotide sequence identity to the *ctxAB* operon encoding CT, eltA and eltB encode LTA and LTB protein. The end of *eltA* gene overlaps with the start of *eltB* gene by four nucleotides (Yamamoto et al., 1987). These genes are also referred to as *ctxAB* or *toxAB* in older studies (Sandkvist et al., 1987). Based on the similar subunit structure and catalytic activity, the nucleotide sequences of the genes for CT and LT show high homology to each other.

Sequence analysis indicates that horizontal transfer from *V. cholerae* may acquire LT encoding genes around 130 million years ago (Yamamoto et al., 1987). In most ETEC isolates, *eltAB* is found flanking by partial or intact insertion sequence (IS) elements and locating on an extrachromosomal virulence plasmid called pEnt (Yamamoto et al., 1987). These results suggest a common mechanism for the transferring of virulence-related genes between species. Moreover, non-pathogenic *E. coli* strains can produce enterotoxins by acquiring entire pEnt plasmids (Scotland et al., 1983).

Similar to CT, early studies considered the periplasm is the final place for LT. A mutant *V. cholerae* strain which cannot secrete CT was also unable to secrete LT indicate CT and LT share a common pathway (Neill et al., 1983). LTB is the secretion-competent substrate. A mutation (E11K) shows that the secretion efficiency of LT and CT was reduced (Mudrak and Kuehn, 2010). Later research shows that type II secretion system found in gram-negative bacteria is required for the secretion of LT (Johnson et al., 2006). In 2002, an operon in ETEC H10407 coding for a functional type II system was found and further study indicate the type II system mediates the release of LT (Tauschek et al., 2002). Furthermore, the results that the type II secretion system in ETEC is able to secrete CT are consistent with results from the expression of LT in *V. cholerae* (Mudrak and Kuehn, 2010). However, in some reports, the secretion of LT is associated with outer membrane vesicles (OMVs) released by ETEC (Turner et al., 2006).

Strains expressing LT have an advantage in colonizing the intestinal tract, for the expression of LT is essential to colonize the epithelial cells and LT promotes the adherence to enterocytes in vitro (Johnson et al., 2009). The host receptor for LT and CT is monosialoganglioside GM1. The terminal galactose residues of GM1 have the most contacts with the toxins and are conserved in their sequence (Merritt et al., 1994). After the binding of the pentameric B subunit to GM1 gangliosides on the host cell surface, it triggers endocytosis of the holotoxin (Tsai et al., 1987), the A1 portion of the A subunit is translocated across the intracellular membrane. Then LTA interact with an intracellular guanine nucleotide protein ADP-ribosylate Gsα (Tsai et al., 1987). Inhibition of GsαGTPase activity result in the constitutive activation of adenylate cyclase, thus leading to intracellular cAMP produced uncontrollably. Increasing cAMP level activate cAMP-dependent protein kinase A (PKA) and the cystic fibrosis transmembrane regulator (CFTR) chloride leads to the secretion of electrolytes and water that result in diarrhea (Sack et al., 1971). Increasing cAMP also activates NF-κB and ERK1/2, JNK and p38 MAPK which promotes ETEC adherence (Johnson et al., 2009; Wang et al., 2012).

**Heat-stable toxin**

ST, small cysteine-rich peptides secreted by ETEC, includes Sta/STI usually associated with human and STb/STII usually found in porcine (Lortie et al., 1991).

Based on the host and amino acid sequence, STa can be subdivided as StaP and StaH. StaP has 18 amino acids and was first identified in porcine, whereas 19-amino-acid STaH was isolated from human. Although there were differences in amino acid sequences, Sta molecules share a core structure of 13 amino acids with 3 disulfide bonds. Both StaH and StaP are encoded in plasmids (So and McCarthy, 1980). Initially synthesized as 72 amino acid precursor peptides, they are exported through the outer membrane by the trimeric ToIC protein exporter (Yamanaka et al., 1998).

STa binds to the extracellular domain of guanylate cyclase C (GC-C) on the brush border of the intestinal epithelium. Then the activation of the GC-C intracellular guanylyl cyclase catalytic domain converts GTP to cGMP and results in intracellular cGMP accumulation (Schulz et al., 1990). Elevated cGMP level activates cGMP-dependent protein kinase II which leads to the phosphorylation of the CFTR (Chao et al., 1994). In addition, elevated cGMP levels inhibit phosphodiesterase 3 resulting in cAMP increase and activation of PKA. Thus, STa induces secretory diarrhea via stimulation of the CFTR channel leading to over-secretion of Cl- and HCO3-, followed by an osmosis-driven electrolytes and water releasing by the cells.

Although STb has been reported in many human isolates, it is most associated with porcine ETEC (Dubreuil, 1997). STb is synthesized as a 48 amino acid peptide composed of two antiparallel α-helices which were separated by a glycine-rich loop (Sukumar et al., 1995). STb contains two disulfide bonds stabilizing the peptide's tertiary structure,
and both these bonds are essential for secretion and toxicity (Sukumar et al., 1995; Arriaga et al., 1995).

The estB gene encodes a 71 amino acid peptide including a 23 amino acid signal peptide (Kupersztoch et al., 1990; Fujii et al., 1991). Synthesized as an 8.1 kDa precursor, STb is converted to a 5.2 kDa mature peptide after secretion (Kupersztoch et al., 1990). According to Zhang et al. (2007), 72.6% of E. coli isolates from diarrhoeic pigs possess the STb enterotoxin gene, indicating that STb is the most prevalent toxin.

STb toxin binds to sulfatide, a glycosphingolipid widely distributed found on the surface of intestinal epithelial cells. After STb is transported into the cell, it activates a GTP-binding regulatory protein and leads to the increase of Ca++ level which activates calmodulin-dependent protein kinase II (CAMKII) (Dreyfus et al., 1993). Moreover, STb activates protein kinase C (PKC) and CFTR which is responsible for Cl– and HCO3– secretion (Fujii et al., 1997). Protein kinase C inhibits Na+ uptake and CAMKII opens a calcium-activated chloride channel. The elevated intracellular Ca++ level affects the activities of phospholipases A2 and C resulting in the formation of PGE2 from membrane lipids and production of 5-HT (or serotonin), PGE2 and 5-HT mediate the transportation of H2O and electrolytes out of the membrane by an unknown mechanism (Peterson and Whipp, 1995).

### EAST1

EAST1 is a 38 amino acids toxin which was first found in an enteropathogenic E. coli from a child. Its coding gene estA was detected in human, bovine, and porcine ETEC, enteropathogenic E. coli and some Salmonella isolates (Paiva de Sousa and Dubreuil, 2001; Savarino et al., 1996). Having 50% with STa, EAST1 also leads to an increase in cGMP. EAST1 has numerous variants and estA is often identified in multiple copies, suggesting functional redundancy of toxins to increase intracellular cGMP level (Yamamoto and Echeverria, 1996).

### Conclusion

Recent researches on enterotoxins have revealed an increasing complexity for which we once believed that the toxins simply enter the cells and leads to the cell lysis. Our knowledge of molecular mechanism underlying ETEC-induced diarrhea will offer us unprecedented opportunities to design new vaccines and therapeutic approaches to this disease.

### Acknowledgement

The Project was full financially supported by king Saud University, through Vice Deanship of Research Chairs.

### REFERENCES


Cite this article as:

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