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Review on pathogenicity mechanism of enterotoxigenic *Escherichia coli* and vaccines against it

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ABSTRACT

Enterotoxigenic *Escherichia coli* (ETEC) is the most common cause of diarrhea in children. Colonization factors (CFs) and LT enterotoxin are the major ETEC candidate vaccines. To cause disease, ETEC must adhere to the epithelium of the small intestine by means of CFs. Watery diarrhea is produced due to the effects of the enterotoxins. Vaccine development against ETEC has been identified as an important primary prevention strategy in developing countries and for travelers to these regions. Mucosal immunization can cause secretory IgA antibody (sIgA) responses that prevents the attachment of bacteria to the intestine and are of particular importance for provide protection against ETEC infection. The design of multivalent ETEC vaccine containing various colonization factors and ETEC toxin may provide protection against a wide range of bacterial strains. In this review, the importance and pathogenesis of ETEC, and the latest ETEC vaccine research results are discussed.

1. Introduction

Diarrheal diseases are one of the most important health problems in different human communities, especially in developing countries [1,2]. Several biological factors, such as bacteria and viruses causing diarrhea diseases, which is led to the deaths of hundreds of thousands of people including children [3,4]. Infectious diarrhea is one of the rate of worldwide diarrhea prevalence caused by *Escherichia coli* strains, especially in areas with lower level of health is very high [5,6]. Based on antigenic differences and mechanism of pathogenicity, *Escherichia coli* causing diarrhea is divided into 6 groups: Enteroaggregative *Escherichia coli* (EAEC), Enterohemorrhagic *Escherichia coli* (EHEC), Enteroinvasive *Escherichia coli* (EIEC), Enteropathogenic *Escherichia coli* (EPEC), Enterotoxigenic *Escherichia coli* (ETEC), and Diffusely adherent *Escherichia coli* (DAEC) [4,7,8]. Of these, a prevalence of diarrhea caused by ETEC is higher, particularly in deprived areas, and people who travel to such areas, that also called traveler's diarrhea [4,7,9].

In 1956 De et al. in Calcutta, isolated a strain of *Escherichia coli* from patients with pseudo-cholera diarrhea and experimentally demonstrated that the isolated strain can causes the accumulation of liquid and electrolyt in closed loops of rabbit intestine [10].

ETEC, is one of the most important causes of bacterial diarrhea in developing countries [2,6,11]. The bacteria cause 15 to 20% of diarrhea in children under 5 years of age in poor countries and it is the major common cause of traveler's diarrhea in persons who travel to Africa, Asia and Latin America [12]. In many of these countries, much higher prevalence rate has been reported in infants under 12 months [12,13]. About 60% of traveler's diarrhea caused by ETEC [13]. Approximately 10 million traveler's diarrhea cases have been reported worldwide per year [14,15]. One interesting point is that 10 to 14% of people with traveler's diarrhea caused by ETEC show symptoms of Bowel syndrome later [3].

Incidence of ETEC strains causing diarrhea in persons over 5 years of age is decreased, however it is seen that older people are also prone to diarrhea caused by ETEC [3,16].

In the world health organization reports, the death toll from diarrhea caused by ETEC is estimated about 157,000 persons a year, roughly equivalent to 9% of deaths from diarrhea [5,17]. In 2013, an average of 42,000 reported deaths due to diarrhea caused by ETEC in children under 5 years of age has attracted a lot of attention [3,18]. In the same year in Africa and South Asia, more than 89,000 deaths were recorded also for persons over the 5 years of age [3,18].

A point of note in the reports is that prevalence in people aged older than 5 years reached 44 million cases, which it is higher in comparison to 6 million with typhoid and 3 million with *cholera* [3,12,18].

Diarrhea caused by ETEC can be mild or severe case with plenty of water disposal, abdominal pain, nausea and vomiting and rarely with fever and headache [15,19,20]. The incubation period varies between 1

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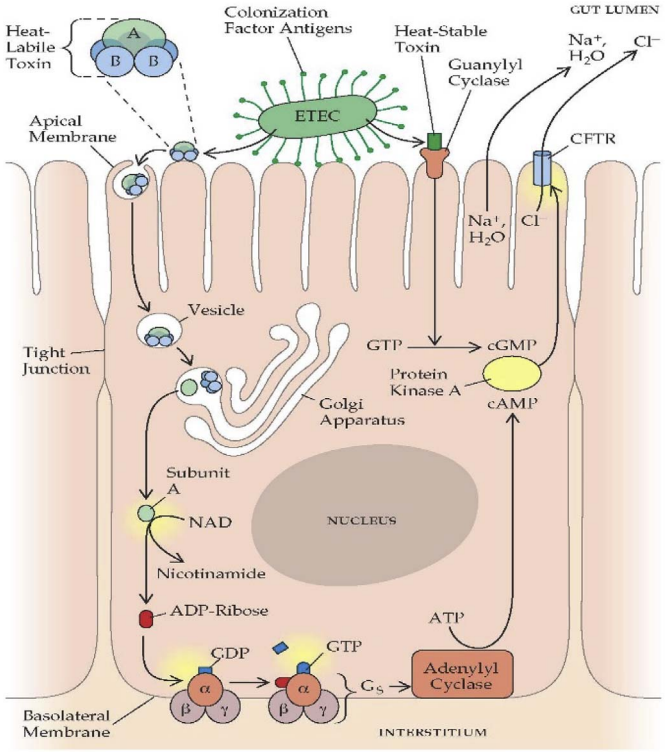

Fig. 1. Mechanisms of disease producing by enterotoxigenic *E. coli* [30]. Pathogenesis of ETEC bacteria invasion involves two steps which are intestinal colonization, followed by elaboration of diarrheagenic enterotoxins. Activation of adenylyl and guanylate cyclase lead to formation of cAMP and cGMP, stimulates water and electrolyte secretion by intestinal endothelial cells.

and 2 days and after onset of the disease, it may be possible to dispose 10 L of water daily in the form of loose stools. In this case, patients need hospitalization and intensive care [21–23].

2. ETEC virulence factors

The disease caused by ETEC is spread by swallowing 10^6 to 10^{10} numbers of the bacteria. When the bacteria reached the small intestine, infection is established. Bacteria by means of surface colonization agents attach to the intestinal epithelium and colonization occurs on the surface of small intestine cells [9,22,24]. After attachment and colonization of bacteria, enterotoxins produced and affected the epithelial cells in the area. Producing heat-labile or heat stable enterotoxins is the major factor of ETEC virulence. The toxins may cause diarrhea independently of each other. ETEC strains could produce simultaneously only ST, LT or both types of toxin [11,25].

In addition to enterotoxins, other virulence factors from adhesion and colonization factors are also proposed. The condition that leads to diarrhea caused by bacteria is shown in Fig. 1 [26].

Escherichia coli produce heat-labile type I and II enterotoxins that is differentiated by of the genetic, biochemical and immunological properties. The heat-labile enterotoxin, type I (LT-I), is an 84 kDa heterohexamer composed of pentameric B subunit and an A subunit [27]. A subunit is made of two domains, which are linked by sulfid bond. A_1 domain is the active portion of the toxin and A_2 domain with helical shape is placed inside pentamer B subunit [27]. The structure of *Escherichia coli* heat-labile toxin is shown in Fig. 2 [28].

The heat-labile enterotoxin induces its toxic effect via binding to ganglioside GM1 at the apical surface of intestinal cells. B subunit link to the ganglioside GM1 at the host cells causing toxin endocytosis. A subunit passes through the cell membrane and reacts with ADP ribosylating factor. Avoiding GTPase activity of the Gsα protein leads to

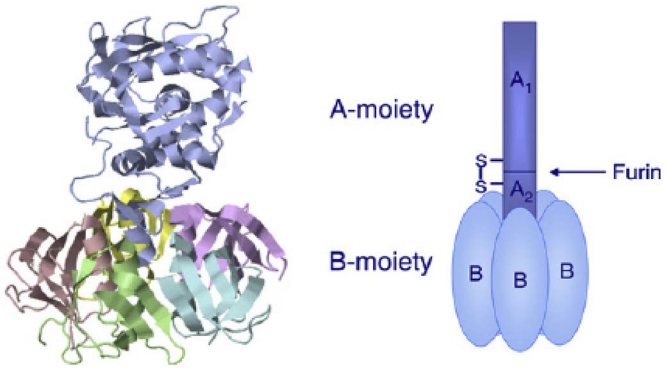

Fig. 2. The structure of *Escherichia coli* heat-labile toxin A and B subunits [33].

continuous activity of adenylate cyclase enzyme and cAMP (3′,5′-cyclic adenosine monophosphate) increasing. The cAMP increase causes activation of cAMP-dependent kinase protein. This enzyme also causes phosphorylation and stimulates chloride channels at the apical membrane. Following these changes, secretion of electrolytes and water into the intestine, resulting in diarrhea [21,22,29].

Another type of LT toxin, which is found in some *E. coli* strains, has been named heat-labile toxin II (LT-II). The toxin, has no immunological cross-reactivity with cholera toxin. The similarity of amino acid sequence of this toxin type with the amino acid sequence of the cholera toxin and and heat-labile toxin type I reaches less than 14% [29,30].

LT-II toxin do not cause fluid accumulation in the adult rabbit's intestine and do not bind to ganglioside GM1. Moreover, genetic information related to the toxin is on the bacterial chromosome. The importance of toxin type II in pathogenicity in humans is not well-known [30].

In addition to toxin LT, enterotoxigenic *Escherichia coli* produce heat-stable enterotoxins, which are cysteine-rich small peptides. The ETEC heat-stable enterotoxins in terms of sequence and three-dimensional structure is very similar to guanylin and uroguanylin (Fig. 3).

This toxin secretes and binds to the extracellular portion of guanylyl cyclase enzyme located on the surface of intestinal epithelial cells. After this binding, the functional intracellular portion of guanylyl cyclase protein is activated, which eventually causes accumulation of cGMP in the cell. The increasing of intracellular cGMP leads to activation of cGMP-dependent Protein Kinase II. Also by phosphorylation the channels' regulator, kinase enzyme causes chloride secretion and inhibition of sodium chloride absorption. In this condition, epithelial cells dehydrate and diarrhea occurs in patient [21,31].

Type I and II heat-stable enterotoxin produced by ETEC. Toxin STI (STa) that is binds to guanylyl cyclase, is divided into two types (ST-P) ST-Ia and (ST-H) ST-Ib [21,31]. The prevalence of LT, ST or ST/LT toxin phenotypes was remarkably varied between different regions in the world (Fig. 4).

One of the most important pathogenic factor in enterotoxigenic *Escherichia coli*, producing one or more of colonization factors (CFs) that usually have fimbrial or fibrillar structure. These surface proteins are one of the first virulence factors that is well-known in ETEC and considered as a target for vaccine production [3,32]. Today about 25 different types of CF is known, which mostly encode by plasmid [3,5].

Colonization factors are of protein in three different groups. The first group or CFA/I-like group include CFA/I, CS1, CS2, CS4, CS14 and CS17. The second is CS5-like group, which includes CS5, CS7, CS18 and CS20 binding agents. An amino acid sequence CS5, CS13, CS18 and CS20 is genetically similar to subunits animal strains fimbriae such as K88 and f41. The third group or special group, contains CS3, CS6 and CS10-12. These binding factors do not have many similarities, and due to the non-uniformity with the two other groups have been placed in a separate category [5,33,34].