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Enterotoxigenic *Escherichia coli* targeting intestinal epithelial tight junctions: An effective way to alter the barrier integrity

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ABSTRACT

Enterotoxigenic *Escherichia coli* are responsible for causing secretory diarrhea in animal(s), including human(s). This group of microorganisms is classified on the basis of production of toxins acting on the intestinal epithelium of the small intestine. Various enterotoxins, heat-labile and heat-resistant, are produced by distinct strains of ETEC. Although the mechanisms of action of ETEC enterotoxins were shown to involve diverse ion channels recent data suggest that these molecules could also be involved in disruption of the permeability barrier of the intestinal epithelium. More precisely, the tight junctions directly responsible for the selective permeability of the intestinal tissue could be affected. Studies indicating a change in TJ following exposure of cell monolayers or animal models either to pure enterotoxins or to ETEC strains producing one or more of these toxic molecules will be discussed.

1. Introduction

A variety of bacteria lives symbiotically in the intestinal lumen. The majority of these bacteria contributes to the maintenance of intestinal function and health [1]. On occasion, pathogenic bacteria intrude into the intestines and sometimes into the tissues to cause infections. Successful enteric, non-invasive pathogens, have to colonize, compete for nutrients and interact with target eukaryotic cells in order to induce secretion of water and electrolytes [2]. In order to have an effective outcome, pathogens must possess highly specialized attributes that enable them to activate eukaryotic cell pathways leading to secretion. The interaction between enteric pathogens and the host intestinal cells can involve toxins. Many enterotoxins responsible for secretion and the resulting diarrhea have been described. Enteric bacterial pathogens interacting with the intestinal epithelial cells can disturb the intestinal tight junction (TJ) barrier [3]. These bacterial pathogens can disrupt the barrier function by direct binding to epithelial cells and also through production of toxins. The result is abnormal electrolytes and water secretion sometimes with mild tissue inflammation [4]. This review focuses on ETEC and the toxins they elaborate in relation to their action on the TJ complex.

2. The epithelial barrier

The gastrointestinal (GI) epithelium forms the body's largest interface with the external environment. Intestinal epithelial cells represent polarized cells with distinct apical microvilli and crypts that interact with the basal lamina. Adjacent cells attach together through intercellular junctions. A key function of the intercellular junctions is the formation of selective barriers [5]. Enterocytes are joined to each other by four different types of junctions. This specialized complex consists of tight junctions (TJ), adherens junctions (AJ), desmosomes, and gap junctions. Three sub-components form the individual junctions of the apical junction complex; 1) transmembrane proteins, 2) cytoskeletal components, and 3) cytoplasmic scaffolding proteins that attach the two together.

The AJ, along with the desmosomes, provide strong adhesive bonds between the epithelial cells and also aids in intracellular communication, but does not determine paracellular permeability [6,7]. As the most apical intercellular membranes, TJ represent the major barrier within the paracellular pathway. TJ are dynamic structures and can readily adapt to changes. The mechanisms involved in this adaptation are still incompletely understood.

3. Tight junction structure

Tight junctions are multiprotein structures consisting of transmembrane proteins, linked to the cell cytoskeleton through transmembrane proteins [8]. Four integral transmembrane proteins, claudin, occludin, junctional adhesion molecules (JAMs), and tricellulin contribute to the semi-permeable barrier. The intracellular domains of these transmembrane proteins interact with cytosolic scaffold proteins, such as zonula occludens (ZO) proteins, which in turn anchor the transmembrane proteins to peri-junctional actomyosin ring. Cytosolic

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proteins link membrane components to the cytoskeleton and participate in signaling between TJs and the cell nucleus. TJs are regulated in their molecular composition, ultrastructure and function by intracellular scaffolding proteins and cytoskeleton. Experimental infections in cell culture often measure transepithelial resistance (TER) as a marker of functional barrier. Damage corresponds to an increased porosity of the TJ barrier with observable passage of molecules such as dextran, mannitol, and inulin. Nevertheless, we have learned that porosity does not necessarily parallel TER alterations [9]. In fact, permeability measurements are carried out using rather large molecules that does not exclude significant permeability changes to molecules of smaller size including water.

3.1. Claudins

Numerous studies have shown that claudins are the key component and backbone of TJ. Claudins play critical roles in barrier formation and paracellular permeability and selectivity in various tissues. At least 27 claudin members have been identified to date [10]. Claudins modulate paracellular transport in the intestinal epithelium [11]. In the intestines, claudins-1, -3, -5, -8, -9, -11, and -14 can be categorized as barrier forming claudins, while -2, -7, -12, and -15 are pore-forming claudins.

An increased expression of these proteins leads to a very tight epithelium, coinciding with an increased TER and increased solute impermeability across the epithelial monolayer. Claudins enable strict control over paracellular flux of cations and anions. Some of the claudins of neighboring cells interact to form intercellular connections while other members of the claudins do not. They are crucial for the barrier function of TJs [12]. Some claudins are phosphorylated and phosphorylation is associated with localization and paracellular permeability.

3.2. Occludin

Occludin is an integral membrane TJ protein presenting four transmembrane domains [13]. Movement of occludin from TJ into cytoplasmic vesicles occurs frequently altering epithelial permeability in barrier function loss [14]. Occludin expression has an important role in TJ structure and in maintaining the barrier function of the intestinal epithelia [15]. The phosphorylated form of occludin appears to be the major form within the TJ, whereas the less phosphorylated forms are found in the basolateral membrane and in the cytosol.

The long C-terminal domain interacts with several intracellular TJ proteins, such as ZO proteins, which are required to link occludin to the actin cytoskeleton [16]. Occludin has a crucial role in TJ structure and permeability in the intestinal epithelia [17] and the hemophilic interdomains of the extracellular loops interact with adjacent cells to create a barrier against macromolecules but not against small ions [15].

3.3. Junctional adhesion molecules

The JAM family belongs to the immunoglobulin (IG) superfamily and is characterized by the extracellular IG domains, one transmembrane and one extracellular domain. JAMs are involved in multiple functions including TJ assembly, regulation of endothelial and epithelial paracellular permeability [10].

3.4. Zonula occludens

Three ZO proteins (ZO-1, ZO-2, and ZO-3) have been identified to date [10]. They are cytoplasmic proteins and are required for regulation and maintenance of TJ structure. ZOs are members of the large family of membrane-associated guanylate kinase proteins. ZOs form a complex on the cytoplasmic side of TJ. ZO-1 is localized in the vicinity of the plasma membrane of TJ in both epithelial and endothelial cells. It interacts with the actin cytoskeleton. ZO-2 and ZO-3 are peripheral proteins and have been identified as ZO-1 binding proteins. Many TJ proteins bind to the N-terminal half region of ZO proteins, while the C-terminal region interacts with the actin cytoskeleton [18]. ZO-1 localizes to the nascent cell-cell contacts in both cell cultures and animal models. It has been proposed that ZO proteins may mediate the early assembly of TJ proteins to form cell-cell contact.

3.5. Tricellulin

Tricellulin is a TJ protein forming a linkage between three adjacent cells [19]. Tricellulin is a tetraspan protein with four transmembrane domains and two extracellular loops. Studies have indicated that tricellulin plays an important role in epithelial TJ regulation at both tricellular and bicellular junctions.

3.6. Actin

Structural and functional linkage was determined between the actin cytoskeleton and the TJ complex. The architecture of the actin cytoskeleton appears to be critical for TJ function. Actin filaments are important in maintaining the cell shape and the integrity of the epithelial barrier. In polarized cells, the apical compartment is rich in actin filaments and these are assembled in several structures. Myosin and actin constitute a ring (perijunctional actinomyosin ring) that encircles the cells at the level of TJ and AJ [20].

4. Enteric pathogen

Non-invasive enteric bacterial pathogens have to adhere effectively to the target eukaryotic cell to colonize and compete for nutrients. Specialized attributes enable them to activate one or more eukaryotic intracellular pathways leading to intestinal water and electrolyte secretion. Some bacterial toxins have evolved to exploit the regulation of water and electrolytes between the environment and the host. This interaction between the pathogen and the host intestine is often brought about by enterotoxins elaborated by the pathogen although other bacterial structures can also be implicated [21].

Permeability defects in intestinal epithelia can be brought about by enteric pathogens by altering TJ proteins through the activity of enterotoxins or using effector proteins [22]. Some pathogens can impair intestinal barrier function by disruption of TJs and initiation of inflammatory cascades [3]. TJ alterations have also been proposed to be involved in diarrhea via a mechanism in which water enters the lumen of the intestine through the passive movement of both water and ions following a break in the intestinal barrier [23].

5. Enterotoxigenic *Escherichia coli*

Most *E. coli* are commensals but some pathotypes related to the virulence groups can cause serious health problems in humans. Serological classification is done according to a modified Kauffman scheme based on the O (somatic or lipopolysaccharide, about 200 serotypes), H (flagellar, 56 serotypes), F (fimbrial, more than 22 serotypes), and K (capsular polysaccharide, about 60 serotypes) antigens. ETEC are characterized by the ability to produce two types of virulence factors; 26 adhesins that promote binding and colonization of the intestinal epithelium and three enterotoxins responsible for fluid secretion [24]. Once established in the animal small intestine, ETEC produces enterotoxin(s) leading to diarrhea. The pathotypes are related to the pathogenicity potential based on the presence of colonization factors (CFs) as well as production of toxins. ETEC is the most common cause of *E. coli* diarrhea in farm animals.