Bacterial interference with host epithelial junctional complexes: Probiotic bacteria vs. A/E lesion-forming Escherichia coli

Topouzova-Hristova Tania, Stephanova Elena, Stoitsova Stoyanka

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Tania Topouzova-Hristova [1]
Elena Stephanova [1]
Stoyanka Stoitsova [2]

Bacterial interference with host epithelial junctional complexes: Probiotic bacteria vs. A/E lesion-forming *Escherichia coli*

Authors' addresses:
[1] Faculty of Biology, Sofia University, Sofia-1000, Bulgaria.
[2] Institute of Microbiology "Stephan Angeloff", Bulgarian Academy of Sciences, Sofia-1113, Bulgaria.

Correspondence:
Stoyanka Stoitsova
Institute of Microbiology "Stephan Angeloff",
Bulgarian Academy of Sciences,
Acad. G. Bonchev St., Bl. 26
Sofia-1113, Bulgaria
Tel.: +359-2 9793159
e-mail:
stoitsova_microbiobas@yahoo.com

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ABSTRACT

During colonization, enteropathogenic (EPEC) and enterohaemorrhagic (EHEC) *Escherichia coli* are capable to manipulate host cytoskeleton and colonize gut epithelia by a specific mode of attachment known as the attaching and effacing lesion (A/E lesion). While actin rearrangements during A/E lesion formation have been extensively investigated, the possible alterations of other cytoskeletal elements like those comprising the intercellular junctional complexes (JC) of polarized cells during infection have only lately attracted attention. The present mini-review addresses the opposite effects of two groups of bacteria, A/E lesion-forming pathogenic *E. coli* and probiotic bacterial strains, on JC. JC are important in maintaining gut barrier functions. EPEC and EHEC can disrupt JC which as a consequence leads to reduction in the transepitelial electrical resistance (TER) and an increase of the permeability to macromolecules. Probiotic bacteria on the other hand stabilize JC thus increasing TER and reducing permeability to macromolecular markers. Probiotic strains can protect JC integrity of polarized cells from the damage caused by EPEC or EHEC. Together with the promise of these results, of concern is the fact that the outcome of the studies can differ dependent on experimental protocols. Studies with living bacteria and different strain combinations have also put forward strain specific effects. Therefore, an important practical item for future studies is the identification of the molecules synthesized by probiotic bacteria that may be active on JC stability.

Key words: EPEC, EHEC, probiotic bacteria, epithelial junctional complexes

Introduction

Enteropathogenic and enterohaemorrhagic *Escherichia coli* (resp., EPEC and EHEC) are adapted to colonize gut epithelia by a specific mode of attachment known as attaching and effacing lesion (A/E lesion). This is produced due to the capacity of A/E pathogens to manipulate host cytoskeleton. Using their Type III secretion machinery, the bacteria inject into the eukaryotic cell a translocated intimin receptor (Tir). Tir is then integrated in the host cell membrane and interacts with intimin molecules available at the bacterial surface thus enforcing the pathogen's adherence. Epithelial microvilli in such loci lose their typical structure and form pedestals where actin accumulates under the elevated cell membrane (Frankel & Philips, 2008).

This type of lesion has been known for decades, and the molecular mechanisms by which EPEC and EHEC manipulate the host-cell actin cytoskeleton have been extensively examined. Meanwhile the putative alterations of other cytoskeletal components have been more or less neglected.

One of the basic disease symptoms that accompany EPEC and EHEC infections is diarrhea. An important pathogenic phenomenon related with diarrhea is the damage of the barrier functions of the intestine leading to uncontrolled influx of water and solutes. These barrier functions in the healthy intestine are maintained due to the integrity of the epithelial junctional complexes (JC). Nevertheless, it was only lately that the effects of EPEC and EHEC on JC have drawn attention.

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As an opposite, recent studies have shown the beneficial effects of probiotic bacteria on JC integrity. The present review will focus on the mode of JC damage by A/E lesion-forming *E. coli* and the promise of the probiotic application against this.

Junctional complexes and epithelial barrier damage by A/E pathogens

Interaction of A/E bacteria with the polarized (epithelial) host cells is accompanied by a complex set of events related to cytoskeleton rearrangement and impairment of the epithelial barrier function. The epithelial barrier function is maintained by two large membrane domains (apical and basolateral), the junctional complex between adjacent cells, composed of the tight junction (TJ), adherens junction (AJ), and desmosomes (D). Bundles of actin filaments support the subapical membrane and some specialized membrane structures such as microvilli and junctional complexes (TJ and AJ).

The main cellular structure that maintains cell polarity and does not allow lateral diffusion of apical proteins as well as pericellular permeability of large soluble molecules is the **tight junction**. Tight junctions are composed of a branching network of transmembrane proteins (claudins and occludins) embedded in both contact membranes, with extracellular domains joining to one another. The cytoplasmic actin directly interacts with claudins and with the zonula occludens (ZO) family of cytoplasmic proteins (Delorme-Axford & Coyne, 2011). ZO-1 binds numerous transmembrane and cytoplasmic proteins and is required for the assembly of both adherens and tight junctions. Depletion of ZO-1 leads to reversible loss of junctional integrity and barrier function and alterations in transepithelial permeability (Van Itallie et al., 2009; Yu et al., 2010).

Another junction, important for epithelial integrity sustaining, localized near the TJ, is the **adherens junction** (AJ). The adhesive properties of AJ are determined by specialized integral membrane proteins comprising the protein family of cadherins. E-cadherin is a protein specific for epithelial cells, and is functionally linked to the generation of a polarized epithelial phenotype. The extracellular region of this protein binds to cadherins of adjacent cells and the intracellular regions of e-cadherins interact with cytoplasmic actin by catenins and other regulatory proteins (Tian et al., 2011). In vitro studies have shown that E-cadherin is not only necessary for adherens junction formation but is also essential for the assembly of other junctional complexes such as desmosomes, gap junctions and tight junctions (Tunggal et al., 2005).

During certain disease states, including viral or A/E pathogens infection, the ability of the intestinal epithelium to regulate absorption and secretion is severely disturbed, resulting in diarrhea (Shifflett et al., 2005; Delorme-Axford & Coyne, 2011). Despite the advances made in understanding of EPEC and EHEC pathogenesis at the molecular level, how the infection triggers diarrhea is still unclear. Both EPEC and EHEC alter intestinal epithelial barrier function by TJ disruption and loss of interaction of the transmembrane TJ proteins occludin and claudin-1 with the cytosolic plaque protein ZO-1, which links them to cortical F-actin (Hanajima-Ozawa et al, 2007). EHEC lower transepithelial electric resistance (TER), increase the paracellular flux of mannitol, and alter ZO-1 distribution by the signaling pathways including myosin light chain kinase and conventional protein kinase C (PKC) (Philpott et al., 1998; Berkes et al., 2003). In contrast, conventional PKCs do not appear to participate in the EPEC-associated disruption of TJs (Berkes et al., 2003). *In vitro* studies have demonstrated that the EPEC effector protein EspF plays a central role in decreasing TER and altering the intestinal epithelial TJ structure. Specifically, EPEC induce disruption of TJ architecture as evidenced by a loss of TJ protein–protein interactions, redistribution of TJ proteins, and the appearance of aberrant TJ strands in the lateral membrane (Shifflett et al., 2005; Hanajima-Ozawa et al, 2007). Surprisingly, recruitment of ZO-1 only does not lead to the loss of TER hence other proteins may be involved in these pathological conditions. The knockout of ZO-1 in mouse epithelial cells causes a pronounced delay in junction assembly, but has no effect on already mature junctions so apparently ZO-1 is not essential for tight junction formation (McNeil et al., 2006).

In a recent study on *in vivo* effect of EHEC infection on murine intestinal barrier function, no alterations of ZO-1 distribution was detected, but reduction of TER, redistribution of occludin and claudin-3 and increased expression of claudin-2 was observed in the colon of infected mice 5-10 days after infection (Roxas et al., 2010). These controversial results need more comprehensive studies on the molecular mechanisms of infection-induced JC alterations.

An important factor known to regulate epithelial monolayer permeability and TJ stability is the adherens junction, composed mainly of the transmembrane protein E-cadherin and cytosolic catenins. It has been shown that EPEC infection induces significant changes in the adherens

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junctions of Caco-2 monolayers, accompanied by significant phosphorylation of PKC-α associated with cadherins, leading to the dissociation of the cadherin/beta-catenin complex (Malladi et al., 2004). Some pathogenic bacteria, such as enteropathogenic *Escherichia coli*, *Shigella flexneri*, and *Campylobacter jejuni*, cleaved E-cadherin of host cells to disrupt the epithelial barrier, but it remained unknown whether this maybe a general virulence mechanism (Hoy et al., 2012).

Probiotic bacteria can counteract A/E lesion-forming pathogenic bacteria by stabilizing junctional complexes and epithelial barrier function

According to the FAO/WHO definition, probiotics are live non-pathogenic microorganisms that have beneficial effects on human health:

http://www.fao.org/es/esn/food/foodandfood_probio_en.stm

The mechanisms by which different probiotics achieve this are variable, numerous and are beyond the scope of this review.

Lately, the capacity of live probiotic bacteria to promote gut barrier function has been the scope of an increased number of studies and the focus of several reviews (Liévin-Le Moal & Servin, 2006; Walter, 2008; Guttman & Finlay, 2009; Lutgendorff et al., 2009.; Kim et al., 2010; Ohland & MacNaughton, 2010; Ahrne et al., 2011; Gill et al., 2011; Ulluwishewa et al., 2011). Using *in vitro* models of cell culture monolayers, probiotic strains have been shown to increase TER, reduce permeability to macromolecules and stimulate the expression of JC proteins. This was monitored in various experiments by TER measurements, Western blotting, qRT-PCR and microarray techniques. On the cellular level, the probiotic bacteia when applied alone have been shown to promote the synthesis of JC components by the cultured cells. This kind of stabilization, in particular that of the TJ, could be a most probable reason for maintaining TER and permeability values even after prolonged co-cultivation with cell cultures in the absence of antibiotics. Table 1. provides several examples on the mechanisms of the action of probiotic bacteria: *Lactobacillus plantarum, L. rhamnosus, L. casei* and *E. coli* Nissle 1917, on cultured-cell monolayers.

These effects further proved beneficial in the presence of diarrheagenic *E. coli* from several pathotypes (Table 1). Importantly, in the strain and dose combinations used in the cited studies, the probiotic bacteria had no antibacterial activity against the pathogens. Hence the effects on JC stability can be considered as pivotal for the observed outputs. While very promising, these data are nevertheless not easy to compare with one another due to the variety of applied experimental protocols (Table 1). Bacteria in the test systems were used in different amounts and proportions. Some data imply that pre-treatment with the probiotic strain was decisive for the beneficial effect (Sherman et al., 2005; Johnson-Henry et al., 2008). In other cases, co-cultivation of the strains was performed (Qin et al., 2009; Liu et al., 2010, Zyrek et al., 2007). Only a few data have shown that the probiotic, if applied after the EPEC, can result in some restoration of TER values and reduction of the JC damage (Parassol et al., 2005). And finally, strain-specific effects have to be underlined as well, and this concerns both the *E. coli* and the probiotic strains (Stöber et al., 2010).

In vivo evidence for effects of probiotic bacteria on uninfected or infected animal models are even sparser. In rats, *L. acidophilus* has been shown to increase occludin expression and maintain gut epithelial tight junctions (Qin et al., 2005). In germfree mice, *L. reuteri* ATCC PTA 6475 protected the animals from disease manifestation caused by EHEC (Eaton et al., 2011).

Metabolic products and molecular mechanisms related with the effects of probiotic bacteria and A/E lesion-forming pathogens on epithelial JC: questions and perspectives

How do these *in vitro* results rely to situations of heavy infectious diarrheas?

Together with strain-specific differences, when the application of live probiotic bacteria is considered usually time is needed to settle the correct type of ecosystem that will ensure the probiotic' benefits. Unfortunately, in infections with A/E lesion-forming pathogens there is rarely a lot of time available. A/E infections are acute, and especially in cases of Shiga toxin- producing strains there is a high risk. Antibiotics are undesirable since massive death of bacteria will result in release of high amounts of toxins thus increasing greatly the danger for the patient. Prospects were sought in the application of bacteriophages, bacteriocins, and low molecular-weight inhibitors, but for the time being in trials none of the interventions proved superior to supportive

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Table 1. *Protective mechanisms of live probiotic bacteria on epithelial barrier integrity in the absence or presence of pathogenic E. coli.*

Probiotic strain	In interaction with:		Effects on TER and junctional complexes	References
	Eukaryote model	*E. coli* strain		
L. plantarum MB452	Caco-2	-	TER increase (bacterial amount-dependent); increase in expression of TJ-related genes, incl. genes for occluding, ZO-1, ZO-2 and cingulin	Anderson et al., 2010
L. plantarum CGMCC 1258	Caco-2; NCM460	*E. coli* O124:NM ATCC 43893 (EIEC); *E. coli* O111:NM ATCC 43887 (EPEC)	Co-cultivation of the probiotic and pathogenic strains protects the cells from the EIEC- and EPEC-provoked damage (i.e., suppression of occluding, claudin-1, JAM-1 and ZO-1 synthesis and TER decrease)	Qin et al., 2009 ; Liu et al., 2010
L. rhamnosus OLL2838	Isolated mouse intestinal epithelial cells	-	Increased expression of ZO-1 and myosin light-chain kinase	Miyauchi et al., 2009
L. rhamnosus R0011	Hep-2, T84	*E. coli* O157:H7 (EHEC); *E. coli* O127:H6 (EPEC)	Preincubation with live *L. rhamnosus* R0052 reduce *E. coli* adherence and number of FAS-positive foci; prevent TER decrease and protect TJ integrity	Sherman et al., 2005
L. rhamnosus GG	MDCK-1 and T84	*E. coli* O157:H7 CL56	Pre-treatment with *L. rhamnosus* diminished EHEC-induced barrier disorders (evidenced by TER and macromolecular permeability /dextran/); prevented TJ disorders evidenced by CLSM after labeling for ZO-1 and claudin 1	Johnson-Henry et al., 2008
L. casei DN-114 001	T84	*E. coli* E2348/69 (EPEC)	When applied before the EPEC, *L. caesei* prevented TER decrease and TJ damage; when applied after the EPEC, *L. caesei* in dose-dependent manner diminish EPEC-caused damage	Parassol et al., 2005
L. acidophilus, *L. fermentum*, *L. gasseri*, *L. rhamnosus*	T84	-	Influence regulation of E-cadherin and β-catenin (DNA microarray and qRT-PCR), modulate epitheliai barrier function	Hummel et al., 2012
E. coli Nissle 1917	T84	-	Differential expression of miRNAs targeting TJ proteins	Veltman et al., 2012
E. coli Nissle 1917	T84	*E. coli* E2348/69 (EPEC)	Co-incubation of EPEC and *E. coli* Nissle 1917 abolished barrier disruption (evidenced by TER values) and enhanced ZO-2 expression	Zyrek et al., 2007

Legend: TER - transepithial electric resistance; TJ - tight junction; EIEC - enteroinvasive *E. coli*; FAS - Fluorescent actin staining test for actin accumulation. Cells shaded green: probiotic strains alone; white cells – protective effects of probiotic strains against *E. coli*-induced damage.

therapy alone (Muniesa et al., 2012).

Provided the beneficial effects of probiotic strains on JC stability and epithelial barrier function, one reasonable task would be the identification of the molecules responsible for these effects. Such molecules may prove useful in therapy by themselves, without the need to apply the live probiotics and wait for the ecosystem to settle.

To check for JS-stabilizing activity of released products, cell-free supernatants of probiotic bacteria were applied to cultured cells. It was shown that cell-free supernatant of *Bifidobacterium lactis* and *B. infantis* reduced TER and prevented the EHEC-induced damage of tight junctions (Ewaschuk et al., 2008; Putaala et al., 2008). Apart from the stabilization of epithelia, released products (total supernatants and selected fractions from them) from *L. acidophilus* La-5 and *L. reuteri* ATCC 55730 were shown to influence EHEC virulence by reducing extracellular concentrations of autoinducer-2 and suppressing quorum sensing-regulated expression of virulence-related genes (Medellin-Peña et al., 2007; Jelčić et al., 2008; Medellin-Peña & Griffiths, 2009). Still of concern, when working with spent culture media from probiotic strains there remains the possibility of strain-specific responses of the A/E-forming pathogens.

The promise in these types of studies is the expectation for a further more precise identification of the molecules responsible for JC stabilization.

One group of such molecules that lately attracted attention is the surface proteins of lactobacilli. Thus, the surface layer adhesive protein (SLAP) from *L. plantarum* CGMCC 1258, while inhibiting EPEC adhesion to Caco-2 cells, stimulated the expression of TJ- associated proteins and promoted the maintenance of TER and TJ structure, the levels of extracellular signal-regulated kinase (EPK) and EPK phosphorylation (Liu et al., 2011b).

Another surface protein in focus is the micro integral membrane protein (MIMP) from *L. plantarum* CGMCC 1258. NCM460 cells transiently infected with MIMP are characterized by increased synthesis of the TJ protein claudin-1 by NCM460 cells. Together with this the protein reduced the attachment of EPEC to the cultured cells (Liu et al., 2011a).

Conclusion

While still quite new, the studies on the interactions between A/E lesion-forming bacteria, probiotic bacteria and epithelial junctional complexes, due to the promise for practical applications, put in focus the need of extensive studies, including more strain combinations, culture cell lines and *in vivo* models. For the time being, we have already some clarity on the cellular targets (e.g., the opposite action of probiotic bacteria and A/E lesion forming bacteria on JC and their components). Yet, by measuring the effects on TER, or JC molecules amounts or expression, we still address the output and not the mechanisms. And these seem to be the correct address for future work.

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