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REVIEW

Enteropathogenic *E. coli*: breaking the intestinal tight junction barrier [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

Enteropathogenic *E. coli* (EPEC) causes acute intestinal infections in infants in the developing world. Infection typically spreads through contaminated food and water and leads to severe, watery diarrhea. EPEC attaches to the intestinal epithelial cells and directly injects virulence factors which modulate multiple signaling pathways leading to host cell dysfunction. However, the molecular mechanisms that regulate the onset of diarrhea are poorly defined. A major target of EPEC is the host cell tight junction complex which acts as a barrier and regulates the passage of water and solutes through the paracellular space. In this review, we focus on the EPEC effectors that target the epithelial barrier, alter its functions and contribute to leakage through the tight junctions.

Keywords

Enteropathogenic *E. coli*, tight junctions, paracellular permeability.

Open Peer Review

Approval Status

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version 2 (revision) 04 May 2016	view	
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Any reports and responses or comments on the article can be found at the end of the article.

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Introduction

EPEC causes diarrhea in infants in developing countries and is responsible for more than a million deaths annually^{1,2}. EPEC infection results in excessive loss of water and electrolytes from the body leading to dehydration and death³. However, the underlying molecular mechanisms are not completely understood. In intestinal epithelial cells, tight junctions regulate the passage of water and solutes and are targeted by EPEC⁴. EPEC directly injects virulence factors into the host cells which target multiple signaling pathways and some have been linked to tight junction disruption⁵. In this review, we focus only on the EPEC effectors reported to be involved in the disruption of tight junctions.

Discussion

EPEC attaches to the apical surface of the intestinal epithelial cells and effaces the microvilli causing localized lesions^{6,7}. EPEC uses a type three secretion system to inject at least twenty five effector proteins into the host cells⁸. Many of the effectors are encoded by genes located on a pathogenicity island called the locus of enterocyte effacement (LEE) although non-LEE encoded effectors have also been identified⁹. One of the effectors, Tir (Translocated intimin receptor) is inserted into the host plasma membrane where it serves as a receptor for intimin, the outer membrane protein of EPEC¹⁰. The LEE- and non-LEE- encoded effectors disrupt host cell function by cytoskeletal reorganization, mitochondrial dysfunction, protein transport defects, suppression of immune responses and epithelial barrier disruption⁸. Since the intestinal tight junctions regulate paracellular permeability, EPEC-mediated disruption of this complex likely contributes to the onset of diarrhea.

Tight junctions seal adjacent epithelial cells and act as (i) a gate that selectively regulates the passage of ions and solutes through the paracellular space and (ii) a fence that prevents the intermixing of apical and basal plasma membrane proteins thereby maintaining cell polarity^{11,12}. The tight junction complex consists of transmembrane proteins, adaptor proteins, small GTPases, kinases, phosphatases, transcriptional and post-transcriptional regulators^{11,12}. The transmembrane proteins regulate cell-cell adhesion and are linked to the cytoskeleton through the adaptor proteins^{11,12}. Paracellular permeability is regulated by the transmembrane proteins belonging to the Marvel-domain containing protein family (occludin, Tricellulin/MarvelD2 and MarvelD3) as well as by members of the claudin family^{11,13}. Additionally, permeability is regulated by tight junction-associated guanine-nucleotide exchange factors for Rho GTPases through the modulation of the actin cytoskeleton¹¹⁻¹³.

Leakage through tight junctions occurs in diarrhea and the EPEC effectors EspF, Map, EspG/G2 and NleA have been implicated in the disruption of host cell tight junctions¹⁴⁻¹⁶.

EspF is a multifunctional effector which disrupts the tight junction barrier¹⁴. The N-terminus of EspF contains mitochondrial and

nucleolus targeting sequences which direct EspF to the mitochondria and nucleolus respectively where it alters their functions¹⁷. The C-terminus of EspF contains three proline rich repeats with binding sites for eukaryotic sorting nexin 9 (SNX9) and neuronal Wiskott-Aldrich syndrome protein (N-WASP)¹⁷. Binding of EspF with SNX9 is required for its recruitment to the plasma membrane but is not sufficient for tight junction disruption as EspF mutants deficient in SNX9 binding also disrupt tight junctions¹⁸. Binding of EspF with N-WASP triggers the activation of the Arp2/3 complex leading to actin polymerization¹⁸. EspF inactivates the sodium-D-glucose co-transporter SGLT-1 through co-operative actions of other EPEC effectors Map, Tir and intimin and also inhibits the activity of the Na⁺/H⁺ exchanger NHE3^{17,19}. EspF from the rabbit EPEC strain E22 has been shown to bind actin and recruit the tight junction proteins ZO-1 and ZO-2 into actin pedestals²⁰ while EspF from the mouse EPEC strain *C. rodentium* is involved in the internalization of claudin-1, -3 and -5 *in vivo*²¹ causing tight junction disruption.

The EPEC effector Map cooperates with EspF in the disruption of tight junctions¹⁴. Like EspF, Map is also targeted to the mitochondria where it alters mitochondrial functions¹⁴. Map activates the Cdc42 GTPase by functioning as its GEF (guanine-nucleotide exchange factor) leading to the formation of transient filopodia²². The C-terminus of Map contains a TRL (Thr-Arg-Leu) motif through which it interacts with EBP50 (ERM-binding phosphoprotein 50) also called Na⁺/H⁺ exchanger regulatory factor 1 (NHERF1)²³. Binding with EBP50 recruits the actin scaffold protein ezrin to this complex linking Map to the actin cytoskeleton. Map also interacts with Na⁺/H⁺ exchanger regulatory factor 2 (NHERF2) and in association with EspF inactivates SGLT-1^{19,24}. The precise mechanism through which Map disrupts tight junctions is not known but it likely occurs through modulation of the actin cytoskeleton.

Another EPEC effector that binds with NHERF2 is NleA²⁴. NleA has been reported to increase paracellular permeability by disrupting the tight junction proteins ZO-1 and occludin¹⁶. NleA inhibits protein secretion from the ER (endoplasmic reticulum) to the Golgi by direct interaction with Sec24, a subunit of the coat protein complex II (COP-II)²⁵. It has been proposed that NleA inhibits the transport of newly synthesized tight junction proteins disrupting the barrier.

The EPEC effector EspG and its homolog EspG2 have been shown to increase the permeability of small tracers through the tight junctions¹⁵. EspG binds the Golgi matrix protein GM130 disrupting the Golgi structure²⁶. Additionally, EspG deregulates the small GTPases Arf1/6 (ADP ribosylation factor 1/6) and Rab1 causing arrest of protein trafficking from the ER to the Golgi^{27,28}. EspG also binds p21-activated kinase at the same site required for Rab1 binding^{28,29} linking the microtubules to this signaling axis indicating that EspG selectively regulates multiple signaling pathways ultimately disrupting tight junctions.

Conclusions

Coordinated actions of the EPEC effectors EspF, Map, NleA and EspG disrupt tight junctions. With the exception of EspF, which forms a complex with ZO-1/ZO-2, none of the other effectors have been reported to interact with any tight junction protein. Therefore, their role in the disruption of tight junctions and onset of diarrhea is possibly due to (i) modulation of the actin cytoskeleton (EspF, Map) or the microtubules (EspG); (ii) inhibition of protein trafficking from the ER to the Golgi (EspG, NleA); (iii) interference with the functions of the Na⁺/H⁺ exchanger (EspF), SGLT-1 (EspF, Map), NHERF-1, -2 (Map, NleA) and (iv) deregulation of aquaporins (EspF, EspG)³⁰.

Author contributions

Both authors contributed in the writing of this manuscript and approved the final version.

Competing interests

No competing interests were disclosed.

Grant information

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Fernando Navarro-Garcia

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This is an interesting review, and I think it makes a positive review to the current knowledge about the effects of EPEC on the tight junctions. However, it will be interesting to note the observations posted by the first reviewer (Dr. Dudeja).

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 17 Apr 2016

Saima Aijaz, Jawaharlal Nehru University, New Delhi, India

Thank you for your comments. I have now amended the review to incorporate the suggestions of Prof. Dudeja.

Competing Interests: No competing interests.

Reviewer Report 05 October 2015

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Pradeep Dudeja

Division of Gastroenterology and Hepatology, University of Illinois at Chicago, Chicago, IL, USA

1. The first sentence conveys the wrong message. EPEC does not cause 1 million deaths but as quoted reference 1, it causes an average of 79000 death/year. The overall deaths due to diarrheal diseases are about 1 million/year.
2. The authors should first outline the mechanisms of EPEC induced diarrhea including recent work on NaCl absorption than focus on tight junctions.
3. The authors should refer to the recent papers on EPEC effects on tight junctions e.g. [Glutfelty et al. Cell Microbiol. 2014](#)

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 06 Oct 2015

Saima Aijaz, Jawaharlal Nehru University, New Delhi, India

Thank you for your comments Prof. Dudeja. We are working on addressing the issues raised by you and will incorporate them in the revised version.

Competing Interests: No competing interests were disclosed.

Author Response 17 Apr 2016

Saima Aijaz, Jawaharlal Nehru University, New Delhi, India

Thank you for your valuable comments. I have now addressed the concerns you had and have incorporated the literature and references related to Cl⁻ absorption as well as references by Glutfelty et al. I appreciate your comments as they have helped me to broaden the focus of this review and helped to highlight the importance of the disruption of ion channels and transporters by EPEC.

Competing Interests: No competing interests.

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