

REVIEW ARTICLE

Virulence factors impair epithelial junctions during bacterial infection

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Abstract

Epithelial cells are typically connected through different types of cell junctions that are localized from the apical membrane to the basal surface. In this way, epithelium cells form the first barrier against pathogenic microorganisms and prevent their entry into internal organs and the circulatory system. Recent studies demonstrate that bacterial pathogens disrupt epithelial cell junctions through targeting junctional proteins by secreted virulence factors. In this review, we discuss the diverse strategies used by common bacterial pathogens, including *Pseudomonas aeruginosa*, *Helicobacter pylori*, and enteropathogenic *Escherichia coli*, to disrupt epithelial cell junctions during infection. We also discuss the potential of targeting the pathogenic mechanisms in the treatment of pathogen-associated diseases.

KEYWORDS

adherens junction, bacterial pathogen, cell junction, epithelial cell, epithelium, infection, tight junction, virulence factor

1 | INTRODUCTION

The epithelium is a continuous sheet of interconnected cells that forms a barrier between the internal and external environments. The two main epithelial types are simple squamous (eg, pulmonary) epithelium and simple columnar (eg, gastric mucosa and intestinal villous) epithelium. The protective function of this cell monolayer depends on a highly organized structure that is maintained by cell junctions connecting adjacent cells and various cytoskeletal components. There are five types of cell junctions, namely tight junctions, adherens junctions, gap junctions, desmosomes, and hemidesmosomes.^{1,2}

The mucosal epithelium consists of one or more cell layers with specialized apical and basolateral surfaces separated by tight junctions that regulate the exchange of substances between the extracellular environment and lumen and between adjacent cells, respectively.³ Claudins and occludin are the two main transmembrane

proteins of tight junctions.⁴ These transmembrane proteins directly bind to cytoplasmic adaptor proteins, such as the zonula occludens (ZO) proteins (ZO-1, ZO-2, and ZO-3) that interact with the actin cytoskeleton.⁵ ZO-1 links tight and adherens junctions by binding to α -catenin and afadin.⁶ Classical cadherins, including E-cadherin, are the main type of adherens junction protein.^{7,8} Desmosomes are composed of desmoglein and desmocollin, which interact with the armadillo family proteins, plakoglobin and plakophilin.^{9,10}

The epithelium forms the first barrier against the microbiota and pathogenic microbes.^{11,12} Under normal conditions, mucosal epithelial cells, mucus-secreting cells, and immune cells form a protective barrier against invading pathogens.¹³ Epithelial cell junctions maintain cell polarity and control diffusion through the epithelium by allowing the selective permeation of substances and excluding bacterial and other types of toxin. Their disruption (eg, under conditions of disease) increases intercellular permeability, resulting in the entry of bacteria, viruses, endotoxins, and macromolecules into

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the systemic circulation.^{8,14-16} This review focuses on the different mechanisms of how exposure of epithelial cells to bacterial pathogens perturb cell junctions, which will be facilitated on the selection of therapeutic target to prevent the invasion of pathogens.

2 | PSEUDOMONAS AERUGINOSA, HELICOBACTER PYLORI, AND ENTEROPATHOGENIC ESCHERICHIA COLI

The world's annual death toll from infectious diseases accounts for about a quarter of deaths. Some of these diseases are caused by pathogenic bacterial infections.¹⁷ For example, acute nosocomial infections like pneumonia are mainly caused by *Pseudomonas aeruginosa* infections.¹⁸ In general, *Helicobacter pylori* and its secretory factors will destroy the gastric barrier and cause the inflammation and proliferation of gastric cell. The excessive cell proliferation increases the risk of gastric cancer.¹⁹ The mortality rate of infantile diarrhea is mainly caused by pathogenic *Escherichia coli*.²⁰ These bacterial pathogens interact with epithelial cells to cause pathology.

3 | PSEUDOMONAS AERUGINOSA SECRETES VIRULENCE EFFECTORS TO DISRUPT CELL JUNCTIONS

During infection, *P. aeruginosa* enters epithelial cells of the mucosal barrier and endothelial cells of the vascular lumen by modulating their cytoskeleton,²¹⁻²³ thereby evading immune surveillance and creating a microenvironment that promotes its proliferation and invasion into deeper tissues. The destruction of the epithelial barrier integrity by changing cell junction components facilitates the intercellular transport of *P. aeruginosa*.²³ Meanwhile, *P. aeruginosa* virulence factors

destroy tight and adherens junctions between epithelial cells, which alter cytoskeletal structure and epithelial cell polarity and increase mucosal permeability, potentially leading to disease.^{22,24}

Effectors of the type II secretion system (T2SS), including proteases along with quorum sensing system signals in bacteria, contribute to the destruction of cell junctions (Figure 1). The extracellular protease LasB, released through the T2SS, degrades junctional proteins between endothelial cells, such as vascular endothelial cadherin and occludin.²⁵ *P. aeruginosa* elastase (PE) increases paracellular permeability and transiently blocks the association between the tight junction proteins claudin-1 and claudin-4, occludin, and tricellulin.²⁶ However, ZO-1, ZO-2, and the adherens junction proteins E-cadherin and β -catenin are unaltered in human nasal epithelial cells treated with PE. On the other hand, exposure to the quorum sensing system signal 3-oxo-C12-HSL decreases total intracellular ZO-1, ZO-3, and junctional adhesion molecule levels and induces occludin dephosphorylation, resulting in tight junction disruption.^{27,28} In addition, 3-oxo-C12-HSL reduces the level of E-cadherin/ β -catenin in the cell through phosphorylation-dependent mechanism, thereby destroying the adherens junction.²⁹

The T3SS undermines the epithelial cell barrier by various means. Pathogenic bacteria inject T3SS toxins through the apical surface of host epithelial cells.³⁰ The secreted T3SS effectors ExoS, ExoT, ExoY, and ExoU destroy the cytoskeleton and cause cell retraction or rounding, eventually leading to cell shedding.³¹⁻³³ Additionally, ExoU induces cell death.^{34,35} The T6SS also plays an important role in the pathogenic mechanism of *P. aeruginosa*.³⁶ Phospholipase D type A (PldA) and PldB activate phosphatidylinositol 3-kinase (PI3K)-Akt signaling to enable epithelial cell invasion. Recent studies have shown that the effector, VgrG2b, simultaneously interacts with α -tubulin, β -tubulin, and the microtubule-organizing center γ -tubulin ring complex to alter the microtubule structure.^{21,37} However, the mechanisms by which *P. aeruginosa* T6SS compromises the epithelial barrier are not fully understood. The

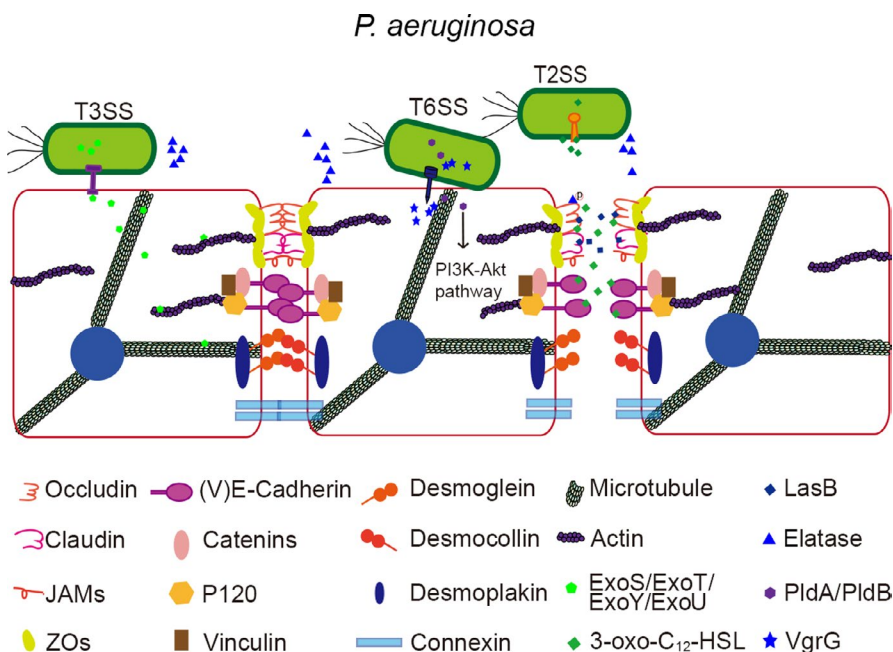


FIGURE 1 Virulence factors of *P. aeruginosa* target epithelial cell junctions during infection. The extracellular protease LasB released through T2SS temporarily blocks the binding of tight junction proteins. The quorum sensing system signal 3-oxo-C12-HSL induces the removal of occludin phosphorylation. The secreted T3SS effectors ExoS, ExoT, ExoY, and ExoU act on the cytoskeleton. The secreted T6SS effector VgrG2b interacts with α -tubulin, β -tubulin, and the γ -tubulin ring complex. PldA and PldB activate PI3K-Akt signaling to infiltrate epithelial cells

secretory systems of *P. aeruginosa* especially T2SS and T3SS toxins play a vital role in disturbing the cytoskeletons and cell junctions. Both the secretory systems are positively regulated by quorum sensing systems, so the quorum sensing systems and its signal molecules can be selected as targets to downregulate the level of secretory toxins generally.

4 | *HELICOBACTER PYLORI* SYSTEMATICALLY DESTROYS CELL JUNCTIONS

Helicobacter pylori colonizes gastric mucosa in about 50% of the human population. Its discovery more than 30 years ago altered the diagnosis and treatment of gastroduodenal disease.³⁸ The perturbation of adherens junctions by *H pylori* infection is a risk factor for gastric cancer,³⁹ which is characterized by the downregulation of E-cadherin. *H pylori* has three major virulence factors that weaken the gastric epithelial barrier, including the cytotoxin VacA, the secreted T4SS system, and effector CagA (Figure 2).⁴⁰

Genes that encoding CagA and T4SS components are both located in the Cag pathogenicity island of pathogenic *H pylori* strains.⁴¹ T4SS facilitates contact-dependent cytoplasmic translocation of CagA and membrane-associated transporter complexes into gastric epithelial cells via interaction with integrin $\alpha 5\beta 1$, which is expressed at the basolateral surface and is protected by tight and adherens junctions.⁴² The serine protease HtrA mediates this interaction while displacing E-cadherin and the interaction between the tight junction proteins occludin and claudin-8, as demonstrated by in vitro experiments using recombinant proteins.⁴³ The HtrA inhibitor, P1 peptide, present at E-cadherin cleavage sites, blocks CagA translocation and phosphorylation and consequently, *H pylori* transmigration.⁴⁴

CagA participates in multiple intracellular processes including inflammation and prevents actin-mediated intracellular trafficking as well as intercellular tight junction maintenance.⁴⁵ CagA activity is regulated by phosphorylation. Non-phosphorylated CagA disrupts adherens junctions in polarized gastric epithelial cells by interacting with E-cadherin, which prevents formation of the E-cadherin/ β -catenin complex. This leads to cytoplasmic and nuclear accumulation and activation of β -catenin, followed by malignant transformation of the cells.^{46,47} Phosphorylated CagA interacts with host proteins such as growth factor receptor-bound protein 2 (GRB2) and ZO-1, resulting in the over-proliferation and migration of gastric epithelial cells.⁴⁸ Most of the effective virulence factors of *H pylori* are protease. Screening the specific inhibitor of these proteases is a potential strategy to inhibit the intruding of *H pylori*.

5 | ENTEROPATHOGENIC *E COLI* DISRUPTS CELL JUNCTIONS THROUGH T2SS EFFECTORS

As a leading cause of infant diarrhea in developing countries, enteropathogenic *E coli* colonizes intestinal epithelial cells and uses

T3SS to inject virulence-associated effector proteins into host cells (Figure 2).²⁰ Genes encoding T3SS components are present in the locus for enterocyte effacement (LEE) pathogenicity island.⁴⁹ One T3SS-dependent virulence mechanism of enteropathogenic *E coli* involves the perturbation of intestinal tight junctions, which is associated with multiple events in host cells, including decreased trans-epithelial resistance of polarized cell monolayers, phosphorylation of myosin light chain and ezrin, dephosphorylation of occludin, dissociation of occludin and ZO-1 from tight junctions, and migration of basolateral proteins such as $\beta 1$ integrin to the apical membrane.^{50,51}

The LEE-encoded T3SS effectors EspF and Map are required for enteropathogenic *E coli*-mediated tight junction destruction.⁵² EspF associates with ZO-1 and ZO-2 scaffold proteins to sequester profilin and prevent actin polymerization, with subsequent rearrangement of occludin and claudin.^{50,53} The interaction between microtubule-associated protein and myosin II regulates the assembly and disassembly of tight junctions, ultimately increasing membrane permeability.⁵⁴ In contrast, the T3SS effectors EspG1 and its homolog EspG2 disrupt tight junctions by targeting microtubules.^{55,56} Like Map and EspF, EspG1/G2 also induces the translocation of occludin from the membrane to the cytosol, which alters tight junction structure.⁵⁰ During enteropathogenic *E coli* infection, EspG1/G2 can prevent the restoration of tight junctions and the epithelial barrier through targeting calcium switch of calmodulins.⁵⁵ NleA may play a role in this process, which is along with anti-inflammatory factors that disrupt the tight junction proteins occludin and ZO-1.^{57,58} T3SS effectors are the mainly factors that involved in the destruction of tight junctions. Inhibition the releases of these effectors through damaging the normal structure of T3SS is a strategy to reduce toxic reaction on epithelial cells.

6 | CONCLUDING REMARKS

It is essential that in the process of preventing microbial infections, epithelial cells can separate the inside of the host from the external environment.⁵⁹ Mucosal epithelia are targeted by pathogenic microorganisms as a means of adhesion, internalization, and exploitation of host cell properties.⁶⁰ During infection, pathogens release virulence factors that destroy tight and adherens junctions, which increases paracellular permeability and compromises epithelial barrier functions through modulation of microtubule and actin filaments. Additionally, interaction with cell junctions allows pathogens to persist at the epithelial cell surface and activate receptors that induce inflammation and promote host cell invasion (Table 1).⁶¹ Tight junctions are also involved in immunity; activation of mucosal immune cells and inflammation have been implicated in the development of pathogen-mediated diseases.⁶⁰

Clarifying the mechanisms by which bacteria disrupt cell junctions can provide insight into the etiology of many diseases and a basis for the development of more effective treatments. For the pathogens that possessing various virulence factors, the upstream regulation system of these factors is a potential therapeutic

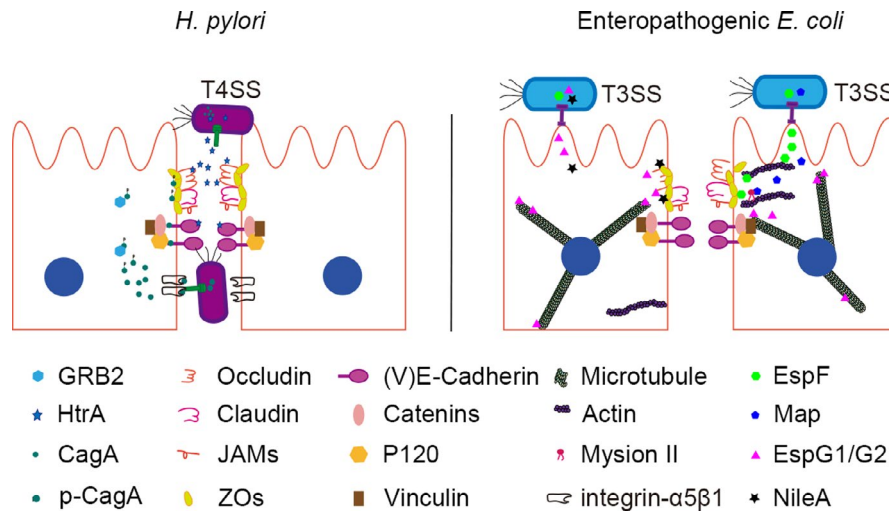


FIGURE 2 Disruption of cell junctions by *H. pylori* and enteropathogenic *E. coli*. A, The T4SS effector serine protease HtrA cleaves E-cadherin and acts on the tight junction proteins occludin and claudin-8. The effector CagA destroys polarized gastric epithelial cells by interacting with E-cadherin. Phosphorylated CagA (p-CagA) interacts with GRB2 and ZO-1. B, EspF and Map are secreted through the T3SS effector system. EspF binds to the ZO-1 and ZO-2 scaffold proteins. The interaction between Map and myosin II regulates the tight junction. In addition, the T3SS effector EspG1 and its homolog EspG2 destroy tight junctions by targeting microtubules, and NileA may destroy occludin and ZO-1

Bacterial Pathogens	The role of bacterial pathogens during infection
<i>Pseudomonas aeruginosa</i>	Alter cytoskeletal structure and epithelial cell polarity; changing cell junction components to destruct the epithelial barrier integrity and increase mucosal permeability
<i>Helicobacter pylori</i>	Inhibition of actin-mediated intracellular trafficking; interact with E-cadherin to prevent formation of junctional complex; weaken the gastric epithelial barrier
Enteropathogenic <i>Escherichia coli</i>	Decreases transepithelial resistance of polarized cell monolayers; disrupt the formation of junctional complex; interrupt the migration of basolateral proteins to the apical membrane

TABLE 1 The role of common bacterial pathogens in disrupting epithelial cell junctions during infection

target, such as the quorum sensing (QS) system in *P. aeruginosa*. Overexpression of the QS quenching system and degradation of the signaling molecules of QS are feasible to reduce the levels of virulence factors which will inhibit the hijack of tight junctions. Some pathogens destroy cell junctions through proteases which are interacted with tight junctions related proteins directly. For example, HtrA and CagA interact with E-cadherin directly during the invasion of *H. pylori*. Small molecules or proteins that block this interaction can be selected as a potential target in the treatment of *H. pylori* associated diseases. Secretory systems are involved in many virulence factors that participated in the destroy of tight junction. Obstruction of the normal secretory systems may interfere this process during the pathogens invasion. Changes in cell junctions may be an important step in the pathogenesis of infectious diseases. Future research needs to target the virulence factors that related to the junctions disruption rather than the microbes, which may be more sensitive and effective.⁵⁹

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

MZ wrote the manuscript and drew the figures. SS and ML conceived the study and revised the manuscript. All authors read and approved the final version of the manuscript.

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