Bacteria and host interactions in the gut epithelial barrier

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The gut mucosa acts as a barrier against microbial invaders, whereas resident commensal and foreign invading bacteria interact intimately with the gut epithelium and influence the host cellular and immune systems. The epithelial barrier serves as an infectious foothold for many bacterial pathogens and as an entry port for pathogens to disseminate into deeper tissues. Enteric bacterial pathogens can efficiently infect the gut mucosa using highly sophisticated virulence mechanisms that allow bacteria to circumvent the defense barriers in the gut. We provide an overview of the components of the mucosal barrier and discuss the bacterial stratagems that circumvent these barriers with particular emphasis on the roles of bacterial effector proteins.

Virulence strategies

Bacterial pathogens have evolved highly sophisticated protein export systems that have been classified into seven types (types I–VII). Many Gram-negative bacterial pathogens have a T3SS, a type IV secretion system (T4SS) or both. T3SSs are evolutionarily and structurally related to flagellar export systems, whereas T4SSs are related to bacterial conjugation systems that translocate DNA. The T3SS consists of approximately 20 highly conserved proteins that form a multiprotein complex composed of the following distinctive parts: (i) a basal body, which is the channel spanning the bacterial membrane periplasm; (ii) a needle structure, which is the core T3SS projection that spans the bacterial membranes and the extracellular space; and (iii) a needle tip, which orchestrates the insertion of the translocon that links the needle to the host membrane. T3SS assembly is a tightly regulated and ordered process in which the basal body is formed in the inner and outer membranes before the needle structure is generated. When the needle is completely

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molecular patterns (PAMPs), which activate the innate immune response to limit and clear the infection. However, *Salmonella* sense the acidification of the SCVs, which occurs as a result of TLR-response to limit and clear the infection. However, *Salmonella* molecular patterns (PAMPs), which activate the innate immune response to limit and clear the infection.

**Microbiota as a major luminal barrier**

The mammalian intestine contains approximately $10^{14}$ commensal bacteria, representing approximately 1,000 species. These luminal commensal bacteria are a predominant part of the microbiota (hereafter we refer to commensal bacteria as the microbiota), and they contribute to intestinal digestive function, inflammation epithelial metabolism and stimulate both epithelial cell proliferation and gut immunity as well as competing with enteric pathogens. Thus, these bacteria can directly or indirectly promote resistance to pathogenic bacterial colonization.

**Microbiota limit bacterial colonization and stimulate epithelial turnover, which occurs at a two-fold faster rate in conventional mice than in germ-free mice.** Comparing the transcriptional profiles of the intestinal epithelia of germ-free and conventional piglets similarly shows that conventional epithelia expresses proteins that contribute to epithelial turnover and are involved in the biosynthesis of mucin, an important component of the gut mucosal barrier, and in immune system priming. Both of these outputs are important for resistance to pathogen colonization. Short-chain fatty acids (SCFAs), such as acetate, propionate, formate or butyrate, are produced as end metabolites by the microbiota and profoundly influence gut barrier function, host immunity, epithelial proliferation and bacterial pathogenesis (Fig. 2a). *Bifidobacteria* species that produce high concentrations of acetate as an end product of carbohydrate metabolism can prevent enteric infection and the release of Shiga toxin, a crucial factor in lethal infection, from the lumen to the blood stream of the host. Examining the role of microbiota in chemically induced colitis in germ-free mice containing selective gut microbiota (called gnotobiotic mice) showed that acetate production by commensal bacteria or acetate administration stimulated the G protein–coupled receptor 43 (GPR43), an acetate chemoattractant receptor on immune cells that regulates inflammatory responses and allows the gnotobiotic mice to efficiently recover from colitis.

Likewise, butyrate, produced mainly by *Fecalibacterium prausnitzii*, *Eubacterium rectale* and *Roseburia* species, prevents bacterial infection by directly affecting virulence gene expression, upregulating the expression of the epithelial antimicrobial peptide LL-37 and providing energy for the colonic epithelium, which in turn allows epithelial proliferation and injury repair. For instance, rabbits that were pretreated with butyrate and infected with *Shigella* had reduced colonic inflammation and bacterial loads in the stool because butyrate upregulates the expression of the *Cap18* gene, which encodes the rabbit homolog of LL-37 and enhances bactericidal activity. Microbiota contribute to the development and function of the immune system. In the intestines of germ-free mice, Peyer’s patches, which are aggregated lymphatic follicles, are poorly developed. In addition, germ-free mice have altered compositions of CD4+ T cells and IgA-producing B cells in the lamina propria, the underlying tissue of epithelial mucosa, compared to conventional mice and rats. The induction of T-lymphocyte subsets is augmented by distinctive species of the luminal microbiota. Segmented filamentous bacteria (SFB), for instance, adhere to the luminal surface of Peyer’s patches in the mouse intestine and strongly stimulate the differentiation of T helper 17 (Th17) cells in the lamina propria, which contribute to resistance against colonization by pathogens. Although the precise mechanism of SFB-promoted Th17 differentiation...
remains partly unclear, SFB adherence to Peyer’s patches seems to induce serum amyloid A, an apolipoprotein produced by the epithelial cells in Peyer’s patches that influences cell adhesion, migration, and proliferation. In another example, the colonization of germ-free mice with a defined mix of Clostridium strains revealed that some Clostridium species stimulate the accumulation of Foxp3+ T regulatory (Treg) cells in the colonic lamina propria, resulting in an amelioration of colitis in chemically induced mouse models of the disease (Fig. 2b). The Clostridium species form a thick bacterial colonizing layer on the epithelium, where they stimulate the production of the active form of TGF-β, which promotes Treg cell differentiation and accumulation in the lamina propria. Some Clostridium species promote the release of the active form of TGF-β, which promotes Treg cell differentiation and accumulation in the colonic lamina propria, thereby preventing inflammatory responses. (c) S. Typhimurium induces inflammation that causes the production of reactive oxygen species that react with luminal thiosulfate to form tetrathionate. Salmonella can use tetrathionate as a terminal respiratory electron acceptor.

Figure 2 | Interaction between the microbiota and gut pathogens. (a) SCFAs produced as end metabolites by the microbiota are important elements that prevent the colonization of bacterial pathogens. (b) The epithelial cell layer separates the gut lumen from the lamina propria, the tissue beneath the mucosal epithelium that contains various myeloid and lymphoid cells. The lamina propria contains a large number of immune cells and acts as an effector site for IgA production and T-cell responses. Luminal antigens are transported through M cells to the subepithelial dome of Peyer’s patches, which contain dendritic cells, T cells and B cells. SFB adhere to the luminal surface of Peyer’s patches, and Th17 cells differentiate in the lamina propria. Some Clostridium species stimulate the release of the active form of TGF-β, which promotes Treg cell differentiation and accumulation in the colonic lamina propria, thereby preventing inflammatory responses. (c) S. Typhimurium induces inflammation that causes the production of reactive oxygen species that react with luminal thiosulfate to form tetrathionate. Salmonella can use tetrathionate as a terminal respiratory electron acceptor.

Mucin as a major epithelial cell surface barrier

The gut epithelium is covered by a thick mucus layer that acts as a frontline defense barrier against the microbiota and pathogenic bacteria. However, enteric bacterial pathogens have evolved mechanisms to circumvent this mucus barrier and directly access the epithelial surface. The mucus layer is largely composed of mucin, which contains various digestive enzymes and antimicrobial peptides as well as immunoglobulins. Mucins are produced and secreted by goblet cells throughout the intestinal tract, and they help remove the gut contents and intruding microbes. Epithelial cells and Paneth cells secrete antimicrobial peptides that help prevent bacteria from penetrating the inner mucus layer. Bacterial colonization is therefore limited to the outer loose mucus layer, where the bacteria interact with the oligosaccharides of secreted mucin glycoproteins, whereas the inner layer is devoid of bacteria (Fig. 3a).

Although mucin is constitutively secreted at a basal level, mucin secretion can change in response to luminal conditions or bacterial infection. Mucin secretion by goblet cells is regulated by a variety of secretagogues, including microbial products, inflammatory...
mediators, hormones, signaling mediators, growth factors and infectious bacteria. Mucin expression can also be controlled at the transcriptional level through several mechanisms. For example, the recognition of PAMPs via TLRs and NOD-like receptors activates the downstream inflammatory signaling pathways that induce the transcription of mucin genes. During *Pseudomonas aeruginosa* infection of the colonic epithelium, lipopolysaccharide (LPS) induces *Muc2* gene transcription by activating a TLR4-dependent pathway (Fig. 3b). In contrast, infection with the Gram-positive bacteria *Staphylococcus aureus* induces the transcription of the *Muc2* gene through a TLR-independent pathway. The bacterial lipoteichoic acid activates platelet-activating factor receptor, which is a G protein-coupled receptor, and transduces signals to the epidermal growth factor receptor (EGFR) via ADAM10 metalloprotease activity. Activation of this pathway, in turn, stimulates the Ras–MAPK–NF-κB pathway and activates mucin gene transcription (Fig. 3b).

The mucus layer acts as a protective barrier against colonization. For instance, inoculation of *Muc1*−/− mice (*Muc1* encodes a cell-surface mucin) but not wild-type mice with *Campylobacter jejuni* results in rapid systemic infection. Similarly, approximately five-fold more *H. pylori* colonize *Muc1*−/− mice than colonize wild-type mice. Wild-type mice develop only mild gastritis after *H. pylori* infection for 2 months, whereas *Muc1*−/− mice develop severe atrophic gastritis and lose gastric parietal cells. Intriguingly, gastric epithelial cells can shed Muc1 in response to *H. pylori* infection, and the released Muc1 acts as a decoy that binds pathogens, preventing bacterial adhesion to the epithelial surface (Fig. 3b). Thus, the interaction of pathogens with mucin can contribute to pathogen elimination due to rapid mucin secretion and mucus shedding. Cell-surface mucins can limit the colonization of bacterial pathogens.

Enteric bacterial pathogens have evolved mechanisms to sense and circumvent the mucus barrier and reach the epithelial cell surface. Although the mechanism remains unclear, *C. jejuni* uses Muc2 as an environmental cue to modulate the expression of genes involved in colonization and pathogenesis. The flagella and chemotaxis systems are used by many enteric pathogens to traverse the mucus layer and access the epithelial cell surface. Disrupting flagella function reduces pathogenicity, highlighting the ability of bacterial motility to promote infection. Some enteric pathogens deploy enzymes, such as Pic, a serine protease that degrades mucin (Table 1), StcE, a zinc metalloprotease that cleaves mucin-type O-glycosylated proteins (Table 1); Hap, a zinc metalloprotease; TagA, a Hap homolog with metalloprotease activity that is distinct from Hap (Table 1), or mucin-degrading enzymes, which degrade mucin oligosaccharides and reduce mucus viscosity and the release of antimicrobial peptides. Thus, although the mucus layer prevents bacterial pathogens from accessing and breaching the epithelial lining, pathogens have developed specific mechanisms to penetrate the mucus barrier.

**Epithelial integrity**

Cell-cell and cell–basement membrane interactions in the epithelium form a barrier that prevents bacteria from translocating to the subepithelial layer. Epithelial cell–cell adherence is sustained by tight junctions (apical multiprotein complexes that form a selectively permeable seal between cells), adherens junctions (junctures subjacent to tight junctions that form a strong interaction with junctional molecules between cells), gap junctions (paired connexin hemichannels) and desmosomes (adhesive junctions between cells). The apical junctions, composed of tight junctions and adherens junctions, consist of transmembrane and cytoplasmic scaffolding proteins that associate with actin filaments and regulate epithelial paracellular permeability.

Tight junctions are composed of zonula occludens (ZO-1 and ZO-2) and junctional adhesion molecules (JAM-1, claudin and occludin), and they functionally segregate the apically expressed membrane proteins from those expressed on the basolateral membrane on polarized epithelial cells (Fig. 4a). However, tight junctions are highly dynamic structures, and their permeability is regulated by several physiological and pathophysiological conditions. For example, inflammatory cytokines can disrupt tight junctions and impair gut barrier integrity. Treating epithelial monolayers with TNF-α or IL-1β increases tight junction permeability by stimulating transcription and activation of myosin light chain kinase (MLCK). The phosphorylation of MLC by MLCK stimulates perijunctional actomyosin contraction, leading to the distortion of transmembrane tight junction strands and increased paracellular permeability. Therefore, the breakdown of tight junctions during bacterial infection results in gut barrier failure, often termed leaky gut, which subsequently facilitates the translocation of bacteria and the luminal

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**Figure 3 | The mucus layer as a gut barrier.** (a) The mucus layer serves as a frontline defense against intruding bacterial pathogens. (b) Pathogenic bacterial infection causes Muc2 production through TLR-dependent or TLR-independent NF-κB pathways, whereas some bacterial pathogens have mucolytic enzyme activities that destroy these mucus layers. PACR, platelet-activating factor receptor.
contents across the damaged epithelial lining and further promotes barrier damage and disease progression.

Bacterial pathogens use tight junctions to facilitate their interactions with the epithelium and penetrate into deeper tissues. Because tight junction components interact with the actin cytoskeleton to sustain the integrity of cell-cell adherence, gastrointestinal pathogens subvert several signal pathways that regulate tight junctions such as the protein kinase C pathway, which regulates actin filaments, and the Rho GTPase pathway, which modulates actin cytoskeleton rearrangement46 (Fig. 4b).

T3SS effectors or toxins from enteric pathogens can subvert host signal pathways that regulate actin organization and tight junctions. Some effectors directly modulate Rho GTPases by acting as guanine exchange factors (GEFs), which convert inactive GDP-bound Rho GTPases to the active GTP-bound forms and disrupt tight junction structure and function. Because the components of tight junctions are constantly recycled between the plasma membrane and cytosol, regulating tight junction components is important to maintain epithelial integrity. To this end, Rho GTPases play pivotal roles: RhoA triggers actomyosin assembly and contraction through Rho-associated kinase (ROCK)-mediated MLC phosphorylation, and Rac1 and Cdc42 activate the PAK–LIMK–cofilin pathway, which leads to actin filament stabilization and tight junction modification (Fig. 4b). Because signaling of Rho GTPases is tightly interconnected, the activation or inactivation of one Rho GTPase can cause an imbalance in other networks, resulting in actin disorganization and tight junction disruption47. For example, EPEC disrupts tight junctions by altering actin cytoskeleton remodeling through the T3SS effectors EspM, Map and NleA (Fig. 4c and Table 1)47. Map activates Cdc42, whereas EspM activates RhoA through its GEF activity and alters the localization of tight junctions48–50. NleA binds to and interferes with COPII-dependent protein trafficking, thus altering tight junction components and permeability51. C. rodentium infection of murine intestinal epithelial cells results in diminished barrier function associated with disrupted tight junctions52. C. rodentium secretes a lymphocyte inhibitor factor called lymphostatin, which can upregulate RhoA and downregulate Cdc42. When mice were infected with C. rodentium lacking the lfpA genes, which encode lymphostatin, the intestinal barrier mediated by tight junctions was not compromised53.

The gastrointestinal pathogen Vibrio parahaemolyticus delivers one effector that induces cell rounding, VopS (Table 1), via the T3SS54. VopS catalyzes the AMPylation—the transfer of AMP from ATP to the threonine residues—of proteins such as Rac1, Cdc42 and RhoA via a phosphodiester bond55, thereby blocking Rho GTPase signaling and reducing actin cytoskeleton remodeling. This ultimately disrupts the integrity of the actin cytoskeleton in epithelial cells56 (Fig. 4d).

S. Typhimurium also delivers effectors that disrupt tight junctions57–58 (Fig. 4e). SopE indirectly activates Rho GTPases, whereas SopE and SopE2 act as GEFs that activate Rac1 and Cdc42 (ref. 6). Activation of Rho GTPases by the S. Typhimurium effectors facilitates the uptake of bacteria by epithelial cells, which also affects tight junction structure and function. Tight junction disruption by S. Typhimurium can be blocked with an inhibitor of geranylgeranylation, a function required for Rho GTPase activation, indicating that Rho GTPase activation by these effectors is a cue for tight junction disruption56. S. Typhimurium SopE-mediated intestinal inflammation is abrogated in Casp–1/−, Il1r1/− or Il18/− mice, indicating

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Figure 4 | Bacterial pathogens breach epithelial integrity. (a) A schematic of junction proteins in polarized epithelial cells. Tight junctions form a semipermeable barrier that restricts the paracellular passage of water and nutrients and maintains cell polarity. Tight junctions are composed of ZO-1, JAM, claudin and occludin. Adhesion junctions are composed of members of the cadherin family. AJ, adherence junction. (b) PKC and Rho GTPases trigger tight junction disruption by modulating the actin cytoskeleton. The phosphorylation of MLC by MLCK induces the contraction of perijunctional actomyosin and breaches the tight junction barrier. (c) Bacterial pathogens target tight junctions and breach epithelial integrity to promote colonization, obtain nutrients and access the underlying tissues. Some bacterial pathogens deliver effectors that modulate Rho GTPases. (d) V. parahaemolyticus delivers VopS, which has AMPylation activity, and targets Rho GTPase. AMPylated Rho GTPase blocks signaling and reduces actin cytoskeleton remodeling, thereby inducing cell rounding. (e) The SipA, SopB, SopE and SopE2 effectors of S. Typhimurium disrupt tight junctions by activating the Rho GTPase.

that caspase-1 activation and cytokine production are involved in the disruption of tight junctions. These studies suggest that S. Typhimurium manipulate actin filaments and intestinal inflammation, allowing the bacteria to disrupt epithelial tight junctions.

H. pylori targets cell-cell junctions, and disrupting these allows the bacteria to directly access basolaterally localized receptors such as β1-integrin or c-Met. H. pylori CagA and VacA (Table 1) can be delivered into the gastric epithelium, where they disrupt tight junctions and adherence junctions. H. pylori delivers CagA via a T4SS, which targets ZO-1 and the PAR1 (also called MARK) polarity-regulating kinase and attenuates gastric epithelial barrier function by altering epithelial cell polarity. CagA triggers the mislocalization of ZO-1 from tight junctions to the bacterial attachment sites. In addition, CagA binds to PAR1 and inhibits its activity, thereby disrupting epithelial integrity. Although the mechanism of tight junction disruption during H. pylori infection is still speculative, VacA also helps open tight junctions. In addition, H. pylori urease (Table 1) stimulates MLCK in the stomach through unknown mechanisms, leading to the dysregulation of tight junctions. H. pylori upregulates the phosphorylation of IL-1 receptor type I, which subsequently activates ROCK and disrupts tight junctions through a process that is independent of CagA and VacA. Thus, H. pylori effectors can disrupt gut integrity via multiple mechanisms.

Intestinal epithelial cells are tightly linked to neighboring cells via cell-cell junctions and form defensive barriers to prevent bacterial intrusion. However, many bacterial pathogens can breach epithelial integrity, expose the basolateral cell surface and alter epithelial polarity to promote colonization and access the underlying tissues.

Epithelial cell death and shedding

Gut epithelial cells undergo cell death and shedding under physiological and pathophysiological conditions. As many as ~3% of total epithelial cells from the villi, which are composed of finger-like projections that protrude from the epithelial lining of the intestinal wall, are extruded in the lumen in the human and mouse intestine. Therefore, a balance among epithelial cell proliferation, differentiation, migration, death and exfoliation is required to maintain homeostasis and epithelial integrity; disrupting this balance can lead to pathological conditions. For instance, inflammatory diseases such as ulcerative colitis increase tight junction permeability as well as epithelial apoptosis, proliferation and turnover. In mice, TNF-α, a cytokine that is elevated in inflammatory bowel disease, promotes both epithelial cell apoptosis and shedding. During bacterial infection, epithelial cell death is a host defense response that eliminates damaged cells as well as pathogens and ultimately emits alarm signals that activate the innate immune system.

Some enteric pathogens can either induce or prevent cell death to gain a survival advantage in the gut. When these bacteria colonize the epithelium, they first prevent cell death to preserve their replicative foothold, whereas later, they induce the demise of host cells to facilitate egress from their niches. Shigella invasion of epithelial cells results in mitochondrial dysfunction—mediated necrosis-like cell death, but the cell death response is antagonized until the bacteria have fully replicated through activation of prosurvival pathway via the recognition of PAMPs (peptidoglycan) by Nod1 (ref. 72). Cell death signaling becomes dominant through a BNI3- and cyclophilin D-dependent process, which leads to mitochondrial permeability and cell death.

S. Typhimurium infection of porcine intestines or Salmonella typhimurium serovar Dublin infection of human intestinal epithelial cells induces caspase-3-dependent and caspase-3-independent cell death, which occurs only after prolonged exposure to the pathogens, implying that the pathogen can suppress cell death at the initial stage of infection. The Salmonella AvrA and SopE effectors counteract epithelial cell death. AvrA, an acetyltransferase that
Figure 5 | Bacterial countermeasures against epithelial cell death and turnover. (a) The gut epithelium undergoes continuous self-renewal to maintain tissue homeostasis and eliminate damaged cells. The epithelial cells that line the gastrointestinal lumen are constantly renewed through a process in which stem cells generated in the crypts migrate to the tip of the villi and ultimately peel off into the lumen. (b) Epithelial cell death and turnover are highly dynamic responses to bacterial infection and limit persistent bacterial colonization, whereas some bacterial pathogens deploy countermeasures against rapid epithelial turnover to prolong their survival. Selected effectors are shown.

modulates MAPKs and inhibits the JNK signaling pathway, helps dampen inflammatory and cell death responses75,76 (Fig. 5b). SopB, an inositol phosphatase, activates the serine-threonine kinase Akt, which plays a critical role in cell survival and proliferation, allowing Salmonella to counteract epithelial cell apoptosis77,78. EPEC delivers a subset of effectors (Table 1) via the T3SS, and these effectors have an impact on a variety of host cell-signaling pathways that are involved in cytoskeletal rearrangement, disruption of epithelial intercellular junctions, epithelial cell death and immune modulation79. EspF disrupts the mitochondrial membrane potential, resulting in cytochrome c release and apoptosis80,81. EspF interacts with Abc12, an antiapoptosis factor, and reduces its expression within the mitochondria, thereby inducing apoptosis82. H. pylori colonization causes apoptosis of gastric pit cells by inducing oxidative stress, in which p38 activation and Bax oligomerization have a key role. VacA-mediated translocation of Bax oligomers into the mitochondria in response to H. pylori infection results in decreased mitochondrial membrane potential and apoptosis83.

Epithelial cell death at the early stage of infection is predominantly a consequence of host defense mechanisms. However, some pathogens elicit epithelial cell death, whereby they breach the epithelial barrier, access the underlying tissues and obtain nutrients. After the bacteria fully propagate within epithelial cells, their death facilitates bacterial egress and spread to other host tissues.

Epithelial turnover

The gut epithelium undergoes continuous self-renewal to maintain tissue homeostasis and eliminate damaged cells. There is a balance between the elimination of damaged cells and the generation of new cells from a stem cell population in the intestinal crypt (Fig. 5a). Aside from physiological renewal, epithelial turnover can accelerate (or decelerate) in response to changes in the luminal environment. Epithelial turnover of the Drosophila melanogaster gut accelerates in response to infection with Erwinia carotovora, Pseudomonas spp. or Serratia marcescens80,84. In mice, C. rodentium stimulates β-catenin signaling via upregulating casein kinase 1ε (CK1ε) production. As a result, C. rodentium stimulates the proliferation of cryptic stem cells, leading to hyperplasia and increased crypt length85. These studies show that increased epithelial cell turnover in response to bacterial pathogens is a host defense mechanism that eliminates infected cells, limits persistent bacterial colonization and maintains tissue homeostasis.

Some bacterial pathogens, such as H. pylori, Shigella or EPEC, counteract rapid epithelial turnover and maintain epithelial cells as a replicative niche. Colonization of the gastric superficial epithelium by H. pylori causes an imbalance between epithelial cell proliferation and apoptosis86. H. pylori CagA hijacks multiple cell signaling pathways to promote persistent colonization of the gastric epithelium by activating transcription factors such as nuclear factor of activated T cells, serum response factor, NF-κB and MAPKs which modulates MAPPKs and inhibits the JNK signaling pathway, helps dampen inflammatory and cell death responses75,76. This results in increased epithelial cell proliferation and apoptosis86. H. pylori CagA stimulates the production of the ubiquitin-like protein NEDD8) to regulate epithelial cell survival and proliferation via a T3SS into progenitors. In HeLa cells and rabbit ileal-loop infection models, Ipab is shown to negatively regulate cell-cycle progression by directly binding to Mad2L2, an anaphase-promoting complex inhibitor, to promote bacterial colonization88.
cycle by causing both G1-S and G2-M arrest93-95. Cif selectively binds to Nedd8 and deamidates Gln-40, inhibiting the activity of the Nedd8-conjugated Cullin-RING E3 ubiquitin ligases96 (Fig. 5b). Inhibition of these enzymes may induce formation of actin stress fibers and contribute to cell-cycle arrest97. During EPEC infection, the effector NleH (Table 1) inhibits apoptosis by binding to the endoplasmic reticulum six-transmembrane protein Bax inhibitor (BI-1). Independent of its kinase activity, NleH inhibits several cellular responses associated with apoptosis, including elevation of cytoplasmic Ca2+ concentrations, nuclear condensation and activation of caspase-3 (ref. 95). In a mouse model of C. rodentium infection, NleH inhibited procaspase-3 cleavage at C. rodentium colonization sites in the intestine98. Although the exact role of NleH in EPEC infection remains partly speculative, it is likely that NleH facilitates EPEC pathogenesis by reducing enterocyte loss, which sustains the replicative niche.

These findings suggest that epithelial turnover changes in response to bacterial infection to limit persistent bacterial colonization. Enteric pathogens can deploy countermeasures to prevent rapid turnover of epithelial cells in order to maintain epithelial cells as a replicative niche.

Conclusions and perspectives

Mutualism between the gut mucosa and microbiota is the most important factor in sustaining gut homeostasis. Enteric bacterial pathogens have highly evolved mechanisms to shift the balance of host-microbiota mutualism for the pathogen's benefit. Many enteric Gram-negative pathogens deploy T3SS effectors that promote the survival and colonization of these pathogens within the gut by disrupting microbiota-mediated colonization resistance, circumventing the innate barriers of the gut and modulating mucosal innate immune responses. T3SS effectors are bacterial executioners that can directly or indirectly modify the target host proteins and subvert host cellular and immune functions. The development of various new technologies, bioinformatics, animal and other eukaryotic model systems will help elucidate the unidentified mechanisms of effector proteins and provide insights that can be used to develop new antimicrobial drugs and therapeutics as alternatives to antibiotics. Antibiotics have been extensively used to selectively target bacterial pathogens to treat and prevent many infectious diseases99,100. However, antibiotics also disrupt the composition of the microbiota, often leaving an imprint on the composition of the microbiota after the antibiotic treatment has been discontinued, thereby promoting the emergence of antibiotic-resistant bacteria. Thus, we need to generate new drugs that target bacterial pathogenesis rather than kill pathogenic bacteria99,100. In this regard, T3SS inhibitors have been recently considered new drugs, and several small-molecule compounds (both synthetic compounds and natural products) have been identified as T3SS inhibitors. For example, a group of salicyliden acylhydrazides inhibits the T3SS activity of many bacterial pathogens by blocking the assembly of the T3SS needle complex, thereby preventing T3SS effector secretion and virulence99,100.

New insights are providing a blueprint for the dynamic networks among the commensal (and pathogenic) microbes and the gut immune system in condition of both health and disease. In recent years, there has been an explosion of new analytical tools that help to identify the microbial and host factors affecting the mutualistic relationship between the microbes and gut. One relatively new approach, systems biology, allows us to comprehensively understand how the gut commensal (and pathogenic) bacteria establish replicative niches and how the host immune system responds to bacterial infection. We envision that an understanding of the mechanisms by which bacteria disrupt the mutualism between the mucosa and microbiota as well as promote infection of the gut epithelium will provide avenues to develop new therapeutics that control microbial infection, excessive host inflammatory responses, microbiota composition and gut homeostasis.

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This study, in addition to ref. 23, shows the benefits of intestinal inflammation during S. Typhimurium infection. S. Typhimurium use tetrathionate, which is produced as a result of inflammation, as an electron acceptor and gains a growth advantage to overcome the host microbiota.


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Competition financial interests
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