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<Review>

Physiological understanding of host-microbial pathogen interactions in the gut

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Abstract : The gut epithelial barrier, which is composed of the mucosal layer and the intestinal epithelium, has multiple defense mechanisms and interconnected regulatory mechanisms against enteric microbial pathogens. However, many bacterial pathogens have highly evolved infectious stratagems that manipulate mucin production, epithelial cell–cell junctions, cell death, and cell turnover to promote their replication and pathogenicity in the gut epithelial barrier. In this review, we focus on current knowledge about how bacterial pathogens regulate mucin levels to circumvent the epithelial mucus barrier and target cell-cell junctions to invade deeper tissues and increase their colonization. We also describe how bacterial pathogens manipulate various modes of epithelial cell death to facilitate bacterial dissemination and virulence effects. Finally, we discuss recent investigating how bacterial pathogens regulate epithelial cell turnover and intestinal stem cell populations to modulate intestinal epithelium homeostasis.

Keywords : epithelial cells turnover, intestinal epithelium, intestinal stem cells, mucosal layer, pathogens, tight junction

Introduction

The gastrointestinal mucosal surface not only is critical for intestinal homeostasis but also serves as the first line of defense against many foreign antigens and pathogens on the intestinal epithelium and luminal content [20]. Underneath the mucus layer, a layer of intestinal epithelium is well sustained by tight cell-cell junctions to maintain the physical and physiological intestinal barrier [75]. The intestinal epithelium is composed of approximately 250 simple cells that include stem cells which are inserted with paneth cells at the crypt base and various functional cells on the villi, such as enterocytes, tuft cells, goblet cells and enteroendocrine cells [75] (Fig. 1). The stem cells continuously generate proliferating transit-amplifying (TA) cells, which differentiate into the various functional cells on the villi to replace the epithelial cells which are lost via apoptosis at the villus tip. This intestinal epithelial cell turnover occurs every 3–5 days in the small intestine and every 5–7 days in the colon to prevent the accumulation of dead or damaged cells [11].

Enteric bacterial pathogens have various bacterial infectious stratagems by which to circumvent the intestinal epithelium barrier of the gut [6]. The attachment of pathogens to an appropriate target site of the intestinal epithelium is a crucial initial step to resist the fluid flow of the luminal contents and the peristalsis of intestinal contraction [52]. Once bound to the epithelial surface, bacterial pathogens may colonize and modify all physiological mechanisms of the intestinal epithelium to exploit mucosal host defenses for their own benefit by facilitating the invasion of pathogenic microorganisms and by producing virulence factors [52]. Virulence factors of bacteria are mainly proteins or other molecules that have evolved to affect the host [24]. Many pathogens possess a specific set of virulence factors that are injected into the host cells by special secretion machines such as the type 3 secretion system. Other bacterial pathogens also directly produce virulence factors to manipulate the host protective and stress responses [24]. Hence, discovering virulence factors is important when attempting to understand bacterial pathogenesis and the interactions between bacteria and hosts, which may also have implications with regard to novel targets in the area of drug and vaccine development [80].

In this review, we focus on recent studies that have investigated the underlying molecular mechanisms of host-pathogen interactions as well as the biological significance of these interactions in the mucosal layer and the intestinal epithelium.

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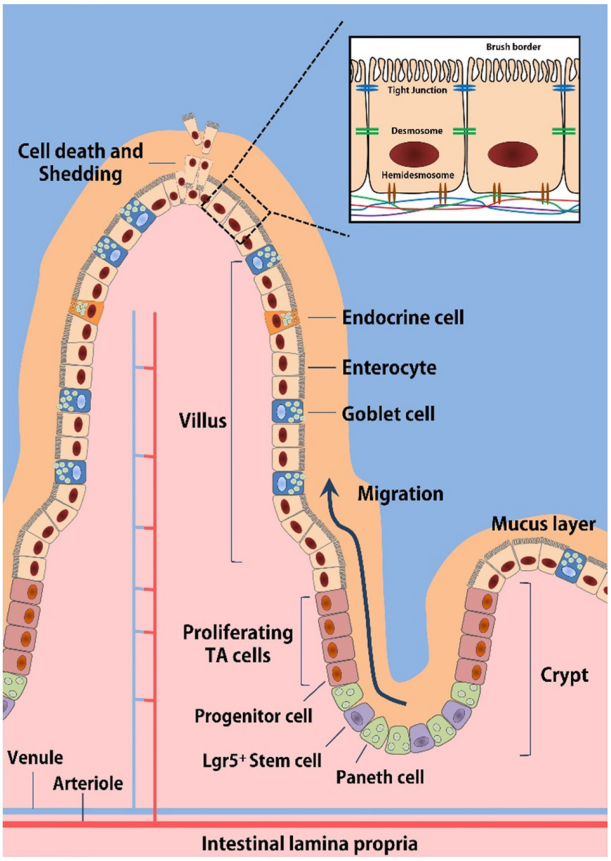

Fig. 1. Intestinal epithelial organization and turnover. The gut epithelial barrier composed of mucosal layer and intestinal epithelium. The intestinal epithelium is organized into the villus and crypt regions. Intestinal stem cells, characterized by high $Lgr5^{+}$ expression, continuously self-renew and generate rapidly dividing transit-amplifying (TA) daughter cells via asymmetric cell division at the small intestinal crypt base. The TA progenitor cells differentiate into paneth cells that migrate downward to the crypt bottom, as well as into absorptive enterocytes, enteroendocrine cells, and mucin-secreting goblet cells that migrate upwards to the tops of the villi. During the differentiation and migration process, tight/adherens junctional proteins are formed at the cell-cell contact sites in order to form continuous defensive barriers. At the villous tips, the migrating cells eventually undergo apoptosis and are shed into the lumen. Thus, intestinal epithelium exerts great efforts to keep the balance between cell death and shedding of damaged cells at the villus tip and the generation of new cells in the crypt.

Distinct bacterial stratagems to circumvent the epithelial mucus barrier

Gastrointestinal mucosal surfaces serve as the frontline defense barrier by promoting the clearance of bacterial pathogens and by separating them from the intestinal epithelium [28]. Damage and impairment of the mucus layer facilitate intestinal epithelium-pathogen interactions and the invasion of pathogenic microorganisms to destabilize the homeostasis of immune responses of the host [59]. The mucus layer mainly contains mucins together with various digestive enzymes and antimicrobial peptides as well as immunoglobulins. In contrast to the outer loose mucus layer that is usually colonized by commensal bacteria interacting with the oligosaccharides of secreted mucin glycoproteins, the inner layer, which is protected from bacteria penetration, is firmly attached to the intestinal epithelial layer and covered with a membrane-bound mucin glycoprotein coating called the glycocalyx. The glycocalyx possesses membrane-specific domains which are involved in cell signaling and cell interaction with intestinal epithelial cells, paneth cells, endocrine cells, and stem cells to maintain a bacteria-free shield [21]. Thus, the organization of the inner mucus layer of the gut is very important for intestinal epithelium homeostasis, as defects in or loss of this allows bacteria to reach the epithelium**.** In addition, mucins are rapidly secreted by goblet cells throughout the intestinal tract during a bacterial infection, and they serve as a decoy that binds pathogens to prevent bacterial adhesion to the intestinal epithelium, thereby limiting the colonization of bacterial pathogens.

The major component of this intestinal mucus layer is the *O*-glycosylated mucin (Muc)2 in the human small intestine and colon, and with Muc5 as the predominant component in the stomach, they play an important role as a physiological barrier via the formation of an enormous net-like mucus polymer barrier [59]. Indeed, *Muc2* deficiency in mice, which lack an inner mucus layer, causes the spontaneous development of inflammation, gross bleeding and increased paracellular permeability by unusual commensal bacteria colonization [30, 71]. *Muc1*- or *Muc2*-deficient mice also show enhanced susceptibility to enteric bacterial infections such as *Campylobacter jejuni*, *Helicobacter* (*H.*) *pylori*, *Salmonella* (*S.*) *enterica serovar Typhimurium*, and *Citrobacter rodentium* [54, 85]. Moreover, a lack of β1,3-N-acetylglucosaminyltransferase, which synthesizes *O*-glycans on mucins in mice, results in a thinner mucus layer that is more prone to enteric bacterial infections [85] and dextran sodium sulfate-induced colitis [2]. In addition, altered expressions of multiple mucins, *Muc1*, *Muc3*, *Muc4*, and *Muc5*, are observed in patients with ulcerative colitis and Crohn's disease [64]. Thus, an intact mucosal layer producing mucins plays a critical role in providing protection against the multiple inflammatory responses induced by invading pathogens and toxins in order to maintain the intestinal barrier function.

Bacterial pathogens use the following two distinctive strategies to regulate mucin levels: the enzymatic degradation of mucin proteins and the transcriptional regulation of mucin genes. Although the mucus layer protects the intestinal epithelial lining from bacterial infection, enteric pathogens have evolved to produce mucus-degrading enzymes such as glycosydases, which break down mucin oligosaccharides and digest mucus barriers to reach the host epithelial layer. For example, Pic, a serine protease produced by *Shigella*, is known to degrade mucin directly [26]. StcE, a zinc metalloprotease pro-

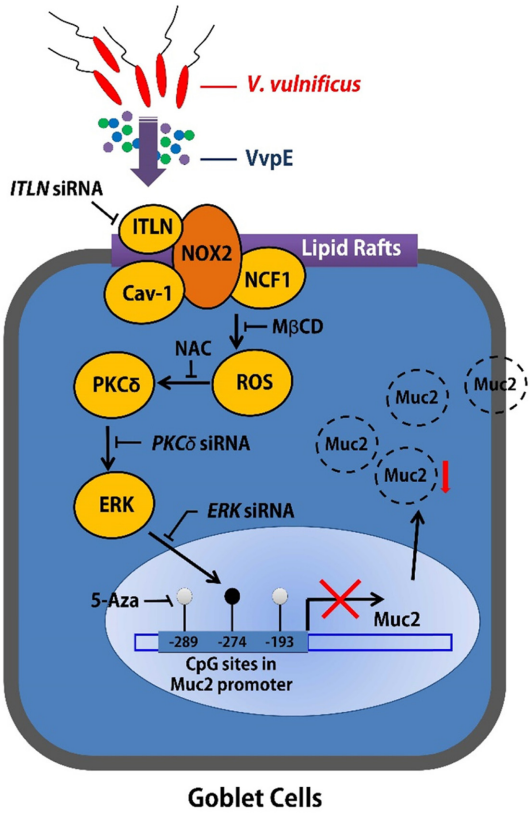

Fig. 2. *Vibrio* (*V.*) *vulnificus* VvpE induces the transcriptional repression of Muc2. VvpE is an important element of *V. vulnificus* for transcriptional repression of *Muc2* in mucus-secreting HT29-MTX cells. VvpE in acting through lipid raft-associated intelectin-1b (ITLN) facilitates the production of reactive oxygen species (ROS) via recruitment of NADPH oxidase (NOX) enzymes. The bacterial signaling of rVvpE through ROS production is uniquely mediated by the phosphorylation of PKCδ and ERK that are responsible for the region-specific methylation of the *Muc2* promoter at the –274 site to repress Muc2 production in mucus-secreting HT29-MTX cells. Cav-1, Caveolin-1; NCF1, neutrophil cytosolic factor 1; MβCD, methyl-β-cyclodextrin; PKCδ, protein kinase C δ; ERK, extracellular signal-regulated kinases; 5-aza, 5-azacytidine.

duced by *enterohemorrhagic E. coli* (EHEC) [60], along with Hap and TagA produced by *Vibrio (V.) cholerae* [73] have also shown to cleave mucin oligosaccharides and reduce mucus viscosity. On the other hand, enteric bacterial pathogens also have specific various bacterial infectious stratagems through which to control mucin gene expression by regulating growth factors [77], transcription factors [25], and the methylation status [27]. Pathogenic bacteria, including *Pseudomonas (P.) aeruginosa*, and *Staphylococcus (S.) aureus*, induce the transcription of the *Muc2* gene through the Src-Ras-MAPK-NF-κB pathway via Toll-like receptors (TLR)4, a family of pattern-recognition receptors mediating the defensive responses to bacterial pathogens [51] or the TLR-independent Ras-MAPK-NF-κB pathway via the activation of the epidermal growth factor receptor (EGFR) [50]. In addition, HofF, an adhesion molecule produced by *H. heilmannii*, plays a critical role in IL-1β-induced gastric *Muc13* expression [19]. In contrast, we recently reported that *V. vulnificus*, VvpE, induces the transcriptional repression of *Muc2* and that VvpE acting through lipid-raft-associated intelectin inhibits *Muc2* expression by stimulating the methylation of the *Muc2* gene promoter through the reactive oxygen species (ROS)-dependent activation of the PKCδ/ERK pathway in mucus-secreting HT29-MTX cells [45] (Fig. 2). These results indicate that enteric bacterial pathogens distinctively regulate intestinal Muc2 expression with different modes of infection mechanisms to circumvent the epithelial mucus barrier of the gut. Thus, the development of new agents that can neutralize bacterial toxin activity or that highlight new pathogenic signaling pathways involved in Muc2 repression may provide potential therapeutic strategies for bacterial pathogen infections in the intestinal epithelium.

Bacterial pathogens alter the structure and function of the intestinal tight/adherens junction (TAJ) barrier

Below the mucous layers, intestinal epithelial cells are tightly joined by tight and adherens junctions (TAJ) in order to form a continuous defensive barrier against bacterial invasions [61]. The TJ is established through the transmembrane proteins occludin and claudin and by the cytoplasmic scaffolding proteins ZO-1, -2, and -3. The AJ consists of the transmembrane protein E-cadherin and the intracellular components p120-catenin, β-catenin and α-catenin. However, enteric bacterial pathogens have various bacterial infectious stratagems to circumvent the epithelial barrier of the gut [6, 17]. Many bacterial pathogens have developed specific mechanisms with which to invade deeper tissues by disrupting defensive barriers, exposing the basolateral cell surface, and altering the degree of paracellular permeability. Thus, TAJ plays structurally and functionally important roles in bacterial pathogen interaction with the intestinal epithelium.

During a bacterial infection, TAJ dysregulation is mainly caused by dysregulation of Rho GTPase activity or by the directly targeting of TAJ proteins [6]. Concerning the regulation of Rho GTPases, *i.e.*, Rho, Rac, and Cdc42, Rho preferentially regulates TJ by controlling the contraction of apical actin-myosin filaments through Rho-associated kinase (ROCK)-mediated MLC phosphorylation, whereas Rac and Cdc42 mainly coordinate the assembly–disassembly of AJ components via the PAK-LIMK-cofilin pathway [61]. The enzymatic modification of Rho GTPases by bacterial toxins can cause an imbalance in the dynamic TAJ structures linked to the actin cytoskeleton and manipulate the focal adhesion, intracellular signaling, and transcription regulation processes [9]. For example, *V. parahaemolyticus* delivers the virulence factors VopS and VopO to catalyze the covalent modification of Rho GTPases, leading to the disruption of the organiza-

tion of the actin cytoskeleton and the epithelial barrier function [32, 84]. *Enteropathogenic E. coli* (EPEC) injects different effectors into the host cytoplasm, Map and EspM. Map preferentially regulates Cdc42, whereas EspM modulates the nucleotide state of RhoA to activate it as guanine nucleotide exchange factors and disrupts TAJ by altering the actin cytoskeleton remodeling process [3]. In contrast, *Citrobacter rodentium* disrupts TAJ by producing lymphostatin, which inhibits Cdc42 activity and increases RhoA activity [7]. Thus, these results suggest that bacterial pathogens distinctively subvert the host signal pathways that regulate actin organization and tight junctions by manipulating the Rho GTPases.

On the other hand, many pathogens directly target epithelial TAJ proteins. With regard to AJ proteins, fragilysin is an example of a toxin produced by *Bacteroides* (*B.*) *fragilis* that disrupts the TAJ barrier by the proteolytic degradation of the AJ molecule E-cadherin [81]. *Listeria* (*L.*) *monocytogenes* also target E-cadherin on the basolateral side of exposed neighboring cells to induce bacterial internalization [10]. On the other hand, a pathogenic protein of *H. pylori*, CagA, has also been shown to interact with the TJ protein ZO-1 and with Par1, a regulator of cell polarity, thereby triggering TAJ disruption [1, 8]. This effector protein also targets E-cadherin to promote the release of β-catenin from the adherence complex to disrupt the TAJ barrier [62]. *H. pylori* has profound effects on ROCK activation through unknown mechanisms, leading to TJ dysregulation [17, 43]. In addition, the pathogenic proteins known as metalloprotease HA/P of *V. cholerae* have also been shown to degrade the extracellular domain of the TJ protein occludin [82]. These results indicate that TAJ proteins are common targets of pathogens to promote TAJ dysregulation and ultimately to invade deeper tissues.

In addition, the tight junction has been characterized as a platform for intercellular receptor complexes, including Annexin A2 (ANXA2), in the mediation of physiological signal transduction [44, 69]. Specifically, ANXA2 is known to mediate the tight junction assembly, possibly by linking juxtaposed exoplasmic leaflets to build up the lipid platform [44]. The importance of ANXA2 in microbial pathogenesis is underscored by the finding that EPEC adherence induces the concentration of cholesterol and glycosylphosphatidylinositol-anchored proteins at sites of bacterial contact, where ANXA2 is recruited to the cytoplasmic membrane surface, possibly stabilizing raft patches and their links to the actin cytoskeleton beneath adhering to EPEC [86]. Thus, ANXA2 plays a critical role as a host mediator of bacterial pathogens in the lipid raft of tight junctions [47, 48]. These results are further supported by a previous study in which several enteric toxins that interact with lipid rafts, including *H. pylori* vacuolating toxin [22, 41] and *C. perfringens* enterotoxin [76], showed the ability to regulate the aggregation of many signaling molecules via lipid raft clustering as an initial attachment platform, thus having a virulence effect on intestinal pathogenesis. Therefore, the modulation of the host proteins that interact with bacterial toxins and the identification of the functional toxins of bacterial pathogens which alter the structure and function of the intestinal TAJ barrier could lead to the development of an important therapeutic strategy for bacterial infections.

Bacterial pathogens elicit a diverse array of cell death responses

Intestinal epithelial cell death and shedding are host defense responses that eliminate senescent epithelial cells as well as enteric pathogens to maintain intestinal tissue homeostasis and proper barrier function [6]. Intestinal epithelium exerts great efforts to maintain the balance between cell death and the shedding of damaged cells at the villus tips and to manage the generation of new cells in the crypt [49, 79]. During a bacterial infection, therefore, intestinal epithelial cell death and shedding are critical events which eliminate instances of bacterial colonization and spreading by exfoliating infected epithelial cells or readily replenishing neighboring epithelial cells [79]. However, many bacterial pathogens are equipped with highly evolved infectious stratagems that manipulate intestinal epithelial cell death to promote their replication and pathogenicity [35, 37, 57, 58] (Fig. 3). During the early stages of infection, bacterial pathogens prevent cell death to preserve their colonization activity and survival, whereas later, they induce cell death to facilitate bacterial dissemination and propagate the virulence effects. For instance, *Salmonella* prevents epithelial cell death by producing AvrA and SopB [35, 39]. To gain a survival advantage for *Salmonella* in the gut, AvrA reduces inflammatory and cell death responses through the inhibition of the JNK signaling pathway [35], and SopB plays a critical role in cell survival and proliferation [39] (Fig. 3A). After prolonged exposure to these pathogens, however, *Salmonella* eventually induces the caspase-3–dependent and caspase-3–independent cell death, implying that this pathogen can counteract epithelial cell death at the initial stage of an infection [58, 67].

Specifically, the host-cell death response stemming from a bacterial infection results in various modes of cell death, including apoptosis, necrosis and pyroptosis. Many enteric bacterial pathogens, such as *S. typhimurium* [35, 58], *H. pylori* [36, 37], and EPEC [57], are known to induce apoptosis through unique cellular mechanisms that regulate intrinsic/extrinsic environmental factors such as oxidative stress, the MAPK signaling pathway, mitochondrial damage, and caspase-3 activation (Fig. 3B). In apoptosis, bacteria are retained within apoptotic bodies/blebs that are engulfed by phagocytes [33]. On the other hand, necrosis is associated with caspase-independent inflammation accompanied by nuclear swelling and the release of cellular contents upon a bacterial infection [4] (Fig. 3D). Pyroptosis has been characterized as a type of programmed cell death coordinated by inflammasome-mediated caspase-1 activation that results in

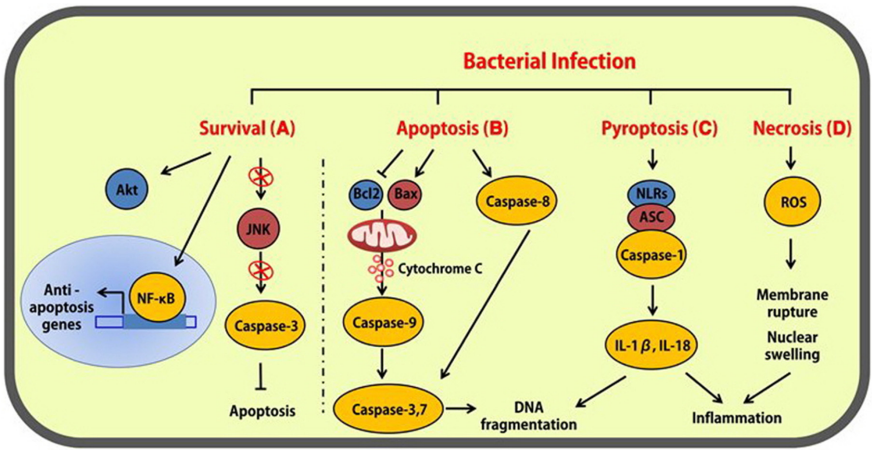

Fig. 3. Host cell survival and death pathways induced by bacterial infection. Bacterial pathogens prevent cell death through the activation of Akt and NF-κB signaling pathways and the inhibition of the JNK signaling pathway (A). However bacterial pathogens are capable of creating many host-cell death pathways, including apoptosis, pyroptosis, and necrosis. Apoptosis is associated with caspase-3 activation that is regulated by two different pathways, the mitochondria-mediated pathway and receptor-mediated caspase-8 activation pathway (B). Pyroptosis is coordinated by multiprotein oligomer consisting of NLRs, ASC, and caspase-1 that result in the maturation of the pro-inflammatory cytokines IL-1β and IL-18 (C). Necrosis is associated with caspase-independent inflammation and ROS production accompanied by nuclear swelling and the release of cellular contents upon a bacterial infection (D). JNK, c-Jun N-terminal kinases; NF-κB, nuclear factor-kappa B; Bax, Bcl2 associated X protein; NLRs, nucleotide-binding oligomerization domain receptors; ASC, apoptosis-associated speck-like protein containing a CARD; IL, Interleukin.

the maturation of the pro-inflammatory cytokines IL-1β and IL-18 [4, 23] (Fig. 3C). Pyroptosis is crucial for controlling microbial infections and can be regulated by the production of ROS or by potassium efflux or lysosomal damage [4, 23, 55]. For instance, an invasion by *Shigella flexneri* of epithelial cells results in necrosis-like cell death through a BNIP3- and cyclophilin D-dependent process [16], whereas an *L. monocytogenes* infection in murine macrophages results in caspase-1-dependent pyroptosis [66].

Although bacterial pathogens are capable of creating many host-cell death pathways, studies have indicated that the mitochondrial cell death pathway, the NF-κB-dependent cell death pathway, and the inflammasome-mediated cell death pathway are the major pathogenic strategies for many bacterial pathogens [4]. The mechanisms by which bacterial pathogens generate mitochondrial cell death pathways have been extensively documented and are generally recognized as involving the blocking or delaying of cell death signals to promote bacterial survival and replication. EPEC postpones host cell death by producing NleH, which interacts with Bax inhibitor-1 [31]. *H. pylori* activate EGFR via an unknown mechanism and stimulate anti-apoptotic signaling involving Akt and Bcl2 [83]. In contrast, we recently found that *V. vulnificus* produces the VvhA to stimulate an imbalance of the Bcl-2/Bax ratio, the release of mitochondrial cytochrome c, and caspase-3/-9 activation during its promotion of apoptotic cell death [46] (Fig. 4). This indicates that *V. vulnificus* effectively generates the mitochondria-mediated cell death pathway by producing VvhA with modes of action that differ from those of EPEC and *H. pylori*.

The cell death pathways mediated by NF-κB and inflam-

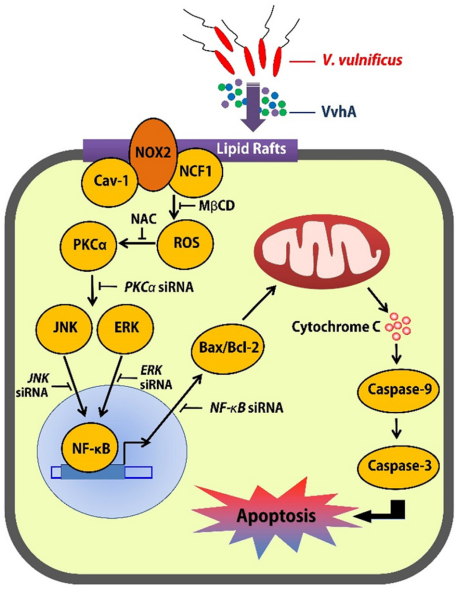

Fig. 4. *V. vulnificus* VvhA triggers the mitochondrial cell death pathway. VvhA, an extracellular haemolysin pore-forming toxin produced by *V. vulnificus*, is a functional virulence factor which triggers the mitochondrial cell death pathway in intestinal epithelial cells. VvhA acting on lipid rafts induces NOX2-mediated ROS production, with this being necessary for PKCα/ERK/JNK activation in intestinal epithelial cells. It thereby stimulates the NF-κBp65-mediated Bcl-2/Bax imbalance to facilitate the cytochrome c-mediated caspase-9/-3 activation in promoting mitochondrial cell death. NOX2, NADPH oxidase 2; NCF1, neutrophil cytosolic factor 1; NAC, N-acetylcysteine; PKCα, protein kinase C alpha.

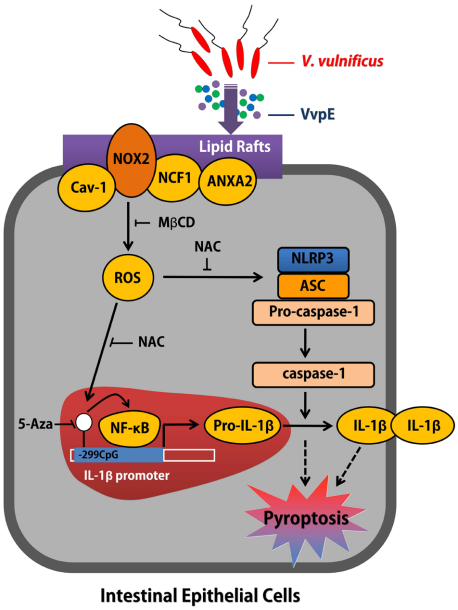

Fig. 5. *V. vulnificus* VvpE induces IL-1β expression and pyroptosis. VvpE is a critical virulence factor of *V. vulnificus* regulating intestinal epithelial cell death pathways mediated by NF-κB and inflammasome activation. VvpE induces the recruitment of NOX2 and NCF1 into membrane lipid rafts coupled with ANXA2 to facilitate the production of ROS. The bacterial signaling of rVvpE through ROS production is concurrently mediated by the transcriptional regulation of NF-κB to promote the production of pro-IL-1β as well as the activation of NLRP3 inflammasome to stimulate caspase-1-meidated pro-IL-1β maturation, and it thereby triggers the pyroptotic cell death. ANXA2, annexin A2; NLRP3, NOD-, LRR- and pyrin domain-conatining 3; IL-1β, Interleukin-1 beta.

masome activation are also used as targets for bacterial pathogens. *V. vulnificus* produces VvhA and VvpE, where VvhA induces NF-κB-dependent cell death via lipid raft-mediated ROS production by the distinct activation of PKCα and ERK/JNK in intestinal epithelial cells [46] (Fig. 4), while VvpE stimulates region-specific transcriptional occupancy by NF-κB in the IL-1β promoter, with the ability to induce pyroptosis via the NLRP3 inflammasome [48] (Fig. 5). However, *Shigella flexneri* inhibits cell death by interacting with the bacterial sensing Nod-like receptor (NLR) family NOD1 to activate the Rip2-NF-κB-Bcl2 pathway. *Yersinia* infections also result in the prevention of inflammasome activation and pyroptosis through the secretion of YopK, which interferes with the T3SS recognition process by NLRP3 and NLRC4 [15]. Therefore, the most prominent feature of host-pathogen interaction is to elicit a diverse array of cell death responses by regulating the mitochondrial cell death pathway, the NF-κB-dependent cell death pathway, and the inflammasome-mediated cell death pathway.

In addition to the roles of bacterial pathogens in activating cell death pathway, autophagy has been proposed as alternative cell death pathway that is manipulated by bacterial infections [42]. Autophagy is a tightly regulated catabolic process consisting of several steps, including initiation, autophagosome formation, fusion with lysosomes, and degradation. It serves to maintain cellular homeostasis and protect the host cell from harmful stimuli [29, 38]. Autophagy is essential for destroying bacteria within lysosomal compartments by engulfing the bacteria within autophagosomes that induce lysosomal fusion and degradation [29, 38]. However, autophagic cell death (ACD), which is referred to as a form of type II programmed cell death, is caused when the excessive autophagy flux is presented in the cells, when the cell is devastated due to an infection, or when apoptosis or pyroptosis is inhibited [42]. For instance, *S. aureus* and *L. monocytogenes* have been shown to utilize autophagy for their replication and dissemination during the process of killing the host cell [12, 53, 68]. We recently reported that VvhA produced by *V. vulnificus* upregulates autophagy flux through c-Src-mediated ROS production and ERK/eIF2α phosphorylation in promoting the necrotic cell death of intestinal epithelial cells [74]. In contrast, *S. typhimurium* and *M. tuberculosis* invade host cells and dwell within cytosolic vacuoles for their replication, which is targeted by autophagy for bacterial restriction and host cell survival [13, 14]. Importantly, *Shigella* was shown to avoid autophagy by producing IcsB, which prevents Atg5–VirG interaction and the recruitment of LC3 [5, 40]. These results indicate that bacterial pathogens have various infectious stratagems by which to modify the autophagic process in manipulating the cell death mechanism. Many pathogens manipulate the autophagosome machinery for their replication and control their phagocytosis using immune cells to facilitate their dissemination into the blood stream [18]. Thus, understanding the process of autophagy may reveal why certain cells may be more or less susceptible to pathogen-induced cell death and may reveal novel therapeutic targets.

Host-bacterial pathogen interactions in intestinal stem cells and epithelial cell turnover

During a bacterial infection, intestinal stem cells (ISCs) continuously replace lost or damaged intestinal epithelial cells to maintain a state of intestinal epithelium homeostasis [75]. Although ISCs reside in specific microenvironments called niches that are important for modulating stem-cell fate and epithelial differentiation, few studies have reported bacterial interactions with ISCs in intestinal crypts. Recent success with regard to the identification and isolation of human intestinal epithelial stem cells harboring leucine-rich repeat-containing G-protein coupled receptor 5 ($Lgr5^{+}$) from the small intestine and colon has led to the culturing of functional intestinal epithelial units termed organoids or enteroids [65]. A recent report published by Nigro *et al.* [56] showed that the bacterial peptidoglycan motif muramyl-dipeptide (MDP) induces the survival of intestinal organoids by interacting with the Nod receptor (NOD), which is the host recep-

tor that recognizes the bacterial product. Moreover, the authors illustrate that $Lgr5^+$ intestinal epithelial stem cells expressing Nod2 trigger stem cell survival, leading to sufficient cytoprotection from oxidative stress, independent of the responses of Paneth cells. In contrast, a more recent report published by Sigal *et al*. [72] showed that *H. pylori* directly colonize the surfaces of gastric stem and progenitor cells, driving the activation of $Lgr5^+$ stem cells to manipulate the chronic inflammatory response. Correspondingly, a recent study has also shown that EHEC has the ability to colonize on human colonoids derived from adult proximal colonic stem cells, where EHEC targets a main component of the colonic mucus, Muc2 and a microvillar resident protein, protocadherin 24, affecting the colonic mucus level and the brush border cytoskeleton [34]. These data therefore provide the first evidence of epithelial cell regeneration and turnover as a consequence of the interaction of ISCs and bacterial products and pathogens, also showing that bacterial colonization on the surface mucus layer and in ISCs niche are required for stem cell activation in the gut.

Aside from the direct interaction between ISCs and bacterial products/pathogens, the intestinal epithelium undergoes continuous self-renewal and turnover through stem cell population and epithelial cell proliferation, differentiation, migration, death and exfoliation along the crypt-villus axis to replace the epithelial cells which are lost via apoptosis at the villus tip [75]. Many pathogens use distinctive strategies to control epithelial turnover. *Citrobacter rodentium* accelerates the proliferation of cryptic stem cells via β-catenin signaling, leading to hyperplasia accompanied by an increased crypt length [70], suggesting that the host increases epithelial cell turnover to compensate for the cell death induced by the bacterial pathogens and thus limits persistent colonization. In contrast, earlier work showed that an intestinal infection of *H. pylori* inhibits cell proliferation without affecting cell migration, thereby delaying epithelial cell turnover [63]. Instead *H. pylori* promotes the persistent colonization of the gastric epithelium by activating many transcription factors [78], providing evidence that enteric pathogens can manipulate epithelial cell turnover to promote their own growth. Thus, host-pathogen interactions during the intestinal epithelial cell turnover process may reflect the altered stem cell population, epithelial proliferation, migration, and cell death of the intestinal epithelium.

Conclusion

In this review, we highlight the underlying molecular mechanisms of host-pathogen interactions in the regulation of the mucus barrier function, tight/adherens junctions, cell death mechanism, intestinal stem cell population, and epithelial cells turnover. However, the intestinal epithelium is not only colonized by the enteric microbial pathogens but also co-exists with the trillions of beneficial commensal microorganisms, referred to as the microbiota. A symbiotic relationship exists between microbiota and the mucosal layer and intestinal epithelium to reinforce the gut barrier function. Conversely, enteric microbial pathogens have various infectious stratagems that allow bacteria to promote their replication and colonization in the presence of competing microbiota. Hence, a comprehensive understanding of the interactions between bacterial pathogens and their hosts and the microbiota could aid in the development of new therapeutic strategies to control bacterial infections, ultimately providing deeper insight into various intestinal disorders.

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