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Review

Bacterial Interactions with the Host Epithelium

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DOI 10.1016/j.chom.2010.06.006

The gastrointestinal epithelium deploys multiple innate defense mechanisms to fight microbial intruders, including epithelial integrity, rapid epithelial cell turnover, quick expulsion of infected cells, autophagy, and innate immune responses. Nevertheless, many bacterial pathogens are equipped with highly evolved infectious stratagems that circumvent these defense systems and use the epithelium as a replicative foothold. During replication on and within the gastrointestinal epithelium, gastrointestinal bacterial pathogens secrete various components, toxins, and effectors that can subvert, usurp, and exploit host cellular functions to benefit bacterial survival. In addition, bacterial pathogens use a variety of mechanisms that balance breaching the epithelial barrier with maintaining the epithelium in order to promote bacterial colonization. These complex strategies represent a new paradigm of bacterial pathogenesis.

Introduction

The interplay between bacteria, the gut epithelium, and host innate defense responses are among the most critical factors that determine the fate of bacterial infections and disease outcomes. The gut epithelium is constantly exposed to dietary and environmental antigens, as well as many resident and foreign bacterial pathogens that establish an infectious foothold in the gut. In some cases, bacterial pathogens breach the epithelial lining to feed on nutrients and disseminate deeper into tissues. In defense against these pathogens, the epithelium has multiple layers of microbial sensing and intrinsic defense systems built in that act as countermeasures against microbial intruders. In the gut epithelium, the elements that provide defense against infection include: commensal microbiota, epithelium integrity, rapid epithelial cell turnover, quick epithelial cell exfoliation and sealing, and innate immune systems. The commensal bacterial flora in the lumen can compete with foreign bacterial intruders and interfere with their colonization of the epithelial surface. In addition, the intestinal flora can help balance immune tolerance with immune activation as well as influence epithelial metabolism, proliferation of stem cells in the crypt, and production of a mucus layer (Leser and Mølbak, 2009; Neish, 2009; Sansonetti and Medzhitov, 2009). Mucosal immunity consists of innate and acquired immune systems and plays a paramount role as the major host defense mechanism against microbial survival and dissemination inside the host (Macdonald and Monteleone, 2005). Both the epithelial monolayer and the mucosal surface act as physical and biological barriers against microbial invaders. The integrity of the epithelial monolayer is sustained by tight cell-cell junctions, and the mucosal surface is covered by a mucin layer containing various digestive enzymes, Muc2 (a major large gel-forming mucin), secreted IgA, and many other antimicrobial agents, including β-defensins, cathelicidins, bactericidal/permeability-increasing protein, and chemokines (Leser and Mølbak, 2009; Mason and Huffnagle, 2009). To maintain epithelial integrity and tissue homeostasis and to avoid the accumulation of damaged or dead cells, gut epithelial cells are constantly renewed throughout our lifespan by a constant supply of progenitors from cryptic stem cells (Radtke and Clevers, 2005). In the gut epithelium, well-differentiated epithelial cells constantly undergo cell death and cell shedding, which contributes to epithelial cell turnover and prevents persistent bacterial colonization. In addition, epithelial cells that are damaged by microbial infection, exogenous and endogenous stresses, or immune disorders are rapidly exfoliated from the epithelium and then readily replenished by neighboring epithelial cells. This epithelial cell turnover helps expel colonized pathogens, confine bacterial spreading, and localize inflammation (Chichlowski and Hale, 2008; Radtke and Clevers, 2005).

In order to overcome these host defensive mechanisms and establish successful infection, many bacterial pathogens use highly evolved infectious stratagems. Many bacteria can subvert and usurp host signaling cascades and defensive functions, are equipped with versatile mechanisms that modulate and circumvent the host defense systems, and have highly evolved intracellular adaptive and survival systems. In the past decade, our knowledge of bacterial infections has greatly increased, and we have a better understanding of the mechanisms that bacteria use to invade and colonize their hosts, such as adherence and invasion of host cells, intracellular multiplication and trafficking, inter- and intracellular spread, and circumvention of the innate immune response. However, we have relatively limited understanding of the bacterial strategies that circumvent epithelial barrier functions, especially those that prevent rapid epithelial turnover, epithelial cell death, epithelial exfoliation, and autophagic clearance. In this review, we focus on the relationship between epithelial barrier functions and bacterial countermeasures used during infection with specific emphasis on bacterial mechanisms that counterbalance epithelial damage with epithelial maintenance to promote bacterial colonization.

Epithelial Cell Turnover

The balance between eliminating dead (and damaged) cells and supplying new cells is an important mechanism to ensure perpetual renewal and sustained tissue homeostasis. If these

mechanisms are uncoupled during the renewal of the gastrointestinal lumenal epithelium, there is an increased risk of microbial infection, microulcers, tissue injury, hyperplasia, and in some cases tumorigenesis (Loktionov, 2007; Radtke and Clevers, 2005). The rates of epithelial cell turnover vary greatly among tissues. In some tissues, such as the lung and bladder, the epithelium is basically quiescent until injury. In contrast, the epithelial cells that line the gastrointestinal lumen rapidly and routinely turn over. These cells 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. This process takes 4–5 days in the intestine but only 2–3 days in the gastric epithelium. In addition to this basal level of renewal, the epithelium can accelerate (or dampen) this turnover in response to various stimuli, immune disorders, bacterial infection, and gut microbiota. For example, the dense population of intestinal microbiota has a marked impact on the host epithelium with many influences on the host gut physiology (Leser and Mølbak, 2009). Savage et al. showed that intestinal epithelial turnover was twice as fast in conventional mice compared to germ-free mice (Savage et al., 1981). In addition, Bates et al. noted that the presence of microbiota promoted zebrafish gut differentiation (Bates et al., 2006). Chowdhury et al. performed transcriptome profiling of the small intestinal epithelium in germ-free and conventional piglets and showed that resident microbiota induced the expression of genes that contribute to intestinal epithelial cell turnover, mucus biosynthesis, and priming of the immune system (Chowdhury et al., 2007). Chowdhury et al. showed that microbiota not only induced a subset of genes that encode receptors and transcriptional factors related to IFN-inducible genes but also anti-inflammatory genes that prevent excessive inflammation (Chowdhury et al., 2007). This study confirmed that the interplay between the intestinal epithelium and microbiota has evolved to maintain a physiological state of inflammation during continuous microbial exposure and that this basal level of inflammation helps maintain intestinal barrier function and homeostasis while preventing excess inflammatory responses (Leser and Mølbak, 2009; Neish, 2009; Stecher and Hardt, 2008). The intestinal epithelium is closely involved in modulating innate and adaptive defense systems because it interacts with lumenal microbial- and dietary-derived antigens as well as immune cells such as macrophages, dendritic cells, and γδ intraepithelial T lymphocytes (Leser and Mølbak, 2009). Furthermore, activation of lumenal pathogen-recognition receptors (PRRs), such as Toll-like receptors (TLRs), in response to microbiota induces moderate NF-κB activation, while activation of basolateral PRRs, such as TLR5 by flagellin, induces a robust inflammatory response (Ishii et al., 2008; Leser and Mølbak, 2009). Therefore, disrupting the relationship between the gut microbiota and the host or intrusion of bacterial pathogens into the epithelial lining can cause acute and chronic intestinal inflammatory diseases.

Epithelial Turnover Is Responsive to Bacterial Infection

Bacterial pathogens are capable of intimately adhering to the apical surface of epithelial cells and/or invading spaces that are usually devoid of bacteria, such as the epithelial cell cytoplasm, epithelial interstitial spaces, intestinal crypts, and lamina propria, where these pathogens elicit robust defense responses. A murine colonic hyperplasia model of *Citrobacter rodentium* is the most reliable model to study the attaching and effacing (A/E) of bacteria, such as enterohemorrhagic *Escherichia coli* (EHEC) and enteropathogenic *E. coli* (EPEC). This model has been used to discover dramatic increases in the levels of casein kinase I ε in response to *C. rodentium* infection, which can stimulate β-catenin signaling (Sellin et al., 2009), which activates the stem cell compartment. Consequently, *C. rodentium* stimulates the proliferation of cryptic stem cells, which leads to hyperplasia and an increase in the length of the crypts between 6 and 20 days after infection of the mouse intestine (Sellin et al., 2009).

Similar epithelial responses to bacterial infection have been recently characterized using the *Drosophila* gut as an emerging model to study the responses of stem cells to bacterial infection (Buchon et al., 2009a, 2009b; Pitsouli et al., 2009). Under a normal physiological state, the epithelial cells in the fly gut undergo a basal level of cell renewal, which takes approximately 1 week. However, this turnover rate can accelerate in response to epithelial cell injury induced by dextran sulfate sodium and bleomycin or by bacterial infection with *Erwinia carotovora*, *Serratia marcescens*, and *Pseudomonas* spp. (Amcheslavsky et al., 2009; Pitsouli et al., 2009). During chemical-induced epithelial damage of the fly gut, the intestinal stem cells proliferate, and gut tissue regeneration can be upregulated by stimulating the insulin receptor signaling pathway (Amcheslavsky et al., 2009). However, when tissue damage is caused by bacterial infection, epithelial turnover is accelerated due to the oxidative burst that occurs in gut epithelial cells. This oxidative burst is a major defense response of the *Drosophila* gut that stimulates the JAK-STAT (Janus kinase-signal transducers and activators of transcription) and JNK (c-Jun NH_2-terminal kinase) signaling pathways in intestinal stem cells (Buchon et al., 2009a, 2009b). Jiang et al. showed that the Unpaired cytokines (Udp, Udp2, and Udp3), which were upregulated by JNK-mediated stress signaling, activated JAK-STAT signaling in stem cells to promote rapid proliferation and renewal of the gut epithelium (Jiang et al., 2009).

Aside from the gut and intestine, the basal level of tissue renewal, such as the urogenitor epithelium, is very low, and the process takes up to ~40 weeks in adult mice (Jost, 1986). However, regeneration of the superficial urothelium in response to uropathogenic *E. coli* (UPEC) infection can be rapidly accelerated and completed in 7 days (Mysorekar et al., 2002). Recent studies have provided a mechanism for the proliferation of urothelial stem cells in response to UPEC infection in a mouse bladder model. These studies have shown that Bmp4 signaling, a member of the TGF-β family that is a key element in stem cell proliferation, is a key negative regulator in the upregulation of urothelial renewal (Mysorekar et al., 2009). Previous studies used a knockout mouse that lacks the Bmp4 receptor gene, *bmpr1a*, to show that complete ablation of the Bmp4 signaling pathway can lead to a substantial decrease in urothelial proliferation in response to UPEC infection (Mysorekar et al., 2009) (Figure 1).

Increased turnover of the intestinal epithelium was also noted during parasitic infections of the mouse large intestine (Cliffe et al., 2005). Upon infection of the large intestine with *Trichuris muris*, a cecal-dwelling parasitic nematode that actively penetrates and colonizes the epithelium and is used as a model for human whipworm, intestinal epithelial cell turnover accelerates

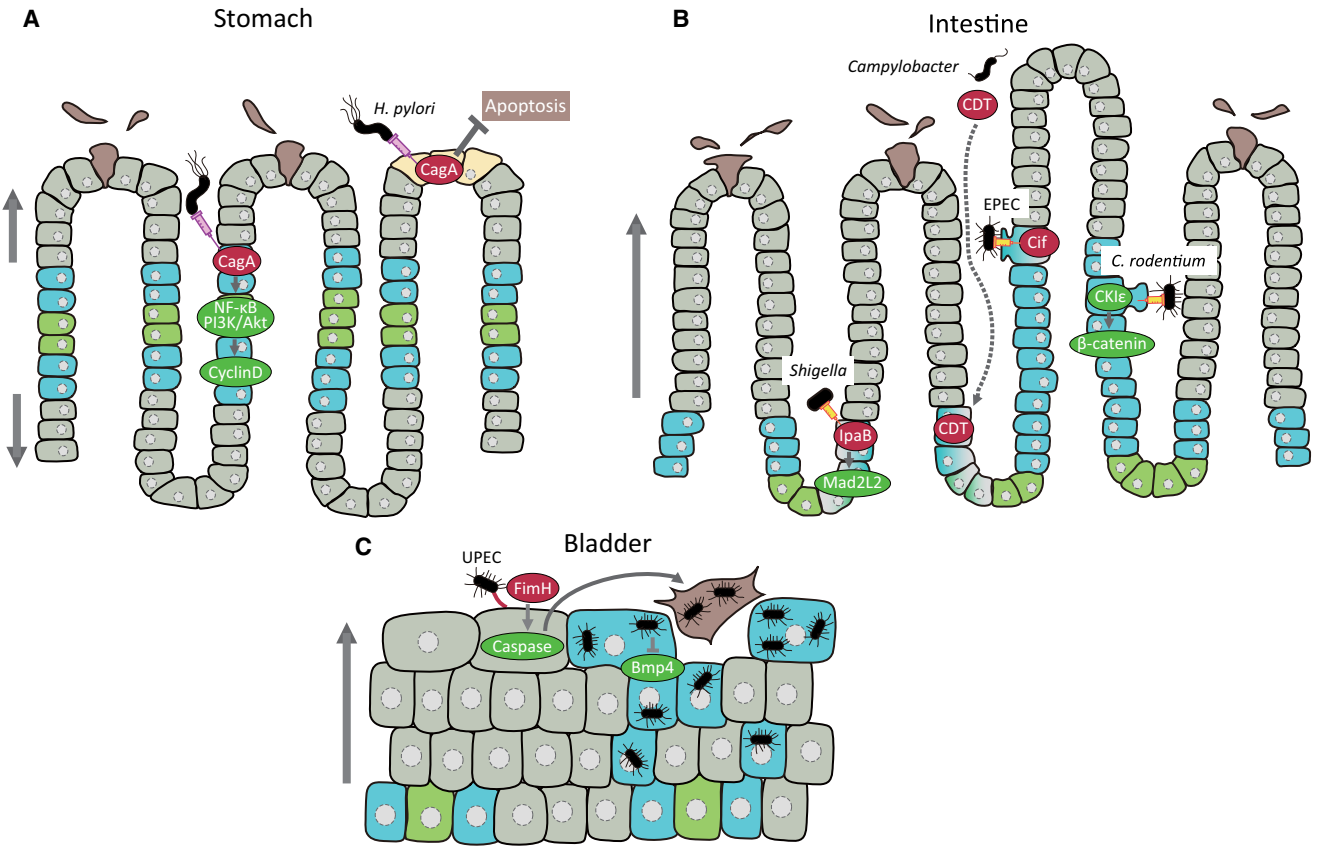

Figure 1. Bacterial Countermeasures against Epithelial Renewal

(A) *H. pylori* delivers CagA via the T4SS to hijack host signal pathways that promote cell motility, disrupt tight junctions and cell polarity (see Figure 3), and activate transcriptional factors. CagA interacts with many cellular factors and activates a variety of signaling pathways, including the MEK/ERK, p38, JNK, and PI3K/Akt pathways. In addition, CagA can activate a subset of transcriptional factors, including nuclear factor of activated T cells (NFAT), serum response factor (SRF), NF-κB, and T cell factor/lymphoid enhancer factor (TCF/LEF), through MEK/ERK kinase activation, β-catenin signaling, and other unknown pathways. As a result, *H. pylori* upregulates Cyclin D1 production, which stimulates gastric progenitor proliferation and MCL-1 expression and dampens rapid turnover of the gastric epithelium.

(B) During EHEC and EPEC infection, intimin, an outer membrane protein, interacts with Tir, an effector secreted via the T3SS into epithelial cells, which allows the bacterium to tightly attach to the epithelial surface by forming an actin pedestal. *C. rodentium* is often used as a model pathogen for EHEC and EPEC, and this bacterium upregulates the level of casein kinase I ε in epithelial cells that stimulates β-catenin signaling and cryptic stem cell proliferation. Although *Shigella* initially enters epithelial cells via M cell entry, after the middle stage of infection, these bacteria directly access the crypts, where they invade nonpolarized epithelial progenitor cells. *Shigella* delivers IpaB via the T3SS, and IpaB targets Mad2L2, an anaphase-promoting complex (APC) inhibitor. The interaction between IpaB and Mad2L2 causes unscheduled activation of APC, which results in cell-cycle arrest at the G2/M phase and allows the pathogen to inhibit epithelial renewal and promote bacterial colonization of the intestinal epithelium. Some EPEC strains deliver Cif via the T3SS into epithelium, which may inhibit intestinal epithelial cell mitosis. Other cyclomodulins may influence epithelial renewal. For example, CDT is delivered from *C. jejuni* into the host cell nucleus and has a deoxyribonuclease-I-like activity that induces limited DNA damage and cell-cycle arrest.

(C) UPEC strains expressing a FimH-type I pili adhere to bladder superficial epithelial cells, which causes epithelial cell apoptosis. In response to UPEC infection, Bmp4, a key negative regulator of proliferation in various stem cells, is downregulated in urothelial stem cells. The reduction in Bmp4 stimulates stem cell proliferation and contributes to urothelial renewal. Cells in green and blue represent stem cells and progenitor cells, respectively, while cells in beige represent apoptotic cells. Dashed lines represent our speculations in the model.

to expel the nematode. In a bromodeoxyuridine pulse-chase experiment, Cliffe et al. showed that the rate of intestinal epithelial cell turnover increased at the initial stage of infection under immune control by IL-13 and CXCL10, and this process was sufficient to expel the nematode (Cliffe et al., 2005). As the nematode infection progressed, the proliferation of cryptic progenitor cells increased, eventually resulting in crypt hyperplasia, a hallmark of nematode infection (Cliffe et al., 2005).

These studies corroborate that epithelial turnover is dynamically altered in response to various stimuli, including bacterial infection. Furthermore, this epithelial activity is essential to maintain tissue homeostasis and limit persistent bacterial colonization while allowing lumenal microbiota to persist. This intricate balance provides strong physical, mucosal, and antibacterial barriers.

Bacterial Countermeasures against Rapid Epithelial Turnover

Recent studies indicated that some bacterial pathogens, such as *S. flexneri* and *H. pylori*, use countermeasures to prevent rapid epithelial turnover in order to maintain epithelial cells as a replicative niche (Iwai et al., 2007; Mimuro et al., 2007) (Table 1). *H. pylori* possess several adhesins that are each specific for glycan groups that are on gastric epithelial cell surfaces and in the overlying mucus. In addition, *H. pylori* feed on exudates

Table 1. Bacterial Proteins Implicated in the Countermeasure against Host Epithelial Barrier Functions

Pathogen's commitment	Pathogens	Bacterial proteins	Host target	Bacterial function	Reference
Epithelial turnover	*C. jejuni*	CDT	DNA	Cell-cycle arrest	Nougayrède et al., 2005
	EPEC	Cif	?	Cell-cycle arrest	Nougayrède et al., 2005
	H. pylori	CagA	GRB2, CRK/ Ras-MAPK/MCL1	Antiapoptosis	Mimuro et al., 2007
	Shigella	IpaB	Mad2L2	Cell-cycle arrest	Iwai et al., 2007
Epithelial-cell shedding	EHEC	OspO1-1, OspO1-2	ILK	?	Kim et al., 2009
	H. influenzae	?	CEACAM	Enhancement of cell adhesion	Muenzner et al., 2005
	M. catarrhalis	?	CEACAM	Enhancement of cell adhesion	Muenzner et al., 2005
	N. meningitidis	Opa_{CEA}	CEACAM	Enhancement of cell adhesion	Muenzner et al., 2005
	Shigella	OspE	ILK	Enhancement of cell adhesion	Kim et al., 2009
	S. Typhimurium	EspO1	ILK	?	Kim et al., 2009
Disruption of cell-cell junction and polarity	EPEC	EspF	N-WASP, SNX9	Tight junction disruption	Alto et al., 2007; Weflen et al., 2009
	EPEC	EspM	?	Tight junction disruption	Arbeloa et al., 2008; Simovitch et al., 2010
	EPEC	Map	Cdc42	Tight junction disruption	Alto et al., 2006; Dean and Kenny, 2004
	EPEC	NleA	Sec23/24	Inhibition of protein trafficking	Kim et al., 2007; Thanabalasuriar et al., 2010
	H. pylori	CagA	E-cadherin, Par1, ZO-1, β-catenin	Bleaching of tight junction and adherens junction, Disruption of cell polarity	Amieva et al., 2003; Bagnoli et al., 2005; Murata-Kamiya et al., 2007; Suzuki et al., 2005; Saadat et al., 2007
	H. pylori	Urease	MLCK, Occuludin	Feeding on nutrient?	Wroblewski et al., 2009
	H. pylori	VacA	?	Feeding on nutrient?	Papini et al., 1998
	P. aeruginosa	?	PI3K	Redistribution of membrane proteins	Kierbel et al., 2007
	S. Typhimurium	SopB, SopE, SopE2, SipA	?	Tight junction disruption	Boyle et al., 2006
Epithelial- cell death	*C. trachomatis*	CPAF	Pumaα, Pumaβ, Bik	Antiapoptosis	Pirbhai et al., 2006
	EPEC	NleH	Bax inhibitor-1 (Bi-1)	Antiapoptosis	Hemrajani et al., 2010
	H. pylori	VacA	?	Antiapoptosis	Willhite et al., 2003; Yamasaki et al., 2006; Galmiche et al., 2000
	N. gonorrhoeae	type IV pili	Bad, Bim	Antiapoptosis	Howie et al., 2008
	N. meningitidis	PorB	VDAC	Antiapoptosis	Massari et al., 2003
	S. Typhimurium	AvrA	MAPKKs	Anti-inflammatory and anti-cell death	Jones et al., 2008
Autophagy	*B. pseudomallei*	BopA	?	Escape from autophagy	Cullinane et al., 2008
	L. monocytogenes	ActA	Arp2/3, Ena/VASP, F-actin	Escape from autophagy	Yoshikawa et al., 2009
	Shigella	IcsB	Atg5	Escape from autophagy	Ogawa et al., 2005

from epithelial cells that have been damaged by inflammatory responses, reactive oxygen species (ROS), apoptotic responses, and ruptured cell-cell junctions. *H. pylori* deliver CagA, a major virulence factor, via a type IV secretion system (T4SS) into gastric epithelial cells. CagA has profound biological activities that usurp cell signaling and function to promote persistent colonization of the gastric epithelium. CagA interacts with many cellular factors that activate a variety of transcriptional factors such as nuclear factor of activated T cells (NFAT), serum response factor (SRF), NF-κB, and T cell factor/lymphoid

enhancer factor (TCF/LEF), as well as other unknown pathways (Amieva and El-Omar, 2008; Fischer et al., 2009; Wessler and Backert, 2008). Upregulation of these transcriptional factors results in Cyclin D1 production and promotes epithelial cell proliferation (Chang et al., 2006). Using a Mongolian gerbil infection model, *H. pylori* was shown to be capable of dampening apoptosis of matured gastric epithelial cells that are normally shed every 2–3 days (Mimuro et al., 2007). When etoposide was used to induce gastric epithelial apoptosis in the gerbil stomach, *H. pylori* upregulated ERK, a prosurvival factor, and MCL-1, an antiapoptotic protein, in a CagA-dependent manner, and this CagA activity promoted the colonization of the gerbil stomach (Mimuro et al., 2007). These findings suggest that CagA counterbalances host defenses by dampening rapid gastric epithelial turnover and accelerating epithelial proliferation (Figure 1).

Shigella deploys a special tactic to modulate epithelial cell turnover (Iwai et al., 2007). This activity is executed by IpaB, also known as a type III secretion system (T3SS) translocator that is secreted via the T3SS from infected intestinal progenitors. In a rabbit ileal loop model, *Shigella* was shown to directly access the crypts at the middle stage of infection, which allowed the bacteria to invade nonpolarized progenitors. At the middle stage of wild-type *Shigella* infection, there are fewer PCNA (proliferation cell nuclear antigen, representing progenitors)-positive cells in the crypts than after infection with the *ipaB* mutant. An in vitro study demonstrated that IpaB secreted by intracellular *Shigella* into HeLa cells causes cell-cycle arrest at the G2/M phase by targeting Mad2L2, an anaphase-promoting complex inhibitor, in an IpaB-Mad2L2-dependent manner. Compared to wild-type *Shigella*, the isogenic mutant in which IpaB and Mad2L2 cannot interact had a much lower colonization rate (Iwai et al., 2007) (Figure 1).

Recent studies have shown that many bacterial pathogens produce and secrete small compounds, toxins, and effectors that interfere with host cell-cycle progression. These factors are called cyclomodulins and have been proposed to be a new class of virulence-associated factors (Nougayrède et al., 2005). The biological impact of cyclomodulins such as Cif (cell-cycle-inhibiting factors), secreted by EPEC and CDT (cytolethal distending toxins) from *Campylobacter jejuni*, on bacterial infection and the host cell types that are targeted by these factors is still unknown, but their biological activities suggest that cyclomodulins help prolong the bacterial infectious foothold (Figure 1).

Epithelial Cell Shedding

Epithelial cells are tightly bound together and attached to the basal lamina, which is composed of the extracellular matrix (ECM) and thus bears most of the mechanistic stress. Therefore, disturbing the balance in epithelial cell proliferation, differentiation, migration, death, and shedding, which are essential to maintain homeostasis and integrity, is associated with various intestinal diseases, including diarrhea, inflammatory colitis, necrotizing enterocolitis, hyperplasia, and microerosions. For example, in ulcerative colitis, epithelial breaching due to microerosions is thought to be due to increased epithelial apoptosis. Schulzke et al. indicated that Th1 cytokines produced from the epithelium in response to a combination of IFN-γ and TNF-α, which are known to stimulate epithelial shedding, was a prominent pathological background that led to damage of the mucosal lining in Crohn's disease (Schulzke et al., 2009). In addition, the intestinal epithelium undergoes cell shedding and transient gap formation following cell detachment, which constantly occurs under physiological conditions. In studies that examined confocal images and scanning electron micrographs of the intestinal villus, it was determined that as many as ~3% of cells from the shaft and tip of the villus are exfoliated in human and mouse intestines (Madara, 1990; Watson et al., 2009). Intriguingly, the local barrier at the gaps remains intact because the gaps are filled with an uncharacterized material that maintains permeability and may provide a protective barrier (Watson et al., 2009). Whenever these gaps are created under physiological or pathophysiological conditions (discussed later), the gaps are used by many bacterial pathogens, including EPEC, EHEC, *Salmonella*, *Shigella*, *Yersinia*, *L. monocytogenes*, and *H. pylori*, to adhere or enter from the basolateral side of exposed neighboring cells. For example, *L monocytogenes* can induce bacterial internalization when internalinA (InlA) and InlB on the bacterial surface interact with E-cadherin and the hepatocyte growth factor receptor (c-Met) expressed on the basolateral surface, respectively (Barbuddhe and Chakraborty, 2009; Cossart and Sansonetti, 2004). Pentecost et al. provided evidence that *L. monocytogenes* target the intact epithelium by accessing multicellular junctions, which are created through spontaneous epithelial cell shedding at the lumenal surface of the intestinal epithelia (Pentecost et al., 2006). In addition to natural epithelial cell shedding, epithelial cell exfoliation is an intrinsic host defense to bacterial infections in which damaged host cells and colonized pathogens are quickly expelled from the epithelial lining. Foundational work by Mulvey et al. showed that UPEC infection of the urinary bladder causes a rapid sloughing of epithelial cells. The bladder transitional epithelium, which is 3–4 cell layers deep, undergoes a low level of turnover under normal physiological conditions (Mulvey et al., 2000). However, Mulvey et al. revealed that the superficial bladder epithelial cells undergo exfoliation via apoptosis with host cell DNA fragmentation and caspase activation when they are infected with UPEC expressing FimH-type I pili (Mulvey et al., 1998). In a C57BL/6 mouse bladder model of UPEC infection, the bladder underwent massive cell ablation, and neutrophils infiltrated into the tissue and lumen, a hallmark of urinary tract infection in human patients (Mulvey et al., 2000). Although our knowledge of cell exfoliation in response to bacterial infection is limited, these studies clearly indicate that the expulsion of infected epithelial cells is an important intrinsic defense system against bacterial colonization.

Bacterial Circumvention of Host Cell Detachment

The exfoliation of infected epithelial cells and inflammatory response are problematic for bacterial pathogens that replicate on or within the gastrointestinal tract, urinary tract, or respiratory tract (Mulvey et al., 2000; Vance et al., 2009). Despite these various host defenses, many bacterial pathogens are capable of colonizing the epithelium, suggesting that they have developed mechanisms to antagonize and/or circumvent host defense systems, including epithelial detachment (Table 1). Muenzner et al. reported that *Neisseria gonorrhoeae* cause epithelial cells to detach from the ECM in vitro. They also showed that some *N. gonorrhoeae* strains expressing colony opacity-associated (Opa) proteins, such as Opa_{CEA}, that recognize

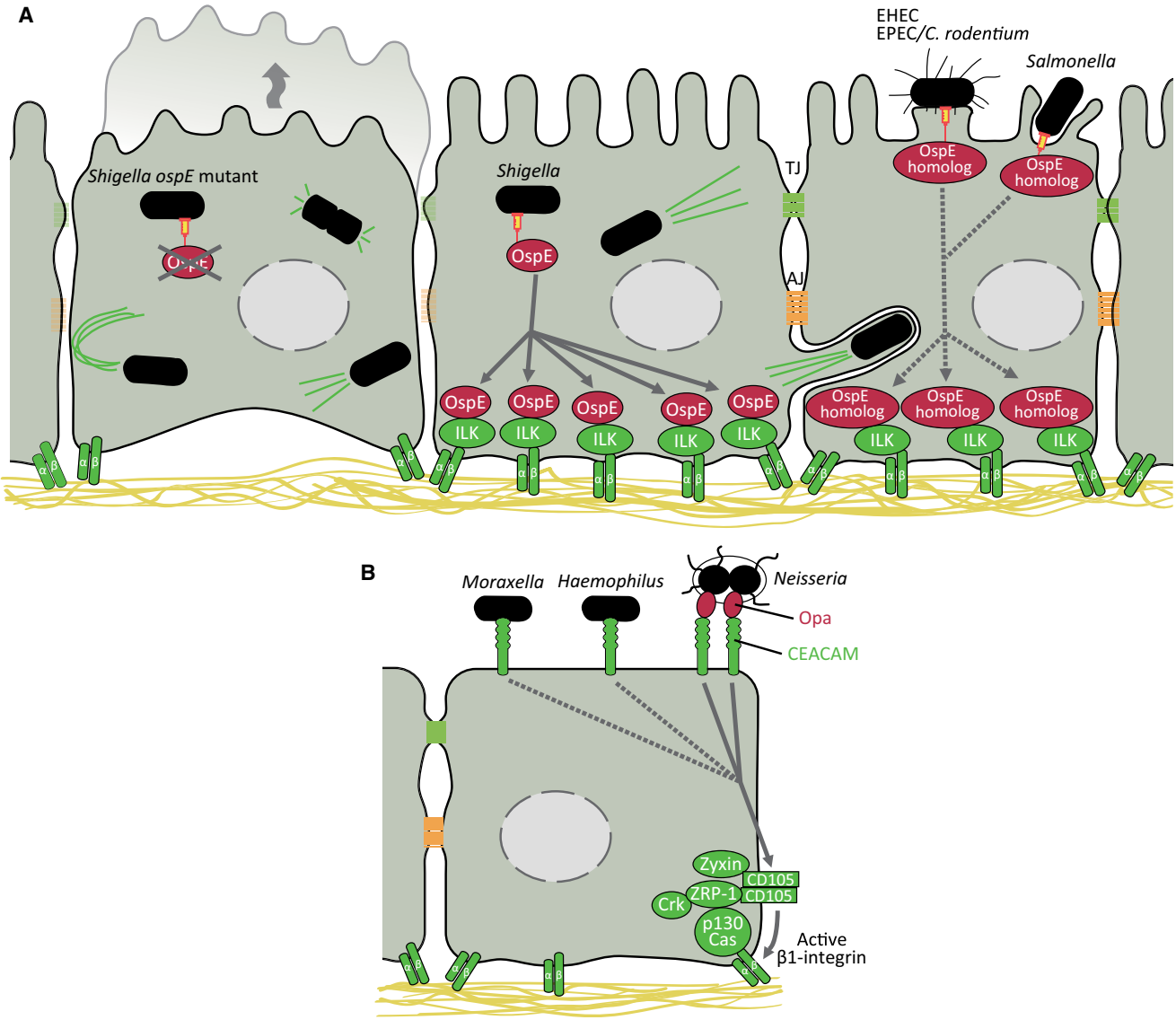

Figure 2. Epithelial Shedding in Response to Bacterial Infection and Bacterial Countermeasures that Inhibit This Defense Mechanism

(A) The intestinal epithelium undergoes rapid cell death and detachment in response to bacterial colonization. *Shigella* delivers OspE via the T3SS during replication within epithelial cells, and OspE targets ILK to reinforce epithelial cell adherence to the ECM. The interaction between OspE and ILK increases the formation of focal adhesions (FA) and surface levels of β1-integrin while suppressing rapid FA turnover, reducing cell motility, and promoting cell adhesion to ECMs. Although the modes of infection differ, OspE-mediated bacterial manipulation of ILK during infection may be widely used by other bacterial pathogens, including EPEC, EHEC, *C. rodentium*, and *S.* Typhimurium. Because these bacteria possess OspE cognate effectors, these effectors are interchangeable with *Shigella* OspE activity.

(B) *N. gonorrhoeae*, *N. meningitidis*, *H. influenzae*, and *M. catarrhalis* enhance CD105 expression in epithelial cells. CD105 is a member of the TGF-β1 receptor family that is linked to β1-integrin via its association with Zyxin and ZRP-1 and therefore reinforces the adherence of epithelial cells to the ECM. Dashed lines represent our speculations in the model. ECMs are shown in yellow.

human carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) were able to enhance expression of CD105, a TGF-β1 receptor family protein, and promote cell adhesion to the ECM, depending on the presence of functional β1-integrins (Muenzner et al., 2005). In particular, they demonstrated that 293T cells that ectopically express CD105 had enhanced adhesion to collagen even in the absence of bacterial infection. These findings indicate that CD105 can counteract the detachment of infected cells through a mechanism that does not involve antiapoptotic effects (Muenzner et al., 2005). Intriguingly, all other bacterial pathogens that bind CEACAM, including *N. meningitidis*, *Haemophilus influenzae*, and *Moraxella catarrhalis*, are able to promote cell adhesion to collagen (Muenzner et al., 2005). These findings suggest that upregulation of CD105 via CECAM engagement may be a widely adopted bacterial strategy to counteract infection-induced exfoliation of epithelial cells (Figure 2).

We recently reported that *Shigella* deploys a unique mechanism to prevent intestinal epithelial cell detachment by delivering the OspE effector via the T3SS. OspE targets a host

integrin-linked kinase (ILK) and reinforces epithelial adhesion to the basal lamina (Kim et al., 2009). OspE-ILK interaction increased the levels of β1-integrin on the epithelial cell surface and dampened the disassembly of focal adhesions, thus greatly stabilizing focal adhesion complexes and reinforcing the adhesion of infected epithelial cells to the ECM (Kim et al., 2009) (Figure 2). At 24 hr after infection, the *ospE* mutant had a lower colonization rate than wild-type *Shigella* in a guinea pig colorectal infection model. Interestingly, the OspE cognate effectors produced from many other bacterial pathogens, including EPEC, EHEC, *C. rodentium*, and *Salmonella* (Tobe et al., 2006), are functionally interchangeable with *Shigella* OspE in their ability to reinforce focal adhesions, suggesting that this bacterial activity is shared with other enteropathogens (Kim et al., 2009). We proposed a model in which *Shigella*, including OspE-producing bacteria, secure an infectious foothold by reinforcing the adherence of infected epithelium to the basal lamina to counteract the host defense system (Figure 2).

Epithelial Cell-Cell Junctions and Cell Polarity

Many bacterial pathogens target tight junctions by perturbing their structure and function. Some bacteria open cell-cell junctions, while others disrupt epithelial cell polarity, which exposes the basolateral surface and breaches the epithelial lining (Guttman and Finlay, 2009). The integrity of the gut epithelium is sustained through cell-cell sealing mediated by tight junctions, adherence junctions, gap junctions, and desmosomes, which act as barriers against foreign antigens and microbes (Shen, 2009). The tight junction is the most lumenal cell-cell junction and is composed of scaffolding proteins, including zonula occludens (ZO)-1, junctional adhesion molecule (JAM)-1, Claudin, and Occludin. The tight junction acts as a permeable barrier and functionally segregates the apically expressed membrane proteins from those expressed on the basolateral membrane in polarized epithelial cells (Shen, 2009). A recent study indicated that the tight junction is a highly dynamic structure. Live-cell fluorescence imaging and analyses of frozen jejunum sections from mice revealed that occludin and ZO-1 concentrate at tight junctions but are dynamically endocytosed from these cell-cell junctions upon treatment with TNF-α, an agonist that stimulates the disruption of cell-cell junctions (Schwarz et al., 2007; Shen et al., 2008). Intensive studies on the molecular mechanisms that regulate tight junctions revealed that actomyosin contraction, which is regulated by myosin II regulatory light chain (MLC) phosphorylation through MLC kinase (MLCK), is a major force that regulates acute tight junctions under physiological conditions (Shen, 2009). There is clear evidence that links the disruption of intestinal tight junctions with inflammation. Several studies indicated that proinflammatory cytokines such as TNF-α and IL-1β induce tight junction dysfunction in an MLCK-dependent manner in both cultured epithelial cells and mouse intestines (Al-Sadi et al., 2008; Ma et al., 2005). In addition, MLCK has been implicated in human diseases such as inflammatory bowel disease (IBD) based on evidence that patient samples have increased MLC phosphorylation and MLCK expression. These findings suggest that MLCK-mediated breaching of cell-cell junctions in IBD patients is a major pathogenic element in disease progression (Blair et al., 2006).

Bacterial Strategies to Promote Inflammation and Breach Cell-Cell Junctions

Gastrointestinal bacterial pathogens have evolved various systems to target and disrupt tight junctions (and in some case adherence junctions) by regulating Rho GTPases and MCLK-mediated signaling in infected epithelial cells. Targeting these pathways affects the organization of the actin cytoskeleton and, in turn, the junction architecture and eventually simulates the innate immune response. Numerous studies reported that EHEC and EPEC modify the epithelial cell surface architecture and interfere with the apical junctions of epithelial cells, leading to a loss of epithelial cell polarity and reduced barrier functions (Guttman and Finlay, 2009; Lapointe et al., 2009) (Table 1). EPEC delivers several effectors via a T3SS that target tight junctions, alter the permeability of the epithelial lining, and decrease *trans*-epithelial resistance (TER), a hallmark for epithelial integrity. Map, which activates the Cdc42 signaling pathway (Alto et al., 2006; Dean and Kenny, 2004), and EspF, which binds to the Neural Wiskott-Aldrich Syndrome protein (N-WASP) and the endocytic regulators sorting nexin 9 (SNX9), modify the structure of tight junctions (Alto et al., 2007; Weflen et al., 2009). EspM activates the RhoA signal pathway and alters the localization of tight junctions (Arbeloa et al., 2008; Simovitch et al., 2010), while NleA interferes with COPII-dependent protein trafficking in the host cell (Kim et al., 2007; Thanabalasuriar et al., 2010). In mouse intestines infected with *C. rodentium*, the function of EspF was confirmed to involve tight junction disruption (Guttman et al., 2006a) and intestinal hyperplasia (Nagai et al., 2005) (Figure 3). Importantly, a recent study that examined mouse intestines infected with *C. rodentium* showed that the inflammatory response to this pathogen at the early stage of infection had no clear effect on the morphology or barrier function of tight junctions, implying that the ability of A/E pathogens to disrupt tight junctions benefits pathogenesis (Guttman et al., 2006a, 2006b). Although none of the bacterial effectors could directly manipulate the tight junction complex, the synergistic activities of each effector seem to be a major force in breaching epithelial junctions.

S. Typhimurium delivers many effector proteins into host cells (Galán, 2009), and some of these effectors, notably SopB, SopE, SopE2, and SipA, disrupt tight junctions and induce the inflammatory response (Boyle et al., 2006; Bruno et al., 2009; Müller et al., 2009) (Table 1). Recent studies have indicated that *Salmonella*-mediated activation of Rho GTPases may be involved in breaching epithelial junctions (Bruno et al., 2009; Guttman and Finlay, 2009; Müller et al., 2009). *S.* Typhimurium triggers gut inflammation by delivering effectors via the T3SS-1 and T3SS-2 encoded by SPI-1 and SPI-2. Among the effectors secreted via T3SS-1, SopE, SopE2, and SopB, which are required for *S.* Typhimurium to invade host cells, were shown to alter the host inflammatory response. Boyle et al. showed that the disruption of tight junctions by *Salmonella* in vitro can be prevented with an inhibitor of host protein geranylgeranylation, confirming that Rho GTPase activation by these effectors is involved in barrier disruption (Boyle et al., 2006). In addition, these effectors affect epithelial cell polarity. *Salmonella* infection redistributed GP135 and E-cadherin to the apical and basolateral surfaces, respectively, in a SopB-, SopE-, SopE2-, and SipA-dependent manner (Boyle et al., 2006). In mice,

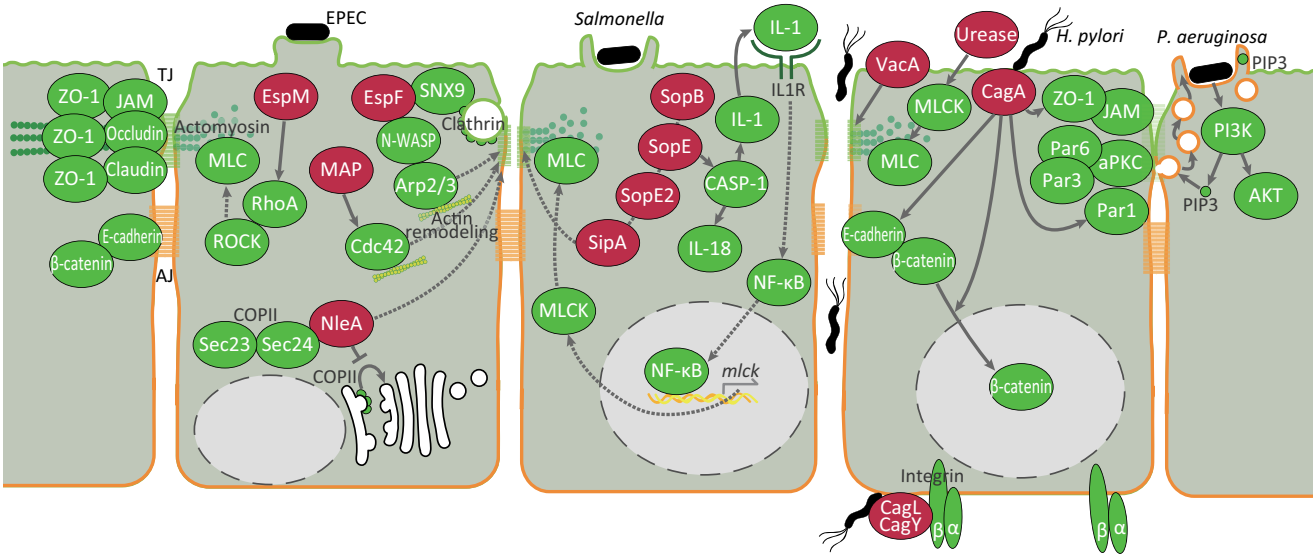

Figure 3. Bacterial Strategies to Breach Cell-Cell Junctions

Bacterial pathogens target TJ (*H. pylori* targets TJ and AJ) to gain nutrients and facilitate bacterial interactions with the epithelium. The TJ complex consists of JAM, Occludin, Claudin, and ZO-1, and the AJ complex consists of E-cadherin and β-catenin. Bacteria-induced remodeling of cell-cell junctions is executed through the following pathways. (1) MLC activation: MLC activity is controlled via MLC phosphorylation, MLCK activation and/or increased expression levels of MLCK that regulates actomyosin remodeling. EPEC delivers EspM via the T3SS, which stimulates RhoA/ROCK activation and may induce MLC phosphorylation. Urease produced by *H. pylori* abundantly surrounds the bacterial surface and activates MLCK/MLC by an unknown mechanism. *S.* Typhimurium delivers SopE, SopE2, SopB, and SipA via the T3SS. SopE activates the IL-1 signal pathway via caspase-1 (CASP-1), which results in the eventual activation of the NF-κB pathway and may influence *mlck* expression. (2) Modulation of Rho GTPase activity: the *S.* Typhimurium effectors SopE, SopE2, and SopB activate Rho GTPases, while SipA manipulates F-actin assembly to disrupt TJ. EPEC targets TJ by delivering Map, EspF, and EspM via the T3SS. Map and EspM activate Cdc42 and RhoA, respectively, while EspF binds to N-WASP and SNX9, which influences the architecture of F-actin and alters the localization of TJ. (3) Direct or indirect targeting of TJ/AJ components: *H. pylori* CagA targets Par1 and ZO-1 to disrupt TJ and deregulate epithelial cell polarity. CagA deregulates the localization of β-catenin beneath the AJ complex by directly interacting with the E-cadherin complex and promoting AJ disruption. The interaction-induced loss of cell-cell junctions allows *H. pylori* to access β1-integrins located on the basolateral surfaces through CagL and CagY of the T4SS. (4) Modulation of membrane trafficking: EPEC delivers NleA via the T3SS, and NleA modifies TJ by interacting with the Sec23/24 complex, which contains components of the COPII protein coat that shape intracellular protein transport vesicles that exit the ER. *P. aeruginosa* activates PI3K and modulates PIP3 to redistribute the basolateral membrane (orange line) to the apical membrane (green line) and create a local microenvironment suitable for colonization. Dashed lines represent our speculations in the model. TJ, tight junction; A, adherence junction.

S. Typhimurium elicits a strong inflammatory response by 8 hr after infection (Hapfelmeier and Hardt, 2005), and SopE, which acts as a GEF for Rac1 and Cdc42, was identified as a key effector (Hapfelmeier et al., 2004). Muller et al. showed that *Salmonella* triggers mucosal inflammation in wild-type mice but not caspase-1$^{-/-}$, IL-1R$^{-/-}$, or IL-18$^{-/-}$ mice in a SopE-dependent manner, suggesting that caspase-1 activation and IL-1β production are involved (Müller et al., 2009). As a result, the activation of GTPases by these effectors leads to the stimulation of the NF-κB, JNK, and p38 pathways and subsequent heightened inflammatory and stress responses (Bruno et al., 2009) (Figure 3). These studies indicate that the pathogen stimulates intestinal inflammation and breaches cell-cell junctions, which is presumed to be a tactic that *Salmonella* uses to obtain nutrients (Stecher et al., 2007).

H. pylori alter epithelial cell-cell junctions, cell polarity, and cell proliferation by hijacking host epithelial cell surface receptors and intracellular signal pathways (Figure 3) (Table 1). *H. pylori* CagA and VacA are major translocatable virulence factors that enter the gastric epithelium and help breach tight junctions and adherence junctions (Fischer et al., 2009). CagA promotes the disruption of E-cadherin/catenin-containing adherence junctions. CagA is recruited to E-cadherin in adherence junctions and stimulates the release of β-catenin from the adherence complex (Murata-Kamiya et al., 2007; Suzuki et al., 2005). CagA interacts with ZO-1 and PAR1/MARK polarity-regulating kinase to disrupt tight junctions and promote the loss of epithelial cell polarity and cell migration (Amieva et al., 2003; Bagnoli et al., 2005; Saadat et al., 2007). VacA assembles into oligomeric structures, inserts into lipid bilayers to form membrane channels, and targets multiple cell surface components (Cover and Blanke, 2005). These VacA-mediated activities were reported to help open tight junctions, although the mechanism of this disruption is still unclear (Papini et al., 1998). In addition, *H. pylori* urease was shown to stimulate Occludin internalization and MLCK activation, leading to the dysregulation of tight junctions (Wroblewski et al., 2009) (Figure 3). These activities appear to promote bacterial colonization because the surface-exposed T4SS needle components CagL and CagY target β1-integrin. This interaction facilitates bacterial attachment to the basolateral surface, allowing CagA to be injected into the gastric epithelium, which may help the bacteria gain nutrients (Jiménez-Soto et al., 2009; Kwok et al., 2007; Wessler and Backert, 2008; van Amsterdam and van der Ende, 2004; van Amsterdam et al., 2006).

A similar bacterial activity that relocates the basolateral membrane components to the apical membrane has also been reported in polarized MDCK cells infected with *Pseudomonas aeruginosa* (Kierbel et al., 2007). Kierbel et al. demonstrated

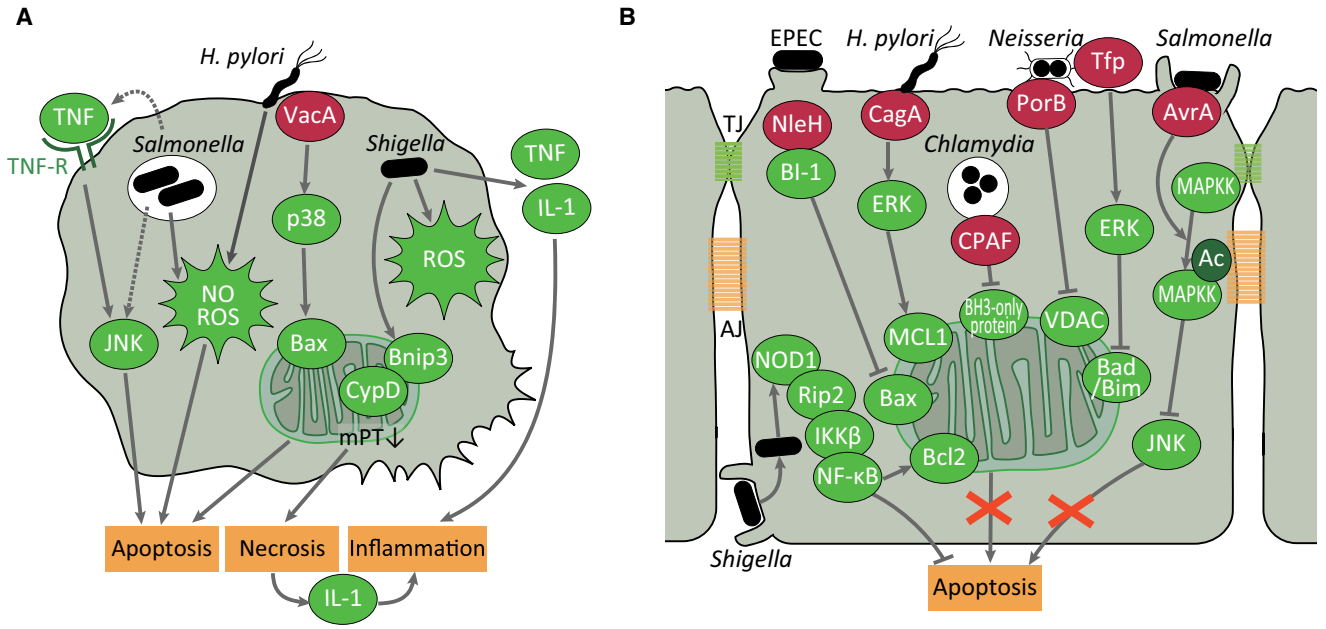

Figure 4. Bacterial Measures that Counterbalance Epithelial Cell Death and Cell Survival

(A) Epithelial cell death induced by bacterial infection. *Salmonella* infection causes delayed apoptosis in epithelial cells via TNF-α and nitric oxide productions. Although the mechanism is still unclear, TNF receptor-mediated apoptosis occurs as a result of prolonged JNK activation in epithelial cells. *Shigella* invasion of the epithelium induces TNF-α and IL-1 production and stimulates oxidative stress, which induces necrosis-like cell death. *Shigella*-induced epithelial cell death also occurs, depending on the balance between the mitochondrial membrane permeabilization-dependent cell death pathway and the NOD1/Rip2/IKKβ/NF-κB prosurvival pathway. In response to oxidative cell stress, death signaling depends on the BH3-only protein Bnip3 and CypD. *H. pylori* VacA activates p38 and stimulates the translocation of Bax to the mitochondria, thus decreasing mitochondrial membrane potential and inducing apoptosis.

(B) Bacterial countermeasures against epithelial cell death. *Salmonella* delivers AvrA via the T3SS to modulate inflammatory and epithelial cell death responses. AvrA exerts acetyltransferase activity toward MAPKKs to suppress the intestinal inflammatory response. AvrA can suppress epithelial cell death during natural infection of the mouse intestine by inhibiting JNK stress signaling. *Shigella* infection of the epithelium leads to oxidative stress and a regulated form of necrotic cell death by decreasing mitochondrial membrane permeability. *Shigella* upregulates the Nod1/Rip2/NF-κB/Bcl-2 prosurvival pathway to antagonize the epithelial cell death response. EPEC delivers NleH via the T3SS, and NleH interacts with Bax inhibitor 1 (BI-1) and blocks epithelial apoptosis. *Neisseria* and *Chlamydia* neutralize host cell death by modifying mitochondrial permeability. *Neisseria* such as *N. meningitides* adhere to nasopharyngeal epithelial cells via type IV pili (Tfp). The PorB outer membrane protein, which is translocated into epithelium, targets VDAC and allows these pathogens to dampen epithelial apoptosis. *N. gonorrhoeae* is capable of activating two prosurvival signaling pathways in epithelial cells through the ERK pathway. Stimulation of the ERK pathway downregulates production of Bad and Bim, thus augmenting the cytoprotective effects. *Chlamydia* secretes CPAF, which helps degrade the proapoptotic BH3-only proteins, and blocks the release of cytochrome *c* from the mitochondria. The dashed lines represent our speculations in the model.

that when *P. aeruginosa* bind to the apical surface, many basolateral proteins together with PI3K and actin are rapidly redistributed to the apical surface, but tight junctions are not disrupted (Kierbel et al., 2007) (Figure 3). Clearly, various bacterial pathogens have evolved strategies to usurp the apical surface by disrupting cell-cell junctions in order to potentially expand the epithelial surface, translocate bacteria deeper into tissues, and feed on nutrients in host body fluids.

Epithelial Cell Death

Epithelial Cell Death Is Caused by Various Stimuli during Bacterial Infection

Although the outcome varies depending on the targeted host cell type (myeloid cells versus nonmyeloid cells) and the stage of infection (early stage versus late stage), bacterial pathogens use various mechanisms to modulate epithelial cell death (Pitsouli et al., 2009). The epithelial cell death response to microbial infection is pivotal for both bacterial pathogens and the host. During the early stages of infection, bacteria that are colonizing on or within the epithelium need to prevent cell death to preserve their replicative foothold. By contrast, the epithelium needs to eliminate infected cells in order to minimize tissue damage and localize inflammation. Despite the pivotal role of epithelial cell death in infections, the impact of the host defense systems and the mechanisms of epithelial cell death are poorly documented. Studies have indicated that epithelial cell death is caused by various stimuli during bacterial infection, including oxidative stress, ER stress, local inflammatory responses, and reorganization of the actin cytoskeleton. During these processes, infected epithelial cells undergo apoptosis and in some cases necrosis, which leads to the sloughing off of infected cells.

An early study reported that human intestinal epithelial cell lines infected with *S.* Dublin, *S.* Typhimurium, or enteroinvasive *E. coli* have delayed apoptosis, in which TNF-α and nitric oxide production induced upon bacterial infection lead to the death of infected epithelial cells (Kim et al., 1998) (Figure 4). Paesold et al. reported that *S.* Dublin infection of human epithelial cells induced caspase-3 activation through a mechanism that required the SPI-2 locus and *spv* locus and led to delayed (~24 hr after infection) epithelial cell apoptosis (Paesold et al., 2002). Schauser and Larsson studied the pathophysiological effects of *S.* Typhimurium infection in the porcine intestine and showed that both caspase-3-dependent and caspase-3-independent programmed

cell death increased (Schauser and Larsson, 2005). Although the mechanism is still partly speculative, recent studies hypothesized that immune-mediated (and TAK1-dependent) apoptosis through prolonged JNK activation in epithelial cells plays a role in inducing cell death during *Salmonella* infection (Ventura et al., 2006; Weston and Davis, 2007).

Upon infection of the epithelium, *Shigella* induces TNF-α and IL-1α production (Arondel et al., 1999; Jung et al., 1995) and stimulates oxidative stress, whereby bacterial infection causes necrosis-like cell death in a caspase-1-independent manner (Carneiro et al., 2009). Carneiro et al. determined the mechanism of *Shigella*-induced epithelial cell death, which occurs as a consequence of the balance between the mitochondrial membrane permeabilization-dependent cell death pathway and the NOD1/Rip2/IKKβ/NF-κB prosurvival pathway (Carneiro et al., 2009). Death signaling was dependent on the BH3-only protein Bnip3 and Cyclophilin D (CypD), which are key regulators of mitochondrial permeability and cell death in response to oxidative cell stress (Carneiro et al., 2009). Therefore, *Shigella* can stimulate two compensatory signaling pathways in the epithelium: the necrotic cell death pathway triggered by the Bnip3-mediated mitochondrial permeability transition and the cytoprotective (also proinflammatory) pathway (Carneiro et al., 2009; Galluzzi and Kroemer, 2009) (Figure 4). Kinetic data on the activation of cell death and cytoprotective signaling suggested that cytoprotective signaling is dominant at the early stages of intracellular *Shigella* replication within the epithelium, while cell death signaling is induced at the later stages to benefit both the invading pathogen and the host (Carneiro et al., 2009).

Additional studies indicate that other bacteria also induce epithelial cell death. For example, *H. pylori* infection of the gastric epithelium induces oxidative stress and subsequent apoptosis of the gastric superficial pit cells (Fischer et al., 2009). Many studies indicate that the VacA toxin delivered from the pathogen into the gastric epithelium plays a major role in inducing gastric cell apoptosis (Galmiche et al., 2000; Willhite et al., 2003; Yamasaki et al., 2006). During VacA-mediated apoptosis, p38 activation and Bax dimerization play a critical role in epithelial cells expressing VacA. The proapoptotic protein Bax exists as a monomer in nonstimulated epithelial cells but forms oligomers and translocates into the mitochondria upon apoptotic stimulation, thus causing a decrease in mitochondrial membrane potential (Ki et al., 2008) (Figure 4).

Bacterial Countermeasures against the Epithelial Cell Death Response

Salmonella infection of the gut epithelium triggers profound inflammation, which can induce epithelial cell death. However, *Salmonella* have evolved mechanisms to neutralize the host innate immune response. *S.* Typhimurium delivers several effectors, including AvrA, SspH1, SseL, and SipC, that modulate the host inflammatory response (Valdez et al., 2009). AvrA is thought to play a major role in modulating both inflammatory and epithelial cell death responses. AvrA possesses acetyltransferase activity toward MAP kinase kinases (MAPKKs) (Jones et al., 2008), which allows *Salmonella* to suppress the intestinal inflammatory response (Du and Galán, 2009; Jones et al., 2008). Using transgenic *Drosophila* and mouse mucosal ex vivo models, Jones et al. demonstrated that AvrA can suppress epithelial cell death during natural infection. Furthermore, Jones et al. confirmed that *Salmonella* interfere with NF-κB activation by specifically inhibiting JNK stress signaling and downstream apoptosis in an AvrA-dependent manner (Jones et al., 2008), and these phenotypes are consistent with the pathogenic features of *Salmonella* infection in the gut (Figure 4).

During EPEC (and *C. rodentium*) infection, EspF is delivered via the T3SS to target the mitochondria, decrease mitochondrial membrane potential, and elicit intestinal epithelial cell apoptosis (Nagai et al., 2005; Nougayrède and Donnenberg, 2004). Recently, Hemrajani et al. reported that T3SS-mediated NleH in EPEC antagonizes the apoptotic response by interacting with Bax inhibitor-1 (BI-1) (Hemrajani et al., 2010) (Figure 4).

Shigella also uses countermeasures to inhibit apoptosis. *Shigella* enters the epithelial cell cytoplasm via M cell entry, and the epithelial cell cytoplasm is subsequently overwhelmed with numerous bacteria that are in close proximity to the mitochondria (Mantis et al., 1996). As mentioned above, *Shigella* infection of the epithelium leads to oxidative stress and a regulated form of necrotic cell death (Carneiro et al., 2009). To dampen epithelial cell death and preserve host cells, *Shigella* interferes with the cell death pathway through both T3SS-dependent (Mantis et al., 1996) and T3SS-independent mechanisms (Carneiro et al., 2009). *Shigella* upregulates the NOD1/Rip2/IKKβ/NF-κB/Bcl-2 prosurvival pathway, which counteracts the epithelial cell death response (Carneiro et al., 2009) (Figure 4). Although there is no direct evidence, it is hypothesized that prosurvival signals through NOD1 may be stimulated by epithelial membrane remnants that are generated when bacteria enter the epithelium (Dupont et al., 2009). However, after this stage, NOD1 may be stimulated by peptidoglycan (PGN) released from the bacterium (Girardin et al., 2003). If this hypothesis is correct, the balance between epithelial cell death and epithelial cell survival may be accidentally achieved during epithelial cell infection.

Other nonenteric pathogens, such as *N. meningitidis*, *N. gonorrhoeae*, and *Chlamydia trachomatis*, neutralize host cell death by modifying mitochondrial permeability. *N. meningitidis*, a causative agent of meningitis and sepsis, adheres primarily to nasopharyngeal epithelial cells via pili and the outer membrane adhesion molecules Opa and Opc and then enters epithelial cells (Virji, 2009). *N. meningitidis* (and *N. gonorrhoeae*) encode a membrane porin, PorB, that is translocated from the bacterial outer membrane into the host epithelial cell membrane upon bacterial contact with the host cell membrane. PorB targets a mitochondrial voltage-dependent anionic channel (VDAC) and dampens epithelial apoptosis (Massari et al., 2003) (Figure 4). *N. gonorrhoeae* initially infect the mucosal surface of the urogenital tract via a type IV pili (Tfp) and Opa, which are required for the pathogenesis that leads to gonorrhea. *N. gonorrhoeae* activate two stress-responsive prosurvival signaling pathways in epithelial cells through the ERK pathway (Howie et al., 2008). The pathogen stimulates the ERK pathway when the Tfp contacts epithelial cells, which in turn downregulates the production of the mitochondria-associated proapoptotic proteins Bad and Bim and augments cytoprotective effects (Howie et al., 2008). *Chlamydia* species, such as *C. trachomatis,* are obligate intracellular pathogens that have evolved a unique intracellular life cycle with remarkable antiapoptotic activities that are required to evade host defense mechanisms and

maintain the replicative niche (Miyairi and Byrne, 2006). Studies indicate that antiapoptotic activity associated with *Chlamydia* correlates with the inhibition of the activation of proapoptotic protein such as Bax and Bak, thus blocking cytochrome *c* release from the mitochondria (Fan et al., 1998; Fischer et al., 2004; Xiao et al., 2004). This antiapoptotic activity is mediated by a *Chlamydia*-secreted protein called CPAF (chlamydial protease/proteasome-like activity factor), which contributes to the degradation of the proapoptotic BH3-only proteins (Pirbhai et al., 2006) (Figure 4). Although the molecular mechanisms that these pathogens use to manipulate signaling involved in epithelial cell death and survival pathways are not fully understood, these studies clearly indicate that the host epithelium uses mechanisms to expel infected cells, while pathogens use various countermeasures to prevent epithelial cell death (and damage) and promote bacterial replication and survival (Table 1).

Autophagy in the Epithelium

Autophagy Acts as a Microbial Surveillance and Clearance System

Autophagy, a ubiquitous bulk degradation system in eukaryotic cells, is an essential cytoplasmic mechanism by which host cells take up and, by lysosome fusion, degrade damaged organelles, misfolded protein aggregates, and invading cytoplasmic bacterial pathogens. Undesirable cytoplasmic protein aggregates and organelles are entrapped within double- (or multiple-) layered isolation membranes, which are the foundation for subsequent autophagosome formation. The enclosed materials are then delivered to autophagosomal compartments and degraded upon autophagosome-lysosome fusion (Deretic and Levine, 2009). Autophagy detects, apprehends, and destroys cytoplasmic invading pathogens, and some autophagic proteins were recently determined to modulate PRR downstream signaling (Delgado et al., 2008; Sanjuan et al., 2007; Shi and Kehrl, 2008; Xu et al., 2007).

Epithelial cells are equipped with both extracellular and intracellular sensing systems that detect intruding microbes by PRRs. Upon ligand binding, PRRs transmit downstream signals that induce cellular activities that mediate the early and effective clearance of pathogens (Delgado et al., 2009). Until recently, there were no reports on NOD1, NOD2, or NLR family proteins in relationship to autophagy; however, Travassos et al. have now provided evidence that links NOD1 and NOD2 with autophagy. They have shown that NOD1 and NOD2 are recruited together with Atg16L1 to the cytosolic face of the cytoplasmic membrane at the *Shigella* entry site and activate autophagy through a mechanism that is independent of the RIP2 and NF-κB (Travassos et al., 2010). They also determined the functional link among NOD2, Atg16L1, and autophagy; a Crohn's disease-associated NOD2 mutant failed to recruit Atg16L1 to the cytoplasmic membrane at the *Shigella* entry site and take up the invaded bacterium into autophagosomes (Travassos et al., 2010). Although the mechanism is still unclear, Cooney et al. showed that NOD2 stimulation by muramyldipeptide in dendritic cells can induce autophagy and that this process requires RIP2, Atg5, Atg7, and Atg16L1, which enhance bacterial clearance and generate MHC II-specific $CD4^+$ T cell responses in the dendritic cells (Cooney et al., 2010). They argued that infection with bacteria, such as *Shigella*, may increase the level of pyroptosis (Suzuki et al., 2007) when the induction of autophagy is defective due to Crohn's disease-associated variants Nod2 or Atg16L1 (Cooney et al., 2010). These studies suggest the existence of a functional link between NLR-mediated immune signaling and the autophagic pathway in macrophages and epithelial cells. In addition, Criollo et al. reported that the IKK complex, composed of IKKα, IKKβ, and IKKγ/NEMO, effectively triggers autophagy in epithelial cells through a process that is independent of NF-κB activation (Criollo et al., 2010). Criollo et al. recently presented evidence that IκB kinase has a central role in inducing autophagy through a canonical pathway driven by p53 depletion, mTOR inhibition, AMPK and JNK1 activation, and the release of Beclin1 (the proautophagic protein) from its inhibitory interaction with Bcl-2 (Criollo et al., 2010). Furthermore, a recent study indicated that autophagy may occur as a direct result of bacterial internalization. Specifically, it was shown that *Yersinia*-induced autophagy in macrophages occurs when the β1-integrin receptor is engaged, but *Yersinia* can prevent autophagy activation through a T3SS-dependent mechanism (Deuretzbacher et al., 2009). GAS efficiently binds and is internalized into epithelial cells. GAS can target CD46, a typical type I glycoprotein that is expressed by all nucleated human cells and serves as receptor that binds *Neisseria*, measles virus Edmonston strain, human herpes virus 6, and adenoviruses B and D (Cattaneo, 2004). During GAS replication, the bacteria are efficiently entrapped in autophagosomes and destroyed upon fusion with lysosomes (Nakagawa et al., 2004). Joubert et al. recently reported that the binding of GAS to CD46 can stimulate autophagy. This interaction links CD46-Cyt-1, one of the two C-terminal splicing variants of CD46, through its interaction with GOPC to the autophagosome formation complex containing Beclin1 and Vps34 (Joubert et al., 2009). Although the downstream events following activation of the CD46-Cyt1/GOPC/Beclin1-Vps34 pathway are still unclear, this study provides additional evidence for a receptor that is used for bacterial internalization and is directly linked with known autophagy-triggering machinery. These studies further confirm that autophagy not only detects invading cytoplasmic bacterial pathogens but also senses the invasion of extracellular bacterial pathogens.

Bacterial Evasion of Autophagic Recognition

Recent studies have established that the interplay between autophagy and bacterial infection is an important process that determines the fate of intracellular bacteria. Some bacterial pathogens are taken up by autophagy and degraded within autolysosomes, while others usurp autophagy for their benefit, and some evade autophagic recognition (Deretic and Levine, 2009; Lerena et al., 2010; Levine and Kroemer, 2008). Among invasive bacterial pathogens, *Shigella*, *L. monocytogenes*, and *Burkholderia pseudomallei* (and perhaps other intracellular pathogens, such as *Rickettsia* and *Mycobacterium marinum*) are capable of inducing actin-based motility in myeloid and non-myeloid cells and have evolved mechanisms to escape autophagic recognition. Without these mechanisms, these pathogens are incapable of multiplying within epithelial cells and spreading from cell to cell, both of which are important to expand their replicative niches. Recent studies suggest that they have evolved mechanisms to evade autophagic recognition, but not to inhibit canonical autophagy (Table 1). *Shigella* can escape autophagy

through a mechanism that requires the IcsB effector delivered via the T3SS during intracellular multiplication. Bacteria that do not produce IcsB are taken up by autophagosomes and degraded by autolysosomes (Ogawa et al., 2005). VirG (IcsA), an outer membrane protein that accumulated at one pole of bacterium and was required for actin-based intracellular bacterial motility, was identified as a target of autophagy (Ogawa et al., 2005). In addition to binding N-WASP, Toca-1, and Vinculin and inducing actin polymerization, the VirG protein also interacted to Atg5, an essential autophagic protein. However, wild-type *Shigella* IcsB could bind to VirG and competitively inhibit the interaction with Atg5, indicating that IcsB acts as an anti-Atg5 binding protein and disguises the target VirG protein from autophagy recognition (Ogawa et al., 2005). A recent study reported that vacuolar membrane remnants generated from *Shigella* invasion can also stimulate the autophagic pathway. Membrane remnants around the bacterial surface recruit LC3 and p62 (Dupont et al., 2009), suggesting that there are two sources that can stimulate autophagy when epithelial cells are infected with *Shigella*.

L. monocytogenes multiplies within macrophages and epithelial cells and moves within and among host cells by recruiting the Arp2/3 complex, Ena/VASP, through the surface protein ActA and inducing actin polymerization (Barbuddhe and Chakraborty, 2009). A recent study in *Drosophila* showed that one of the fly PRRs, PGRP-LE, recognizes listerial diaminopimelic acid-type PGN and stimulates autophagy (Yano et al., 2008). In addition, membrane remnants that are generated upon *L. monocytogenes* invasion and listeriolysin O-mediated membrane rupture can stimulate autophagy (Meyer-Morse et al., 2010), further supporting the current concept that pore-forming bacterial products can act as autophagic agonists (Dupont et al., 2009; Gutierrez et al., 2007; Terebiznik et al., 2009). Several studies have shown that multiple bacterial mechanisms are required to escape autophagy (Birmingham et al., 2007; Py et al., 2007). In addition, we recently determined the mechanism by which *L. monocytogenes* ActA helps evade autophagy through a mechanism that is independent of bacterial motility (Yoshikawa et al., 2009). Analyses of *L. monocytogenes*-expressing ActA mutants that are unable to recruit host proteins showed that these mutants were ubiquitinated, after which they colocalized with p62/SQSTM1 and LC3 and then were taken up by autophagosomes. These findings suggest that coating the entire bacterial surface with ActA-associated host proteins, including the Arp2/3 complex, Ena/VASP, and F-actin, in the host cell cytoplasm allows the pathogen to evade autophagic recognition (Yoshikawa et al., 2009).

Although the precise mechanisms are unclear, *B. pseudomallei* invades macrophages, replicates intracellularly, and delivers BopA via the T3SS, which is shown to play a role in evading autophagy recognition (Cullinane et al., 2008). Cullinane et al. showed that the colocalization of LC3 with the bacteria increased when autophagy was stimulated in macrophages and MEFs. However, blocking bacterial protein synthesis with chloramphenicol increased autophagosomal uptake of bacteria, suggesting that evading autophagy is an active process that requires newly synthesized bacterial proteins. The molecular mechanisms by which bacterial infection induces autophagy are not fully understood, but studies strongly suggest that pathogens are unable to directly control the activation of canonical autophagy but use highly evolved mechanisms to avoid autophagic recognition.

Conclusion

In this review, we highlight the intrinsic defensive systems that protect the epithelial lining from bacterial invasion, including epithelial cell turnover, epithelial cell exfoliation, cell death, autophagy, and the innate immune response, and discuss how bacterial pathogens counteract these defense systems. As exemplified in this review and others (Mulvey et al., 2000; Pitsouli et al., 2009), defenses at both the cellular and tissue levels are intrinsic elements that are essential to maintain tissue homeostasis. On the other hand, bacterial pathogens have evolved mechanisms to defeat the host innate defense systems and usurp and subvert host cellular and immune functions. These strategies allow the bacteria to obtain nutrients from the host, compete with lumenal microbiota, and successfully colonize the host. At the same time, these bacterial activities inflict cellular stresses, tissue injury, and inflammatory and mechanical damages on the epithelium and are occasionally accompanied by cell death, cell exfoliation, cell proliferation, and epithelial breach, resulting in the disruption of the bacterial replicative niche. Therefore, mucosal bacterial pathogens have evolved strategies to renovate damaged epithelium, renew epithelium, dampen cell death, and reinforce epithelial cell shape. These intricate strategies represent a compromise between the pathogen and the host. Although we are still in the process of understanding the pathogenic impact of these bacterial strategies on infectious diseases, future studies will greatly impact our knowledge of these bacterial mechanisms. Molecular and cellular studies of the dynamic interplay between pathogens and the mucosal epithelium will not only uncover uncharacterized bacterial pathogenic strategies, but also provide many insights into the biological impact of host innate defensive elements on the development of inflammatory diseases and cancer.

ACKNOWLEDGMENTS

We thank the members of the Sasakawa laboratory for their advice. This work was supported by a Grant-in-Aid for Young Scientists (B) (20790330, 21790414, and 21790413), a Grant-in-Aid for Scientific Research (B) (20390123), a Grand-in-Aid for Scientific Research (S) (20229006), a Grand-in-Aid for Exploratory Research (20659067), a Grant-in-Aid for Scientific Research on Priority Areas (18073003), and a Contract Research Fund for the Program of Founding Research Centers for Emerging and Reemerging Infectious Diseases from the Ministry of Education, Culture, Sports, Science and Technology (MEXT). Part of this work was supported by grants from the Naito Foundation, the Waksman Foundation of Japan Inc., the Senri LifeScience Foundation, the Uehara Memorial Foundation, and the Yakult Bio-Science Foundation.

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