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Salmonella interaction with and passage through the intestinal mucosa: through the lens of the organism

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Salmonella enterica serotypes are invasive enteric pathogens spread through fecal contamination of food and water sources, and represent a constant public health threat around the world. The symptoms associated with salmonellosis and typhoid disease are largely due to the host response to invading *Salmonella*, and to the mechanisms these bacteria employ to survive in the presence of, and invade through the intestinal mucosal epithelia. Surmounting this barrier is required for survival within the host, as well as for further dissemination throughout the body, and subsequent systemic disease. In this review, we highlight some of the major hurdles *Salmonella* must overcome upon encountering the intestinal mucosal epithelial barrier, and examine how these bacteria surmount and exploit host defense mechanisms.

Keywords: *Salmonella*, intestinal mucosa, tight junctions

SALMONELLA BIOLOGY

Salmonella enterica are Gram (−) bacteria responsible for causing typhoid disease and gastroenteritis. *S. enterica* serovar Typhi (*S.* Typhi) is the primary cause of typhoid fever, while non-typhoidal *Salmonella* (NTS) strains can cause *Salmonella*-induced food poisoning called salmonellosis. While young children, the elderly, and immuno-compromised individuals are most at risk for complications, people at any age are susceptible to the diarrhea, intestinal cramping, and intestinal epithelial erosion associated with salmonellosis. The disease is primarily spread by the contamination of water and food items with fecal matter from infected hosts and is often self-limiting, but can cause prolonged complications (Graham et al., 2000).

Salmonella possess *Salmonella* pathogenicity islands (SPI), or collections of pathogenesis-related genes acquired horizontally. At least 21 SPIs have been identified in *S.* Typhimurium and *S.* Typhi combined (for review of functions, see Sabbagh et al., 2010). In *S.* Typhimurium and in *S.* Typhi, SPI-1 and SPI-2 contain genes for two type-three secretion systems (T3SS). Specific to Gram (−) bacteria, the T3SS likely evolved from the flagella basal body, and is composed of a motor, needle complex, and translocon through which secreted effectors are injected into host cells (Stebbins and Galan, 2003). The effectors provide various functions, including promoting bacterial entry, controlling inflammatory responses, and regulating bacterial survival within the cell (summarized in **Table 1**). The SPI-1 T3SS (T3SS1) is primarily associated with invasion (Galan, 1996). Effectors secreted through the SPI-2 T3SS (T3SS2) seem to primarily promote the intracellular survival of *Salmonella*, although they may not be absolutely required for *S.* Typhi survival within human macrophages (Forest et al., 2010). However, in *S.* Typhi infections of humanized non-obese–diabetic mice, the loss of some SPI-2 genes caused a competitive disadvantage (Libby et al., 2010). Also, SPI-2 genes are up-regulated during *S.* Typhi infection of macrophages *in vitro* (Faucher et al., 2006). While the functions of SPI-1 and SPI-2 effectors have traditionally been considered distinct from each other, there is increasing evidence suggesting overlap in the times at which effectors from each system are required (Lawley et al., 2006; Brawn et al., 2007). These data highlight the intricate level of coordination between SPI-1 and SPI-2 involved with *Salmonella* pathogenesis. Nonetheless, recent data challenges the dependence of T3SS1 in *Salmonella* invasion (Radtke et al., 2010). SPI-1 mutant strains of *Salmonella* were capable of invading HT-29 3D intestinal cells to a higher degree than HT-29 monolayers, although the level of invasion was less than that of the wild-type strains. Additionally, SPI-1 was required for wild-type levels of intracellular replication over a 24-h period. The additional loss of SPI-2 may enhance these phenotypes. These data suggest that various pathways are required for wild-type levels of invasion and intracellular replication. Other types of secretion systems that may promote pathogenicity have been identified in additional SPI loci (Sabbagh et al., 2010).

SALMONELLA INTERACTION WITH THE INTESTINAL MUCOSAL EPITHELIA

The gastrointestinal tract is the largest mucosal surface in the human body, with the epithelial monolayer surface area alone measuring 400 m^2 (MacDonald and Monteleone, 2005; Turner, 2009). Regulating the host microbiota, immune responses, and barrier functions is paramount to providing timely and controlled retaliation to pathogenic assaults, while simultaneously maintaining a healthy, balanced, intestinal environment (**Figure 1**).

GUT MICROBIOME

The human intestine is home to a plethora of mutualistic microorganisms, dominated mostly by *Lactobacillus*, *Bacteroides*, and *Firmicutes* (Backhed et al., 2005). Among other roles, these organisms assist immune system development and promote epithelial homeostasis (Rakoff-Nahoum et al., 2004; Mazmanian et al., 2005). Beyond

Table 1 | *Salmonella* effectors and their roles in pathogenesis.

Effector	Location	Function	Targets	T3SS apparatus
AvrA	SPI-1	Controls *Salmonella*-induced inflammation (Collier-Hyams et al., 2002; Ye et al., 2007).	IkBa, beta-catenin	1
SipA	SPI-1	Mediates invasion at apical surface by inducing actin bundling and promotes neutrophil migration to the apical surface. Also suspected to promote SCV formation. Undergoes cleavage by CASPASE-3 (Zhou et al., 1999; Brawn et al., 2007; Wall et al., 2007; Srikanth et al., 2010)	Actin	1
SipB	SPI-1	Component of T3SS1 translocon (Kaniga et al., 1995; Hayward et al., 2000)	Cholesterol	1
SipC	SPI-1	SPI-1 translocon component, induces actin bundling to promote invasion (Kaniga et al., 1995; McGhie et al., 2001)	Actin	1
SipD	SPI-1	Component of T3SS1 translocon (Lara-Tejero and Galan, 2009)		1
SopA		E3 ubiquitin ligase that may promote escape from SCV and promotes neutrophil migration. Also required during invasion (Wood et al., 2000; Raffatellu et al., 2005; Zhang et al., 2006)		1
SptP	SPI-1	Similar to GAPs, and is a tyrosine phosphatase. Reverses pro-inflammatory responses due to other *Salmonella* effectors (Stebbins and Galan, 2000)	Cdc42, Rac-1	1
SopB	SPI-5	Inositol polyphosphate phosphatase that promotes macropinocytosis, regulates SCV localization, and promotes fluid secretion (Norris et al., 1998; Hernandez et al., 2004)	Inositol phosphates	1
SpiC	SPI-2	Helps regulate T3SS2 secretion (Yu et al., 2002)	Hook 3, TassC	2
SseF	SPI-2	SCV regulation (Abrahams et al., 2006)	Microtubules	2
SseG	SPI-2	SCV positioning (Abrahams et al., 2006)	Microtubules	2
SopD		Promotes invasion and fluid secretion (Zhang et al., 2002; Raffatellu et al., 2005)		1/2
SopE	Bacteriophage	Promotes membrane ruffling and disrupts tight junctions (Hardt et al., 1998; Boyle et al., 2006)	Rac-1, Cdc42	2
SopE2		Promotes membrane ruffling and disrupts tight junctions (Stender et al., 2000; Boyle et al., 2006)	Cdc42	2
SspH1	Bacteriophage Gifsy-3	E3 ubiquitin ligase (Rytkonen and Holden, 2007)		1/2
SspH2	SPI-12	E3 ubiquitin ligase (Quezada et al., 2009)		2
PipB2		Promotes Sif extension (Knodler and Steele-Mortimer, 2005)	Kinesin-1	2
SifA		Sif formation and membrane integrity (Stein et al., 1996; Beuzon et al., 2000)	SKIP, Rab7	2
SopD2		Sif formation and promotes bacterial replication in mouse macrophages (Jiang et al., 2004)		2
SseJ		Negatively regulates Sifs and antagonizes SifA-mediated stability of SCV (Ruiz-Albert et al., 2002)	Cholesterol	2
SseL		Cysteine protease and has de-ubiquitinating activity. Helps attenuate *Salmonella* virulence (Rytkonen et al., 2007)		2
SteC		A kinase that promotes F-actin meshwork formation (Poh et al., 2008)		2
SpvB	pSLT (in *S.* Typhimurium)	Depolymerizes actin filaments *in vitro* (Lesnick et al., 2001)	Actin	2
SpvC	pSLT (in *S.* Typhimurium)	A phosphothreonine lyase required for complete virulence in murine models (Mazurkiewicz et al., 2008)		1/2

A summary of effectors whose functions in Salmonella pathogenesis have been identified.

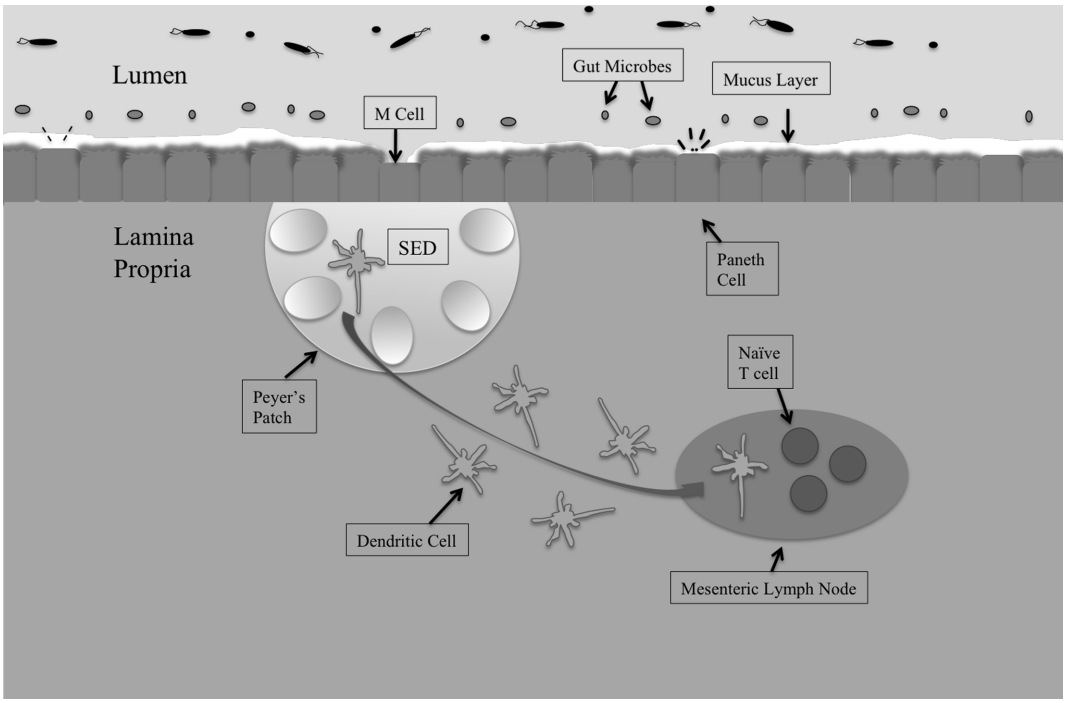

FIGURE 1 | The intestinal mucosal epithelium is home to various interacting cell types that come together to maintain intestinal homeostasis and protect against invading pathogens. The first line of defense is the host microbiota, populations of commensal organisms that compete with invading pathogens for nutrients and space. The mucus layer protects against *Salmonella* invasion of epithelial cells, and the bacteria must adhere to mucus components in order to remain in the intestines. The epithelial monolayer underlying the mucus layer contains distinct cell types with different roles. M cells sample intestinal antigens and are the preferred route of entry by *Salmonella*. Underlying the M cells is the subepithelial dome (SED) that houses Peyer's patches. Peyer's patches contain germinal centers and have associated dendritic cells. Dendritic cells take whole bacteria to the mesenteric lymph node (MLN), from which *Salmonella* can escape to promote systemic disease.

these regulatory roles, the microbiome also functions as a critical barrier to invading pathogens. The population of host microbes in the gut physically blocks pathogen access to the epithelial layer, and also outcompetes pathogens for nutrients, thus reducing the survival and invasiveness of intestinal pathogens. Nevertheless, *S.* Typhimurium is capable of maneuvering through the microbiome to reach intestinal epithelial cells. In mouse experiments with wild-type and avirulent *S.* Typhimurium, Stecher et al. demonstrate that an inflammatory state in the intestines not only permits wild-type *S.* Typhimurium to outcompete the microbiome during colonization, but also allows a normally avirulent strain to colonize the intestines in the presence of intestinal microbiota (Stecher et al., 2007). These data unveil an intriguing concept that *S.* Typhimurium utilizes inflammation to outcompete host microbiota in mouse models of salmonellosis.

Host microbiota may also regulate the degree of *S.* Typhimurium shedding by infected mice. Following infection with 10^8 CFU of bacteria, mice were classified into three groups corresponding to the level of fecal shedding: supershedders (>10^8 CFU/g feces), moderate shedders (10^4–10^8 CFU/g feces), and low shedders (<10^4 CFU/g feces). Treating low shedders with streptomycin converted these mice to supershedders (Lawley et al., 2008), suggesting that the absence of host microbes may have permitted *Salmonella* to colonize more heavily, thus elevating the level of shed bacteria. The significance of a gut microbiota barrier to *Salmonella* infection was also explored by Croswell et al. (2009). The authors found that treatment of mice with streptomycin, streptomycin–bacitracin, or ampicillin–vancomycin–neomycin–metronidazole (AVNM) reduced gut microflora populations, and that each treatment was subsequently associated with an increase in *Salmonella* colonization and *Salmonella*-induced inflammation compared to untreated mice. The authors further show that allowing 3 weeks between antibiotic treatment and infection still predisposed mice to greater levels of inflammation in response to *Salmonella* infection than was seen in mice that were never treated with antibiotics. These results suggest that antibiotics have long-lasting effects on gut microflora populations, and that the presence and composition of gut microfloral populations may play an important role in controlling *Salmonella* colonization.

Additionally, Barman et al. (2008) showed that salmonellosis may alter the microfloral population in FvB mice. Infected mice showed disrupted numbers of *Bacteroides, Lactobacillus/Enterococcus*, and *Eubacterium rectale/Clostridium coccoides* groups and of the *C. perfringens* group compared to uninfected mice. However, *Salmonella* did not replace these populations, and wild-type population numbers returned after *Salmonella* clearance, suggesting any effect salmonellosis has on the intestinal microbiota is not permanent.

MUCOSAL LAYER

Key to the mucosal epithelium is the formation of a mucus layer along the luminal lining of the gastrointestinal tract. Mucosal epithelial cells, specifically goblet cells, secrete glycosylated transmembrane

proteins called mucins at the cell surface. These proteins generate a layer of large complexes containing thread-like structures and oligosaccharides. The resulting gelatinous layer blocks contact between the underlying epithelial monolayer and large particles, including bacteria. The mucus layer also contains trefoil factors, which are peptides produced in tissues containing mucus-producing cells, such as the intestinal epithelia. Their various proposed functions include limiting intestinal inflammation (Playford et al., 1996) and regulation of immune system responses (Baus-Loncar et al., 2005). Adhering to the mucosal layer would be necessary for *Salmonella* to avoid being washed out of the intestines. Indeed, there is evidence of *Salmonella* binding to mucus, although increased adherence does not necessarily correlate with increased mucosal penetration (Nevola et al., 1987; McCormick et al., 1988; Vimal et al., 2000).

EPITHELIAL MONOLAYER: M CELLS AND THE ASSOCIATED PEYER'S PATCHES

The epithelial monolayer mediates interactions between triggers of immune responses, and the gut-associated lymphoid tissue (GALT), where immune responses originate (MacDonald and Monteleone, 2005; Turner, 2009). A follicle-associated epithelial (FAE) layer of columnar epithelial cells blankets lymphoid tissue within the intestinal wall. The intestinal mucosa contains a variety of cell types with unique functions including enterocytes, entero-endocrine cells, goblet cells, Paneth cells, and microfold (M) cells (**Table 2**). While all the unique epithelial cells found within the intestine are important for establishing the functional intestinal mucosal epithelium, in mice the M cells are a key route of invasion by *Salmonella*, and are thus discussed in greater detail here.

Microfold cells are specialized intestinal epithelial cells that are found in the FAE overlying mucosa-associated lymphoid tissue (MALT). Their primary role is to sample mucosal contents and transfer antigens from the lumen to underlying Peyer's patches (Tam et al., 2008), and thus act as sentinels of the intestinal epithelium. The overall structure of M cells differs in various ways from that of enterocytes. For example, the apical (mucosal) surface of M cells is not covered by the mucus layer observed over other cells in the intestines (Frey et al., 1996). Additionally, the apical brush border characteristic of enterocytes is absent from M cells, which instead contain microfolds. These features promote the sampling role of M cells, but also inadvertently provide opportunities for enteropathogen docking and invasion.

An additional trait of M cells that promotes both their role as intestinal sentinels and promotes enteropathogen invasion is their use of a transcytotic pathway to shuttle luminal contents to lymphoid tissue (Kraehenbuhl and Neutra, 2000). Following endocytosis of extra-cellular material (including invading pathogens), M cells transfer the endosomal contents to the basolateral (serosal) surface and to the underlying MALT. This action results in appropriate immune responses to infection. Likewise, bacteria engulfed at the apical surface can escape the M cell through this same pathway and disseminate to other areas.

M cells provide a key route of invasion by *S.* Typhimurium (Jensen et al., 1998). SPI-1 genes help regulate *Salmonella* invasion through M cells, although SPI-1-independent processes are also proposed to regulate M cell invasion (Clark et al., 1996). For example, *Salmonella* defective for *invA* are less able to invade M cells (Clark et al., 1998), but SPI-1 mutants are still capable of invading M cells (Martinez-Argudo and Jepson, 2008). These results suggest that while SPI-1 genes are needed for wild-type levels of invasion, SPI-1-independent mechanisms are also important for *Salmonella* invasion through M cells.

The lymphoid tissue underlying M cells contains Peyer's patches, which are large aggregates of B lymphocyte follicles that contain germinal centers, and can be found along the length of the intestine. Here, the antigens translocated by M cells are processed by local dendritic cells. Lysozyme-producing dendritic cells associated with Peyer's patches mediate uptake of *S.* Typhimurium (LeLouard et al., 2010). Peyer's patches also play a role in up-regulating intestinal IgA as a result of *Salmonella* infection (Hashizume et al., 2008).

EPITHELIAL MONOLAYER: STRUCTURE AND *SALMONELLA* ENTRY

The barrier function of the epithelial monolayer results from a variety of proteins that maintain close intercellular interactions. Desmosomes are composed of cadherins, which form an adhesive

Table 2 | Cells within the intestinal epithelial monolayer.

Cell type	General function	*Salmonella* infection impact
Goblet cell	Sustained and environmentally triggered mucin production (Deplancke and Gaskins, 2001)	Loss of goblet cells reported as a result of *S.* Typhimurium-mediated colitis in mice (Hapfelmeier et al., 2004, 2005), although this observation is not restricted only to colitis caused by *Salmonella*. Invasion of goblet cells reported in *Salmonella* infections of pig ileal loops (Meyerholz et al., 2002; Meyerholz and Stabel, 2003).
Paneth cell	Secrete the anti-microbial peptides lysozyme and alpha-defensin (Ayabe et al., 2000)	*S.* Typhimurium may modulate the production of anti-microbial peptides (Salzman et al., 2003)
M cells	Transfer antigens to Peyer's patches (Tam et al., 2008)	M cells are the preferred route of invasion by *Salmonella* (Jensen et al., 1998)
Enterocytes	*Absorptive/villus enterocytes*: Nutrient absorption *Crypt enterocytes*: Chloride and IgA secretion	*Salmonella* invasion of absorptive enterocytes reported in calf ileal loops (Frost et al., 1997), and in pig ileal loops (Meyerholz et al., 2002; Meyerholz and Stabel, 2003)
Entero-endocrine	Secretion of various hormone molecules, promote food digestion	*Salmonella*-specific responses undetermined

interface (Koch and Franke, 1994; Green and Simpson, 2007), and varying structural proteins, notably plakoglobin and desmoplakin (Hatsell and Cowin, 2001), all of which help disperse forces from physical stress. Meanwhile, the paracellular pathway, or area between adjacent epithelial cells, is kept closed by tight junctions and adherens junctions. Bacteria attempting to penetrate the epithelial monolayer must possess mechanisms for overcoming these strong interactions. As such, *Salmonella* possess several effectors that mediate disruption of the epithelial barrier and subsequent uptake into the non-phagocytic epithelial cells lining the intestinal lumen. These actions are carried out largely through the alteration of junctional protein localization and of Rho GTPase activity. The latter mechanism is especially important in the *Salmonella*-mediated modulation of tight junctions. Dis-regulating the Rho-GTPases leads to changes in organization of junctions and of actin at the cell membrane, which subsequently alters the membrane morphology in a manner referred to as "ruffling" (Finlay et al., 1991), and promotes *Salmonella* entry (**Figure 2**).

Interactions between classical (Ca^{2+} dependent) cadherins and catenin family proteins are the foundation of adherens junctions. E-cadherin (epithelial cadherin) is a single pass transmembrane protein and its ability to homodimerize with E-cadherins on neighboring cells makes it a key component of adherens junctions (Takeichi, 1991). E-cadherins form small, intercellular clusters that quickly associate with actin and, overtime, expand into larger bundles that strengthen cell–cell adhesion (Adams et al., 1998). p120-catenin stabilizes E-cadherin at the cell surface, and its loss induces down-regulation of E-cadherin (Davis et al., 2003). Further, beta-catenin, a transcription factor involved in cell proliferation and differentiation, can bind to the cytoplasmic domain of E-cadherin (Hartsock and Nelson, 2008), and direct the localization of E-cadherin from the ER to the plasma membrane (Chen et al., 1999). Phosphorylation of beta-catenin promotes its ubiquitination and subsequent degradation (Aberle et al., 1997). Duan et al. (2007) demonstrated that cultured epithelial cells infected with *S.* Typhimurium display increased phosphorylated beta-catenin. This increase in phosphorylated beta-catenin resulting from *Salmonella* infection could increase the level of beta-catenin degradation, and thus could limit E-cadherin translocation to the plasma membrane. Such an action would result in weakened adherens junctions and promote *Salmonella* invasion through the epithelial monolayer.

The tight junction is an important regulator of epithelial monolayer permeability (Martinez-Palomo and Erlij, 1975), and of cell polarity by preventing mixing of apical (mucosal) and basolateral (serosal) components. The "leak pathway" permits passage of larger solutes, such as bacterial peptides (but not whole bacteria), while the "small pore pathway" excludes solutes larger than 4 Å, and exhibits some charge selectivity (Forster, 2008; Turner, 2009). The core of tight junction complexes is composed of occludin, ZO, and claudin proteins. Occludin binds to several crucial tight junction proteins including ZO-1 (Furuse et al., 1994), ZO-2 (Itoh et al., 1999), and ZO-3 (Haskins et al., 1998) and its activity is regulated by PKC-mediated phosphorylation (Andreeva et al., 2001). ZO-1, ZO-2, and ZO-3 are members of the membrane-associated guanylate kinase (MAGUK) family (Gonzalez-Mariscal et al., 2000). ZO-1 and ZO-2 assist in the polymerization of claudins, which permits extension of tight junctions, and also recruit ZO-3 to the tight junction (Umeda et al., 2006; Tsukita et al., 2009). A specific role for ZO-3 has yet to be determined, although it is shown to not be required for tight junction formation (Adachi et al., 2006). Claudins, which are tetraspan transmembrane proteins, are key structural elements, as they recruit occludin (Furuse et al., 1998). Another class of tight junction proteins include junction-associated adhesion molecules (JAMs), or which are integral membrane proteins that belong to the immunoglobin superfamily.

Cytoskeletal regulators of tight junctions include myosin ATPase, AMP-activated protein kinases, and especially Rho-GTPases (Turner, 2009). Rho-GTPases regulate various cell functions and

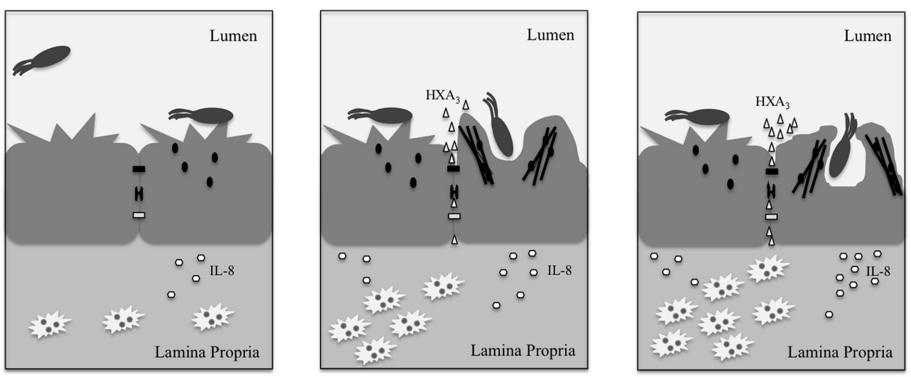

FIGURE 2 | Upon interacting with the epithelial cell, *Salmonella* secrete effectors (small black ovals) that promote release of the neutrophil chemoattractant, HXA_3 (small triangles), and membrane ruffling as a result of actin (black lines) re-organization. The release of HXA_3 from the apical (mucosal) surface creates a concentration gradient across tight junctions (small black rectangle), desmosomes (small black bracket), and adherens junctions (small white rectangle) and through the paracellular space. Neutrophils (jagged white ovals) are recruited via basolateral secretion of IL-8, and subsequently migrate from the basolateral surface to the apical surface to the point of infection due to the HXA_3 gradient. The actin re-organization alters the morphology of the cell membrane at the apical surface in a manner that promotes *Salmonella* uptake via macropinocytosis. Once inside the cell, *Salmonella* reside within a compartment termed the SCV that forms as a result of macropinocytosis.

are activated by guanine-nucleotide exchange factors (GEFs), which facilitate the active, GTP-bound state. GTPase activating protein (GAPs) inhibit Rho-GTPases by activating GTP hydrolysis, thereby inducing the inactive, GDP-bound state (Etienne-Manneville and Hall, 2002). Rho-GTPases are regulated by several proteins, the best-studied being Rho, Cdc42, and Rac. Rho is required for formation of focal adhesions and stress fibers, while constitutive Rac causes formation of plasma membrane extensions (Ridley and Hall, 1992; Ridley et al., 1992). Cdc42 activity promotes finger-like extrusions called filopodia (Nobes and Hall, 1995).

The tight junction is a key target of *Salmonella*, as infection with *S.* Typhimurium induces altered localization of ZO-2 and claudin-1, degradation of ZO-1, and promotes dephosphorylation of occludin in T84 cells (Kohler et al., 2007). *Salmonella* SPI-1 effectors SopB, SopE, SopE2, and SipA each play various roles in inducing ruffling and bacterial uptake by changing the localization and expression of ZO-1 and occludin, although SipA and SopB alone are not sufficient for this process (Boyle et al., 2006). These same effectors were also associated with altered epithelial cell polarity. Specifically, SopB (or SopB/SigD) is a phosphoinositide phosphatase that promotes formation of macropinosomes (Hernandez et al., 2004). This effector is capable of indirectly stimulating Cdc42-dependent cytoskeletal rearrangements (Zhou et al., 2001), which can lead to extension of the cell membrane around the bacterium. Similarly, SopE and SopE2 act like GEFs, and activate Cdc42 and Rac1 GTPases to induce cytoskeletal rearrangements that favor bacterial uptake (Stender et al., 2000). SipA and SipC associate with actin and promote bundling of actin filaments so as to facilitate macropinocytosis of *Salmonella* (McGhie et al., 2001).

As control mechanisms, *Salmonella* also secrete SptP and AvrA. SptP acts like a GAP, and essentially reverses the cytoskeletal rearrangements that occur during *Salmonella* uptake (Fu and Galan, 1999). AvrA, which stabilizes tight junctions (Liao et al., 2008) and inhibits NF-kB activation (Collier-Hyams et al., 2002), keeps the *Salmonella*-induced inflammation response in check.

IMMUNE RESPONSE

PAMP Receptors

Intestinal epithelial cells can recognize invading pathogens by capturing common viral or bacterial components, known as pathogen-associated molecular patterns (PAMPs). Toll-like receptors (TLRs) are a well-studied family of PAMP receptors. Several types of intestinal epithelial cells have been shown to express TLRs (Elphick and Mahida, 2005; Tyrer et al., 2006; Palazzo et al., 2007; Gribar et al., 2008), and each type of receptor recognizes a unique PAMP. TLR4 recognizes lipopolysaccharide (LPS) from Gram (−) bacterial cell walls, TLR2 in concert with TLR1 or TLR6 recognizes triacyl or diacyl bacterial lipopeptide, respectively, and TLR5 recognizes flagellin protein from bacterial flagella. The binding of a TLR to its cognate antigen triggers activation of a signaling cascade that ultimately, via NF-kB activation, induces production of pro-inflammatory cytokines. TLR5-flagellin interaction during *S.* Typhimurium in mouse infections was shown to regulate the early stages of *Salmonella* infection and immune response. Peyer's patches and mesenteric lymph nodes (MLN) in TLR5-deficient mice accumulate more *Salmonella* than wild-type littermates, and aflagellate *Salmonella* accumulate more in Peyer's patches and MLN of wild-type mice than do wild-type *Salmonella* (Fournier et al., 2009). As *Salmonella* encounter Peyer's patches after traversing the epithelial monolayer, regulation at this stage would be key to preventing further bacterial dissemination.

NLRs, or nucleotide-binding and oligomerization domain (NOD)-like receptors, are another established family of PAMP receptors that recognize components of bacterial peptidoglycan. The NLR family includes Nod1 and Nod2, both of which are expressed intracellularly by intestinal epithelial cells (MacDonald and Monteleone, 2005). Nod1 recognizes – D-glutamyl-*meso*-diaminopimelic (DAP) acid, expressed predominantly by Gram (−) bacteria (Chamaillard et al., 2003), while Nod2 recognizes muramyl dipeptide (MDP), which is common to Gram (−) and Gram (+) bacteria (Girardin et al., 2003). Binding between Nod1 and Nod2 and their specific PAMPs is followed by association with the adaptor protein Rip2, which initiates production of NF-kB-dependent pro-inflammatory cytokines. A recent paper suggests the role of Rip2-mediated inflammatory responses is key during *Salmonella* infection only when SPI-2 effectors are present (Geddes et al., 2010). As SPI-2 effectors are associated predominantly with intracellular survival, Rip2, and thus Nod1 and Nod2, may be required for dampening the ability of *Salmonella* to survive after invading host cells.

NEUTROPHIL RECRUITMENT

The release of IL-8 resulting from pathogen-induced immune signaling stimulates the recruitment of neutrophils from blood vessels to the basolateral (serosal) surface (**Figure 2**). Neutrophils are phagocytic white blood cells that are among the first line of the innate immune defense in response to pathogens. Recruitment of neutrophils to the apical (mucosal) surface is mediated by release of hepoxilin A_3 (HXA_3), a potent neutrophil chemoattractant, and a metabolite of the arachidonic acid pathway (McCormick, 2007). SipA at the apical surface induces a lipid signal cascade that includes the activation of PKC, which causes the release of arachidonic acid from the plasma membrane (Wall et al., 2007). Arachidonic acid is then converted to HXA_3 by 12-lipoxygenase activity. Release of HXA_3 is facilitated by the ABC transporter, MRP2 (Pazos et al., 2008), and generates a concentration gradient across the tight junction and through the paracellular space. The recruitment of neutrophils superficially seems solely an act of host defense; however the rapid migration of these cells through the epithelial monolayer may actually loosen the epithelial cell–cell interactions, and thus create space through which *Salmonella* can invade, further facilitating PMN infiltration (Kohler et al., 2007). Indeed, PMN transmigration has been demonstrated to reduce epithelial monolayer resistance *in vitro* (Nash et al., 1987).

SALMONELLA RESIDE IN THE *SALMONELLA* CONTAINING VACUOLE

The *Salmonella* containing vacuole (SCV) is an intracellular vacuole that forms via macropinocytosis of *Salmonella*. The SopB effector helps direct the maturation of the SCV into a compartment

suitable for bacterial survival and replication (Bakowski et al., 2008), and may function to steer the compartment away from the endocytic pathway (Hernandez et al., 2004). After formation of the SCV, many T3SS2 effectors assist in formation of *Salmonella*-induced filaments (Sifs), localization of the SCV, and intravacuolar replication.

SifA, SseF, SseG, and SopD2 are involved with Sif formation, which results in the extension of tubules outward from the SCV after fusion with late endosomal compartments (Brumell et al., 2001). SifA is required for Sif formation (Stein et al., 1996), as its absence precludes formation of any Sif structures. SseF and SseG are also required for proper Sif formation, as mutants lacking either of these effectors only form Sif-like structures that differ in composition from fully formed Sifs (Kuhle et al., 2004). SopD2 has recently been shown to balance the effects of SifA, as its loss in SifA mutants restores SCV stability in the presence of other effectors (Schroeder et al., 2010). The role of Sifs in *Salmonella* survival and replication has yet to be determined. To date, these structures have not been observed *in vivo*. SseF and SseG also localize with, and promote bundling of, microtubules in the cytoplasm and help position the SCV near the microtubule organizing center (MTOC), which is close to the Golgi network (Kuhle et al., 2004; Ramsden et al., 2007). Proximity to the Golgi network appears necessary for efficient bacterial replication within the SCV, as disruption of the Golgi network and loss of SseG diminish bacterial growth (Salcedo and Holden, 2003).

Once *Salmonella* enter the host epithelial cell, they seem to form two populations with distinct doubling rates. One population, referred to as "hyper-replicating," doubles approximately every 20 min while the population as a whole doubles approximately every 95 min (Knodler et al., 2010). Interestingly, at least a third of the hyper-replicating population was found in the cytosol and express T3SS1 genes, while the slower replicating population was found in SCVs, and was expressing T3SS2 genes. These findings suggest that while *Salmonella* are capable of replicating within the SCV, cytosolic replication may be more efficient.

SALMONELLA INDUCES ILEAL SECRETION

One of the hallmarks of salmonellosis is a severe, watery diarrhea caused by high levels of fluid secretion into the intestines. Various T3SS1 effectors are proposed to mediate the level of fluid secretion into the gut, likely due to their ability to induce inflammation, including SopA, SopB, SopD, SopE2, and SipA (Zhang et al., 2002). Additionally, the loss of some genes encoded on SPI-5, including SopB, significantly reduced the level of *S. dublin*-induced fluid secretion from ligated ileal loops (Wood et al., 1998). One theory is that the rapid influx of neutrophils, in response to the inflammation caused by secreted effectors, impairs the epithelial barrier to an extent that results in leakage of extravascular fluids (Zhang et al., 2003). Neutrophil migration could also lead to chloride secretion by epithelial cells (Madara et al., 1993), an event that is compensated for by the subsequent secretion of water in an attempt by the host to restore ion balance.

MECHANISMS OF *SALMONELLA* ESCAPE FROM EPITHELIAL CELLS AND DISSEMINATION

Salmonella Typhi, using the same initial invasion mechanisms as *S.* Typhimurium can disseminate in humans to cause systemic disease through infection of the gallbladder, liver, spleen, and bone marrow (Gonzalez-Escobedo et al., 2011). In mice, *S.* Typhimurium is capable of invading and destroying M cells, allowing penetration of the intestinal epithelium (Jones et al., 1994), and subsequent phagocytosis by macrophages. *S.* Typhimurium survival within the macrophages is mediated by SPI-2 effectors (Cirillo et al., 1998) and is essential for dissemination.

The role of the MLN in bacterial dissemination was closely explored by Voedisch et al. Here, the authors show that dendritic cells are major suppliers of *S.* Typhimurium to the MLN, but they alone are not regulating dissemination. After permitting re-circulation of intestinal lymph following removal of the MLN (mesenteric adenectomy), the authors observed increased liver colonization by *S.* Typhimurium in wild-type mice, and increased colonization of liver and spleen in NRAMP1+ 129Sv mice. Wild-type levels of liver and spleen colonization were observed in Rag2-deficient mice, suggesting the observations in the adenectomized mice were not artifacts of a weak adaptive immune response (Voedisch et al., 2009). These results suggest that while dendritic cells supply the MLN with *S.* Typhimurium, the MLN is the final barrier to dissemination in this pathway, as its presence is needed to limit the circularization of infected dendritic cells.

Although most NTS infections are self-limiting, NTS strains are capable of inducing prolonged infection, particularly in those with weakened immune systems. To cause prolonged infection, *Salmonella* must have a mechanism in place to facilitate escape from infected host cells. A recent paper suggests *Salmonella* escape their intracellular niche by co-opting the epithelial cell shedding process, a host mechanism used to remove dying epithelial cells. During this process, called extrusion, cells adjacent to a dying cell contract and force the dying cell out into the lumen (Madara, 1990; Mayhew et al., 1999). The authors found that during *Salmonella* infection, extrusion rates were increased, and about 10% of infected cells underwent extrusion, followed by inflammatory cell death. In contrast, less than 1% of uninfected cells underwent extrusion, and those that were extruded did not exhibit inflammatory cell death (Knodler et al., 2010). Escape into the lumen can permit *Salmonella* to infect additional cells, or to exit the host completely as part of its transmission cycle.

CONCLUDING REMARKS

Salmonella employ various mechanisms to overcome host defense mechanisms. By evolving ways to subvert, mimic, antagonize, or exploit a defense strategy, *Salmonella* maintain their ability to infect vertebrate hosts. The present and future research endeavors aimed at better understanding the tools *Salmonella* use to invade and traverse the mucosal intestinal epithelia will provide invaluable knowledge that will help devise ways to better treat and prevent *Salmonella* infections.

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