16

Salmonella: Invasion, Evasion & Persistence

Belal Chami and Shisan Bao
The University of Sydney, Bosch Institute
Australia

1. Introduction

The gastrointestinal tract, home to a large flora of commensal bacteria, performs the essential role of degradation and absorption of food via gastrointestinal epithelia. The semi-permeability of the epithelial barrier allows for food-derived nutrients to pass through and enter the bloodstream, while simultaneously acting as a physical and chemical barricade towards microbes. Despite this, many bacteria have evolved the means to elude the gastrointestinal tract and migrate to underlying host tissue, causing disease. These disease-causing bacteria are largely thought to obtain their virulence through horizontal gene transfer, which has enabled the bacterium to overcome the epithelial barrier and disguise itself from the intestinal immune system (Flannagan et al., 2009).

Salmonella species are facultative, gram-negative intracellular bacteria harbouring over 2000 serovars; however, only a handful is associated with disease in humans. While *Salmonella* remains the leading cause of bacterial gastroenteritis, it is also one the most extensively studied and well characterised bacterial species. Worldwide, *Salmonella* is thought to cause ~1.3 billion cases of human disease, including diarrhoea and typhoid fever (McGhie et al., 2009). Moreover, 3-5% of patients with typhoid fever develop persistent infection of the gallbladder which acts as a crucial reservoir to the human-specific *Salmonella enteric serotype* typhi (S. Typhi). The recent development of the streptomycin pre-treated murine model of *Salmonella* enterica serovar typhimurium (S. Typhimurium) has become the pinnacle in gaining critical insight into the pathogenesis of diseases caused by *Salmonella*. More specifically, it has enabled scientists to investigate molecular mechanisms of invasion, evasion, intracellular survival and persistence of *Salmonella* in host tissue.

In this chapter, we aim to decipher the pathogenesis of *Salmonell*a by breaking down and exploring the effects of various virulence factors in *Salmonella*. The knowledge of the molecular basis of host-pathogen interactions will bring help bridge the gap between molecular events and clinical presentations of Salmonellosis.

2. Internalization of Salmonella

2.1 M cells

Membraneous epithelilal (M) cells are sparsely distributed, specialized cells characterized by an irregular microvilli apical surface and a basolateral cytoplasmic invagination (or an intraepithelial pocket) which can harbour lymphocytes or macrophages. M cells are not

randomly distributed; rather they reside in the specialised follicle-associated epithelium (FAE) overlying lymphoid aggregates such as Peyer's patches. The molecular features of the apical surface of M cells promote adherence, uptake and sampling of inert particles and microbes within the lumen, which are transferred to the underlying lymphoid tissue where immunological responses are initiated (Kraehenbuhl & Neutra, 2000). In most cases, only antigens that adhere to the surface of M cells induce strong immunological responses. However, several types of bacteria and viruses are known to exploit M cells by selectively adhering to the apical surface, internalising themselves and evading mucosal immune responses (Neutra et al., 1996; Sansonetti & Phalipon, 1999). Numerous studies have shown both S. Typhi (Kohbata et al., 1986; Pascopella et al., 1995) and S. Typhimurium (Clark et al., 1994; Jones et al., 1994) preferentially target and invade mouse M cells and that *S.* Typhimurium also invades M cells in calf ileal loops (Frost et al., 1997). Despite these reports, there is a lack of direct evidence that human M cells are a major site of *Salmonella* invasion, although it is widely speculated that sites of *Salmonella* evasion in humans parallel mice and calf studies. Supporting this is the presence of ulcerations corresponding to the position of Peyer's patches in human typhoid (Owen, 1994).

Studies of S. Typhimurium invasion in murine and calf M cells have revealed active processes of internalization. Membrane ruffles are a crucial determinate of salmonella invasion, as demonstrated in cultured epithelial cell lines (Galan & Collmer, 1999). The membrane 'ruffler' formation results from extensive rearrangement of M cell apical membranes due to the redistribution of polymerised actin to form membrane protrusions (Clark et al., 1994; Frost et al., 1997; Jones et al., 1994). This process is triggered by a active process in which the ultimate result is engulfment of *Salmonella.* It should be noted that not all bacterial uptake by M cells involves specialised adaptive processes. Some of the bacteria entry, such as in the case of *Salmonella*, is performed in a passive fashion by taking advantage of the M cell sampling and uptaking abilities. Although M cells appear to play a major role in salmonella internalization and evasion, this highly adaptive pathogen is able to exploit other routes that all amount to the pathogenesis of *Salmonella.*

2.2 Macrophages

Professional antigen presenting cells, such as macrophages are endlessly sampling luminal contents, phagocytising potential pathogens and presenting antigens to adaptive immune cells, in a process of maintaining host protection against potentially harmful microbes. Phagocytic uptake of gram-negative bacteria (such as *Salmonella)* is a complex mechanism which involves various receptors and interactions. Of particular importance are Pattern-recognition receptors that recognise pathogen-associated molecular patterns that include lipopolysaccharides and flagellin. Macrophages are thought to readily recognise and engulf *Salmonella* in a independent fashion, thus offering an alternative independent point of entry.

2.3 Enterocytes

Enterocytes are simple columnar epithelial cells found in the intestine which offer the first line of defence against microbes in the lumen. Despite employing a vast array of protective features, many microbes including *Salmonella* have evolved strategies to secure their survival in host cells by manipulating cellular structures, as observed in M cells. Therefore, *Salmonella* also invades enterocytes by employing membrane ruffles.

2.4 Dendritic cells (DC)

DCs are a heterogeneous group of cells that are categorized primarily by their resident tissue. They are best known for their ability to capture and present antigens and have been heavily implicated in host immune responses and self-tolerance. *Salmonella* appears to possess the ability to cross the gut epithelial via DCs. It has been proposed that *Salmonella* from the intestinal lumen is absorbed via dendritic projections that pass between adjacent intestinal epithelial cells without disturbing the epithelial barrier integrity (Rescigno et al., 2001). This is through the utilization of fimbriae which selectively adheres *Salmonella* to dendritic projections, allowing *Salmonella* to invade DCs, thereby utilizing DCs as an additional entry point, as demonstrated in a recent study (Guo et al., 2007).

3. Virulence genes

The presence of virulence determinants in S. Typhimurium, and the somewhat related pathogenic *E.coli,* are thought to be acquired by lateral gene transfer (Boyd & Brussow, 2002; Schmidt & Hensel, 2004). Pathogenicity islands (PAI) describe chromosomal regions that contain virulence genes which are otherwise absent in non-pathogenic or closely related strains species (Hacker et al., 1990; Lavigne & Blanc-Potard, 2008). They are typically inserted adjacent to tRNA genes and their overall base composition differs markedly from the native bacterial chromosome, thereby implying they are acquired from a foreign source (Daigle, 2008). According to Schmidt and Hensel, 2004., five major PAI's have been identified in S. Typhimurium, which are referred to as *Salmonella* pathogenicity islands (SPI) (Schmidt & Hensel, 2004). The human-specific S. Typhi contains a chromosomal region of virulence, SPI-7, not present in S. Typhimurium which causes unique clinical presentations in patients affected, such as typhoid fever.

3.1 SPI-1

T3SS-1, encoded by SPI-1, is required for SPI-1-dependant internalisation of non-pathogenic cells, such as M cells and epithelial cells, and mediates the translocation of effector proteins into eukaryotic cells (Ehrbar et al., 2002; Lostroh & Lee, 2001; Mills et al., 1995). At least 15 effectors can be translocated by T3SS-1, most of which are encoded on the SPI-1, such as SptP, SipA, SipB and AvrA (McGhie et al., 2009). Until recently, T3SS-1 effectors were thought only important for the internalization of *Salmonella*; however recent of studies have revealed that many effectors of T3SS-1 have important roles in post-invasion processes that are discussed later in this chapter.

3.2 SPI-2

T3SS-2, encoded by SPI-2, is required for intracellular replication and immune evasion (Ochman et al., 1996; Shea et al., 1996; Waterman & Holden, 2003). Known effectors of T3SS-2, encoded on SPI-2, include SpiC, SseF, SseG, whereas SifA, SifB, PipB, PipB2, SseI, SseJ, SseL, SspH2, GogB are other effectors of T3SS-2 which are encoded on a different loci (Lavigne & Blanc-Potard, 2008). To date, the effectors of T3SS-2 remain less understood than effectors of T3SS-1, although it is widely established that T3SS-2 is required for survival within host cells (Hensel et al., 1998). Several studies have also highlighted the importance of SPI-2 in systemic infection, while noting its contribution in intestinal disease and

inflammation (Bispham et al., 2001; Coburn et al., 2005; Coombes et al., 2005; Hapfelmeier et al., 2005). T3SS-2 evasion processes are discussed in greater detail later in this chapter.

3.3 SPI-3

SPI-3 encodes virulence factors that are important in intestinal colonization and intracellular survival. MgtC is an inner membrane protein, common to several intracellular pathogens, required for intramacrophage survival (Alix & Blanc-Potard, 2007; Blanc-Potard & Groisman, 1997). SPI-2 also encodes MisL, a type V secretion system (T5SS) (Blanc-Potard et al., 1999; Henderson et al., 2004). Although not much is known about MisL, a recent study of S. Typhimurium demonstrates that MisL is important for intestinal colonization by promoting S. Typhimurium binding to fibronectin (Dorsey et al., 2005).

3.4 SPI-4

SPI-4 encodes a type I secretion system (T1SS) and also a substrate of T1SS, SiiE, a large (600kDa) surface-associated non-fimbrial adhensin that contributes to invasion and adhension to eukaryotic cells (Gerlach et al., 2007). Although encoded by SPI-4, SiiE is also coregulated by SPI-1. Little is known about the precise function of SPI-4, however a study in both calf and mouse models have revealed a role of SiiE in colonization and intestinal inflammation (Morgan et al., 2007).

3.5 SPI-5

Many additional effectors of T3SS-1, including SopA, SopB, SopD, SopE, SopE2, Ssph1 and Slrp, are not encoded on SPI-1; rather they are encoded by other horizontally acquired elements, such as on SPI-5 (Lavigne & Blanc-Potard, 2008; Wood et al., 1998). Some of these effectors, namely SopB and SopE/SopE2 induce dramatic rearrangement of the actin cytoskeleton in host cells, which results in large membrane ruffles and subsequent internalization of *Salmonella* (McGhie et al., 2009).

3.6 SPI-7

SPI-7 is a large 134 kb segment that encodes the Vi capsular polysaccharide antigen, Type IV pili and SopE (Hornick et al., 1970). The *viaB* locus is a 14Kb region within SPI-7 that prevents host recognition of *Salmonella* by TLR4 and TLR5 (Raffatellu et al., 2005). The locus contains genes that synthesize, regulate and export the Vi capsular antigen. The presence of the Vi antigen contributes greatly to the virulence in *S. Typi*, which is required for intracellular survival in phagocytes and has been implicated in system dissemination by virtue of its role in serum resistance (Hirose et al., 1997; Looney & Steigbigel, 1986). It should be noted that SPI-7 is expressed in S. Typhi and is absent in S. Typhimurium.

3.7 Fimbriae & biofilm

Four different types of fimbriae are known to be expressed by *Salmonella* that include type 1 fimbriae, plasma-encoded fimbriae, long polar fimbriae and thin aggregrative fimbriae (curli), all of which seem to have specificities for different cell types (Darwin & Miller, 1999). A recent study has shown fimbriae, namely type 1 fimbrial adhesion FimH, mediates *Salmonella* uptake

into murine DCs in a T3SS-independent fashion (Guo et al., 2007). It is thought that 13 fimbrial loci exist in *Salmonella*, which are thought to aid the internalization process through biofilm formation, attachment to host cells and colonization (Humphries et al., 2001). Conversely, an earlier study has demonstrated a more invasive behavior of an S. Typhi mutant strain carrying a deletion of the *fim* genes (encoding type 1 fimbriae) compared to its wild-type strain (Miyake et al., 1998). It is thought that in the absence of type 1 fimbrial adhesions, various interactions between bacterial surface proteins and host cells may become more enhanced and result in a higher degree of invasion. BapA, a large cell-surface protein is also required for biofilm formation and subsequent evasion and colonization, similar to FimH (Latasa et al., 2005). BapA is secreted through T1SS (BapBCD) and its expression is orchestrated with genes encoding other fimbriae, suggesting important interplay in overall biofilm formation. The significance of the biofilm stems from the extreme environmental conditions the bacteria are subjected to, and their successful survival lies in their ability to grow in surface-attached biofilms protected by an extracellular matrix (Costerton et al., 1995; Davey & O'Toole G, 2000). The formation of biofilms, particularly in the gall bladder, contributes greatly to pathogenesis of chronically infected S. Typhi individuals.

3.8 Flagella

The role of flagella in *Salmonellosis* remains controversial due, in part, to seemingly conflicting studies and poorly understood intracellular mechanisms. For example, the evasiveness of *Salmonella* is increased via flagellar-based motility, despite the fact several studies have demonstrated that flagellin monomers are potent inducers of innate immunity (Franchi et al., 2006; Miao et al., 2006; Schmitt et al., 2001). Conversely, it has been well demonstrated that flagellin interacts with T3SS-1 from *Salmonella*-infected macrophages and is translocated into the cytosol resulting in activation of the inflammasome and subsequent cell death via caspase-1 pathway (discussed later in the chapter) (Miao et al., 2007; Ren et al., 2006; Sun et al., 2007).

4. Evasion of salmonella

4.1 The *Salmonella*-containing vacuole (SCV)

Following internalization, *Salmonella* becomes engrossed within a vacuole in which it is able to survive and replicate intracellularly. These vacuoles, termed *Salmonella*-containing vacuole (SCV) are characterised by the transformation of cell markers as the vacuole matures, displaying late endosome and lysosome markers, particularly lysosomes glycoprotein markers (Steele-Mortimer et al., 1999). Concurrent to marker transformation post-invasion, SCVs migrate from the periphery plasma membrane to a juxtanuclear position at the microtubule-organising centre (Deiwick et al., 2006; Salcedo & Holden, 2003). In some cell types, the onset of intracellular replication are marked by the presence of *Salmonella*-induced filaments (Sifs) which are branched membrane tubules expelling from the SCV (Drecktrah et al., 2008). Within the SCV, various processes occur, such as *Salmonella* transformation and immune modulation, which are imperative for survival, replication and dissemination of *Salmonella*. Following internalisation of *Salmonella* and translocation of SPI, SPI-effectors are localized to different cellular compartments, such as the Golgi apparatus and lysosomes (Freeman et al., 2003; Haraga & Miller, 2003; Knodler et al., 2003; Salcedo & Holden, 2003). The differential localisation pattern indicates the ability of SPI-effectors and thereby *Salmonella* to manipulate various aspects of host cell function.

4.2 Maturation and trafficking of SCV

Initially, when SCV form, they are characterized by early endocytic markers, such as transferrin receptor (TfnR), early endosomal antigen 1 (EEA1) and several Rab GTPases, such as Rab4/5 & 11. In an effort to curb or delay lysosomal fusion, SCV deviates from the endocytic pathway, thereby reducing the likelihood of lysis of *Salmonella*. (Steele-Mortimer, 2008). Maturing SCVs are characterised by replacement of earlier endocytic markers by later markers including Rab7, vacuolar ATPase (v-ATPase) and lysosomal membrane glycoproteins (1pgs), such as LAMP-1 (Steele-Mortimer, 2008).

Several studies have implicated the effectors of T3SS-1, namely SopE and SopB in the maturation of SCV and replication of *Salmonella* within. SopE and SopB are required for the recruitment of Rab5 within the SCV, which binds the phosphatidylinositol 3-kinase (PI(3)P) Vps34 required for LAMP-1 recruitment (Mallo et al., 2008; Mukherjee et al., 2001; Steele-Mortimer, 2008). Vps34 also acts via PI(3)P on the SCV membrane, which enables recruitment of EEA1(Mallo et al., 2008). SopB acts by inhibiting the degradation of epidermal growth factor receptor (EGFR) by lysosomes (Dukes et al., 2006). In a recent study, SopB has been attributed to the disappearance of late endosomal markers by recruiting sorting nexin-1 (SNX-1) which is speculated to down-regulate mannose 6-phosphate receptor from maturing SCV (Bujny et al., 2008). Collectively, the current data pins a role for SopB in diverting SCV trafficking from the endosomal maturation pathway. In addition to this, SopB has also been demonstrated to indirectly increase intracellular *Salmonella* replication, via activation of Akt, which in turn deactivates Rab14 GAP, AS160 (Layton & Galyov, 2007). The activated Rab14 increases intracellular *Salmonella* replication most likely by delaying SCV-lysosomal fusion (Kuijl et al., 2007). On the same note, SpiC, an effector of T3SS-2 is thought to prevent fusion of macrophage-late lysosomes with SCV (Steele-Mortimer, 2008).

SseJ, another effector of T3SS-2 on the SPI-2, is localised to the SCVs and SIFs. A study in SseJ null mutant mice has revealed that SseJ is required for full virulence of *Salmonella* (Ruiz-Albert et al., 2002). More specifically, an *in vitro* study has revealed deacylase activity of SseJ, while portraying its role in esterification of cholesterol, which is enriched in SCV (Brumell et al., 2001; Catron et al., 2002; Garner et al., 2002; Ohlson et al., 2005). It is thought the esterification of cholesterol could disrupt cell-signalling platforms or inhibit molecular interactions of complexes on the SCV, ultimately interfering with vesicular trafficking (Ohlson et al., 2005).

SopA, an effector of SPI-1, promotes bacterial escape from the SCV in HeLa cells (Diao et al., 2008). It is one of several effectors that is structurally and functionally similar to HECT E3 ubiquitin ligases and is thought to have a role in disrupting SCV membrane integrity (Steele-Mortimer, 2008; Zhang et al., 2006).

4.3 Sif biogenesis

As mentioned previously, maturing SCVs migrate from the peripheral towards the perinuclear region of the host cell, strategically positioning themselves adjacent to the Golgi apparatus. This seems to be important for promoting bacterial replication and survival, and, as a recent study suggests, the close proximity of SCV to the Golgi apparatus may enable interception of endocytic and exocyclic transport vesicles to stockpile nutrients and/or

membrane materials (Ramsden et al., 2007). In line with this, exocytic transport vesicles are able to be averted from their normal path under the influence of SifA, SseG and SseF towards the SCV (Kuhle et al., 2006).

In epithelial cells, SseG and SseF are required for the maintenance of SCV at the perinuclear region, while also appearing to be important for intracellular replication (Deiwick et al., 2006). SseG and Ssef are thought to either form 'tethers' from the SCV to the Golgi apparatus or manipulate dynein activity in an attempt to 'glue' the SCV to the microtubule-organising centre (MTOC) (Kuhle & Hensel, 2002; Ramsden et al., 2007; Salcedo & Holden, 2003). SopB is also required for the retention of the SCV within the perinuclear region by mediating the phosphorylation of actin-associated motor myosin II light chain (MLC) (Wasylnka et al., 2008).

Once the SCV has positioned itself near the perinuclear region of the host cell, replication is initiated and is characterised by LAMP-rich specialised tubulovesicular structures called *Salmonella*-induced filaments (Sifs) that project from the SCV. The fusion of late endosome with the SCV is thought to induce Sif formation, however the precise role of Sifs remains elusive (McGhie et al., 2009; Steele-Mortimer, 2008). SifA is essential for the formation and maintenance of Sifs, by which SifA and two other T3SS-2 effectors, PibB2 and SseJ, cooperate to induce and drive extensions of Sif tubules from the juxtanuclear SCV towards the periphery of the host cell (Brumell et al., 2002; Henry et al., 2006; Knodler & Steele-Mortimer, 2005). SifA is localised to the SCV by the TS33-1 effector SipA, where it forms a functional complex with the host protein SKIP (SifA and kinesin-interacting protein) (Brawn et al., 2007). SKIP has been shown to bind to the PibB2-induced microtubule-based motor kinesin-1 that drives Sif extensions (Boucrot et al., 2005; Brawn et al., 2007; Henry et al., 2006). SKIP also directly interacts with various GTPases such as rab9 which is implicated in lysosome positioning and function, potentially displacing it from this complex (Barbero et al., 2002; Ganley et al., 2004; Jackson et al., 2008). In addition to this, SifA is also able to bind to rab7, uncoupling it from RILP and recruiting RhoA, another GTPase that promotes membrane tubulation when activated in the presence of SKIP and SseJ (Harrison et al., 2004; Lossi et al., 2008; Ohlson et al., 2008; Ramsden et al., 2007). Moreover, SseF and SseG are believed to augment Sif formation via modulation of aggregated endosomal compartments. In studies of *Salmonella* mutants lacking SseF and and SseG, SCVs induced fewer Sifs compared to wild type bacteria (Ramsden et al., 2007).

Salmonella within the SCV have evolved mechanisms that allow it to escape the ubiquitination process by employing three T3SS-2 effectors that interfere with host ubiquitin pathways (Quezada et al., 2009). SseL is a deubiquitinase that acts via modulation of NF-KB, although the downstream effects are unclear (Coombes et al., 2007; Le Negrate et al., 2008; Rytkonen et al., 2007). SspH1 and SspH2 are both members of the ubiquitin E3 ligases family. More research is required to clearly define a role for these two effectors. Nevertheless a study has identified that SspH1 can ubiquitinate ubiquitin (Rohde et al., 2007).

5. Surviving intracellularly

Salmonella has successfully evolved several strategies to manipulate and supress cellular immune responses. SptP GAP and tyrosine phosphatase activities play critical roles in

reversing MAPK activation while AvrA acetyltransferase activity towards MAPK kinases inhibits Jnk activation (Jones et al., 2008; Lin et al., 2003; Murli et al., 2001). Moreover, SpvC has been shown to directly inhibit Erk, Jnk and p38 MAPKs via its phosphothreonine lyase activity (Li et al., 2007; Mazurkiewicz et al., 2008).

The deubiquitinase activity of SPI-2 effector, SseL, is able to suppress NFκB activation by impairing IκBα ubiquitination and degradation (Le Negrate et al., 2008). This is thought to be an additional measure by which *Salmonella* is able to target transcription factors downstream from MAPK pathways to ensure immune suppression. AvrA also has a reportedly redundant role to SseL deubiquitinase activity and thereby acts in a similar manner (Ye et al., 2007). Furthermore, SspH1 inhibits NFkB-dependant gene expression, although the precise mechanisms are unclear (Rohde et al., 2007). The production of reactive host species by many host cells is imperative for killing intracellular pathogens. This is essentially through the employment of NADPH oxidase (NOX2) activity found in lysosomes. *Salmonella* is able to evade this activity by utilizing SodCl, a superoxide dismutase, which protects itself from the reactive oxygen species.

Metal availability in host eukaryotic cells is required for full virulence of *Salmonella*. Of many trace elements reportedly utilised by *Salmonella*, iron appears most important. Iron is a cofactor for various fundamental enzymes and metabolic processes in *Salmonella*, which must compete with the host cell to obtain this ion (Schaible & Kaufmann, 2004). Epidemiological evidence from patients with β-thalassaemia has demonstrated an increased susceptibility of *Salmonella* infection (Wanachiwanawin, 2000). Similarly, *Salmonella* virulence was exacerbated in laboratory conditions replicating iron overload (Sawatzki et al., 1983). Further evidence of increased virulence of *Salmonella* in iron favouring conditions stems from the attenuated intracellular growth of mutant S. Typhi whose iron uptake abilities are impaired (Furman et al., 1994).

In light of the importance of iron in *Salmonella* virulence, it is not surprising that *Salmonella* has evolved strategies in acquiring various metals for intracellular survival. The iron availability in eukaryotic hosts is limited due to activity of transferrin and natural resistance-associated macrophage protein one (Nramp1) (Nairz et al., 2009). Additionally, during bouts of inflammation, the antimicrobial protein lipocalin-2 further inhibits bacterial iron acquisition, creating an environment highly unfavourable for *Salmonella* and other intracellular bacteria (Raffatellu et al., 2009). *Salmonella* therefore employs two siderophores, enterobactin and salmonchelin, when acquiring iron in highly competitive conditions (Muller et al., 2009). Salmonechelin, a glucoslyated derivative of enterobactin, is thought to resist or inhibit lipocalin-2 function (Raffatellu et al., 2009). Furthermore, SCVs in macrophages contain enough iron to affect activity of metal-responsive promoters, independently of Nramp1 (Taylor et al., 2009).

Magnesium is also significant for intracellular survival of *Salmonella* and is delivered by three distinct systems for uptake: CorA, MgtA and MgtB (Blanc-Potard & Groisman, 1997). Zinc and potassium are also implicated in intracellular survival of *Salmonella*. In low zinc conditions, ZnuABC Zn^{2+} uptake system demonstrates its importance intracellularly, while further studies with ZnuABC mutants have supported the importance of zinc acquisition for virulence of *Salmonella* (Ammendola et al., 2007). Finally, the Trk complex system functions as a low-affinity K^+ transporter and resist host antimicrobial peptides(Parra-Lopez et al., 1994).

6. Effector modulation of host immunity

Many SPI effectors can induce symptoms that are the hallmark feature of salmonellosis, such as acute intestinal inflammation and diarrhoea, through activation of immune cells and release of cytokines. During *Salmonella* invasion, transcription factors AP-1 and NFκB are activated via stimulation of Cdc42 by SPI-1 effectors SopE/SopE2 and SopB. More specifically, Erk, Jnk and p38 mitogen-activated protein kinase (MAPK) are unregulated in a Raf1-dependant fashion that ultimately result in transcription of AP-1 and NFκB to release proinflammatory cytokines such as IL-8, recruiting polymorphonuclear leukocytes (PMNs) (Layton & Galyov, 2007; Patel & Galan, 2006, 2005). Concomitantly, SipA triggers the Arf6- and phospholipase D signalling cascade, releasing PMN chemoattractant hepoxillin A3 apically via protein kinase Cα (Layton & Galyov, 2007; Wall et al., 2007). As a result, PMN chemoattractant hepoxillin A3 promotes transmigration of PMN across the epithelium into the intestinal lumen. The actions of SopB, SopE, SopE2 and SipA are likely to disturb the integrity in the epithelial barrier and result further in PMN transmigration whilst promoting fluid flux, thereby contributing to diarrhoea (Boyle et al., 2006). SopB is also known to play a role in the induction of diarrhoea due to its inositol phosphatase activity, producing $Ins(1,4,5,6)P_4$, which promotes cellular chloride ion secetion and fluid flux (Layton & Galyov, 2007). Contrary to this, the SPI-1 effector, AvrA,, has been shown to have countering effects on ion secretion by stabilising cell permeability and tight junctions in intestinal epithelial cells (Liao et al., 2008). This action is believed to have a strategic advantage to bacterial survival since disruption of the epithelial lining also increases the inflammatory response. Therefore AvrA may help *Salmonella* survive in the host during by dampening intestinal inflammation.

7. Cell death

'From death comes life' A proverb that seems to rings true during *Salmonella* infection of host cells. The relationship between *Salmonella* invasion and cell death has been long implicated; however the once simplistic model has now evolved to include many different mechanisms in which *Salmonella* prompts cell death.

In many ways the epithelium provides a barrier, both physical and chemical, that demarcates two environments whilst selectively allowing particles to migrate to their new world. The internalization of *Salmonella* alone, mostly by epithelial lining cells, does not guarantee access to the underlying tissue. Rather, the phenomenon of cell death is thought to create the perfect opportunity for *Salmonella* to reach and infect Peyer's patches and disseminate to systemic tissues in the host. Internalized *Salmonella* in cultured epithelial cells is able to exploit and induce apoptosis *in vitro* after 8-12 hours, in a manner that is characteristic of apoptosis, such as activation of apoptotic caspase-3 and caspase-8 (Kim et al., 1998; Paesold et al., 2002; Zeng et al., 2006). Apoptosis is best defined as an active, controlled, genetically regulated and ATP-requiring process that results in cell death in the face of aging or damaged cells (Danial & Korsmeyer, 2004; Kerr et al., 1972). The bacterial invasion and synthesis of bacterial proteins from SPI-2 T3SS-II and *spv* genes are required for intracellular proliferation and induction of apoptosis (Paesold et al., 2002). Additionally, following bacterial invasion, host cellular production of inflammatory mediators, such as TNF-α and nitric oxide, are all thought to contribute to epithelial cell apoptosis. It should be noted that apoptosis of *Salmonella*-infested epithelial cells is a relatively delayed event,

which is thought to favour intracellular reproduction, increasing the overall niche of intracellular *Salmonella* (Kim et al., 1998).

A relatively newly defined form of apoptosis utilized by *Salmonella* and other intracellular residing bacteria is termed pyroptosis. Its unique mechanism, features and inflammatory outcome distinguishes it from our current understanding of apoptosis (Fink & Cookson, 2007; Hersh et al., 1999). Pyroptosis is caspase-1 dependant which is activated in the inflammasome during *Salmonella* infection. This differs from apoptosis in that caspase-3 and not caspase-1 is central to cell death (Brennan & Cookson, 2000; Jesenberger et al., 2000). Additionally, Caspase-3 is not activated in pyroptosis, nor is caspase-6 and caspase-8. Furthermore, the characteristic mitochondrial release of cytochrome *c* in apoptosis does not occur during pyroptosis (Jesenberger et al., 2000). Finally, the unique inflammatory outcome of pyroptosis stems from caspase-1 ability to release proinflammatory cytokines IL-1β and IL-18 by cleaving their precursor molecules (Fantuzzi & Dinarello, 1999).

The SPI-1 effector, SipB has been heavily implicated in host cell death of following *Salmonella* internalization of macrophages, as supported by a study which showed reduced apoptosis following infection of SPI-1 mutant *Salmonella* (Monack et al., 2004). SipB is able to bind and activate caspase-1, triggering rapid pyroptosis. Flagellin is also demonstrated to be necessary in caspase-1-mediated, SPI-1-dependent pyroptosis, as observed in flagellin void *Salmonella* mutants (Franchi et al., 2006; Miao et al., 2006). It should be noted that pyroptosis is not limited in macrophages, as caspase-1-dependant death has also been observed in *Salmonella*-infected DCs (van der Velden et al., 2003). Another described pyroptosis in *Salmonella* infected macrophages, dubbed 'Delayed SPI-2-dependant caspase-1-mediated pyroptosis, is thought to be important during the systemic phase of infection, as SPI-1 effectors and flagellin are both repressed during this stage (Cummings et al., 2005; Fink & Cookson, 2007; Schlumberger & Hardt, 2006). Delayed macrophage death also requires expression of the *spvB* gene, as mutations in this gene prevent induction of delayed macrophage death (Browne et al., 2002; Libby et al., 2000). Despite this, *Spv* genes may not directly affect cytotoxicity as Spv mutants show attenuated intracellular replication and therefore may indirectly affect cytotoxicity through intracellular proliferation of *Salmonella* (Fink & Cookson, 2007). Delayed macrophage pyroptosis may heavily contribute to virulence of *Salmonella*, as dissemination is largely through *Salmonella*-infected macrophages (Fields et al., 1986). Therefore, delaying cell death and the subsequent release of *Salmonella* will allow the bacterium to disseminate further virtually undetected. Finally, an extremely late event of apoptosis in *Salmonella*-infected macrophages has been observed in caspase-1-deficient macrophages, which is caspase-1-indepedant (Hernandez et al., 2003; Jesenberger et al., 2000). SPI-1 and more notably, sipB, are shown to be required for this caspase-1-indepenant apoptosis in macrophages (Hernandez et al., 2003; Jesenberger et al., 2000). This process acts via the release of mitochondrial cytochrome *c* and activation of caspase-2, caspase-3, caspase-6 and caspase-8, resulting in apoptosis (Jesenberger et al., 2000). More research is required to clarify the role of caspase-1-independent apoptosis in the physiological setting of Salmonellosis.

The many failsafe's of *Salmonella* mediated cell death has caused greater debate on the effects in the virulence of *Salmonella*. In light of the fact that pyroptosis produces an inflammatory outcome, one may speculate that this pathway may have important implications in host protection. Contrarily, epithelial cell death via apoptosis is believed to

enhance bacterial migration and dissemination as this would likely result in a breach of the epithelial barrier and thereby allow more *Salmonella* to pass into underlying tissue. Further research in *Salmonella* mediated cell death may help clarify this seemly paradoxical event.

8. Serum resistance

Serum resistance and subsequent typhoid fever is a characteristic phenomenon observed in cases of S. Typhi infection. Unlike S. Typhimurium, S. Typhi is host specific and it does not persist in animals and thus is inherently difficult to study *in vivo* conditions. As a result, volunteer studies have been essential in establishing the pathogenesis of typhoid fever. Following mucosa invasion of S. Typhimurium, host pattern recognition receptors recognise various pathogen-associated molecular patterns (PAMPs) that are unique to bacteria. Such receptors include Toll-like receptor 4 (TLR-4) which recognises lipopolysaccharide (LPS) and TLR-5 that is exclusively expressed on the basolateral surface of epithelial cells which recognises and binds flagellin (Gewirtz et al., 2001; Hayashi et al., 2001; Poltorak et al., 1998). Activation of TLR signalling induces expression of various proinflammatory cytokines such as TNF-α and chemokine IL-8, recruiting neutrophils to the intestinal mucosa. These inflammatory markers are essential to the containment and clearance of *Salmonella* infection in the mucosa (Raffatellu et al., 2006). Contrasting this, S. Typhi invasion of the intestinal mucosa does not initiate effective immune response, nor triggers the neutrophil influx that is characteristic of S. Typhimurium. This is due to the expression of the Vi capsular antigen on the SPI-7 that is absent in S. Typhimurium. The expression of the Vi capsular antigen in S. Typhi is thought to down-regulate the TLR-mediated host response in the intestinal mucosa, ultimately allowing bacteria to escape the immune defence line and disseminate to the liver, bone marrow and gall bladder (Tsolis et al., 2008). In human colonic epithelial cell lines, capsulated S. Typhi reduces IL-8 production, while in a similar study, noncapsulated S. Typhi triggers more IL-8 and TNF-alpha production (Hirose et al., 1997; Raffatellu et al., 2005; Sharma & Qadri, 2004). Capsulated S. Typhi interferes with TLR5 and TLR4/MD2/CD14 stimulation by flagella and LPS, respectively (Miyake et al., 1998). In this view, the Vi capsular antigen appears to deafen the immune system by simply reducing immunological stimulation and subsequent response. An earlier study has revealed an approximate 10,000-fold decrease in the virulence of non-capsulated S. Typhi in an intraperitoneal mouse model of infection (Hone et al., 1988). Similarly, a volunteer study has demonstrated a significant increase in disease activity amongst those infected with capsulated S. Typhi (Hornick et al., 1970). Finally, vaccination with the Vi antigen has confirmed protection during human infection with capsulated S. Typhi (Klugman et al., 1987).

Numerous theories by which the Vi capsular antigen inhibits inflammation have been proposed, two of which are most widely accepted. Primarily, Vi capsular antigen may physically mask PAMPs, thereby interfering with TLR stimulation (Raffatellu et al., 2006). This is supported by studies which found that the Vi antigen blocks the agglutination of S. Typhi with anti-LPS serum (Felix, 1934). Vi antigen also inhibits type 1 fimbriae mediated agglutination in *Saccharomyces cerevisiae*, collectively insinuating that the capsule physically masks surface structures (Miyake et al., 1998). Secondly, it has been suggested that the Vi antigen may attenuate downstream signalling related to IL-8 production (Qadri, 1997; Sharma & Qadri, 2004). Despite its early discovery, many questions regarding Vi capsular antigen mechanisms remain unanswered. This is due to the limitations of directly studying

the Vi capsular antigen *in vivo* in laboratory condition. Vi antigen expression is markedly reduced, if not obliterated, when transferred to the laboratory. This characteristic loss of the Vi capsular antigen is ascribed to the genetic instability of the SPI-7, which frequently is lost in the laboratory passage (Bueno et al., 2004).

S. Typhi blood isolates from infected patients suggests that Vi antigen expression is invariable (Robbins & Robbins, 1984). However, S. Typhi isolates in human stool samples revealed a lack of Vi capsular antigen expression, compared to blood samples in the same patients (James Craigie, 1936). Moreover, recent *in vitro* studies have suggested that S. Typhi downregulates the expression of the Vi capsular antigen in the intestinal lumen as a result of the inherent high osmolarity of the region (Pickard et al., 1994). Concurrently, flagella expression is unregulated, while T3SS-1 genes are expressed (Arricau et al., 1998). These studies have revealed the transient nature of Vi expression during various stages of S. Typhi infection. The environmental shift between the high osmolarity of the intestinal lumen and low osmolarity inside the host tissue appears to promote expression of the Vi capsular antigen, while simultaneously repressing mobility and invasiveness. Upon invasion into the underlying epithelium, S. Typhi encounters low osmolarity within its new environment, thus triggering two component regulatory systems RcsBC and OmpR EnvZ. Both components activate Vi antigen expression while the expression of flagella and T3SS-1 genes are reduced (Arricau et al., 1998; Pickard et al., 1994; Virlogeux et al., 1996). The effects of Vi antigen expression on S. Typhi virulence are demonstrated in recent *in vitro* studies in which capsulated S. Typhi is resistant to phagocytosis (Looney & Steigbigel, 1986). These findings perplex the role virulent T3SS-1 effectors in intracellular survival of macrophages.

9. Typhoid fever or septic shock?

Unlike S. Typhi, S. Typhimurium and other non-typhoidal *Salmonella* serotypes generally do not elicit typhoid fever due to the absence of the SPI-7 and therefore Vi capsular antigen. In immunocompetent individuals, non-typhoidal *Salmonella* serotypes prompt a strong immune response, marked by neutrophil influx and inflammatory diarrhoea which help contain the infection in the intestinal mucosa. Conversely, immunocompromised patients infected with non-typhoidal *Salmonella* serotypes develop fulminant bacteraemia, a clinical presentation markedly different to that of typhoid fever (Tsolis et al., 2008). The failed containment of non-typhoidal *Salmonella* to the intestinal mucous leads to bacterial dissemination to the blood stream, prompting an aggressive immune response towards bacteria associated LPS. LPS, a known inducer of endotoxic shock, binds the TLR4-MD-2-CD14 receptor complex inducing an excessive inflammatory response marked by release of TNF-α, IFN-γ and IL-1β (Hoebe et al., 2004). Endotoxic shock is characterised by a decrease in hypotension and microvascular thrombosis as the result of fibrin deposition in capillaries (Waage et al., 1991). LPS is thought to contribute to septic shock and mortality in patients with non-typhoidal *Salmonella*, in part due to the rapid production of TLR4-dependant TNF-α in macrophages (Engelberts et al., 1991; Wilson et al., 2008). The synergic induction of nitric oxide synthase, by TNF-α, IFN-γ and IL-1β, increases production of nitric oxide which is a powerful vasodilator contributing to hypotension (Petros et al., 1991). In addition to this, TNF-α increases tissue-factor expression on monocytes, resulting in cleavage of serum fibrinogen to fibrin (Carlsen et al., 1988). Deposits of fibrin in microvasculature lead to intravascular coagulopathy, which can cause organ failure (Waage et al., 1991). Contrary to

the robust immunological response to non-typhoidal *Salmonella* serotypes, S. Typhi induces only a weak immune response. This is paralleled in the clinical setting as coagulatory abnormalities are not apparent in patients with typhoid fever (Butler et al., 1978). Furthermore, serum cytokine levels in patients with typhoid fever were relatively lower when compared to patients with gram-negative septic shock (Raffatellu et al., 2006). The impaired identification and host response towards S. Typhi is attributed to its ability to conceal two important molecular signatures, LPS and flagellin. As mentioned previously, the Vi capsular antigen is thought to mask the physical structure of LPS, thereby evading TLR4 recognition and subsequent response. Additionally, *viaB* locus in SPI-7 is also able to attenuate TLR5 signalling, albeit via a different mechanism. TviA, a regulatory protein encoded by the *viaB*, suppresses the transcription of locus *flhC* and *flhD*, both of which encode the master regulator of flagella expression (Winter et al., 2008). As a result, the transcription of the flagellin gene *fliC* is reduced and therefore flagellin is downregulated. This unique mechanism allows S. Typhi to evade TLR5 recognition of flagellin.

10. Persistence

The vast majority of patients with acute typhoid fever recover following adequate treatment; however 3-5% of cases develop a chronic infection in the gall bladder (Levine et al., 1982; Merselis et al., 1964). In light of the fact that S. Typhi is a human restrictive pathogen, these chronically affected individuals form crucial reservoirs for future spread via the faecal and urinal oral route (Bhan et al., 2005; Khatri et al., 2009). Furthermore, chronically affected individuals are almost always asymptomic, making identification of possible carriers a difficult task (Shpargel et al., 1985; Sinnott & Teall, 1987).

Epidemiological studies have revealed a strong association between chronic carriers of S. Typhi and gallstones (Schioler et al., 1983). Moreover, 90% of chronically affected individuals have gallstones (Karaki & Matsubara, 1984). Alongside S. Typhi, many other bacterium have been implicated in the development of gallstones and later cholecystitis, an inflammation and obstruction of the gallbladder (Capoor et al., 2008; Swidsinski & Lee, 2001). Despite much research, it remains unclear whether S. Typhi and other bacterium directly cause cholecystitis, or rather colonise previously damaged gall bladders (Cohen et al., 1987; Vaishnavi et al., 2005; Vogelsang & Boe, 1948).

Being the site of bile storage, the gall bladder possesses a harsh environment and therefore can only be inhabited by organisms resistant to the bile (van Velkinburgh & Gunn, 1999) (Thanassi et al., 1997). S. Typhi is known to form biofilms in abiotic and biotic surfaces and it is therefore not surprising that S. Typhi forms biofilms in the gall bladder (Ledeboer & Jones, 2005). Interestingly, however, formation of *Salmonella* biofilms on gallstones is dependent on the presence of bile (Prouty et al., 2002). Bile is known to interact and manipulate gene expression in *Salmonella* biofilms, such as downregulating SPI-1 genes and mobility genes (Crawford et al., 2010; Prouty & Gunn, 2000). Despite this, the effects are believed to be minimal and may not dramatically affect the abilitiy of *Salmonella's* to invade via effectors of SPI-1 or migrate via flagella. As S. Typhi is a human-restricted pathogen, utilising laboratory models such as S. Typhimurium does not truly mimic the pathogenesis of typhoid fever as S. Typhimurium induces a characteristic neutrophil response that is otherwise absent in S. Typhi. Therefore, much of our current understanding of S. Typhi

resistance in gall bladders stems from *in vitro* biofilm models, such as tube biofilm assays (TBA)(Gonzalez-Escobedo et al., 2011). Earlier studies have established the essential role of biofilm formation on gallstones removed from patients chronically infected with S. Typhi (Prouty et al., 2002). More recently, TBAs have revealed the bile-dependant enhancement of S. Typhi biofilms and specific binding to cholesterol-coated surfaces, such as in some cases of gallstones (Crawford et al., 2008). Furthermore, TBA's have also confirmed that the flagellin subunit *fliCI* is necessary for initial binding to cholesterol-coated surfaces. In addition to this, outer-membrance protein C (OmpC) also affects binding of S. Typhi biofilms to cholesterol (Crawford et al., 2010). Following the initial attachment phase, formation of microcolonies typically precedes the development to mature biofilm. During these two latter stages, extracellular polymeric substances (EPS) help biofilm structural development and cell-cell interaction (Costerton et al., 1999). In S. Typhi infected gallbladders, EPS is primarily composed of cellulose, colanic acid, Vi capsular antigen, curli fimbriae, O antigen capsule and biofilm associated proteins (Gibson et al., 2006; Gonzalez-Escobedo et al., 2011; Jonas et al., 2007; Ledeboer & Jones, 2005). Although the role of some elements in the EPS remain minor, other elements such as cellulose, colonic acid and O antigen capsule are crucial for S. Typhi persistence and biofilm development (Crawford et al., 2008; Prouty & Gunn, 2003; Prouty et al., 2002).

Biofilm development is an important component of bacterial survival and persistence. In order to decipher the complex interaction between S. Typhi biofilm and gallbladder further research is required. Humanised moue models are currently in development and may provide useful data by allowing S. Typhi to be studied directly in biofilm formation and gallbladder persistence (Song et al., 2010).

11. Conclusion

In the last two decades, incredible progress has been made in our understanding of the very complex host-*Salmonella* interactions. Furthermore, current therapeutic developments reflect our current knowledge of molecular events during Salmonellosis. Despite this, existing animal models used to decipher *Salmonella* and host interactions have severe limitations and implications in the clinical scenario. For example, the scarcity of data on S. Typhi, due to no suitable animal models available, has limited our understanding and therefore therapeutic development in human typhoid. Although the recently developed streptomycin pre-treated murine model has greatly enhanced our understanding of S. Typhimurium, we must caution ourselves in over interpreting data as S. Typhimurium mouse model does not always reflect the human disease. Until laboratory based *in vivo* models of S. Typhi are established, scientists must rely on a combination of existing models to help increase our understanding of the infectious processes.

12. References

Alix, E. & A. B. Blanc-Potard (2007). MgtC: a key player in intramacrophage survival. *Trends Microbiol,* Vol.15, No.6, (Jun), pp. 252-256, 0966-842X.

Ammendola, S., P. Pasquali, et al. (2007). High-affinity Zn2+ uptake system ZnuABC is required for bacterial zinc homeostasis in intracellular environments and

contributes to the virulence of *Salmonella* enterica. *Infect Immun,* Vol.75, No.12, (Dec), pp. 5867-5876, 0019-9567

Arricau, N., D. Hermant, et al. (1998). The RcsB-RcsC regulatory system of *Salmonella* typhi differentially modulates the expression of invasion proteins, flagellin and Vi antigen in response to osmolarity. *Mol Microbiol,* Vol.29, No.3, (Aug), pp. 835-850, 0950-382X

Barbero, P., L. Bittova, et al. (2002). Visualization of Rab9-mediated vesicle transport from endosomes to the trans-Golgi in living cells. *J Cell Biol,* Vol.156, No.3, (Feb 4), pp. 511-518, 0021-9525.

Bhan, M. K., R. Bahl, et al. (2005). Typhoid and paratyphoid fever. *Lancet,* Vol.366, No.9487, (Aug 27-Sep 2), pp. 749-762, 0140-6736

Bispham, J., B. N. Tripathi, et al. (2001). *Salmonella* pathogenicity island 2 influences both systemic salmonellosis and *Salmonella*-induced enteritis in calves. *Infect Immun,* Vol.69, No.1, (Jan), pp. 367-377, 0019-9567.

Blanc-Potard, A. B. & E. A. Groisman (1997). The *Salmonella* selC locus contains a pathogenicity island mediating intramacrophage survival. *EMBO J,* Vol.16, No.17, (Sep 1), pp. 5376-5385, 0261-4189

Blanc-Potard, A. B., F. Solomon, et al. (1999). The SPI-3 pathogenicity island of *Salmonella* enterica. *J Bacteriol,* Vol.181, No.3, (Feb), pp. 998-1004, 0021-9193.

Boucrot, E., T. Henry, et al. (2005). The intracellular fate of *Salmonella* depends on the recruitment of kinesin. *Science,* Vol.308, No.5725, (May 20), pp. 1174-1178, 0036-8075

Boyd, E. F. & H. Brussow (2002). Common themes among bacteriophage-encoded virulence factors and diversity among the bacteriophages involved. *Trends Microbiol,* Vol.10, No.11, (Nov), pp. 521-529, 0966-842X.

Boyle, E. C., N. F. Brown, et al. (2006). Salmonella enterica serovar typhimurium effectors SopB, SopE, SopE2 and SipA disrupt tight junction structure and function. *Cell Microbiol,* Vol.8, No.12, (Dec), pp. 1946-1957, 1462-5814.

Brawn, L. C., R. D. Hayward, et al. (2007). Salmonella SPI1 effector SipA persists after entry and cooperates with a SPI2 effector to regulate phagosome maturation and intracellular replication. *Cell Host Microbe,* Vol.1, No.1, (Mar 15), pp. 63-75, 1931-3128

Brennan, M. A. & B. T. Cookson (2000). Salmonella induces macrophage death by caspase-1-dependent necrosis. *Mol Microbiol,* Vol.38, No.1, (Oct), pp. 31-40, 0950-382X.

Browne, S. H., M. L. Lesnick, et al. (2002). Genetic requirements for salmonella-induced cytopathology in human monocyte-derived macrophages. *Infect Immun,* Vol.70, No.12, (Dec), pp. 7126-7135, 0019-9567

Brumell, J. H., P. Tang, et al. (2001). Characterization of Salmonella-induced filaments (Sifs) reveals a delayed interaction between Salmonella-containing vacuoles and late endocytic compartments. *Traffic,* Vol.2, No.9, (Sep), pp. 643-653, 1398-9219

Brumell, J. H., P. Tang, et al. (2002). Disruption of the Salmonella-containing vacuole leads to increased replication of Salmonella enterica serovar typhimurium in the cytosol of epithelial cells. *Infect Immun,* Vol.70, No.6, (Jun), pp. 3264-3270, 0019-9567

Bueno, S. M., C. A. Santiviago, et al. (2004). Precise excision of the large pathogenicity island, SPI7, in Salmonella enterica serovar typhi. *J Bacteriol,* Vol.186, No.10, (May), pp. 3202-3213, 0021-9193

Bujny, M. V., P. A. Ewels, et al. (2008). Sorting nexin-1 defines an early phase of Salmonella-containing vacuole-remodeling during Salmonella infection. *J Cell Sci,* Vol.121, No.Pt 12, (Jun 15), pp. 2027-2036, 0021-9533.

Butler, T., W. R. Bell, et al. (1978). Typhoid fever. Studies of blood coagulation, bacteremia, and endotoxemia. *Arch Intern Med,* Vol.138, No.3, (Mar), pp. 407-410, 0003-9926.

Capoor, M. R., D. Nair, et al. (2008). Microflora of bile aspirates in patients with acute cholecystitis with or without cholelithiasis: a tropical experience. *Braz J Infect Dis,* Vol.12, No.3, (Jun), pp. 222-225, 1413-8670

Carlsen, E., A. Flatmark, et al. (1988). Cytokine-induced procoagulant activity in monocytes and endothelial cells. Further enhancement by cyclosporine. *Transplantation,* Vol.46, No.4, (Oct), pp. 575-580, 0041-1337

Catron, D. M., M. D. Sylvester, et al. (2002). The Salmonella-containing vacuole is a major site of intracellular cholesterol accumulation and recruits the GPI-anchored protein CD55. *Cell Microbiol,* Vol.4, No.6, (Jun), pp. 315-328, 1462-5814

Clark, M. A., M. A. Jepson, et al. (1994). Preferential interaction of Salmonella typhimurium with mouse Peyer's patch M cells. *Res Microbiol,* Vol.145, No.7, (Sep), pp. 543-552, 0923-2508

Coburn, B., Y. Li, et al. (2005). Salmonella enterica serovar typhimurium pathogenicity island 2 is necessary for complete virulence in a mouse model of infectious enterocolitis. *Infect Immun,* Vol.73, No.6, (Jun), pp. 3219-3227, 0019-9567

Cohen, J. I., J. A. Bartlett, et al. (1987). Extra-intestinal manifestations of salmonella infections. *Medicine (Baltimore),* Vol.66, No.5, (Sep), pp. 349-388, 0025-7974

Coombes, B. K., B. A. Coburn, et al. (2005). Analysis of the contribution of Salmonella pathogenicity islands 1 and 2 to enteric disease progression using a novel bovine ileal loop model and a murine model of infectious enterocolitis. *Infect Immun,* Vol.73, No.11, (Nov), pp. 7161-7169, 0019-9567

Coombes, B. K., M. J. Lowden, et al. (2007). SseL is a salmonella-specific translocated effector integrated into the SsrB-controlled salmonella pathogenicity island 2 type III secretion system. *Infect Immun,* Vol.75, No.2, (Feb), pp. 574-580, 0019-9567

Costerton, J. W., Z. Lewandowski, et al. (1995). Microbial biofilms. *Annu Rev Microbiol,* Vol.49, pp. 711-745, 0066-4227.

Costerton, J. W., P. S. Stewart, et al. (1999). Bacterial biofilms: a common cause of persistent infections. *Science,* Vol.284, No.5418, (May 21), pp. 1318-1322, 0036-8075

Crawford, R. W., D. L. Gibson, et al. (2008). Identification of a bile-induced exopolysaccharide required for Salmonella biofilm formation on gallstone surfaces. *Infect Immun,* Vol.76, No.11, (Nov), pp. 5341-5349, 0019-9567.

Crawford, R. W., K. E. Reeve, et al. (2010). Flagellated but not hyperfimbriated Salmonella enterica serovar typhimurium attaches to and forms biofilms on cholesterol-coated surfaces. *J Bacteriol,* Vol.192, No.12, (Jun), pp. 2981-2990, 0021-9193.

Cummings, L. A., S. L. Barrett, et al. (2005). FliC-specific CD4+ T cell responses are restricted by bacterial regulation of antigen expression. *J Immunol,* Vol.174, No.12, (Jun 15), pp. 7929-7938, 0022-1767.

Daigle, F. (2008). typhi genes expressed during infection or involved in pathogenesis. *J Infect Dev Ctries,* Vol.2, No.6, pp. 431-437, 1972-2680 (Electronic) 1972-2680 (Linking).

Danial, N. N. & S. J. Korsmeyer (2004). Cell death: critical control points. *Cell,* Vol.116, No.2, (Jan 23), pp. 205-219, 0092-8674

Darwin, K. H. & V. L. Miller (1999). Molecular basis of the interaction of Salmonella with the intestinal mucosa. *Clin Microbiol Rev,* Vol.12, No.3, (Jul), pp. 405-428, 0893-8512.

Davey, M. E. & A. O'Toole G (2000). Microbial biofilms: from ecology to molecular genetics. *Microbiol Mol Biol Rev,* Vol.64, No.4, (Dec), pp. 847-867, 1092-2172

Deiwick, J., S. P. Salcedo, et al. (2006). The translocated Salmonella effector proteins SseF and SseG interact and are required to establish an intracellular replication niche. *Infect Immun,* Vol.74, No.12, (Dec), pp. 6965-6972, 0019-9567

Diao, J., Y. Zhang, et al. (2008). Crystal structure of SopA, a Salmonella effector protein mimicking a eukaryotic ubiquitin ligase. *Nat Struct Mol Biol,* Vol.15, No.1, (Jan), pp. 65-70, 1545-9985

Dorsey, C. W., M. C. Laarakker, et al. (2005). Salmonella enterica serotype typhimurium MisL is an intestinal colonization factor that binds fibronectin. *Mol Microbiol,* Vol.57, No.1, (Jul), pp. 196-211, 0950-382X.

Drecktrah, D., S. Levine-Wilkinson, et al. (2008). Dynamic behavior of Salmonella-induced membrane tubules in epithelial cells. *Traffic,* Vol.9, No.12, (Dec), pp. 2117-2129, 1398-9219.

Dukes, J. D., H. Lee, et al. (2006). The secreted Salmonella dublin phosphoinositide phosphatase, SopB, localizes to PtdIns(3)P-containing endosomes and perturbs normal endosome to lysosome trafficking. *Biochem J,* Vol.395, No.2, (Apr 15), pp. 239-247, 0264-6021

Ehrbar, K., S. Mirold, et al. (2002). Characterization of effector proteins translocated via the SPI1 type III secretion system of Salmonella typhimurium. *Int J Med Microbiol,* Vol.291, No.6-7, (Feb), pp. 479-485, 1438-4221

Engelberts, I., E. J. von Asmuth, et al. (1991). The interrelation between TNF, IL-6, and PAF secretion induced by LPS in an in vivo and in vitro murine model. *Lymphokine Cytokine Res,* Vol.10, No.1-2, (Apr), pp. 127-131, 1056-5477.

Fantuzzi, G. & C. A. Dinarello (1999). Interleukin-18 and interleukin-1 beta: two cytokine substrates for ICE (caspase-1). *J Clin Immunol,* Vol.19, No.1, (Jan), pp. 1-11, 0271-9142.

Felix, A., and R. M. Pitt (1934). A new antigen of B. typhosus. *The Lancet,* Vol.224, No. 57, (28 July 1934), pp. 186 - 191.

Fields, P. I., R. V. Swanson, et al. (1986). Mutants of Salmonella typhimurium that cannot survive within the macrophage are avirulent. *Proc Natl Acad Sci U S A,* Vol.83, No.14, (Jul), pp. 5189-5193, 0027-8424

Fink, S. L. & B. T. Cookson (2007). Pyroptosis and host cell death responses during Salmonella infection. *Cell Microbiol,* Vol.9, No.11, (Nov), pp. 2562-2570, 1462-5814

Flannagan, R. S., G. Cosio, et al. (2009). Antimicrobial mechanisms of phagocytes and bacterial evasion strategies. *Nat Rev Microbiol,* Vol.7, No.5, (May), pp. 355-366, 1740-1526

Franchi, L., A. Amer, et al. (2006). Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in salmonella-infected macrophages. *Nat Immunol,* Vol.7, No.6, (Jun), pp. 576-582, 1529-2908.

Freeman, J. A., M. E. Ohl, et al. (2003). The Salmonella enterica serovar typhimurium translocated effectors SseJ and SifB are targeted to the Salmonella-containing vacuole. *Infect Immun,* Vol.71, No.1, (Jan), pp. 418-427, 0019-9567

Frost, A. J., A. P. Bland, et al. (1997). The early dynamic response of the calf ileal epithelium to Salmonella typhimurium. *Vet Pathol,* Vol.34, No.5, (Sep), pp. 369-386, 0300-9858.

Furman, M., A. Fica, et al. (1994). Salmonella typhi iron uptake mutants are attenuated in mice. *Infect Immun,* Vol.62, No.9, (Sep), pp. 4091-4094, 0019-9567

Galan, J. E. & A. Collmer (1999). Type III secretion machines: bacterial devices for protein delivery into host cells. *Science,* Vol.284, No.5418, (May 21), pp. 1322-1328, 0036-8075.

Ganley, I. G., K. Carroll, et al. (2004). Rab9 GTPase regulates late endosome size and requires effector interaction for its stability. *Mol Biol Cell,* Vol.15, No.12, (Dec), pp. 5420-5430, 1059-1524.

Garner, M. J., R. D. Hayward, et al. (2002). The Salmonella pathogenicity island 1 secretion system directs cellular cholesterol redistribution during mammalian cell entry and intracellular trafficking. *Cell Microbiol,* Vol.4, No.3, (Mar), pp. 153-165, 1462-5814

Gerlach, R. G., D. Jackel, et al. (2007). Salmonella Pathogenicity Island 4 encodes a giant non-fimbrial adhesin and the cognate type 1 secretion system. *Cell Microbiol,* Vol.9, No.7, (Jul), pp. 1834-1850, 1462-5814

Gewirtz, A. T., T. A. Navas, et al. (2001). Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. *J Immunol,* Vol.167, No.4, (Aug 15), pp. 1882-1885, 0022-1767

Gibson, D. L., A. P. White, et al. (2006). Salmonella produces an O-antigen capsule regulated by AgfD and important for environmental persistence. *J Bacteriol,* Vol.188, No.22, (Nov), pp. 7722-7730, 0021-9193.

Gonzalez-Escobedo, G., J. M. Marshall, et al. (2011). Chronic and acute infection of the gall bladder by Salmonella typhi: understanding the carrier state. *Nat Rev Microbiol,* Vol.9, No.1, (Jan), pp. 9-14, 1740-1526

Guo, A., M. A. Lasaro, et al. (2007). Adhesin-dependent binding and uptake of Salmonella enterica serovar typhimurium by dendritic cells. *Microbiology,* Vol.153, No.Pt 4, (Apr), pp. 1059-1069, 1350-0872

Hacker, J., L. Bender, et al. (1990). Deletions of chromosomal regions coding for fimbriae and hemolysins occur in vitro and in vivo in various extraintestinal Escherichia coli isolates. *Microb Pathog,* Vol.8, No.3, (Mar), pp. 213-225, 0882-4010

Hapfelmeier, S., B. Stecher, et al. (2005). The Salmonella pathogenicity island (SPI)-2 and SPI-1 type III secretion systems allow Salmonella serovar typhimurium to trigger colitis via MyD88-dependent and MyD88-independent mechanisms. *J Immunol,* Vol.174, No.3, (Feb 1), pp. 1675-1685, 0022-1767

Haraga, A. & S. I. Miller (2003). A Salmonella enterica serovar typhimurium translocated leucine-rich repeat effector protein inhibits NF-kappa B-dependent gene expression. *Infect Immun,* Vol.71, No.7, (Jul), pp. 4052-4058, 0019-9567.

Harrison, R. E., J. H. Brumell, et al. (2004). Salmonella impairs RILP recruitment to Rab7 during maturation of invasion vacuoles. *Mol Biol Cell,* Vol.15, No.7, (Jul), pp. 3146-3154, 1059-1524.

Hayashi, F., K. D. Smith, et al. (2001). The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature,* Vol.410, No.6832, (Apr 26), pp. 1099-1103, 0028-0836.

Henderson, I. R., F. Navarro-Garcia, et al. (2004). Type V protein secretion pathway: the autotransporter story. *Microbiol Mol Biol Rev,* Vol.68, No.4, (Dec), pp. 692-744, 1092-2172

Henry, T., C. Couillault, et al. (2006). The Salmonella effector protein PipB2 is a linker for kinesin-1. *Proc Natl Acad Sci U S A,* Vol.103, No.36, (Sep 5), pp. 13497-13502, 0027-8424

Hensel, M., J. E. Shea, et al. (1998). Genes encoding putative effector proteins of the type III secretion system of Salmonella pathogenicity island 2 are required for bacterial virulence and proliferation in macrophages. *Mol Microbiol,* Vol.30, No.1, (Oct), pp. 163-174, 0950-382X

Hernandez, L. D., M. Pypaert, et al. (2003). A Salmonella protein causes macrophage cell death by inducing autophagy. *J Cell Biol,* Vol.163, No.5, (Dec 8), pp. 1123-1131, 0021-9525

Hersh, D., D. M. Monack, et al. (1999). The Salmonella invasin SipB induces macrophage apoptosis by binding to caspase-1. *Proc Natl Acad Sci U S A,* Vol.96, No.5, (Mar 2), pp. 2396-2401, 0027-8424

Hirose, K., T. Ezaki, et al. (1997). Survival of Vi-capsulated and Vi-deleted Salmonella typhi strains in cultured macrophage expressing different levels of CD14 antigen. *FEMS Microbiol Lett,* Vol.147, No.2, (Feb 15), pp. 259-265, 0378-1097.

Hoebe, K., E. Janssen, et al. (2004). The interface between innate and adaptive immunity. *Nat Immunol,* Vol.5, No.10, (Oct), pp. 971-974, 1529-2908

Hone, D. M., S. R. Attridge, et al. (1988). A galE via (Vi antigen-negative) mutant of Salmonella typhi Ty2 retains virulence in humans. *Infect Immun,* Vol.56, No.5, (May), pp. 1326-1333, 0019-9567

Hornick, R. B., S. E. Greisman, et al. (1970). Typhoid fever: pathogenesis and immunologic control. *N Engl J Med,* Vol.283, No.13, (Sep 24), pp. 686-691, 0028-4793

Humphries, A. D., S. M. Townsend, et al. (2001). Role of fimbriae as antigens and intestinal colonization factors of Salmonella serovars. *FEMS Microbiol Lett,* Vol.201, No.2, (Jul 24), pp. 121-125, 0378-1097

Jackson, L. K., P. Nawabi, et al. (2008). The Salmonella virulence protein SifA is a G protein antagonist. *Proc Natl Acad Sci U S A,* Vol.105, No.37, (Sep 16), pp. 14141-14146, 0027-8424.

James Craigie, K. F. B. (1936). The identification of the V and W forms of B. typhosus and the occurrence of the V form in cases of typhoid fever and carriers. *The Journal of Pathology and Bacteriology,* Vol.43, No.2, pp. 249-260.

Jesenberger, V., K. J. Procyk, et al. (2000). Salmonella-induced caspase-2 activation in macrophages: a novel mechanism in pathogen-mediated apoptosis. *J Exp Med,* Vol.192, No.7, (Oct 2), pp. 1035-1046, 0022-1007.

Jonas, K., H. Tomenius, et al. (2007). Roles of curli, cellulose and BapA in Salmonella biofilm morphology studied by atomic force microscopy. *BMC Microbiol,* Vol.7, pp. 70, 1471-2180

Jones, B. D., N. Ghori, et al. (1994). Salmonella typhimurium initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches. *J Exp Med,* Vol.180, No.1, (Jul 1), pp. 15-23, 0022-1007.

Jones, R. M., H. Wu, et al. (2008). Salmonella AvrA Coordinates Suppression of Host Immune and Apoptotic Defenses via JNK Pathway Blockade. *Cell Host Microbe,* Vol.3, No.4, (Apr 17), pp. 233-244, 1931-3128

Karaki, K. & Y. Matsubara (1984). [Surgical treatment of chronic biliary typhoid and paratyphoid carriers]. *Nihon Shokakibyo Gakkai Zasshi,* Vol.81, No.12, (Dec), pp. 2978-2985, 0446-6586

Kerr, J. F., A. H. Wyllie, et al. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer,* Vol.26, No.4, (Aug), pp. 239-257, 0007-0920.

Khatri, N. S., P. Maskey, et al. (2009). Gallbladder carriage of Salmonella paratyphi A may be an important factor in the increasing incidence of this infection in South Asia. *Ann Intern Med,* Vol.150, No.8, (Apr 21), pp. 567-568, 0003-4819.

Kim, J. M., L. Eckmann, et al. (1998). Apoptosis of human intestinal epithelial cells after bacterial invasion. *J Clin Invest,* Vol.102, No.10, (Nov 15), pp. 1815-1823, 0021-9738

Klugman, K. P., I. T. Gilbertson, et al. (1987). Protective activity of Vi capsular polysaccharide vaccine against typhoid fever. *Lancet,* Vol.2, No.8569, (Nov 21), pp. 1165-1169, 0140-6736

Knodler, L. A. & O. Steele-Mortimer (2005). The Salmonella effector PipB2 affects late endosome/lysosome distribution to mediate Sif extension. *Mol Biol Cell,* Vol.16, No.9, (Sep), pp. 4108-4123, 1059-1524.

Knodler, L. A., B. A. Vallance, et al. (2003). Salmonella type III effectors PipB and PipB2 are targeted to detergent-resistant microdomains on internal host cell membranes. *Mol Microbiol,* Vol.49, No.3, (Aug), pp. 685-704, 0950-382X.

Kohbata, S., H. Yokoyama, et al. (1986). Cytopathogenic effect of Salmonella typhi GIFU 10007 on M cells of murine ileal Peyer's patches in ligated ileal loops: an ultrastructural study. *Microbiol Immunol,* Vol.30, No.12, pp. 1225-1237, 0385-5600.

Kraehenbuhl, J. P. & M. R. Neutra (2000). Epithelial M cells: differentiation and function. *Annu Rev Cell Dev Biol,* Vol.16, pp. 301-332, 1081-0706

Kuhle, V., G. L. Abrahams, et al. (2006). Intracellular Salmonella enterica redirect exocytic transport processes in a Salmonella pathogenicity island 2-dependent manner. *Traffic,* Vol.7, No.6, (Jun), pp. 716-730, 1398-9219

Kuhle, V. & M. Hensel (2002). SseF and SseG are translocated effectors of the type III secretion system of Salmonella pathogenicity island 2 that modulate aggregation of endosomal compartments. *Cell Microbiol,* Vol.4, No.12, (Dec), pp. 813-824, 1462-5814

Kuijl, C., N. D. Savage, et al. (2007). Intracellular bacterial growth is controlled by a kinase network around PKB/AKT1. *Nature,* Vol.450, No.7170, (Nov 29), pp. 725-730, 0028-0836.

Latasa, C., A. Roux, et al. (2005). BapA, a large secreted protein required for biofilm formation and host colonization of Salmonella enterica serovar Enteritidis. *Mol Microbiol,* Vol.58, No.5, (Dec), pp. 1322-1339, 0950-382X.

Lavigne, J. P. & A. B. Blanc-Potard (2008). Molecular evolution of Salmonella enterica serovar typhimurium and pathogenic Escherichia coli: from pathogenesis to therapeutics. *Infect Genet Evol,* Vol.8, No.2, (Mar), pp. 217-226, 1567-1348.

Layton, A. N. & E. E. Galyov (2007). Salmonella-induced enteritis: molecular pathogenesis and therapeutic implications. *Expert Rev Mol Med,* Vol.9, No.18, pp. 1-17, 1462-3994.

Le Negrate, G., B. Faustin, et al. (2008). Salmonella secreted factor L deubiquitinase of Salmonella typhimurium inhibits NF-kappaB, suppresses IkappaBalpha ubiquitination and modulates innate immune responses. *J Immunol,* Vol.180, No.7, (Apr 1), pp. 5045-5056, 0022-1767

Ledeboer, N. A. & B. D. Jones (2005). Exopolysaccharide sugars contribute to biofilm formation by Salmonella enterica serovar typhimurium on HEp-2 cells and chicken intestinal epithelium. *J Bacteriol,* Vol.187, No.9, (May), pp. 3214-3226, 0021-9193.

Levine, M. M., R. E. Black, et al. (1982). Precise estimation of the numbers of chronic carriers of Salmonella typhi in Santiago, Chile, an endemic area. *J Infect Dis,* Vol.146, No.6, (Dec), pp. 724-726, 0022-1899.

Li, H., H. Xu, et al. (2007). The phosphothreonine lyase activity of a bacterial type III effector family. *Science,* Vol.315, No.5814, (Feb 16), pp. 1000-1003, 0036-8075.

Liao, A. P., E. O. Petrof, et al. (2008). Salmonella type III effector AvrA stabilizes cell tight junctions to inhibit inflammation in intestinal epithelial cells. *PLoS One,* Vol.3, No.6, pp. e2369, 1932-6203

Libby, S. J., M. Lesnick, et al. (2000). The Salmonella virulence plasmid spv genes are required for cytopathology in human monocyte-derived macrophages. *Cell Microbiol,* Vol.2, No.1, (Feb), pp. 49-58, 1462-5814.

Lin, S. L., T. X. Le, et al. (2003). SptP, a Salmonella typhimurium type III-secreted protein, inhibits the mitogen-activated protein kinase pathway by inhibiting Raf activation. *Cell Microbiol,* Vol.5, No.4, (Apr), pp. 267-275, 1462-5814

Looney, R. J. & R. T. Steigbigel (1986). Role of the Vi antigen of Salmonella typhi in resistance to host defense in vitro. *J Lab Clin Med,* Vol.108, No.5, (Nov), pp. 506-516, 0022-2143

Lossi, N. S., N. Rolhion, et al. (2008). The Salmonella SPI-2 effector SseJ exhibits eukaryotic activator-dependent phospholipase A and glycerophospholipid : cholesterol acyltransferase activity. *Microbiology,* Vol.154, No.Pt 9, (Sep), pp. 2680-2688, 1350-0872.

Lostroh, C. P. & C. A. Lee (2001). The Salmonella pathogenicity island-1 type III secretion system. *Microbes Infect,* Vol.3, No.14-15, (Nov-Dec), pp. 1281-1291, 1286-4579

Mallo, G. V., M. Espina, et al. (2008). SopB promotes phosphatidylinositol 3-phosphate formation on Salmonella vacuoles by recruiting Rab5 and Vps34. *J Cell Biol,* Vol.182, No.4, (Aug 25), pp. 741-752, 0021-9525

Mazurkiewicz, P., J. Thomas, et al. (2008). SpvC is a Salmonella effector with phosphothreonine lyase activity on host mitogen-activated protein kinases. *Mol Microbiol,* Vol.67, No.6, (Mar), pp. 1371-1383, 0950-382X

McGhie, E. J., L. C. Brawn, et al. (2009). Salmonella takes control: effector-driven manipulation of the host. *Curr Opin Microbiol,* Vol.12, No.1, (Feb), pp. 117-124, 1369-5274

Merselis, J. G., Jr., D. Kaye, et al. (1964). Quantitative Bacteriology of the Typhoid Carrier State. *Am J Trop Med Hyg,* Vol.13, (May), pp. 425-429, 0002-9637

Miao, E. A., C. M. Alpuche-Aranda, et al. (2006). Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1beta via Ipaf. *Nat Immunol,* Vol.7, No.6, (Jun), pp. 569-575, 1529-2908.

Miao, E. A., E. Andersen-Nissen, et al. (2007). TLR5 and Ipaf: dual sensors of bacterial flagellin in the innate immune system. *Semin Immunopathol,* Vol.29, No.3, (Sep), pp. 275-288, 1863-2297.

Mills, D. M., V. Bajaj, et al. (1995). A 40 kb chromosomal fragment encoding Salmonella typhimurium invasion genes is absent from the corresponding region of the Escherichia coli K-12 chromosome. *Mol Microbiol,* Vol.15, No.4, (Feb), pp. 749-759, 0950-382X

Miyake, M., L. Zhao, et al. (1998). Vi-deficient and nonfimbriated mutants of Salmonella typhi agglutinate human blood type antigens and are hyperinvasive. *FEMS Microbiol Lett,* Vol.161, No.1, (Apr 1), pp. 75-82, 0378-1097.

Monack, D. M., D. M. Bouley, et al. (2004). Salmonella typhimurium persists within macrophages in the mesenteric lymph nodes of chronically infected Nramp1+/+ mice and can be reactivated by IFNgamma neutralization. *J Exp Med,* Vol.199, No.2, (Jan 19), pp. 231-241, 0022-1007

Morgan, E., Bowen, A.J, et al (2007). SiiE is secreted by the Salmonella enterica serovar Typhimurium pathogenicity island 4-encoded secretion system and contributes to intestinal colonization in cattle. *Infect Immun*. Vol. 75 No. 3, (Mar), pp.1524-33. 0019-9567

Mukherjee, K., S. Parashuraman, et al. (2001). SopE acts as an Rab5-specific nucleotide exchange factor and recruits non-prenylated Rab5 on Salmonella-containing phagosomes to promote fusion with early endosomes. *J Biol Chem,* Vol.276, No.26, (Jun 29), pp. 23607-23615, 0021-9258

Muller, S. I., M. Valdebenito, et al. (2009). Salmochelin, the long-overlooked catecholate siderophore of Salmonella. *Biometals,* Vol.22, No.4, (Aug), pp. 691-695, 0966-0844.

Murli, S., R. O. Watson, et al. (2001). Role of tyrosine kinases and the tyrosine phosphatase SptP in the interaction of Salmonella with host cells. *Cell Microbiol,* Vol.3, No.12, (Dec), pp. 795-810, 1462-5814

Nairz, M., G. Fritsche, et al. (2009). Slc11a1 limits intracellular growth of Salmonella enterica sv. typhimurium by promoting macrophage immune effector functions and impairing bacterial iron acquisition. *Cell Microbiol,* Vol.11, No.9, (Sep), pp. 1365-1381, 1462-5814

Neutra, M. R., A. Frey, et al. (1996). Epithelial M cells: gateways for mucosal infection and immunization. *Cell,* Vol.86, No.3, (Aug 9), pp. 345-348, 0092-8674

Ochman, H., F. C. Soncini, et al. (1996). Identification of a pathogenicity island required for Salmonella survival in host cells. *Proc Natl Acad Sci U S A,* Vol.93, No.15, (Jul 23), pp. 7800-7804, 0027-8424

Ohlson, M. B., K. Fluhr, et al. (2005). SseJ deacylase activity by Salmonella enterica serovar typhimurium promotes virulence in mice. *Infect Immun,* Vol.73, No.10, (Oct), pp. 6249-6259, 0019-9567.

Ohlson, M. B., Z. Huang, et al. (2008). Structure and function of Salmonella SifA indicate that its interactions with SKIP, SseJ, and RhoA family GTPases induce endosomal tubulation. *Cell Host Microbe,* Vol.4, No.5, (Nov 13), pp. 434-446, 1931-3128

Owen, R. L. (1994). M cells--entryways of opportunity for enteropathogens. *J Exp Med,* Vol.180, No.1, (Jul 1), pp. 7-9, 0022-1007

Paesold, G., D. G. Guiney, et al. (2002). Genes in the Salmonella pathogenicity island 2 and the Salmonella virulence plasmid are essential for Salmonella-induced apoptosis in intestinal epithelial cells. *Cell Microbiol,* Vol.4, No.11, (Nov), pp. 771-781, 1462-5814

Parra-Lopez, C., R. Lin, et al. (1994). A Salmonella protein that is required for resistance to antimicrobial peptides and transport of potassium. *EMBO J,* Vol.13, No.17, (Sep 1), pp. 3964-3972, 0261-4189

Pascopella, L., B. Raupach, et al. (1995). Host restriction phenotypes of Salmonella typhi and Salmonella gallinarum. *Infect Immun,* Vol.63, No.11, (Nov), pp. 4329-4335, 0019-9567

Patel, J. C. & J. E. Galan (2005). Manipulation of the host actin cytoskeleton by Salmonella--all in the name of entry. *Curr Opin Microbiol,* Vol.8, No.1, (Feb), pp. 10-15, 1369-5274.

Patel, J. C. & J. E. Galan (2006). Differential activation and function of Rho GTPases during Salmonella-host cell interactions. *J Cell Biol,* Vol.175, No.3, (Nov 6), pp. 453-463, 0021-9525.

Petros, A., D. Bennett, et al. (1991). Effect of nitric oxide synthase inhibitors on hypotension in patients with septic shock. *Lancet,* Vol.338, No.8782-8783, (Dec 21-28), pp. 1557-1558, 0140-6736

Pickard, D., J. Li, et al. (1994). Characterization of defined ompR mutants of Salmonella typhi: ompR is involved in the regulation of Vi polysaccharide expression. *Infect Immun,* Vol.62, No.9, (Sep), pp. 3984-3993, 0019-9567

Poltorak, A., X. He, et al. (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science,* Vol.282, No.5396, (Dec 11), pp. 2085-2088, 0036-8075

Prouty, A. M. & J. S. Gunn (2000). Salmonella enterica serovar typhimurium invasion is repressed in the presence of bile. *Infect Immun,* Vol.68, No.12, (Dec), pp. 6763-6769, 0019-9567

Prouty, A. M. & J. S. Gunn (2003). Comparative analysis of Salmonella enterica serovar typhimurium biofilm formation on gallstones and on glass. *Infect Immun,* Vol.71, No.12, (Dec), pp. 7154-7158, 0019-9567.

Prouty, A. M., W. H. Schwesinger, et al. (2002). Biofilm formation and interaction with the surfaces of gallstones by Salmonella spp. *Infect Immun,* Vol.70, No.5, (May), pp. 2640-2649, 0019-9567.

Qadri, A. (1997). Identification of specific recognition molecules on murine mononuclear phagocytes and B lymphocytes for Vi capsular polysaccharide: modulation of MHC class II expression on stimulation with the polysaccharide. *Immunology,* Vol.92, No.1, (Sep), pp. 146-152, 0019-2805.

Quezada, C. M., S. W. Hicks, et al. (2009). A family of Salmonella virulence factors functions as a distinct class of autoregulated E3 ubiquitin ligases. *Proc Natl Acad Sci U S A,* Vol.106, No.12, (Mar 24), pp. 4864-4869, 0027-8424.

Raffatellu, M., D. Chessa, et al. (2005). The Vi capsular antigen of Salmonella enterica serotype typhi reduces Toll-like receptor-dependent interleukin-8 expression in the intestinal mucosa. *Infect Immun,* Vol.73, No.6, (Jun), pp. 3367-3374, 0019-9567.

Raffatellu, M., D. Chessa, et al. (2006). Capsule-mediated immune evasion: a new hypothesis explaining aspects of typhoid fever pathogenesis. *Infect Immun,* Vol.74, No.1, (Jan), pp. 19-27, 0019-9567

Raffatellu, M., M. D. George, et al. (2009). Lipocalin-2 resistance confers an advantage to Salmonella enterica serotype typhimurium for growth and survival in the inflamed intestine. *Cell Host Microbe,* Vol.5, No.5, (May 8), pp. 476-486, 1931-3128

Ramsden, A. E., D. W. Holden, et al. (2007). Membrane dynamics and spatial distribution of Salmonella-containing vacuoles. *Trends Microbiol,* Vol.15, No.11, (Nov), pp. 516-524, 0966-842X.

Ren, T., D. S. Zamboni, et al. (2006). Flagellin-deficient Legionella mutants evade caspase-1- and Naip5-mediated macrophage immunity. *PLoS Pathog,* Vol.2, No.3, (Mar), pp. e18, 1553-7366

Rescigno, M., G. Rotta, et al. (2001). Dendritic cells shuttle microbes across gut epithelial monolayers. *Immunobiology,* Vol.204, No.5, (Dec), pp. 572-581, 0171-2985

Robbins, J. D. & J. B. Robbins (1984). Reexamination of the protective role of the capsular polysaccharide (Vi antigen) of Salmonella typhi. *J Infect Dis,* Vol.150, No.3, (Sep), pp. 436-449, 0022-1899.

Rohde, J. R., A. Breitkreutz, et al. (2007). Type III secretion effectors of the IpaH family are E3 ubiquitin ligases. *Cell Host Microbe,* Vol.1, No.1, (Mar 15), pp. 77-83, 1931-3128.

Ruiz-Albert, J., X. J. Yu, et al. (2002). Complementary activities of SseJ and SifA regulate dynamics of the Salmonella typhimurium vacuolar membrane. *Mol Microbiol,* Vol.44, No.3, (May), pp. 645-661, 0950-382X

Rytkonen, A., J. Poh, et al. (2007). SseL, a Salmonella deubiquitinase required for macrophage killing and virulence. *Proc Natl Acad Sci U S A,* Vol.104, No.9, (Feb 27), pp. 3502-3507, 0027-8424

Salcedo, S. P. & D. W. Holden (2003). SseG, a virulence protein that targets Salmonella to the Golgi network. *EMBO J,* Vol.22, No.19, (Oct 1), pp. 5003-5014, 0261-4189

Sansonetti, P. J. & A. Phalipon (1999). M cells as ports of entry for enteroinvasive pathogens: mechanisms of interaction, consequences for the disease process. *Semin Immunol,* Vol.11, No.3, (Jun), pp. 193-203, 1044-5323

Sawatzki, G., F. A. Hoffmann, et al. (1983). Acute iron overload in mice: pathogenesis of Salmonella typhimurium infection. *Infect Immun,* Vol.39, No.2, (Feb), pp. 659-665, 0019-9567.

Schaible, U. E. & S. H. Kaufmann (2004). Iron and microbial infection. *Nat Rev Microbiol,* Vol.2, No.12, (Dec), pp. 946-953, 1740-1526

Schioler, H., E. D. Christiansen, et al. (1983). Biliary calculi in chronic Salmonella carriers and healthy controls: a controlled study. *Scand J Infect Dis,* Vol.15, No.1, pp. 17-19, 0036-5548.

Schlumberger, M. C. & W. D. Hardt (2006). Salmonella type III secretion effectors: pulling the host cell's strings. *Curr Opin Microbiol,* Vol.9, No.1, (Feb), pp. 46-54, 1369-5274.

Schmidt, H. & M. Hensel (2004). Pathogenicity islands in bacterial pathogenesis. *Clin Microbiol Rev,* Vol.17, No.1, (Jan), pp. 14-56, 0893-8512.

Schmitt, C. K., J. S. Ikeda, et al. (2001). Absence of all components of the flagellar export and synthesis machinery differentially alters virulence of Salmonella enterica serovar typhimurium in models of typhoid fever, survival in macrophages, tissue culture invasiveness, and calf enterocolitis. *Infect Immun,* Vol.69, No.9, (Sep), pp. 5619-5625, 0019-9567.

Sharma, A. & A. Qadri (2004). Vi polysaccharide of Salmonella typhi targets the prohibitin family of molecules in intestinal epithelial cells and suppresses early inflammatory

responses. *Proc Natl Acad Sci U S A,* Vol.101, No.50, (Dec 14), pp. 17492-17497, 0027-8424.

Shea, J. E., M. Hensel, et al. (1996). Identification of a virulence locus encoding a second type III secretion system in Salmonella typhimurium. *Proc Natl Acad Sci U S A,* Vol.93, No.6, (Mar 19), pp. 2593-2597, 0027-8424

Shpargel, J. S., R. S. Berardi, et al. (1985). Salmonella typhi carrier state 52 years after illness with typhoid fever: a case study. *Am J Infect Control,* Vol.13, No.3, (Jun), pp. 122-123, 0196-6553

Sinnott, C. R. & A. J. Teall (1987). Persistent gallbladder carriage of Salmonella typhi. *Lancet,* Vol.1, No.8539, (Apr 25), pp. 976, 0140-6736

Song, J., T. Willinger, et al. (2010). A mouse model for the human pathogen Salmonella typhi. *Cell Host Microbe,* Vol.8, No.4, (Oct 21), pp. 369-376, 1931-3128

Steele-Mortimer, O. (2008). The Salmonella-containing vacuole: moving with the times. *Curr Opin Microbiol,* Vol.11, No.1, (Feb), pp. 38-45, 1369-5274

Steele-Mortimer, O., S. Meresse, et al. (1999). Biogenesis of Salmonella typhimurium-containing vacuoles in epithelial cells involves interactions with the early endocytic pathway. *Cell Microbiol,* Vol.1, No.1, (Jul), pp. 33-49, 1462-5814

Sun, Y. H., H. G. Rolan, et al. (2007). Injection of flagellin into the host cell cytosol by Salmonella enterica serotype typhimurium. *J Biol Chem,* Vol.282, No.47, (Nov 23), pp. 33897-33901, 0021-9258.

Swidsinski, A. & S. P. Lee (2001). The role of bacteria in gallstone pathogenesis. *Front Biosci,* Vol.6, (Oct 1), pp. E93-103, 1093-4715

Taylor, C. M., D. Osman, et al. (2009). Differential expression from two iron-responsive promoters in Salmonella enterica serovar typhimurium reveals the presence of iron in macrophage-phagosomes. *Microb Pathog,* Vol.46, No.2, (Feb), pp. 114-118, 0882-4010

Thanassi, D. G., L. W. Cheng, et al. (1997). Active efflux of bile salts by Escherichia coli. *J Bacteriol,* Vol.179, No.8, (Apr), pp. 2512-2518, 0021-9193.

Tsolis, R. M., G. M. Young, et al. (2008). From bench to bedside: stealth of enteroinvasive pathogens. *Nat Rev Microbiol,* Vol.6, No.12, (Dec), pp. 883-892, 1740-1526

Vaishnavi, C., S. Singh, et al. (2005). Prevalence of Salmonella enterica serovar typhi in bile and stool of patients with biliary diseases and those requiring biliary drainage for other purposes. *Jpn J Infect Dis,* Vol.58, No.6, (Dec), pp. 363-365, 1344-6304.

van der Velden, A. W., M. Velasquez, et al. (2003). Salmonella rapidly kill dendritic cells via a caspase-1-dependent mechanism. *J Immunol,* Vol.171, No.12, (Dec 15), pp. 6742-6749, 0022-1767

van Velkinburgh, J. C. & J. S. Gunn (1999). PhoP-PhoQ-regulated loci are required for enhanced bile resistance in Salmonella spp. *Infect Immun,* Vol.67, No.4, (Apr), pp. 1614-1622, 0019-9567

Virlogeux, I., H. Waxin, et al. (1996). Characterization of the rcsA and rcsB genes from Salmonella typhi: rcsB through tviA is involved in regulation of Vi antigen synthesis. *J Bacteriol,* Vol.178, No.6, (Mar), pp. 1691-1698, 0021-9193.

Vogelsang, T. M. & J. Boe (1948). Temporary and chronic carriers of Salmonella typhi and Salmonella paratyphi B. *J Hyg (Lond),* Vol.46, No.3, (Sep), pp. 252-261, 0022-1724

Waage, A., P. Brandtzaeg, et al. (1991). Current understanding of the pathogenesis of gram-negative shock. *Infect Dis Clin North Am,* Vol.5, No.4, (Dec), pp. 781-791, 0891-5520

Wall, D. M., W. J. Nadeau, et al. (2007). Identification of the Salmonella enterica serotype typhimurium SipA domain responsible for inducing neutrophil recruitment across the intestinal epithelium. *Cell Microbiol,* Vol.9, No.9, (Sep), pp. 2299-2313, 1462-5814

Wanachiwanawin, W. (2000). Infections in E-beta thalassemia. *J Pediatr Hematol Oncol,* Vol.22, No.6, (Nov-Dec), pp. 581-587, 1077-4114

Wasylnka, J. A., M. A. Bakowski, et al. (2008). Role for myosin II in regulating positioning of Salmonella-containing vacuoles and intracellular replication. *Infect Immun,* Vol.76, No.6, (Jun), pp. 2722-2735, 0019-9567

Waterman, S. R. & D. W. Holden (2003). Functions and effectors of the Salmonella pathogenicity island 2 type III secretion system. *Cell Microbiol,* Vol.5, No.8, (Aug), pp. 501-511, 1462-5814.

Wilson, R. P., M. Raffatellu, et al. (2008). The Vi-capsule prevents Toll-like receptor 4 recognition of Salmonella. *Cell Microbiol,* Vol.10, No.4, (Apr), pp. 876-890, 1462-5814

Winter, S. E., M. Raffatellu, et al. (2008). The Salmonella enterica serotype typhi regulator TviA reduces interleukin-8 production in intestinal epithelial cells by repressing flagellin secretion. *Cell Microbiol,* Vol.10, No.1, (Jan), pp. 247-261, 1462-5814.

Wood, M. W., M. A. Jones, et al. (1998). Identification of a pathogenicity island required for Salmonella enteropathogenicity. *Mol Microbiol,* Vol.29, No.3, (Aug), pp. 883-891, 0950-382X

Ye, Z., E. O. Petrof, et al. (2007). Salmonella effector AvrA regulation of colonic epithelial cell inflammation by deubiquitination. *Am J Pathol,* Vol.171, No.3, (Sep), pp. 882-892, 0002-9440

Zeng, H., H. Wu, et al. (2006). Flagellin/TLR5 responses in epithelia reveal intertwined activation of inflammatory and apoptotic pathways. *Am J Physiol Gastrointest Liver Physiol,* Vol.290, No.1, (Jan), pp. G96-G108, 0193-1857.

Zhang, Y., W. M. Higashide, et al. (2006). The inflammation-associated Salmonella SopA is a HECT-like E3 ubiquitin ligase. *Mol Microbiol,* Vol.62, No.3, (Nov), pp. 786-793, 0950-382X

IntechOpen