Microbiological Research 167 (2012) 199–210

Contents lists available at SciVerse ScienceDirect

Microbiological Research

journal homepage: www.elsevier.de/micres

Review article

Role of antigens and virulence factors of *Salmonella enterica* serovar Typhi in its pathogenesis

Jasmine Kaur, S.K. Jain*

Department of Biotechnology, Hamdard University, Hamdard Nagar, New Delhi 110062, India

ARTICLE INFO

Article history:
Received 20 May 2011
Received in revised form 5 August 2011
Accepted 8 August 2011

Keywords:
S. Typhi
Pathogenesis
Virulence factors
Type III secretion system
SPI's

ABSTRACT

Salmonella enterica serovar Typhi (*S.* Typhi), the aetiologic agent of typhoid fever, is a human restricted pathogen. The molecular mechanism of *Salmonella* pathogenicity is complex. The investigations of the molecular mechanisms of *Salmonella* virulence factors have shown that pathogenic *Salmonella* spp. are distinguished from their non-pathogenic relatives by the presence of specific pathogenicity genes, often organized in so-called pathogenicity islands (PIs). The type III secretion system (T3SS) proteins encoded by two *Salmonella* PIs (SPIs) are associated with the pathogenicity at molecular level. The identification of T3SS has provided new insight into the molecular factors and mechanisms underlying bacterial pathogenesis. The T3SS encoded by SPI-1 contains invasion genes; while SPI-2 is responsible for intracellular pathogenesis and has a crucial role for systemic *S. enterica* infections. These studies reveal a complex set of pathogenic interferences between intracellular *Salmonella* and its host cells. The understanding of the mechanisms by which *Salmonella* evade the host defense system and establish pathogenesis will be important for proper disease management.

Contents

1. Introduction 200
2. Pathogenesis of typhoid fever 200
   2.1. Intestinal mucosal immunity (first line of defense) 200
   2.2. Dissemination from intestinal mucosa's lamina propria 200
3. Host immune defense 200
   3.1. Clinical manifestations 202
4. Virulence factors and protein secretion mechanisms of *S. enterica* 203
   4.1. Type III protein secretion systems (T3SS) 203
   4.2. *Salmonella* pathogenicity island-1 type III secretion system: intestinal invasion and onset of diarrhea 203
   4.3. *Salmonella* pathogenicity island-2 type III secretion system: intracellular survival and systemic infection 205
   4.4. Other SPIs 206
   4.5. Plasmid-encoded virulence 206
5. Apoptosis and the human toll receptor activated in typhoid fever 206
6. Vaccines against typhoid fever 207
7. Conclusion 207
   Disclosure 207
   Acknowledgements 207
   References 207

* Corresponding author. Tel.: +91 11 26059688x5580; fax: +91 11 26059663.
E-mail addresses: skjain@jamiahamdard.ac.in, sk608@rediffmail.com, jasmine.kaur1@yahoo.co.in (S.K. Jain).

0944-5013/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.micres.2011.08.001

1. Introduction

Salmonella enterica serovar Typhi (*S.* Typhi) is a facultative intracellular pathogen that causes typhoid fever in humans (the only known natural hosts and reservoir of infection) (Mweu and English 2008). The organisms are noncapsulated, nonsporulating, Gram-negative anaerobic bacilli, which have characteristic flagellar, somatic, and outer coat antigens (Shahane et al. 2007). Typhoid remains a major health problem, especially in developing world where there is substandard water supply and lack of sanitation (Crum Cianflone 2008; Bhunia et al. 2009; Crump and Mintz 2010; Dougan et al. 2011). *S.* Typhi is transmitted through contaminated food and water, following ingestion, the bacteria spread from the intestine via blood to the intestinal lymph nodes, liver, and spleen where they multiply. Significant morbidity and mortality is associated with this disease possibly affecting over 90 million people globally each year (Majowicz et al. 2010). The risk of acquiring typhoid fever is increased among clinical microbiologists and travelers to regions where the disease is endemic (Levine et al. 2004).

Due to the rapid and widespread emergence of *S.* Typhi serotypes with resistance to multiple antibiotics and changing modes of bacterial presentation, typhoid fever is becoming increasingly difficult to diagnose and treat (Feng 2000; Akinyemi et al. 2005; Yang et al. 2010). The emergence of multidrug-resistant strains of *S.* Typhi has added a sense of urgency to develop more effective typhoid vaccines (Levine et al. 2004). Despite recent advances (Wahid et al. 2007; Tacket et al. 2000), the pace of progress in this area of vaccinology is not fast enough and needs to be accelerated (Sztein 2007). Finally, there is a need of continuous surveillance and sharing of antimicrobial susceptibility data for *Salmonella* among countries worldwide (de Oliveira et al. in press) to ensure the effectiveness of control programmes (Pui et al. 2011).

2. Pathogenesis of typhoid fever

Typhoid is a systemic disease that varies in severity. Recently a novel model has been reported that allows analyses of the pathogenesis of S. Typhi in a humanized non-obese diabetic (NOD) severe combined immune deficient (SCID) mouse model (Libby et al. 2010). The understanding of typhoid fever pathogenesis, especially the cellular and molecular phenomena that are responsible for clinical manifestations of this disease, has greatly increased with several important discoveries (Andrade and Andrade 2003). These include:

(a) Bacterial type III protein secretion system.
(b) The virulence genes of *Salmonella* spp. encoding five different Sips (*Salmonella* invasion protein) namely Sip A, B, C, D and E, which are capable of inducing apoptosis in macrophages.
(c) The function of Toll R2 and Toll R4 receptors present in the macrophage surface (originally discovered in Drosophila). The Toll family receptors are critical in cell signaling mediated through macrophages in association with lipopolysaccharide-binding protein (LBP) and CD14.
(d) The lines of immune defense between intestinal lumen and internal organs.
(e) The fundamental role of the endothelial cells in inflammatory deviation from bloodstream into tissues infected by bacteria.

2.1. Intestinal mucosal immunity (first line of defense)

The infectious dose of *S.* Typhi in volunteers varies between 1000 and 1 million organisms (Hornick et al. 1970). The low gastric pH is an important defense mechanism as the bacteria must survive the gastric acid barrier to reach the small intestine. In the small intestine, bacteria move across the intestinal epithelial cell (CEI) and reach the M cells, thus penetrating in the Peyer's patches (Fig. 1; Denise et al. 2004). The M cells are specialized epithelial cells overlying Peyer's patches that have probably originated from CEI and small pockets in the mucosal surface. After contact with M cells, the infectious bacteria are rapidly internalized and they reach a group of antigen-presenting cells (APCs), being partially phagocytized and neutralized. The infected phagocytes are organized in discrete foci that become pathological lesions, surrounded by normal tissue. Lesion formation is a dynamic process that requires the presence of adhesion molecules such as ICAM1 (Inter-Cellular Adhesion Molecule 1), VCAM-1 (Vascular Cell Adhesion Molecule 1) and the balanced action of cytokines [tumor necrosis factor (TNF)-α, interleukin (IL)-12, IL-18, IL-14, IL-15 and interferon (IFN)-γ]. Failure to form pathological lesions results in abnormal growth and dissemination of the bacteria in the infected tissue. Some bacteria escape this barrier, and reach the developed lymphoid follicles (Peyer's patches); formed mainly by mononuclear cells as T lymphocytes, as well as dendritic cells (DC). DC presents the bacterial antigens to immune cells that provoke activation of T and B lymphocytes.

2.2. Dissemination from intestinal mucosa's lamina propria

The T and B lymphocytes come out from the lymphatic nodules and reach liver and spleen via reticuloendothelial system (Fig. 1). In these organs the bacteria are killed mainly by phagocytosis through the macrophage system. However, *Salmonella* are able to survive and multiply within the mononuclear phagocytic cells (House et al. 2001). At a threshold level, determined by the number of bacteria, the bacterial virulence and the host immune response, the bacteria are released from their sequestered intracellular habitat into the bloodstream. This bacteremic phase of disease is characterized by dissemination of the organisms. The most common sites of secondary infection are the liver, spleen, bone marrow, gallbladder and Peyer's patches in the terminal ileum. In liver, *S.* Typhi provokes Kupffer cell activation. Kupffer cells have high microbicidal power and neutralize the bacteria with oxidative free radicals, nitric oxide as well as enzymes, active in acid pH. The survived bacteria invade hepatocytes and cause cellular death, mainly by apoptosis.

3. Host immune defense

The main host defense against *Salmonella* spp. occurs through the neutrophils, followed by mononuclear cells. These inflammatory cells produce cytokines as TNF-α, IFN-γ, IL-1, IL-2, IL-6 and IL-8. The Kupffer cells are the main TNF-α producer in the liver. Clearance of bacteria from tissues requires the CD28-dependent activation of CD4+, T cell receptor (TCR)-α β T cells and is controlled by Major histocompatibility complex (MHC) class II genes (McSorley and Jenkins 2000; Hess et al. 1996). DC and B-cells are involved in the initiation and development of T-cell immunity to *Salmonella* (Mastroeni et al. 2000; Yrlid et al. 2000). Interaction between B and T-cells is needed for the development of antibody response to *Salmonella* proteins and for isotype switching of antibody response against lipopolysaccharide antigens (Sinha et al. 1997). Resistance to reinfection with virulent *Salmonella* microorganisms (secondary infection) in immunized mice requires the presence of CD4+ dependent Th1 type immunological memory, CD8+ T cells and anti-*Salmonella* antibodies (Mastroeni et al. 1993; Thompson et al. 2009). The development of *Salmonella*-specific CD4 effector responses has been examined in both susceptible and resistant mice. These studies suggest massive expansion of

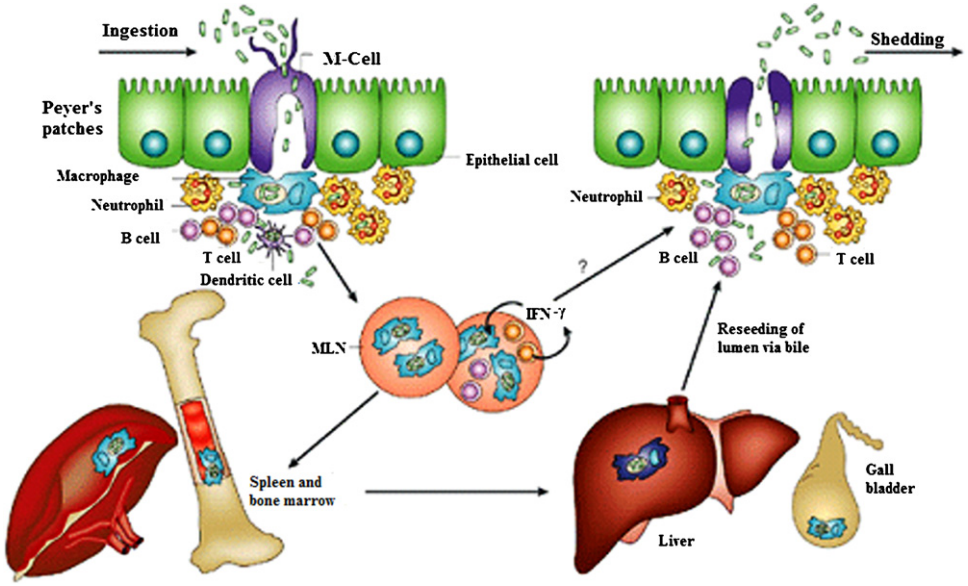

Fig. 1. Schematic representation of persistent infection with *Salmonella enterica* serovar Typhi in humans: bacteria enter the Peyer's patches of the intestinal tract mucosal surface by invading M cells – specialized epithelial cells that take up and transcytose luminal antigens for uptake by phagocytic immune cells. This is followed by inflammation and phagocytosis of bacteria by neutrophils and macrophages and recruitment of T and B cells. In systemic salmonellosis, such as typhoid fever, *Salmonella* may target specific types of host cells, such as dendritic cells and/or macrophages that favour dissemination through the lymphatics and blood stream to the mesenteric lymph nodes (MLNs) and to deeper tissues. This then leads to transport to the spleen, bone marrow, liver and gall bladder. Bacteria can persist in the MLNs, bone marrow and gall bladder for life, and periodic reseeding of the mucosal surface via the bile ducts and/or the MLNs of the small intestine occur, and shedding can take place from the mucosal surface. Interferon-(IFN-γ), which can be secreted by T cells, has a role in maintaining persistence by controlling intracellular *Salmonella* replication. Interleukin (IL)-12, which can increase IFN-γ production and the proinflammatory cytokine tumor-necrosis factor-(TNF-α) also contribute to the control of persistent *Salmonella*.
Adopted from Denise et al. (2004).

Salmonella-specific CD4 T cells and rapid acquisition of Th1 effector functions, namely the enhanced ability to secrete TNF-α, IFN-γ and IL-2 upon restimulation (Johanns et al. 2010; Griffin and McSorley 2011).

Epithelial cells seem to play the central role in coordinating the inflammatory response to intestinal pathogens. The interaction of *Salmonella* spp. with epithelial cells leads to the generation of a great number of biochemical signals by these cells. These include the basolateral release of chemokines (including IL-8) and apical secretion of "pathogen-elicited epithelial chemoattractant" (PEEC). These substances are partially responsible for guiding the recruitment and traffic of PMNs (Polymorphonuclear Leukocytes) across CEIs. After initial localization in resident phagocytes (macrophages) the bacteria associate mainly with PMNs in early phase of infection. It was therefore reasonable to assume that PMNs control early bacterial growth in tissue. However, evidence for a predominant role of mononuclear cells and not the PMNs in early resistance to the disease has been provided (Hormaeche et al. 1990). In a study *S.* Typhimurium (the rodent counterpart of *S.* Typhi that causes salmonellosis in mice/rats with typhoid like symptoms) infection was shown to induce IL-8 secretion by intestinal epithelium that was mediated through increase in intracellular calcium. This phenomenon was found to be NF-κB dependent (Gewirtz et al. 2002). A functional T3SS (type III secretion systems) is required for the induction of PMN transmigration. Furthermore, protein synthesis in both bacteria and epithelial cells is also required for this activity. Invasion of *Salmonella* spp. into epithelial cells is insufficient for trans-epithelial signaling to PMNs, and PMN migration occurs even when *Salmonella* spp. invasion is blocked. The 'inflammatory deviation' that happens when blood leukocytes migrate across endothelial cells into hepatic and spleen tissues is another important event. This phenomenon occurs through the action of adhesion molecules named integrins (chain αβ) in inflammatory cells and selectins in endothelial cells (E and P). Afterward, selectins are substituted by ICAM and VCAM proteins (whose partner in inflammatory cells is VLA4 or α4β1 integrin). The inflammatory microenvironment is completed by chemokines that are capable of stimulating leukocyte motility (chemokinesis) and directed movement (chemotaxis) of neutrophils and mononuclear cells. Chemokines bind to CC and CXC receptors in the surface of inflammatory cells. The chemokines help the blood leukocyte migration directly to host cells infected by bacteria. TNF-α is produced by macrophages and other mononuclear cells and has much antibacterial activity against *Salmonella* spp. Besides the macrophage phagocytosis, TNF-α in association with IFN-γ, IL-2 and other cytokines, is responsible for the neutralization of these invasive bacteria. Bacteria infested Peyer's patches produce strong inflammatory reaction with the recruitment of leukocytes. The potent inflammatory reaction against *Salmonella* species provokes host cell death, as well as apoptosis of both inflammatory and epithelial cells following nutrient deprivation and termination of bacterial replication (Santos et al. 2011). The inflammatory response of the Th1-dominant type is destructive for host cells and for bacteria; it attenuates progressively and coincides with increase of the Th2-immune response. Th2 cells produce IL-4, IL-10, IL-13 and transforming growth factor (TGF) that cause powerful protective effect on host cells (hepatocytes, CEIs, inflammatory cells, etc.) through partial inhibition of cytokines associated with the Th1 response.

Salmonella flagella have been implicated in host early innate immunity against *Salmonella* as these cause intestinal epithelial or macrophage inflammation following infection. Ciacci-Woolwine et al. (1998) and Wyant et al. (1999) reported that *S.* Typhi flagella induced cytokine release from human monocytes and impaired antigen presentation by human macrophages. Flagellin of *Salmonella* suppresses epithelial apoptosis and limits disease during enteric infection (Fig. 2) (Vijay-Kumar et al. 2006). It has been demonstrated that flagellin was the component that activated Toll-like receptor 5 (TLR) (Hayashi et al. 2001; Steiner 2007). Moreover, studies have identified Intracellular IL-1-converting enzyme

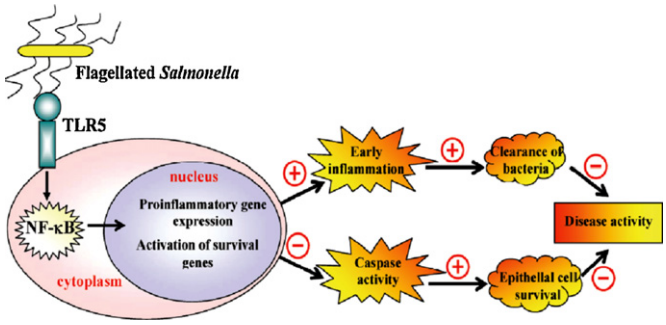

Fig. 2. Flagellin of *Salmonella* bacteria stimulates the activations of TLR5 and NF-κB, suppresses epithelial apoptosis and limits disease during enteric infection. Adopted from Zhang et al. (2008).

protease-activating factor (Ipaf) as an essential sensor for cytoplasmic flagellin (Miao et al. 2006; Franchi et al. 2006), which activates caspase-1 and induces the production of the proinflammatory cytokine IL-18 (Broz et al. 2010).

Using TLR5-deficient mice Feuillet et al. (2006) showed that while TLR5 was crucial for the *in vivo* recognition of flagellin, it may also participate in the detection of systemic infection by *S.* Typhimurium. TLR5 activates NF-κB and the mitogen-activated protein kinases (MAPKs) leading to the secretion of many cytokines, including IL-6, IL-12, and TNF-α, whereas Ipaf permits the activation of caspase-1 and secretion of mature IL-1β.

The activation of macrophages by Lipopolysaccharide (LPS) from *Salmonella* species also results in the release of a variety of inflammatory cytokines, such as IL-6 and IFN-β, which were not detected in macrophages of TLR4 knockout mice (Takeuchi et al. 1999). Following binding to LPS, in association with proteins MD2 and CD14, TLR4 dimerizes and undergoes a conformational change required for the recruitment of downstream Toll/interleukin-1 receptor (TIR) domain-containing adaptor molecules to activate both NF-κB and MAPKs (Mahieu and Libert 2007). Further, TLR4 triggers the early response to *Salmonella* and TLR4 and TLR2 are required sequentially for efficient macrophage function in *Salmonella* infections (Weiss et al. 2004). TLR2 can recognize *Salmonella* lipoproteins and lipoteichoic acid (Takeuchi et al. 1999; Bulut et al. 2001), probably in cooperation with TLR6 and/or TLR1 (Takeuchi et al. 2001; Hemmi et al. 2000). TLR9 is activated by bacterial DNA (detecting unmethylated Cp motifs). Totemeyer et al. (2005) have demonstrated that TLR1, TLR2, and TLR9 are up-regulated while TLR6 is down-regulated which accounts for the plateau phase observed during sublethal *S.* Typhimurium infection in rodents. These results suggest that in addition to TLR4, the TLR2-TLR1 complex and TLR9 may play a role in controlling infection, particularly in the later stages when the bacterial growth is suppressed, possibly at the adaptive phase of the immune response (Zhang et al. 2008).

3.1. Clinical manifestations

S. Typhi is responsible for typhoid fever in humans, a disease with high clinical toxicity and possible evolution to death (Balows et al. 1991; Murray et al. 1995). The clinical symptoms in patients with typhoid fever are related to cellular microbiological phenomena (Cossart et al. 1996). The bacterial invasion of several host cells and the inflammatory response (neutrophils, monocytes-macrophages, T and B lymphocytes) with high cytokine production are important elements causing the clinical manifestations. Cytokines (IL-1, IL-6, TNF-α, IFN-α, β and γ) are also responsible for fever emergence, lasting for four weeks in untreated typhoid cases. The potent inflammatory reaction against *Salmonella* spp. has the inconvenience of provoking host cell death, as well as the apoptosis of both inflammatory and epithelial cells. The increase in cytokines in peripheral blood causes fever after an incubation period of 5–21 days. Initially there is low fever, that rises progressively, and by the second week it is often high and sustained (39–40 °C). The fever occurs in more than 80% of patients (Khan et al. 1998). The classic disease description includes bacteremia and fever during the first week, as well as nonspecific symptoms such as chills, headache, anorexia, sore throat, myalgia, psychosis and mental confusion in 5–10% of the cases. A coated tongue, tender abdomen, hepatomegaly, and splenomegaly are common. In the second week, a few rose spots, blanching erythematous maculopapular lesions, approximately 2–4 mm in diameter, appear in 5–30 percent of cases. These usually occur on the abdomen and chest and more rarely on the back, arms, and legs. A relative bradycardia in relation to fever, intestinal constipation or diarrhea in smaller number of patients (mainly in young children and adults with HIV infection) may occur. Without treatment or correct diagnosis, the typhoid fever may prolong to the third week and the inflammatory lesions become intense in Peyer's patches and intestinal lamina propria (with abundant monocytes, macrophages and lymphocytes). A lymphoid hyperplasia in the ileocecal area followed by ulceration and necrosis (cellular death), with subsequent gastrointestinal bleeding or intestinal perforation occurs. These phenomena result in appearance of several clinical symptoms such as jaundice (due to hepatocyte death and cholangiocyte activation) and increased activity of enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamyl transpeptidase, alkaline phosphatase, etc. Further complications in 10–15 percent of patients may result in death, after the third week of disease. The fever declines in the 4th week of disease in 90% of the survivors, without antibiotic therapy. However, weakness and weight loss may persist for many months. Pyogenic meningitis, acute cholangitis and mural endocarditis are rare manifestations of typhoid fever. The true "sepsis", which constitutes severe typhoid fever disease, is caused by LPS/endotoxin molecules that are abundant at the surface of Enterobacteria such as *Salmonella* spp. and *Escherichia coli*. These molecules are capable of activating the macrophages, through cell surface receptors like CD14, Toll R2 and Toll R4 (in humans). The *viaB*-locus allows *S.* Typhi to modulate host responses by evading innate immune surveillance through TLR5 and TLR4 (Raffatellu et al. 2008). The inflammatory response of the Th1-dominant type is destructive for host cells and for bacteria. The Th1 response attenuates progressively, and this coincides with an increase in the Th2-immune response. Th2 cells produce IL-4, IL-10, IL-13, TGF-β, that cause a powerful protective effect in the host cells through the partial inhibition of the cytokines associated with Th1 cells. The predominance of Th1 immune response can be detected by immunohistochemistry using specific antibodies. Cytokines (as IFN-γ) in peripheral mononuclear cells can also be detected by flow cytometer or "ELISPOT". The ELISPOT technique is a recent discovery that is capable of evaluating cellular frequencies in a quantitative way, through identification of cells producing IFN-γ which characterizes a Th1 immune response.

The deficiency in inhibiting the proinflammatory cytokines TNF-α, IFN-γ, IL-2, etc., will result in cellular destruction predominantly in relation to cellular protection. This phenomenon provokes serious tissue lesions such as intestinal perforation and hemorrhage. The gastrointestinal bleeding should be attributed to endothelial cell activation through the increase of inflammatory cytokines and circulating LPS/endotoxin, primarily in the intestine. This results in high rate of endothelial cell death, and erosion of a necrotic tissue through the enteric vessel wall. The basal membrane of vessels is rich in collagen and is exposed, that allows the adherence of activated platelets. In addition, endothelial cells with the participation of pro and anticoagulation systems also activate the

coagulation cascade. This microenvironment becomes responsible for the bleeding and CEI death, ulcerated lesions and intestinal (usually ileal) perforation. B lymphocytes are found in the central area of lymphoid follicles, and migrate across vascular walls together with T lymphocytes. Some lymphocytes are retained in the mesenteric lymph nodes that become plasma cells in intestinal mucosa's lamina propria. At this stage, these cells become capable of producing IgG and IgM, as well as secretory IgA, which is the first line of intestinal defense. The final molecular junction of dimeric IgA occurs within intestinal lamina propria. In this site, IgA receives a protecting component produced by CEIs and remains resistant to digestive enzymes. Later, IgA is secreted into the intestinal lumen, together with other protectors' elements of intestinal mucosa such as intestinal microbiota and local antimicrobial, the secretory IgA constitutes the main element of the intestinal humoral defense.

One of the major clinical features of salmonellosis is diarrhea, which is caused by SPI-1 T3SS translocated proteins. The SopB protein appears to play an important role in the activation of secretory pathways, the attraction of neutrophils to the sites of infection (thereby increasing inflammation), and an alteration of ion balances within cells (Wallis and Galyov 2000). Additionally, SopB which probably influences the ion balance in cells through the antagonism of chloride channels in the infected cells. The alteration of ion balances within the cells can lead to fluid secretion into the intestinal tract and subsequent diarrhea. Other proteins such as SipA, SopA, SopD, and SopE2 may also play a role in *Salmonella*-associated gastroenteritis (Wallis and Galyov 2000; Zhang et al. 2003).

4. Virulence factors and protein secretion mechanisms of *S. enterica*

The complex pathogenesis of systemic *S. enterica* infections correlates with the presence of a large number of defensive as well as offensive virulence factors (Groisman and Ochman 1997). To date, more than 300 genes have been annotated as regulatory genes in *Salmonella* and of those, 14 regulators including SpvR, FruR, IHF, PhoP/PhoQ, SsrA/SsrB, SlyA, Hnr, RpoE, SmpB, CsrA, RpoS, CRP, OmpR/EnvZ, and Hfq are required for virulence regulation during systemic infection in an acute mouse infection model (Yoon et al. 2009, 2011).

As a facultative intracellular pathogen, *S. enterica* can adapt to the intracellular environment, and the two-component regulatory system PhoPQ appears to be an important sensor for the transition between extra- and intracellular life (Groisman 2001). The PhoPQ system has also been shown to regulate a large number of genes in *Salmonella* (Prost and Miller 2008).

Many of the *Salmonella* virulence factors, such as adhesion, invasion, and toxin genes are clustered in certain areas of the chromosome known as "*Salmonella* pathogenicity islands" (SPI) (Santos et al. 2003). The SPI can be located on the chromosome or on a plasmid, are flanked by repeat sequences, and tend to have a varied G/C composition as compared to surrounding region. SPI are characterized by a base composition different from the core genome and are often associated with tRNA genes and mobile genetic elements, like IS elements, transposons or phage genes (Schmidt and Hensel 2004). By now, 15 SPIs have been identified in *S.* Typhi (Parkhill et al. 2001; Vernikos and Parkhill 2006) two of these, SPI-1 and SPI-2 encode T3SS (Kuhle and Hensel 2004).

Two hallmarks of *Salmonella* pathogenesis, the host invasion and intracellular proliferation, are directly linked to genes in SPI. SPI-1 contains invasion genes; while SPI-2 is required for intracellular pathogenesis and has a crucial role for systemic *S. enterica* infections (Hansen-Wester and Hensel 2001).

4.1. Type III protein secretion systems (T3SS)

Although termed secretion systems, the biological function of T3SS is the translocation of proteins from the bacterial cytoplasm into the host cell, thus functioning as 'molecular syringes' (Hueck 1998). The T3SS is a complex of proteins that allows for the transfer of virulence factors directly into the host cells and is associated with at least 20–30 structural and regulatory proteins involved in cellular invasion, many of which have homology to proteins in the flagellar export apparatus (Marlovits et al. 2004; Gala'n and Wolf-Watz 2006; Marlovits and Stebbins 2010). While over 30 TTSS effectors have been identified to date (McGhie et al. 2009; Thomas and Holden 2009), the list is thought to be an underestimate of the true effector repertoire because several virulence phenotypes are dependent on TTS but are not linked to any known effectors (Lapaque et al. 2009; Srinivasan et al. 2009; Niemann et al. 2011).

In contrast to the type II secretion system, translocation via the T3SS occur independent of an N-terminal conserved *sec*-sequence that is cleaved after secretion. T3SS is an ATP-dependent system. T3SS are restricted to Gram-negative bacteria and are present in a number of different species, where they perform distinct functions ranging from antiphagocytic and cytotoxic effects on host cells (Ysc/Yop system of *Yersinia* spp.), invasion of host cells (*S. enterica* SPI-1 system, *Shigella* spp. Mxi/Spa system), intracellular pathogenesis (*S. enterica* SPI-2 system, *Chlamydia* spp. T3SS) to the establishment of symbiotic relationships such as that observed for the insect endosymbiont *Sodalis glossinidius* and the plant symbiont *Rhizobium* spp. *S. enterica* possesses two distinct T3SS with roles in different phases of pathogenesis. Although *S. enterica* was the first example of a pathogen employing two T3SS, genome sequencing revealed that multiple T3SS also occur in other species such as *Yersinia* spp., *Vibrio parahaemolyticus* and *Burkholderia pseudomallei* (Pallen et al. 2003).

4.2. Salmonella pathogenicity island-1 type III secretion system: intestinal invasion and onset of diarrhea

Salmonella have evolved intricate measures to invade host cells following the epithelial attachment. Upon its interaction with host cells, T3SS facilitates endothelial uptake and invasion (Fig. 3) (Zhang et al. 2008).

The base structure of the T3SS complex spans the cell membrane and the cell wall of *Salmonella*, and a needle structure protrudes from the base that interacts with host cells. Within the base and needle structure is an inner rod that forms the conduit between the bacterial cytoplasm and the host cell membrane (Gala'n and Wolf-Watz 2006). On the cytoplasm side of the T3SS structure, there is a set of export machinery that contains an ATPase complex that facilitates the transport of effector molecules through the inner rod to a translocase structure in the host cell membrane. The T3SS structural genes located on SPI-1 include *prgHIJK*, *spaMNOPQRS*, and *invABCEFGH*, as well as multiple regulatory and effector genes. The major T3SS regulatory protein is HilA, whose expression is mediated by a number of environmental factors important for cell survival (Lostroh and Lee 2001). The assembly of the SPI-1 T3SS appears to be built from the base up. An assembly model starts with the assembly of the inner ring structure, which spans the cell membrane and is assembled from PrgH and PrgK protein subunits (Gala'n and Wolf-Watz 2006). Next, the cytoplasmic export machinery, which is composed of the InvA, InvC, SpaP, SpaQ, SpaR, and SpaS proteins, is assembled. Also, the outer ring structure, composed of InvG and InvH, is assembled in the outer membrane and connected to the inner ring structure and is stabilized with the aid of the regulatory protein InvJ. The completed base structure allows for the assembly of the needle and inner rod structures, which are

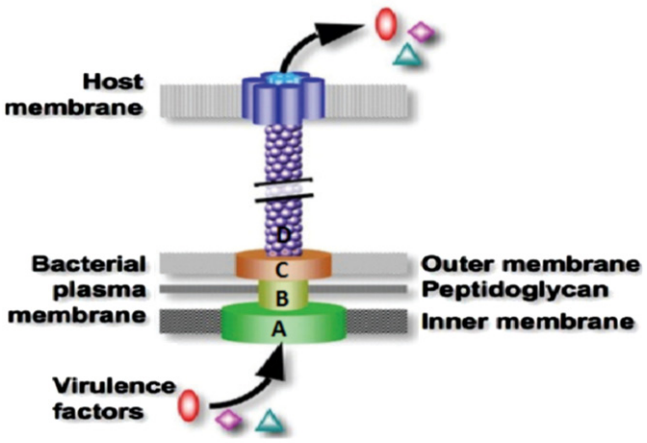

Fig. 3. Schematic representation of the *Salmonella* pathogenicity island 1 associated type III secretion system. The base structure of the T3SS is composed of the inner ring structure (A), which is composed of PrgHK protein subunits; the cytoplasmic export machinery (B), which is composed of the InvAC and SpaPQRS; and the outer ring structure (C), which is composed of InvGH and connected to the inner ring structure. The completed base structure allows for the assembly of the needle (D) and inner rod structures, which are made up of PrgJ and PrgI subunits, respectively, that interact with the host cell membrane.
Modified from Zhang et al. (2008).

made up of PrgJ and PrgI subunits, respectively (Marlovits et al. 2004; Gala'n and Wolf-Watz 2006).

The completed SPI-1 T3SS allows for effector proteins to be translocated from the bacterial cytoplasm to the host cell. In the bacterial cytoplasm, chaperone molecules bind to the effector proteins and accompany the molecule to the export machinery of the T3SS. The SPI-1 secreted effectors SopE and SopE2 act as guanine nucleotide-exchange-factors (GEFs) for the small GTPases Cdc42 and Rac (Thomson et al. 2004) (Fig. 4). Additional SPI-1 translocated effectors of *Salmonella* affect actin dynamics during the invasion process. SipA binds and stabilizes actin and SipC, which forms part of the T3SS delivery pore, can independently cause actin rearrangements via its distinct actin-bundling and actin-nucleating domains that results in membrane ruffling (Lostroh and Lee 2001; McGhie et al. 2001; Myeni and Zhou 2010). In addition to modulating actin, SipC interacts directly with Exo70, a component of the exocyst

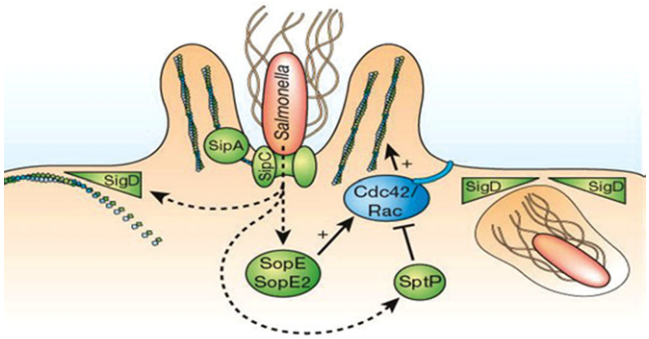

Fig. 4. Entry into host cells is mediated by the T3SS and its effectors encoded by SPI-1. Membrane attachment to the cortical actin cytoskeleton is loosened by SigD/SopB. SopE and SopE2 enhance Cdc42 and Rac1 activity directly by acting as guanine nucleotide-exchange factors. SipA and SipC alter cytoskeletal structure, SipC by nucleating actin and initiating polymerization and SipA by binding actin and modulating actin bundling. These cytoskeletal rearrangements are downregulated by the GAP (GTPase-activating protein) activity of SptP, which inactivates Cdc42 and Rac. SigD also is involved in sealing invaginating regions of the plasma membrane to form intracellular vacuoles.
Adopted from Zhang et al. (2008).

Table 1
Salmonella Pathogenicity Island 1 associated type III secretion system effector proteins.

Effector proteins	Effect on host cell
SipA	Rearrangement of cytoskeleton/neutrophil recruitment
SipB	Actin nucleation/translocation of other effectors
SipC	Translocation of other effectors
SopA	Immune cell recruitment, fluid secretion
SopB	Cytoskeleton rearrangement, neutrophil recruitment, fluid secretion
SopC	Neutrophil recruitment, fluid secretion
SopD	Neutrophil recruitment, fluid secretion
SopE	Rearrangement of cytoskeleton
SptP	Rearrangement of cytoskeleton

Modified from Lostroh and Lee (2001).

complex, which mediates docking and fusion of exocytic vesicles with the plasma membrane (Nichols and Casanova 2010; Malik-Kale et al. 2011). Recent work suggests that SopE can also activate RalA, a GTPase that is required for assembly of the exocyst (Nichols and Casanova 2010) and has been implicated in Fc gamma receptor-mediated phagocytosis (Corrotte et al. 2010). Thus SopE and SipC together appear to direct fusion of exocytic vesicles with the plasma membrane at the site of entry, presumably as a source of membrane for the expanding ruffle or phagocytic cup (Nichols and Casanova 2010). *Salmonella* also alters the actin cytoskeleton, through manipulation of phosphoinositides. The plasma membrane is intimately associated with actin cytoskeleton, and this interaction depends on phosphatidylinositol 4,5-bisphosphate (PtdIns (4,5) P2). SigD/SopB is an SPI-1 translocated inositol phosphatase that induces the rapid disappearance of PtdIns (4,5) P2 from invaginating regions of the membrane during *Salmonella* invasion (Bakowski et al. 2010) and can hydrolyse a number of phosphor inositides and inositol phosphates *in vitro* and has diverse effects on host cells (Patel et al. 2009; Bakowski et al. 2010; Braun et al. 2010). This increases elasticity to facilitate the remodelling of the plasma membrane associated with *Salmonella* entry (Raucher et al. 2000). PtdIns (4,5) P2 has also been implicated in vesicle fission during the creation of phagosomes and clathrin-coated vesicles, and accordingly, SigD is also involved in sealing plasma membrane invaginations to form bona fide vacuoles. After invasion, an additional SPI-1 effector, SptP, acts as a GTPase-activating protein (GAP) for Cdc42 and Rac1, thereby inactivating these G proteins and returning cell morphology to a relatively normal state (Knodler and Steele-Mortimer 2003). SptP is a bifunctional protein, with its GAP domain at the amino terminus, and a protein tyrosine phosphatase domain at the carboxy terminus. A potential target for the tyrosine phosphatase activity of SptP is the intermediate filament protein vimentin, which is recruited to the membrane ruffles stimulated by *Salmonella* (Murli et al. 2001). Other studies have also identified another intermediate filament protein involved in *Salmonella* entry, SipC, which binds cytokeratins and expression of dominant negative cytokeratin-18 inhibits *Salmonella* entry into HEp2 cells (Carlson et al. 2002) (Fig. 4). Some of the translocated proteins and their functions have been summarized in Table 1.

Membrane ruffling is characterized by a rearrangement of cell membrane and cytosol such that the *Salmonella* bacterium is surrounded by the host cell and internalized (Jones et al. 1993; Goosney et al. 1999). Once *Salmonella* is internalized, the microorganism resides in the membrane bound vacuole/*Salmonella*-containing vacuole (SCV; Knodler and Steele-Mortimer 2003). As the SCV matures, it migrates from the luminal border of the cell to the basal membrane where the *Salmonella* interact with and enter into the macrophages associated with Peyer's patches in the submucosal space (Ohl and Miller 2001; Pegues et al. 2005). The formation of SCV occurs separately from

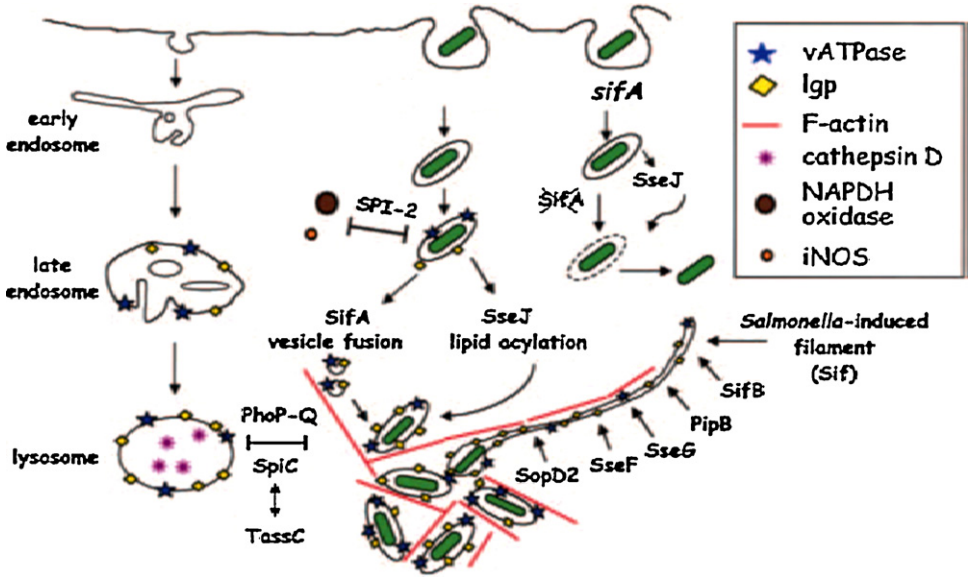

Fig. 5. Attachment and internalization of *Salmonella* in a host cell. After attachment to the host cell surface, *Salmonella* can express a type III secretion system that facilitates endothelial uptake and invasion through the translocation of effector proteins from the bacterial cell to the host cell cytosol. These effector proteins interact with the cytoskeleton, leading to membrane ruffling. Membrane ruffling allows the bacterium to be surrounded by the host cell membrane and internalized in the membrane-bound *Salmonella*-containing vacuole (SCV). SCVs do not interact extensively with late endosomes or lysosomes. In macrophages, evidence implicates the SPI-2 effector protein SpiC (which interacts with the host protein TassC) and the PhoP-Q regulon in this process. SifA, SifB, SseJ, SseF, SseG, PipB and SopD2 are examples of SPI-2 translocated proteins that localize to the SCV and Sifs in epithelial cells. Loss of vacuolar membrane from the *sifA* requires the action of SseJ.
Modified from Waterman and Holden (2003).

the normal endocytic processing pathways present in host cells. Further, it also acquires some of the endosomal markers involved in intracellular processing; though, it does not fuse with lysosomal compartments (Fig. 5; Unsworth and Holden 2000; Alonso and Garcia-del Portillo 2004). More recently, SopB was found to manipulate the SCV surface charge resulting in the inhibition of SCV and lysosome fusion (Bakowski et al. 2010; Jonathan et al. 2011). This separation helps, *Salmonella* to avoid being killed by the normal phago-lysosomal processing pathways (Holden 2002). The SCVs are important for *Salmonella* survival and transport in epithelial cells and play a key role in the survival of the bacterium within phagocytic cells such as macrophages during invasive infections. Therefore, the ability to survive and proliferate in SCV is very important for virulence of *Salmonella* (Tierrez and Garcia-del Portillo 2005; Foley and Lynne 2008).

4.3. *Salmonella pathogenicity island-2 type III secretion system: intracellular survival and systemic infection*

Invasive *Salmonella* infections are associated with T3SS encoded on SPI-2. The SPI-2 T3SS genes are only expressed inside the host cell SCV. Many of the genes encoded on SPI-2 encode for the structure of secretion system apparatus (*ssaG* through *ssaU*), secretion system effector (*sseABCDEF*), secretion system chaperones (*sscAB*), and secretion system regulatory (*ssrAB*) genes required for a functional T3SS (Hensel 2000). The SPI-2 T3SS secretion apparatus expression is regulated through SsrA-SsrB 2-component regulatory system, which is subsequently regulated by a second 2-component regulatory system, OmpR-EnvZ (Lee et al. 2000; Garmendia et al. 2003). A number of environmental conditions have been associated with the induction of the expression of SPI-2 T3SS genes through the OmpR-EnvZ regulatory system, including low osmolarity, low levels of certain nutrients, and acidification of the SCV (Cirillo et al. 1998; Lee et al. 2000). The activated SPI-2 T3SS facilitates the transfer of effector proteins from *Salmonella* across the SCV membrane to interact with targets in the host cells. The first SPI-2 effector protein to be identified and characterized was SpiC (Uchiya et al. 1999). Lee et al. (2002) have identified a novel host cell protein as a target for SpiC, which has been named TassC. TassC was identified by a yeast two-hybrid screen and was also shown to interact with SpiC by pull-down and co-immune precipitation experiments. SpiC appears to exclude TassC from associating with the SCV. Many SPI-2 T3SS effector proteins including, SifA, SifB, SseJ, SseF, SseG, PipB and SopD2 interact with microtubule bundles and their associated motor proteins and are involved in the formation of *Salmonella*-induced filaments (SIF) that extend from SCV (Table 2 and Fig. 5; Waterman and Holden 2003; Abrahams and Hensel 2006). The formation of SIF takes place as the result of the fusion of SCV with other vesicles in the cell (Abrahams and Hensel 2006). They are enriched in lysosomal membrane glycoproteins (lgps) such as LAMP1 (García-del Portillo et al. 1993). The major functions of SIF are not fully understood; however, it is likely that SIF are important for pathogenesis and may play a role in intracellular replication of *Salmonella* because their formation often coincides with replication of the microorganisms (Knodler and Steele-Mortimer 2003). SpiC is translocated into the cytosol of host

Table 2
Salmonella pathogenicity island 2 associated type III secretion system effector proteins.

Effector proteins	Effect on host cell
SpiC	Disruption of vesicular transport
SseF	Aid in *Salmonella*-induced filament formation
SseG	Aid in *Salmonella*-induced filament formation
SifA	*Salmonella*-containing vacuole membrane integrity
SifB	Targeting to *Salmonella* induced filaments
SspH2	Cytoskeleton rearrangement
SrfT	Apoptosis
SseI	Cytoskeleton rearrangement
SseJ	*Salmonella*-containing vacuole membrane dynamics/acyl transferase
PipB	Targeting to *Salmonella* induced filaments
SopD2	Targeting to *Salmonella* induced filaments/late endosomes

Modified from Waterman and Holden (2003).

macrophages, where it interacts with the endomembrane system and interferes with normal secretory pathways of the host (Uchiya et al. 1999). This disruption likely protects organisms from bactericidal compounds, including reactive oxygen and reactive nitrogen molecules that are able to kill bacteria (Hensel 2000). Systemic infections are severe manifestations of salmonellosis. To facilitate systemic infection, intracellular *Salmonella* present in immune cells such as macrophages and DC may be carried from the intestinal tract to other areas of the body. Dendritic cells are important migratory phagocytes that are widely distributed throughout the body in lymphoid and non-lymphoid tissues (Sundquist et al. 2004). The ability of DC to migrate throughout the body potentially facilitates the spread of *Salmonella* to various parts of the body. While in DC, the *Salmonella* do not appear to replicate but remain viable, possibly in a small colony variant state with reduced metabolic activity and increased persistence (Tierrez and Garcia-del Portillo 2005). Genes encoded on SPI-2 T3SS appear to suppress antigen presentation by DC, which limits a robust immune response by the infected cells (Waterman and Holden 2003). A proteomics analysis of *S.* Typhi grown in low pH, low magnesium, minimal media (MgM or LPM) was reported. MgM is designed to approximate the phagosome of infected macrophages and is known to induce expression of SPI-2 virulence genes and other genes related to virulence and intra macrophage survival (Ansong et al. 2008; Shi et al. 2009). The combination of lowered metabolic activity and immunosuppression contributes to the persistence of *Salmonella* within host cells. When macrophages or DC enter the organ systems, the *Salmonella* can spread to adjacent cells and trigger apoptosis, which leads to increased pathology among the infected cells (Sheppard et al. 2003; Tierrez and Garcia-del Portillo 2005).

4.4. Other SPIs

The role of genes that belong to other pathogenicity islands in *S.* Typhi pathogenesis has not been investigated in depth yet. Other than these two major PIs, there are other PIs that have been identified most of which are of utmost importance to the virulence and survival of the bacterium. Such islands are SPI-3, SPI-4 and SPI-5. It has been suggested that when genes associated with SPI-3, SPI-4, and SPI-5 together with SPI-1 and SPI-2 are inactivated, *S.* Typhi loses the ability to express several virulence-associated traits, a factor that could begin to explain the loss of host range of these serovars. The magnesium transport system *mgtBC* is located on SPI-3 and magnesium availability is a key signal for Salmonella in the intracellular environment (Charles et al. 2009; Sheikh et al. 2010). SPI-4 carries genes (*spi4R* and *spi4D*) that are predicted to encode a type I secretion system whereas *pipAB* and *sopB* genes which encodes for TTSS-1 and TTSS-2 effector proteins are located on SPI-5 (Faucher et al. 2006). SPI-6 carries *pagN* and genes that encodes the safA-D and tcsA-R chaperone-usher fimbrial operons. Another important island is SPI-7 which is responsible for the production of the Vi polysaccharide capsule and also carries genes for potential virulence factors such as a phage encoding the *sopE* effector protein of SPI-1, a type IV pilus and a putative type IV secretion system (Helena and Seth-Smith 2008). SPI-8 encodes two bacteriocin pseudogenes and a degenerate integrase; notably, genes conferring immunity to the bacteriocins remain intact. SPI-9, carry genes that encodes a type I secretory apparatus and a single, large repeats in toxin (RTX)-like protein. On SPI-10, the *prpZ* locus encoding for proteins with homology to eukaryotic-type Ser/Thr protein phosphatase and kinases has been found to promote survival in macrophages (Faucher et al. 2008). SPI-11 carries *pagC* and *pagD* genes that encodes for macrophoage survival and serum resistance (Harris et al. 2006). SPI-12 carries genes that encode for TTSS-2 effector proteins (Morgan 2007).

4.5. Plasmid-encoded virulence

In addition to the virulence factors associated with the SPI-1 and SPI-2 T3SS, some factors such as virulence or antimicrobial resistance can be found on plasmids (Rotger and Casadesus 1999; Sheppard et al. 2003). Strains from many serovars lack virulence plasmids; however, some of the most important serovars for human health, including *S.* Typhimurium, *S.* Enteritidis, and *S.* Choleraesuis are known to harbor virulence plasmids (Lu et al. 1999; Villa and Carattoli 2005; Yu et al. 2006). These virulence plasmids have a genetic locus called *Salmonella* plasmid virulence, which contains *spvRABCD* genes. The *spv* genes appear to be important for bacterial multiplication within host cells during extra-intestinal infections (Guiney et al. 1995). Additional virulence genes located on virulence plasmids include those encoding fimbriae (*pef-BACDI*) and serum resistance (*traT*; Rotger and Casadesus 1999). Although most virulence plasmids are not self-transmissible, some appear to contain a full concert of transfer (*tra*) genes that allow the plasmids to be transferred to additional strains by conjugation (discussed subsequently), potentially increasing the virulence of the recipients (Ahmer et al. 1999). Due to their conservation among members of a particular serovar, virulence plasmids provide a significant advantage to the strains harboring these plasmids (Foley and Lynne 2008).

5. Apoptosis and the human toll receptor activated in typhoid fever

Typhoid fever is an important example of severe sepsis, because it presents an enormous amount of cellular death (necroapoptosis) besides severe toxemia. The role of Toll R2 and Toll R4 receptors has been studied in Drosophila. The innate immune system uses Toll family receptors to sign organisms' presence and initiate host defense. Bacterial lipoproteins (BLP) expressed in all bacteria species are potent activators of "Toll-like receptor 2" (Toll R2). The innate immune system includes macrophages and natural killer (NK) cells that act directly on pathogens through cytokines and other stimulatory molecules, which activate the adaptive immune responses (cellular and molecular) through T and B lymphocytes (Modlin et al. 1999). The innate immune system (first line of defense) identifies the pathogens by standard recognition receptors, which attach to microbial macromolecules. In this way, macrophage mannose receptors attach to structures with repeats of polymannose. Before the discovery of the Toll R2 receptors the CD14 receptors, were considered as of fundamental importance to recognize LPS/endotoxin on the surface of Gram negative bacteria. The difference between CD14 and Toll R2 is that the former are a "GPI" (glycosyl-phosphatidylinositol) receptor, that does not cross the cellular membrane, and does not transmit signals to cytoplasm or activate protein chains of macrophages (Hoffmann et al. 1999; Modlin et al. 1999). In contrast, Toll R2 molecules cross the cellular membrane, and transmit sign for the signal transduction chains. The activation of these protein pathways spreads to the transcriptional factor NF-κB that will migrate to the cellular nucleus starting from the cytoplasm. NF-κB recognizes and liberates genes encoding the adhesion molecules, TNF-α and other Th1 cytokines (IFN-γ, IL-2, etc.). The patients with deficient immune response to combat the infection by macrophage activation may develop septic shock (Morrison & Ryan 1987). The pathogens have molecular antigens such as LPS, glycolipid of mycobacteria, lipoteichoic acid, mannans of yeast, and RNAs of virus that are recognized by receptors (Janeway 1989; Medzhitov et al. 1997).

Two LPS binding proteins, BPI (bactericide permeability increasing protein) and LBP (lipopolysaccharide binding protein) are of fundamental importance, because their association with LPS results

in distinct effects. BPI (55 kD) which is present in neutrophils with selective toxicity against Gram-negative bacteria has an antimicrobial role. This protein is more effective when it acts on neutrophils in synergism with defensins (intestinal mucosa's antimicrobial). LBP increases the cell sensitivity to LPS allowing the effector cells to be activated by subpicomolar LPS concentrations (Tobias et al. 1986; Ulevitch and Tobias 1999). LBP also carries out an important role in the bacterial clearance of peripheral blood through CD14.

Studies have indicated that Toll family receptors (TLRs) in association with LBP and CD14 are critical in LPS mediated signaling (Hoffmann et al. 1999). Presently six members of the TLR family are known, two of which, TLR2 and TLR4, are associated with LPS signalizing. Several lines of evidence support the view that TLR4 is the receptor for gram negative bacterial LPS and TLR2 is the receptor for gram positive peptidoglycan and lipoproteins (Schwandner et al. 1999; Takeuchi et al. 1999). TLR2 is the first member of the TLR family that was found to be involved in responsiveness to LPS. Evidence was also provided that TLR2 directly bind LPS (Yang et al. 1998). In addition to TLR2, TLR4 was also found to be involved positive bacteria, in LPS-mediated signaling. Positional cloning of the *Lps* gene, which governs responses to LPS, disclosed the defect in the LPS-hyporesponsive mouse strains C3H/HeJ and C57BL/10ScCr is due to mutation in the TLR4 gene (Poltorak et al. 1998; Qureshi et al. 1999) while overexpression of either TLR2 or TLR4 has been reported to confer responsiveness to LPS in cell lines (Yang et al. 1998; Chow et al. 1999). It was also shown that hTLR2 (Toll-like human receptor 2) and hTLR4 (Toll-like human receptor 4) can interact with CD14 located on the surface of monocytes/macrophages to form the LPS receptor complex. LPS treatment leads to receptor oligomerization and to subsequent recruitment of IRAK (Yang et al. 1999). Toll is a type I transmembrane receptor with an extracellular domain containing leucine-rich repeat (LRR) and a cytoplasmic domain similar to that of mammalian interleukin-1 receptor (IL-1R) (Aliprantis et al. 1999, 2000; Nomura et al. 2000). This domain communicates with an adapting protein MyD88 (myeloid differentiation factor 88) that interacts with TLR4 starting cell signalizing as follows: IL-1R associated kinase (IRAK-1), TNF-α receptor (TNFR) associated factor 6 (Traf-6) and NF-κB inducing kinase (NIK). The kinases IRAK-1 and NIK phosphorylase inactive an complex in the cytoplasm named IkBs (IKKα and IKKβ), causing its degradation through ubiquination-proteasome. This reaction releases the NF-κB/REL (REL – reticuloendotheliosis oncogene, signal responsive transcriptional factor) dimer, and NF-κB migrates to the nucleus resulting in activation of immunomodulatory genes. This, in turn, causes the synthesis of cytokines, adhesion molecules (ICAMs), stress proteins (HSPs), and activation of inhibitors of apoptosis genes (IAPs). TLR2 transmits a proapoptotic signal via death receptors and FADD protein (fan-associated death domain). The FADD contacts a homologous area in the caspase-8 prodomain, that cleaves effective caspases 3 and 7 and activates the apoptosis program (Aliprantis et al. 1999, 2000). Finally there is binding of TLR2 to the cell apoptosis machinery (Aliprantis et al. 2000).

6. Vaccines against typhoid fever

None of the currently available typhoid vaccines is ideal. The first line of parenteral whole cell vaccines has been associated with fever and systemic reactions. Although licensed, it is considered unsuitable for mass immunization and no longer in use (Garmony et al. 2002). Consequently two vaccines have been developed. The first is a live oral vaccine based on Ty21a (an attenuated strain of *S.* Typhi) that is well-tolerated and the other is Vi-based parenteral subunit vaccine (based on the purified capsular polysaccharide *S.* Typhi Vi antigen). Both these vaccines are well tolerated but are only moderately protective.

One of the obstacles in developing of the vaccines has been the variety of typhoidal and non-typhoidal salmonelloses caused by different serovars and strains. Efforts are being made to develop improved attenuated *S.* Typhi vaccine strains. The most promising strains include CVD 908-*htrA*, CVD 906, CVD 909, Ty800, and M01ZH09 (Levine et al. 2001, 2004; Tacket et al. 2000; Hohmann et al. 1996; Jain 2009). These attenuated strains of *S.* Typhi could also be candidates to serve as carrier of foreign antigens from other pathogens.

The outer membrane proteins (OMPs) of *Salmonella* have been exploited as the possible candidate for conferring protection against typhoid. Our laboratory has been able to isolate, purify and characterize a Novel non-porin OMP from the outer membranes of *S.* Typhi with an apparent molecular mass of 49 kDa that is highly immunogenic, evokes humoral and cell-mediated immune responses, and confers 100% protection to immunized rats against challenge with very high doses of *S.* Typhimurium. This OMP provides a promising target for the development of candidate vaccine against typhoid (Hamid and Jain 2008, 2010).

7. Conclusion

Salmonella has been around for long and large number of studies has been carried out in an effort to combat its infections. *Salmonella* has evolved a remarkable mechanism for adaptation to its intracellular environment. Studies on bacterial pathogenesis have allowed insight into the complex process of highly adapted interactions between pathogens and their hosts at the cellular and molecular levels. Immunologists and microbiologists have long appreciated murine infection with *Salmonella* as a model that allows a natural route of challenge and detailed study of bacterial virulence factors and innate and adaptive immunity. Further, the elucidation of the molecules and mechanisms underlying bacterial pathogenesis is a major focus of microbiological research which yields practical applications from refined diagnostics to new antibiotics and improved vaccines against Salmonella infections, to use Salmonella strains as live carriers for recombinant vaccines, and to develop novel forms of treatment that target the function of specific virulence factors.

Disclosure

The authors declared no conflict of interest.

Acknowledgements

We gratefully acknowledge Indian Council of Medical Research (ICMR) for giving financial assistance in the form of Senior Research Fellowship (SRF) to Jasmine Kaur.

References

Abrahams GL, Hensel M. Manipulating cellular transport and immune responses: dynamic interactions between intracellular *Salmonella enterica* and its host cells. Cell Microbiol 2006;8:728–37.

Ahmer BM, Tran M, Heffron F. The virulence plasmid of *Salmonella typhimurium* is self-transmissible. J Bacteriol 1999;181:1364–8.

Akinyemi K, Smith S, Oyefolu A, Coker A. Multidrug resistance in Salmonella enteric serovar typhi isolated from patients with typhoid fever complications in Lagos, Nigeria. Public Health 2005;119(4):321–7.

Aliprantis AO, Yang RB, Mark MR, Suggett S, Devaux B, Radolf JD, et al. Cell activation and apoptosis by bacterial lipoproteins through Toll-like receptor-2. Science 1999;285:736–9.

Aliprantis AO, Yang RB, Weiss DS, Godwski P, Zychlinsky A. The apoptotic signaling pathway activated by Toll-like receptor 2. EMBO J 2000;19:3325–36.

Alonso A, Garcia-del Portillo F. Hijacking of eukaryotic functions by intracellular bacterial pathogens. Int Microbiol 2004;7:181–91.

Andrade DR, Andrade DR. Typhoid fever as cellular microbiological model. Rev Inst Med Trop Sao Paulo 2003;45:185–91.

Ansong C, Yoon H, Norbeck AD, Gustin JK, McDermott JE. Proteomics analysis of the causative agent of typhoid fever. J Proteome Res 2008;7:546–57.
Bakowski MA, Braun V, Lam GY, Yeung T, Heo WD, Meyer T, et al. The phospho inositide phosphatase SopB manipulates membrane surface charge and trafficking of the Salmonella-containing vacuole. Cell Host Microbe 2010;7:453–62.
Balows A, Hausler Jr WJ, Hermann KL, Isenberg HD, Shadomy HJ. Manual of clinical microbiology. 5th ed. Washington: American Society for Microbiology; 1991.
Bhunia R, Hutin Y, Ramakrishnan R, Pal N, Sen T, Murhekar M. A typhoid fever outbreak in a slum of South Dumdum Municipality, West Bengal, India, 2007: evidence for foodborne and waterborne transmission. BMC Public Health 2009;9:115.
Braun V, Wong A, Landekic M, Hong WJ, Grinstein S, Brumell JH. Sorting nexin3 (SNX3) is a component of a tubular endosomal network induced by Salmonella and involved in maturation of the Salmonella-containing vacuole. Cell Microbiol 2010;12:1352–67.
Broz P, Newton K, Lamkanfi M, Mariathasan S, Dixit VM, Monack DM. Redundant roles for inflammasome receptors NLRP3 and NLRC4 in host defense against Salmonella. J Exp Med 2010;207:1745–55.
Bulut Y, Faure E, Thomas L, Equils O, Arditi M. Cooperation of Toll-like receptors 2 and 6 for cellular activation by soluble tuberculosis factor and Borrelia burgdorferi outer surface protein A lipoprotein: role of Toll-interacting protein and IL-1 receptor signaling molecules in Toll-like receptor 2 signaling. J Immunol 2001;167:987–94.
Carlson SA, Omary MB, Jones BD. Identification of cytokeratins as accessory mediators of *Salmonella* entry into eukaryotic cells. Life Sci 2002;70:1415–26.
Charles RC, Harris JB, Chase MR, Lebrun LM, Sheikh A, LaRocque RC, et al. Comparative proteomic analysis of the PhoP regulon in *Salmonella enterica* Serovar Typhi versus Typhimurium. PLoS One 2009;4(9):e6994, doi:10.1371/journal.pone.0006994.
Chow JC, Young DW, Golenbock DT, Christ WJ, Gusovsky F. Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. J Biol Chem 1999;274:10689.
Ciacci-Woolwine F, Blomfield IC, Richardson SH, Mizel SB. *Salmonella* flagellin induces tumor necrosis factor α in a human promonocytic cell line. Infect Immun 1998;66:1127–34.
Cirillo DM, Valdivia RH, Monack DM, Falkow S. Macrophage-dependent induction of the *Salmonella* pathogenicity island 2 type III secretion system and its role in intracellular survival. Mol Microbiol 1998;30:175–88.
Corrotte M, Nyguyen AP, Harlay ML, Vitale N, Bader MF, Grant NJ. Ral isoforms are implicated in Fc gamma R-mediated phagocytosis: activation of phospholipase D by RalA. J Immunol 2010;185:2942–50.
Cossart P, Boquet P, Normark S, Rappuoli R. Cellular microbiology emerging. Science 1996;271:315–6.
Crum Cianflone NF. Salmonellosis and the GI tract: more than just peanut butter. Curr Gastroenterol Rep 2008;10(4):424–31.
Crump JA, Mintz ED. Global trends in typhoid and paratyphoid fever. Clin Infect Dis 2010;50:241–6.
de Oliveira FA, Pasqualotto AP, da Silva WP, Tondo EC. Characterization of *Salmonella enteritidis* isolated from human samples. Food Res Int, in press, doi:10.1016/j.foodres.2010.09.040.
Denise MM, Anne M, Stanley F. Persistent bacterial infections: the interface of the pathogen and the host immune system. Nat Rev Microbiol 2004;2:747–65.
Dougan G, John V, Palmer S, Mastroeni P. Immunity to salmonellosis. Immunol Rev 2011;240:196–210.
Faucher SP, Porwollik S, Dozois CM, McClelland M, Daigle F. Transcriptome of Salmonella enterica serovar Typhi within macrophages revealed through the selective capture of transcribed sequences. Proc Natl Acad Sci U S A 2006;103:1906–11.
Faucher SP, Viau C, Gros PP, Daigle F, Le Moual H. The *prpZ* gene cluster encoding eukaryotic-type Ser/Thr protein kinases and phosphatases is repressed by oxidative stress and involved in *Salmonella enterica* serovar Typhi survival in human macrophages. FEMS Microbiol Lett 2008;281:160–6.
Feng Ying C. The epidemiology of typhoid fever in the Dong Thap Province, Mekong Delta region of Vietnam. Am J Trop Med Hyg 2000;62(5):644.
Feuillet V, Medjane S, Mondor I, Demaria O, Pagni PP, Galan JE, et al. Involvement of Toll-like receptor 5 in the recognition of flagellated bacteria. Proc Natl Acad Sci U S A 2006;103:12487–92.
Foley SL, Lynne AM. Food animal-associated Salmonella challenges: pathogenicity and antimicrobial resistance. J Anim Sci 2008;86:E173–87.
Franchi L, Amer A, Body-Malapel M, Kanneganti TD, Ozoren N, Jagirdar R, et al. Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1β in *Salmonella*-infected macrophages. Nat Immunol 2006;7:576–82.
Gala'n JE, Wolf-Watz H. Protein delivery into eukaryotic cells by type III secretion machines. Nature 2006;444:567–73.
García-del Portillo F, Zwick MB, Leung KY, Finlay BB. *Salmonella* induces the formation of filamentous structures containing lysosomal membrane glycoproteins in epithelial cells. Proc Natl Acad Sci U S A 1993;90:10544–8.
Garmendia J, Beuzon CR, Ruiz-Albert J, Holden DW. The roles of SsrA-SsrB and OmpR-Envz in the regulation of genes encoding the *Salmonella typhimurium* SPI-2 type III secretion system. Microbiology 2003;149:2385–96.
Garmony HS, Brown KB, Titball RW. Salmonella vaccines for use in humans: present and future perspectives. FEMS Microbiol Rev 2002;26:339–53.
Gewirtz AT, Reed KA, Merlin D, Hobert M, Neish AS, James L. Modeling microbial-epithelial interactions in the intestine. Methods Microbiol 2002;31: 377–96.
Goosney DL, Knoechel DG, Finlay BB. Enteropathogenic *E. coli*, *Salmonella*, and *Shigella:* masters of host cell cytoskeletal exploitation. Emerg Infect Dis 1999;5:216–23.
Griffin AJ, McSorley SJ. Development of protective immunity to Salmonella, a mucosal pathogen with a systemic agenda. Mucosal Immunol 2011 Jul;4(4):371–82, doi:10.1038/mi.2011.2.
Groisman EA, Ochman H. How *Salmonella* became a pathogen. Trends Microbiol 1997;5:343–9.
Groisman EA. The pleiotropic two-component regulatory system PhoP-PhoQ. J Bacteriol 2001;183:1835–42.
Guiney DG, Fang FC, Krause M, Libby S, Buchmeier NA, Fierer J. Biology and clinical significance of virulence plasmids in *Salmonella* serovars. Clin Infect Dis 1995;21(2):S146–51.
Hamid N, Jain SK. Characterization of an outer membrane protein of Salmonella enterica serovar typhimurium that confers protection against typhoid. Clin Vaccine Immunol 2008(9):1461–71.
Hamid N, Jain SK. Immunogenic evaluation of a recombinant 49-kilodalton outer membrane protein of Salmonella typhi as a candidate for a subunit vaccine against typhoid. J Infect Dis Immun 2010;2(2):30–40.
Hansen-Wester I, Hensel M. *Salmonella* pathogenicity islands encoding type III secretion systems. Microbes Infect 2001;3:549–59.
Harris JB, Bernal AB, Rollins SM, Alam A, LaRocque RC, Bikowski M, et al. Identification of *in vivo*-induced bacterial protein antigens during human infection with *Salmonella enterica* serovar Typhi. Infect Immun 2006;74(9):5161–8.
Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, et al. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 2001;410:1099–103.
Helena MB, Seth-Smith. SPI-7: *Salmonella*'s Vi-encoding pathogenicity island. J Infect Dev Countries 2008;2(4):267–71.
Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, et al. A Toll-like receptor recognizes bacterial DNA. Nature 2000;408:740–5.
Hensel M. *Salmonella* pathogenicity island 2. Mol Microbiol 2000;36:1015–23.
Hess J, Ladel C, Miko D, Kaufmann SH. *Salmonella typhimurium aroA*-infection in gene targeted immunodeficient mice: major role of CD4+ TCR-alpha beta cells and IFN gamma in bacterial clearance independent of intracellular location. J Immunol 1996;156:3321–6.
Hoffmann JA, Kafatos FC, Janeway Jr CA, Ezekowitz RA. Phylogenetic perspectives in innate immunity. Science 1999;284:1313–8.
Hohmann EL, Oletta CA, Killeen KP, Miller SI. phoP/phoQ-deleted *Salmonella typhi* (Ty800) is a safe and immunogenic single dose typhoid fever vaccine in volunteers. J Infect Dis 1996;173:1408–14.
Holden DW. Trafficking of the *Salmonella* vacuole in macrophages. Traffic 2002;3:161–9.
Hormaeche CE, Mastroeni P, Arena A, Uddin J, Joysey HS. T-cells do not mediate the initial suppression of a Salmonella infection in the RES. Immunology 1990;70:247–50.
Hornick RB, Greisman SE, Woodward TE, DuPont HL, Dawkins AT, Snyder M. Typhoid fever: pathogenesis and immunologic control. N Engl J Med 1970;283:686–91.
House D, Bishop A, Parry CM, Dougan G, Wain J. Typhoid fever: pathogenesis and disease. Curr Opin infect Dis 2001;14:573–8.
Hueck CJ. Type III protein secretion systems in bacterial pathogens of animals and plants. Microbiol Mol Biol Rev 1998;62:379–433.
Jain SK. M-01ZH09, an oral live attenuated Salmonella enterica serovar Typhi vaccine for the prevention of typhoid fever. Curr Opin Mol Ther 2009;11:565–71.
Janeway Jr CA. Approaching the asymptote evolution and revolution in immunology. Cold Spring Harbor Symp Quant Biol 1989;54(1):1–13.
Johanns TM, Ertelt JM, Rowe JH, Way SS. Regulatory T cell suppressive potency dictates the balance between bacterial proliferation and clearance during persistent Salmonella infection. PLoS Pathog 2010;6:e1001043.
Jonathan J, Deepak C, Michael H. Cellular aspects of immunity to intracellular Salmonella enterica. Immunol Rev 2011;240:185–95.
Jones BD, Paterson HF, Hall A, Falkow S. *Salmonella typhimurium* induces membrane ruffling by a growth factor receptor-independent mechanism. Proc Natl Acad Sci U S A 1993;90:10390–4.
Khan AQ, Zhao L, Hirose K, Miyake M, Li T, Hashimoto Y, et al. *Salmonella typhi rpoS* mutant is less cytotoxic than the parent strain but survives inside resting THP-1 macrophages. FEMS Microbiol Lett 1998;161:201–8.
Knodler LA, Steele-Mortimer O. Taking possession: biogenesis of the *Salmonella*-containing vacuole. Traffic 2003;4:587–99.
Kuhle V, Hensel M. Cellular microbiology of intracellular *Salmonella enterica*: functions of the type III secretion system encoded by *Salmonella* pathogenicity Island 2. Cell Mol Life Sci 2004;61:2812–26.
Lapaque N, Hutchinson JL, Jones DC, Meresse S, Holden DW, Trowsdale J, et al. *Salmonella* regulates polyubiquitination and surface expression of MHC class II antigens. Proc Natl Acad Sci U S A 2009;106:14052–7.
Lee AH, Zareei MP, Daefler S. Identification of a NIPSNAP homologue as host cell target for *Salmonella* virulence protein SpiC. Cell Microbiol 2002;4:739–50.
Lee AK, Detweiler CS, Falkow S. OmpR regulates the two-component system SsrA-Ssrb in *Salmonella* Pathogenicity Island 2. J Bacteriol 2000;182:771–81.
Levine MM, Galen JE, Tacket CO, Barry EM, Pasetti MF, Sztein MB. Attenuated strains of *Salmonella enterica* serovar Typhi as live oral vaccines against typhoid fever. In: Levine MM, Kaper JB, Rappuoli R, Liu M, Good M, editors. New generation vaccines. 3rd ed. New York: Marcel Dekker; 2004. p. 479–86.
Levine MM, Tacket CO, Sztein MB. Host-*Salmonella* interaction: human trials. Microbes Infect 2001;3:1271–9.

Libby SJ, Brehm MA, Greiner DL, Shultz LD, McClelland M, Smith KD, et al. Humanized nonobese diabetic-scid IL2rgammanull mice are susceptible to lethal Salmonella Typhi infection. Proc Natl Acad Sci U S A 2010;107:15589–94.
Lostroh CP, Lee CA. The *Salmonella* pathogenicity island-1 type III secretion system. Microbes Infect 2001;3:1281–91.
Lu S, Manges AR, Xu Y, Fang FC, Riley LW. Analysis of virulence of clinical isolates of *Salmonella enteritidis* in vivo and in vitro. Infect Immun 1999;67:5651–7.
Mahieu T, Libert C. Should we inhibit type I interferons in sepsis. Infect Immun 2007;75:22–9.
Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'brien SJ, et al. The global burden of non typhoidal Salmonella gastroenteritis. Clin Infect Dis 2010;50:882–9.
Malik-Kale P, Jolly CE, Lathrop S, Winfree S, Luterbach C, Steele-Mortimer O. Salmonella – at home in the host cell. Front Microbiol 2011;2(June (125))., doi:10.3389/fmicb.2011.00125.
Marlovits TC, Stebbins CE. Type III secretion systems shape up as they ship out. Curr Opin Microbiol 2010;13:47–52.
Marlovits TC, Kubori T, Sukhan A, Thomas DR, Gala'n JE, Unger VM. Structural insights into the assembly of the type III secretion needle complex. Science 2004;306:1040–2.
Mastroeni P, Simmons C, Fowler R, Hormaeche CE, Dougan G. Igh-6$^{-/-}$ (B-cell deficient) mice fail to mount solid acquired resistance to oral challenge with virulent *Salmonella enterica* serovar *typhimurium* and show impaired Th1 T-cell responses to Salmonella antigens. Infect Immun 2000;68:46–53.
Mastroeni P, Villarreal-Ramos B, Hormaeche CE. Adoptive transfer of immunity to oral challenge with virulent salmonellae in innately susceptible BALB/C mice requires both immune serum and T cells. Infect Immun 1993;61:3981–4.
McGhie EJ, Hayward RD, Koronakis V. Cooperation between actin-binding proteins of invasive *Salmonella:* SipA potentiates SipC nucleation and bundling of actin. EMBO J 2001;20:2131–9.
McGhie EJ, Brawn LC, Hume PJ, Humphreys D, Koronakis V. *Salmonella* takes control: effector-driven manipulation of the host. Curr Opin Microbiol 2009;12:117–24.
McSorley SJ, Jenkins MK. Antibody is required for protection against virulent but not attenuated *Salmonella enterica* serovar *typhimurium*. Infect Immun 2000;68:3344–8.
Medzhitov R, Preston-Hurlburt P, Janeway Jr CA. A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. Nature 1997;388:394–7.
Miao EA, Alpuche-Aranda CM, Dors M, Clark AE, Bader MW, Miller SI, et al. Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1β via Ipaf. Nat Immunol 2006;7:569–75.
Modlin RL, Brightbill HD, Godowski PJ. The Toll of innate immunity on microbial pathogens. N Engl J Med 1999;340:1834–5.
Morgan E. Salmonella pathogenicity islands. In: Rhen M, Maskell D, Mastroeni P, Threlfall J, editors. Salmonella: molecular biology and pathogenesis. United Kingdom: Horizon Scientific Press; 2007. p. 67–88.
Morrison DC, Ryan JL. Endotoxins and disease mechanisms. Ann Rev Med 1987;38:417–32.
Murli S, Watson RO, Galán JE. Role of tyrosine kinases and the tyrosine phosphatase SptP in the interaction of *Salmonella* with host cells. Cell Microbiol 2001;3:795–810.
Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Manual of clinical microbiology. 6th ed. Washington, DC: ASM Press; 1995. p. 450–2.
Mweu E, English M. Typhoid fever in children in Africa. Trop Med Int Health 2008;13(4):532–40.
Myeni SK, Zhou D. The C terminus of SipC binds and bundles F-actin to promote Salmonella invasion. J Biol Chem 2010;285:13357–63.
Nichols CD, Casanova JE. Salmonella-directed recruitment of new membrane to invasion foci via the host exocyst complex. Curr Biol 2010;20:1316–20.
Niemann GS, Brown RN, Gustin JK, Stufkens A, Shaikh-Kidwai AS, Li J, et al. Discovery of novel secreted virulence factors from *Salmonella enterica* serovar Typhimurium by proteomic analysis of culture supernatants. Infect Immun 2011:33–43.
Nomura F, Akashi S, Sakao Y, Sato S, Kawai T, Matsumoto M, et al. Cutting edge: endotoxin tolerance in mouse peritoneal macrophages correlates with down-regulation of surface Toll-like receptor 4 expression. J Immunol 2000;164:3476–9.
Ohl ME, Miller SI. Salmonella: a model for bacterial pathogenesis. Annu Rev Med 2001;52:259–74.
Pallen MJ, Chaudhuri RR, Henderson IR. Genomic analysis of secretion systems. Curr Opin Microbiol 2003;6:519–27.
Parkhill J, Dougan G, James KD, Thomson NR, Pickard D, Wain J, et al. Complete genome sequence of a multiple drug resistant *Salmonella enterica* serovar Typhi CT18. Nature 2001;413:848–52.
Patel JC, Hueffer K, Lam TT, Galan JE. Diversification of a Salmonella virulence protein function by ubiquitin-dependent differential localization. Cell 2009;137:283–94.
Pegues DA, Ohl ME, Miller SI. *Salmonella* species, including *Salmonella typhi*. In: Mandell GL, Bennet GE, Dolin R, editors. Principles and practice of infectious diseases. Philadelphia: Churchill Livingstone; 2005. p. 2636–54.
Poltorak A, He X, Smirnova I, Liu MY, Huffel CV, Du X, et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. Science 1998;282:2085–8.
Prost LR, Miller SI. The *Salmonellae* PhoQ sensor: mechanisms of detection of phagosome signals. Cell Microbiol 2008;10:576–82.
Pui CF, Wong WC, Chai LC, Tunung R, Jeyaletchumi P, Noor Hidayah MS, et al. Salmonella: a foodborne pathogen. Int Food Res J 2011;18:465–73.
Qureshi ST, Lariviere L, Leveque G, Clermont S, Moore KJ, Gros P, et al. Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (*Tlr4*). J Exp Med 1999;189:615–25.
Raffatellu M, Wilson RP, Winter SE, Bäumler AJ. Clinical pathogenesis of typhoid fever. J Infect Dev Countries 2008;2(4):260–6.
Raucher D, Stauffer T, Chen W, Shen K, Guo S, York JD, et al. Phosphatidylinositol 4,5-bisphosphate functions as a second messenger that regulates cytoskeleton-plasma membrane adhesion. Cell 2000;100:221–8.
Rotger R, Casadesus J. The virulence plasmids of *Salmonella*. Int Microbiol 1999;2:177–84.
Santos RL, Tsolis RM, Bäumler AJ, Adams LG. Pathogenesis of *Salmonella* induced enteritis. Braz J Med Biol Res 2003;36:3–12.
Santos Andrade JR, Andrade. TNF-α production and apoptosis in hepatocytes after *listeria* monocytogenes and *Salmonella* Typhimurium invasion. Rev Inst Med Trop Sao Paulo 2011;53(2):107–12.
Schmidt H, Hensel M. Pathogenicity islands in bacterial pathogenesis. Clin Microbiol Rev 2004;17:14–56.
Schwandner R, Dziarski R, Wesche H, Rothe M, Kirschning CJ. Peptidoglycan and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. J Biol Chem 1999;274:17406–9.
Shahane V, Muley V, Kagal A, Bharadwaj R. Non-typhoid *Salmonellosis*: emerging infection in Pune. Indian J Med Microbiol 2007;25:173–4.
Sheikh A, Charles RC, Rollins SM, Harris JB, Bhuiyan MS, et al. Analysis of Salmonella enterica serotype Paratyphi A gene expression in the blood of bacteremic patients in Bangladesh. PLoS Negl Trop Dis 2010;4(12):e908, doi:10.1371/journal.pntd.0000908.
Sheppard M, Webb C, Heath F, Mallows V, Emilianus R, Maskell D, et al. Dynamics of bacterial growth and distribution within the liver during *Salmonella* infection. Cell Microbiol 2003;5:593–600.
Shi L, Ansong C, Smallwood H, Rommereim L, McDermott JE, et al. Proteome of *Salmonella Enterica* serotype Typhimurium grown in a low Mg2+/pH medium. J Proteomics Bioinform 2009;2:388–97, doi:10.4172/jpb.1000099.
Sinha K, Mastroeni P, Harrison J, de Hormaeche RD, Hormaeche CE. *Salmonella typhimurium aroA*, *htrA*, and *aroD htrA* mutants cause progressive infections in athymic (nu/nu) BALB/c mice. Infect Immunol 1997;65:1566–9.
Srinivasan A, Nanton M, Griffin A, McSorley SJ. Culling of activated CD4 T cells during typhoid is driven by *Salmonella* virulence genes. J Immunol 2009;182:7838–45.
Steiner TS. How flagellin and toll-like receptor 5 contribute to enteric infection. Infect Immun 2007;75:545–52.
Sundquist M, Rydstrom A, Wick MJ. Immunity to *Salmonella* from a dendritic point of view. Cell Microbiol 2004;6:1–11.
Sztein M. Cell-mediated immunity and antibody responses elicited by attenuated *Salmonella enterica* serovar typhi strains used as live oral vaccines in humans. Clin Infect Dis 2007;45:S15–9.
Tacket CO, Sztein MB, Wasserman SS, Losonsky G, Kotloff KL, Wyant TL, et al. Phase 2 clinical trial of attenuated *Salmonella enterica* serovar Typhi oral live vector vaccine CVD908-*htrA* in US volunteers. Infect Immun 2000;68:1196–201.
Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, Ogawa T, et al. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram positive bacterial cell wall components. Immunity 1999;11:443–51.
Takeuchi O, Kawai T, Mühlradt PF, Morr M, Radolf JD, Zychlinsky A, et al. Discrimination of bacterial lipoproteins by Toll-like receptor 6. Int Immunol 2001;13:933–40.
Thomas M, Holden DW. Ubiquitination – a bacterial 1 effector's ticket to ride. Cell Host Microbe 2009;5:309–11.
Thompson LJ, Dunstan SJ, Dolecek C, Perkins T, House D, Dougan G, et al. Transcriptional response in the peripheral blood of patients infected with Salmonella enterica serovar Typhi. Proc Natl Acad Sci U S A 2009;106:22433–8.
Thomson N, Baker S, Pickard D, Fookes M, Anjum M, Hamlin N, et al. The role of prophage-like elements in the diversity of *Salmonella enteric* serovars. J Mol Biol 2004;339:279–300.
Tierrez A, Garcia-del Portillo F. New concepts in *Salmonella* virulence: the importance of reducing the intracellular growth rate in the host. Cell Microbiol 2005;7:901–9.
Tobias PS, Soldau K, Ulevitch RJ. Isolation of a lipopolysaccharide binding acute phase reactant from rabbit serum. J Exp Med 1986;164:777–93.
Totemeyer S, Kaiser P, Maskell DJ, Bryant CE. Sublethal infection of C57BL/6 mice with *Salmonella enterica* Serovar Typhimurium leads to an increase in levels of Toll-like receptor 1 (TLR1), TLR2, and TLR9 mRNA as well as a decrease in levels of TLR6 mRNA in infected organs. Infect Immun 2005;73:1873–8.
Uchiya K, Barbieri MA, Funato K, Shah AH, Stahl PD, Groisman EA. A *Salmonella* virulence protein that inhibits cellular trafficking. EMBO J 1999;18:3924–33.
Ulevitch RJ, Tobias PS. Recognition of gram-negative bacteria and endotoxin by the innate immune system. Curr Opin Immunol 1999;11:19–22.
Unsworth KE, Holden DW. Identification and analysis of bacterial virulence genes in vivo. Philos Trans R Soc Lond B Biol Sci 2000;355:613–22.
Vernikos GS, Parkhill J. Interpolated variable order motifs for identification of horizontally acquired DNA: revisiting the *Salmonella* pathogenicity islands. Bioinformatics 2006;22:2196–203.
Vijay-Kumar M, Wu H, Jones R, Grant G, Babbin B, King TP, et al. Flagellin suppresses epithelial apoptosis and limits disease during enteric infection. Am J Pathol 2006;169:1686–700.
Villa L, Carattoli A. Integrons and transposons on the *Salmonella enterica* serovar Typhimurium virulence plasmid. Antimicrob Agents Chemother 2005;49:1194–7.

Wahid R, Salerno-Goncalves R, Tacket CO, Levine MM, Sztein MB. Cell-mediated immune responses in humans after immunization with one or two doses of oral live attenuated typhoid vaccine CVD 909. Vaccine 2007;25:1416–25.
Wallis TS, Galyov EE. Molecular basis of *Salmonella* induced enteritis. Mol Microbiol 2000;36:997–1005.
Waterman SR, Holden DW. Functions and effectors of the *Salmonella* pathogenicity island 2 type III secretion system. Cell Microbiol 2003;5:501–11.
Weiss DS, Raupach B, Takeda K, Akira S, Zychlinsky A. Toll-like receptors are temporally involved in host defense. J Immunol 2004;172:4463–9.
Wyant TL, Tanner MK, Sztein MB. *Salmonella typhi* flagella are potent inducers of proinflammatory cytokine. Infect Immun 1999;67:3619–24.
Yang B, Qu D, Zhang X, Shen J, Cui S, Shi Y, et al. Prevalence and characterization of *Salmonella* serovars in retail meats of marketplace in Shaanxi, China. Int J Food Microbiol 2010;141(1–2):63–72.
Yang RB, Mark MR, Gray A, Huang A, Xie MH, Zhnag M, et al. Toll-like receptor-2 mediated lipoplysaccharide-induced cellular signaling. Nature 1998;395:284–8.
Yang RB, Mark MR, Gurney AL, Godowski PJ. Signaling events induced by lipopolysaccharide-activated toll-like receptor 2. J Immunol 1999;163:639–43.
Yoon H, Ansong C, McDermott JE, Gritsenko M, Smith RD, Heffron F, et al. Systems analysis of multiple regulator perturbations allows discovery of virulence factors in Salmonella. BMC Syst Biol 2011;5:100.
Yoon H, McDermott JE, Porwollik S, McClelland M, Heffron F. Coordinated regulation of virulence during systemic infection of Salmonella enterica serovar Typhimurium. PLoS Pathog 2009;5:e1000306.
Yrlid U, Svensson M, Johansson C, Wick MJ. *Salmonella* infection of bone marrow derived macrophages and dendritic cells: influence on antigen presentation and initiating an immune response. FEMS Immunol Med Microbiol 2000;27:313–20.
Yu H, Wang J, Ye J, Tang P, Chu C, Hu S, et al. Complete nucleotide sequence of pscv50, the virulence plasmid of *Salmonella enterica* serovar Choleraesuis SC-B67. Plasmid 2006;55:145–51.
Zhang S, Kingsley RA, Santos RL, Andrews-Polymenis H, Raffatellu M, Figueiredo J, et al. Molecular pathogenesis of *Salmonella enterica* serotype Typhimurium-induced diarrhea. Infect Immun 2003;71:1–12.
Zhang XL, Jeza VT, Pan Q. *Salmonella Typhi*: from a human pathogen to a vaccine vector. Cell Mol Immunol 2008;5(2):9.