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Typhoid fever remains a major public health problem in many regions of the world. The disease is caused by *Salmonella typhi*. There are around 16.6 million cases being reported every year and 600,000 deaths annually (World Health Organization, 2000) with most deaths occurring in South East Asia, Africa and South America. The disease is prevalent in developing countries due to poor hygiene and inadequate sanitation. The countries that continue to have high disease burdens include Indonesia, Vietnam, Pakistan, and Thailand. The severe disease manifestations in these regions add to the high cost of treatment associated with hospitalization. The emergence of multiple-drug-resistant strains of *S. typhi* is thought to have contributed to the high incidence of disease (White *et al.*, 1996). Typhoid is rare in industrialized nations, though travellers to endemic countries may occasionally acquire the disease.

**The Pathogen**

*Salmonella typhi* is a gram negative, rod-shaped, non-spore forming, intracellular bacterium of about 2-4µ by 0.5µ in size and belongs to the family Enterobacteriaceae. *S. typhi* is taxonomically designated as *Salmonella enterica*, subspecies *enterica*, serovar Typhi. It is a facultative, anaerobic and actively motile bacterium with numerous long peritrichous flagella. Almost all the clinical isolates of *S. typhi* are encapsulated on primary isolation. The important antigens of *S. typhi* include:

**Somatic antigen:** The somatic or the O-antigen forms the side chain component of the lipopolysaccharide present in the bacterial cell envelope. The genus *Salmonella* is classified into a number of serotypes based on the presence of characteristic O antigens on the bacterial surface. *S. typhi* possesses O9 and O12 antigenic determinants on its surface (Parker, 1984).

**Flagellar antigen:** The flagellar antigen (flagellin) consists of a single protein of about 60kDa. The filament of each flagellum generally consists of around 20,000 subunits of flagellin. Usually, *Salmonella* serovars have two genes *fliC* and *fljB* that are alternately expressed in a stochastic fashion and code for flagellin of distinct antigenic specificities. In *S. typhi* only *fliC* is expressed and codes for one type of flagellin, designated H:d.

**Capsular antigen:** The capsular antigen of *S. typhi*, commonly referred to as Vi (for Virulence), is a linear polymer of N-acetyl galacturonic acid with variable O-acetylation.
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at C3 positions. The molecular weight of Vi ranges from $5 \times 10^6$ to $20 \times 10^6$ daltons. It has been shown to have two antigenic determinants, one constituted by the O-acetyl galacturonic acid moiety and the other by N-acetyl and carboxyl groups together. Felix and Pitt discovered Vi antigen and described many of the relevant bacteriological, clinical and epidemiological features of the polysaccharide (Felix and Pitt, 1934a). Using a mouse model of parenteral infection, they observed that this antigen appeared to protect the pathogen from the action of antibodies against the O-antigen (Felix and Pitt, 1934b).

Pathogenesis of typhoid

*S. typhi* enters the body through the oral route upon intake of contaminated food or water. It passes through the stomach and invades the gut epithelium, possibly in the distal ileum. From there, the bacteria invade the lymphoid tissues and are phagocytosed by the underlying macrophages and disseminated to distal organs including liver and spleen. The bacteria multiply in the phagocytes, emerge and continue to multiply in blood. During the first week, the patient suffers from headache, fever, abdominal discomfort and general lethargy. Bacteremia occurs at the same time as symptoms appear and there is relatively low mortality during the bacteremic period. Low-level bacteremia is a characteristic feature of typhoid fever. During the second week, the condition of the patient worsens. The organisms invade many tissues including the intestinal mucosa. *S. typhi* thrive and multiply in the bile, organisms from the gall bladder re-infect the intestinal mucosa and Peyer’s patches (Figure 1). Characteristic “rose spots” often appear on the trunk and the abdomen for few days. Abdominal distension and tenderness and enlargement of spleen are common complaints, but diarrhea is usually absent. The symptoms of untreated typhoid fever begin to resolve by the fourth week of infection, although relapse occurs in 10% of individuals apparently recovering from the infection (Hornick et al., 1970). Intestinal complications can occur in the later stages of untreated infection: these include bleeding and perforation that are the result of the inflammatory response in the Peyer’s patches, followed by necrosis and ulceration of the intestinal epithelium (Bitar et al., 1985, Butler et al., 1985). Certain individuals infected with *S. enterica* serovar Typhi become chronic carriers. Others relapse to typhoid disease with the same *S. typhi* strain several months after initial infection (1-5% of antibiotic treated
**Figure 1. S.typhi pathogenesis.** S.typhi infects the body via Peyer’s patches of the small intestine. The bacteria migrate to mesenteric lymph nodes and arrive via blood in the liver and spleen. After multiplication in the above sites, the bacteria migrate back into the Peyer’s patches for the second round of infection and consequently the clinical symptoms are seen. Inflammation in the small intestine leads to ulcers and necrosis. Adapted from Everest, *et al.*, 2001.

individuals) that suggests the presence of a persistent reservoir of bacteria in these individuals. Short-term persistence (lasting several months) by *S.typhi* may involve colonization of immature immune cells in the bone marrow, whereas long-term carriage is associated with infection of the gall bladder from where bacteria can be directly shed into the intestine via the bile duct. The long-term or chronic carriers shed bacteria in their stools for a varied period of time ranging from 1 year to lifetime without any apparent
signs of disease (Vogelsang et al., 1948). Typhoid carriers are of special concern from a public health viewpoint because they are the reservoirs for the spread of infection and disease. *S*. *typhi* is carried for years even in the presence of an immune response; chronic carriers of *S*. *typhi* have high levels of circulating serum antibodies to Vi and flagellar antigens, indicating that the organism has established a privileged niche that is sequestered from the host's immune defenses (House et al., 2001).

**Chemotherapy and vaccines**

Appropriate antibiotic therapy normally resolves *S*. *typhi* infection within 3-5 days and prevents the occurrence of most complications. Chloramphenicol is the antibiotic of choice, but some *S*. *typhi* strains are resistant to it (Gilman et al., 1975; Butler et al., 1973). Owing to the prevalence of multidrug resistant strains, this drug has been widely replaced by ampicillin and co-trimazole. More recently, increasing resistance to the latter antibiotics has prompted the use of quinolone derivatives and third generation cephalosporins.

Vaccination of high-risk population is considered the most promising strategy for the control of typhoid fever. The two currently licensed typhoid vaccines that confer protection without significant side effects are: parenteral vaccine based on purified Vi polysaccharide of *S*. *typhi*, and Ty21a, a live attenuated vaccine that is administered orally. Following administration according to their respective schedules, these vaccines provide protective immunity but for shorter time periods (Typhoid vaccines: WHO, 2000). The vaccines afford some protection to people who must enter areas where typhoid fever is common. Several new vaccines that offer improvements over Ty21a and Vi have been progressing through clinical trials. These include (1) several engineered strains of *S*. *typhi* (Tacket, et al., 2000; Hohmann et al., 1996; Hindle et al., 2002) that appear promising as candidate single-dose vaccine because of their increased immunogenicity over Ty21, and; (2) a parenteral Vi-conjugate vaccine that stimulates higher titers of Vi antibodies than unconjugated Vi polysaccharide and that elicits immunogenic memory (Lin et al., 2001). The best means of protection against typhoid fever is good sanitation.
**Salmonella-host cell interactions**

The entry of *S. typhi* into intestinal epithelial cells is a complex process and involves an intimate interaction of bacteria with host cells. Though typhoid has been a global health problem for a long time, the pathogenesis of the disease still remains poorly understood. Relatively little is known about the bacterium-host cell interactions that occur at different stages of *S. typhi* pathogenesis. This is mainly due the absence of a suitable animal model. Since *S. typhi* fails to establish infection in laboratory animals, most of the research reported on the pathogenesis of typhoid fever is based on *in vitro* studies with human and murine cell lines and infection of mice with *S. typhimurium*.

1. **Salmonella typhimurium as a murine model to study typhoid**

Studies aimed at understanding *S. typhi*-host cell interaction have widely used *S. typhimurium*, as a model since this organism causes disease in mice that resembles human typhoid (Jones and Falkow, 1996). Mice are either Salmonella-sensitive *Ity<sup>5</sup>*, or they carry the dominant *Ity<sup>7</sup>* allele. The *Ity<sup>5</sup>* mice are defective in the natural-resistance-macrophage-associated protein 1 (Nramp-1) (Forbes and Gros, 2001). The bacteria are able to survive inside macrophages in these mice and hence *Ity<sup>5</sup>* mice succumb to overwhelming sepsis following parenteral injection of very few microorganisms. The major effect of *Ity<sup>7</sup>* is an almost complete inhibition of bacterial growth in mice (Benjamin *et al.*, 1990). One advantage of using a mouse strain while studying *Salmonella* immunity is that both humoral and cell-mediated immunity are required for protection in these mice, while innately resistant mice control low doses of virulent *Salmonella* with a moderate, non-specific immune response (Hormaeche *et al.*, 1990; Mastroeni *et al.*, 1993). Studies with *S. typhimurium* have revealed the presence of a large number of invasion promoting genes, which were subsequently shown to exist in *S. typhi* (Galan *et al.*, 1996). Studies with *S. typhimurium* have given a lot of insight into understanding *S. typhi*-host cell interaction.

2. **Salmonella-intestinal epithelial cell interaction**

2.1. *Adhesion and invasion*
After ingestion, *S. typhi* can survive exposure to the low pH of the stomach and arrive in the intestine where it can penetrate the gut epithelium (Galan and Sansonetti, 1996). In small intestine, the bacteria adhere to and invade the M cells ("M" for microfold or membranous cells) embedded in the Peyer's patches. M cells have increased pinocytic activity and deliver microbes and antigen via transepithelial vesicle transport from the gut lumen to macrophages and lymphocytes residing below the epithelium. Active attachment to epithelial cells promoted by the bacterium may be necessary before invasion can occur and this most likely involves adhesion molecules on the bacterium interacting with receptors on the host cells. Long polar fimbriae (lpf) have been shown to mediate attachment of *Salmonella* to Peyer's patches in mouse (Baumler et al, 1996).

Microscopic studies reveal that salmonellae invade epithelial cells by a morphologically distinct process termed bacterial-mediated endocytosis (Francis et al., 1992). Shortly after bacteria adhere to the apical epithelial surface, profound cytoskeletal rearrangements occur in the host cell, disrupting the normal epithelial brush border and inducing formation of membrane ruffles that reach out and enclose adherent bacteria in large vesicles. Following bacterial internalization, a fraction of *Salmonella*-containing vesicles transcytose to the basolateral membrane, and the apical brush border reconstitutes. Studies with *Salmonella typhimurium* have revealed the presence of a large number of invasion promoting molecules secreted by salmonellae which enable them to invade the non-phagocytic epithelial cells, survive intracellularly in tissue macrophages and also deliver signals that result in apoptosis of the macrophages. These effector molecules belong to a novel protein secretion apparatus called the Type III secretion system (Hueck, 1998; Brummel et al., 2001; Galan and Zhou, 2000; Ohl and Miller, 2001).

### 2.1.1. The Type III secretion system

Several Gram-negative pathogenic bacteria have evolved a complex protein secretion system termed type III to deliver bacterial effector proteins into host cells that then modulate host cellular functions for the pathogen's benefit. These bacterial devices are present in both plant and animal pathogenic bacteria and are evolutionarily related to the flagellar apparatus suggesting an evolutionary relation (Galan and Collmer, 1999, Hueck,
Although type III secretion systems are substantially conserved, the effector molecules they deliver are unique for each bacterial species. The hallmark of type III secretion systems is that none of the secreted proteins has a conserved signal sequence. In Salmonellae, clusters of chromosomal virulence genes termed *Salmonella* pathogenicity islands (SPIs) encode the type III secretion apparatus (Ohl and Miller, 2000). At least five pathogenicity islands (SPI-1 to-5) have been found in a range of serovars of *Salmonella enterica*. In *S. enterica* serovar Typhi further five islands with characteristics of SPIs have been identified in the complete genome (Parkhill et al., 2001). The roles of SPI-1 and SPI-2 have been well characterized.

**SPI-1** is a 40kb chromosome locus at centisome 63 in *S. enterica* and encodes genes necessary for invasion of intestinal epithelial cells and induction of intestinal secretory and inflammatory responses. SPI-1 genes encode components of a type III secretion apparatus, regulatory proteins and secreted effector proteins and their chaperones (Darwin et al., 1999). When administered orally, SPI-1 defective mutants of *S. typhimurium* are attenuated in their ability to cause systemic infections, yet they are fully virulent when bacteria are injected intraperitonially (Jones et al., 1996, Baumler et al., 1997, Penheiter et al., 1997). Thus, SPI-1 appears to be necessary for the initial phase of the disease process.

**SPI-2** is a gene cluster at centisome 31 in the *Salmonella* genome. The encoded genes are essential for intracellular replication, and in the mouse enteric fever model, are necessary for establishment of systemic infection beyond the intestinal epithelium (Cirillo et al., 1998; Hensel et al., 1998).

A characteristic feature of the Type III secretion system is the requirement of an activation signal for the secretion of its effector proteins. This signal is provided upon contact with host tissues (Galan, 1996). Also factors such as osmolarity, oxygen tension, divalent cations like calcium etc. can modulate the secretion of these proteins (Galan et al., 1999). A number of components of the type III secretion assemble into an organelle, appropriately termed the "needle complex" that spans both the inner and the outer membrane of the bacterial envelope (Figure 2). This supramolecular complex (Kubori et al, 1998) is composed of two pairs of inner and outer rings that presumably anchor the structure to the inner and outer membrane of the bacterial envelope. The rings are
connected by a rod-like structure, which together form the base of the needle complex. The protein components of the base and the needle substructures have been recently identified (Kubori et al., 2000). PrgH, PrgK and InvG make up the base substructure. The PrgH and PrgK proteins exhibit signature features of lipoproteins, while InvJ belongs to the secretin family of outer membrane exporter proteins. The major component of the needle substructure is PrgI, a low molecular weight protein is also encoded within the

Figure 2. Schematic representation of Salmonella typhimurium needle complex and its putative components. The location of different components is hypothetical. Other proteins not listed in the scheme may also be present.

Adapted from Galan and Collmer, 1999.

Type III secretion-associated cluster of genes in SPI-1. The length of the needle portion is controlled by the function of InvJ, a protein previously shown to be secreted via the type III secretion system (Collazo et al., 1995). Absence of InvJ results in abnormally long needles and the complete absence of type III secretion.

Upon activation (contact with host cells), this system works like ‘molecular syringes’ in delivering the effector proteins inside the host cell cytoplasm. Some of the translocated proteins have been shown to interact directly with the cellular targets that control cytoskeletal organization (Figure 3). For example, it has been shown that Salmonella outer proteins, SopE (Salmonella outer protein E) and SopE2 act as guanine-nucleotide-exchange factors (GEFs) for small GTPases Cdc42 and Rac. Activation of these Rho GTPases is required for actin polymerization at the site of bacterial entry (Stender et al., 2000). Consistent with this activity, microinjection or transient expression
of SopE in cultured cells leads to marked actin cytoskeleton rearrangements and membrane ruffling that resemble the changes induced by *Salmonella* infection. These cytoskeletal rearrangements can be blocked by co-expression of dominant-negative forms of either Cdc42 or Rac-1 (Hardt *et al*., 1995). These results position SopE as a key stimulator of signaling events leading to bacterial entry.

Sip A (*Salmonella* invasion protein A), another bacterial protein translocated into host cells, binds specifically to actin lowering the critical concentration required for its polymerization and for stabilizing actin filaments. This favors the outward extension of the host cell protrusions required for bacterial entry (Zhou, 1999). *Salmonella* also alters the actin cytoskeleton through manipulation of phosphoinositides. The plasma membrane is intimately associated with the actin cytoskeleton and this interaction depends on phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2) (Raucher *et al*., 2000). 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 associated with *Salmonella* entry. PtdIns(4,5)P2 have also been implicated in vesicle fission during the creation of

![Diagram of the Type III secretion system](image)

**Figure 3. The Type III secretion system.**
Modified from Brummel *et al*., 1999
hagosomes, and accordingly, SigD also is involved in sealing plasma membrane invaginations to form vacuoles (Terebiznik et al., 2002).

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 (Fu and Galan, 1999). SptP is a bifunctional protein, with its GAP domain at the amino terminus, and a protein phosphatase domain at the carboxy terminus (Zhou et al., 2001). A potential target for 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). Sip C is another Type III secretion system protein that is involved in Salmonella entry. This protein binds cytokeratins and expression of dominant negative cytokeratin-18 inhibits Salmonella entry into HEp2 cells (Carlson et al., 2002). A category of Salmonella invasion proteins (SipB, SipC and SipD) form secretion channels which connect bacteria and the host cells and deliver effector proteins that are capable of penetrating the mammalian cell membrane (Collazo et al., 1997). It has been reported that InvJ and SpaO are required for the secretion of several other proteins including InvJ, SpaO, SipB and SipC. Therefore, a mutation in either invJ or spaO results in a non-invasive phenotype (Collazo et al., 1996).

The expression of invasion genes is clearly regulated by multiple factors and may be further complicated in S.typhi by the presence of the polysaccharide capsule, Vi. The expression of Vi is dependent upon the osmolarity of the environment and involves the global regulator Rcs-AB (Arricau et al., 1998). Under conditions of low osmolarity, Vi is downregulated, which enhances the secretion of SPI-1 effector proteins and promotes expression of an invasive phenotype. Indeed, deletion of Vi biosynthetic genes enhances the secretion of SPI-1 products and allows expression of an invasive phenotype of S.typhi at any osmolarity (Zhao et al., 2001).

The type III secretion system has also been associated with the assembly of invasomes, appendage-like structures that appear on the bacterial surface upon contact with host cells (Ginocchio et al., 1994). These structures are transient and presumed to be rapidly shed from the bacterial surface. The assembly and subsequent shedding of these structures is dependent on the Type III secretion system and does not require de novo
protein synthesis. Although the actual components of invasome have not been identified, its dependence on the Type III secretion system strongly suggests that it is made up of targets of this secretion apparatus. The role of invasome in *Salmonella* interaction with host cells is not known.

Following invasion, *Salmonella* can survive and replicate within a membrane-bound compartment, the *Salmonella*-containing vacuole (SCV). While it was previously thought that the fusion of SCV with late endocytic compartments was blocked, it was later demonstrated that fusion is merely delayed in epithelial cells (Brumell et al., 2001). Indeed, several hours after infection, the SCV fuses extensively with the late endosome, causing it to elongate into tubules known as *Salmonella*-inducing filaments (Sifs). The formation of Sifs requires SifA (Stein et al., 1996) a bacterial effector protein translocated into host cells by SPI-2 Type III secretion system. Sifs are unique to *Salmonella*-infection of epithelial cells in vitro, though their role during infection in vivo remains unknown.

### 2.2 Repertoire of intestinal epithelial cell responses

Infection of epithelial cells with *Salmonella* causes increased expression and secretion of a number of cytokines with chemoattractant and proinflammatory functions. Thus, stimulated/infected epithelial cells express and secrete relatively high levels of the chemoattractant cytokines IL-8, GROα, GROβ, GROγ and ENA-78 (Eckmann et al., 1993; McCormick et al., 1993; Jung et al., 1995). These molecules belong to the C-X-C family of chemokines and are characterized by their ability to attract and activate polymorphonuclear leukocytes (PMN), suggesting that an important function of intestinal epithelial cells is to initiate the mucosal influx of PMN. Upon infection epithelial cells also secrete albeit at lower levels, a range of C-C chemoattractants including RANTES (regulated upon activation, normal T-cell expressed and secreted), MCP (macrophage chemoattractant protein)-1, MIP (macrophage inflammatory protein)-1β and MIP-3α (Eckmann et al., 2001, Yang et al., 1997). Epithelial production of chemokines such as IL-8 in response to *Salmonella* infection is probably essential for recruitment of PMNs during the disease process (Gewirtz, 2001). The interaction between *S. typhi* with human gut is less inflammatory than that seen with *S. typhimurium* (House et al., 2001). The lack
of acute inflammation and subsequently reduced recruitment of neutrophils may allow *S. typhi* to invade into deeper tissues of the gut, although there is little experimental evidence for this hypothesis. However, *S. typhi* can induce significant levels of IL-6 secretion from intestinal epithelial cells (Weinstein *et al.*, 1997).

3. *Salmonella* –macrophage interaction

Shortly following invasion of the gut epithelium, invasive *Salmonella* serovars encounter macrophages within the gut-associated lymphoid tissue. To move into deeper tissue, these bacteria must be able to avoid and/or survive the nutrient-poor and microbicidal environment of the professional phagocytes following internalization. Antimicrobial activities of the macrophages include production of reactive oxygen and nitrogen species as well as antimicrobial peptides and hydrolytic enzymes. Among the *Salmonella* genes necessary for survival in the macrophages are constituents of a two-component response regulator termed, PhoP/PhoQ (Miller *et al.*, 1989). This regulatory system consists of a membrane-spanning sensor/kinase protein (PhoQ) that transfers a phosphate to the second cytoplasmic component (PhoP) in response to environmental stimuli. This system regulates 40 genes, the expression of which is required for the survival of *Salmonella* inside the macrophage phagosome. Activation of PhoP regulon leads to widespread modifications in the protein and lipopolysaccharide components of the bacterial inner and outer membrane (Guo *et al.*, 1998). These surface modifications promote *Salmonella* survival in the stressful environment of the phagosome in part conferring resistance to the activity of antimicrobial peptides (Guo *et al.*, 1998).

Inside the macrophages, the maintenance of *Salmonella*-containing vacuole (SCV) is accomplished by the actions of SPI-2 effector protein, SifA, which plays a major role in virulence as loss of SCV results in the death of bacteria in the macrophage cytosol. While SifA keeps *Salmonella typhimurium* within a vacuolar niche, other factors alter the fate of this compartment. For example, The SPI-2 type III secretion system has been shown to block assembly and delivery of the NADPH oxidase, a multiprotein superoxide generating complex that plays a major role in innate host defense to SCV (Gallois *et al.*, 2001). By preventing the delivery of the NADPH oxidase, intracellular *Salmonella* is able to minimize its exposure to toxic free radicals and evade oxidative
damage. The NADPH oxidase does not localize to vacuoles containing wild-type Salmonella but localizes to vacuoles containing bacteria with mutations within SPI-2 (Vazquez-Torres et al., 2000). Studies have shown that intracellular S. typhimurium can also employ the TTSS of SPI2 to alter the cellular localization of iNOS to protect itself from the damaging effects of reactive nitrogen intermediates (RNI) and the combined effects of RNI and reactive oxygen intermediates (Chakravortty et al., 2002).

Salmonella survives in macrophages within a distinct phagosomal compartment that diverges from the normal degradative pathway of the host cell (Rathman et al., 1997). Salmonella inhibition of phagosome-lysosome fusion appears to involve secretion of SpiC, which interferes with normal cellular trafficking and thereby hinders maturation of Salmonella-containing phagosome as well as vesicle compartments devoid of microorganism (Uchiya et al., 1999). The invading bacteria almost immediately reside in vacuoles rich in lgp and Rab7 and Rab9, which are members of the Ras-related group of small GTP-binding proteins. These proteins partially confer specificity to membrane targeting and recognition. Salmonella that survive the initial interaction with the macrophage do not encounter cathepsin or mannose-6-phosphate, and they go on to replicate to a limited extent within cells. The capacity of the bacteria to reside in this privileged niche is dependent on the prompt acidification of the Salmonella-containing vacuole.

The interaction between Salmonella and the macrophages results in an alteration in the expression of a number of host genes including those encoding pro-inflammatory mediators (e.g. iNOS, chemokines, interleukin-1β), receptors or adhesion molecules (TNF αR, CD40, ICAM-1) and anti-inflammatory mediators (TGFβ1 and 2) (Rosenberger et al., 2000). Other up-regulated genes include those involved in cell death or apoptosis (e.g. ICE protease, TNFR1 and Fas). SipB, a SPI-1 effector protein, is responsible for the induction of apoptosis in macrophages infected with Salmonella by directly engaging a key component of the macrophage’s apoptotic machinery, caspase-1. It is this interaction that presumably leads to the activation of Caspase-1, thereby promoting downstream events necessary for apoptosis (Hersh et al., 1998). Caspase-1 is unique among caspases because it also directly cleaves the proinflammatory cytokines IL-1β and IL-18 to produce bioactive cytokines. Salmonella-induced caspase-1 mediated
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cytotoxicity can occur in association with, or independently of, the activation of the
initiator caspase, caspase-2 (Jesenberger et al., 2000). Serovar Typhi survives well within
human macrophages while causing only slight macrophages cell death, almost all of
which is orderly and controlled by apoptosis (Schwan et al., 2000). The ability of this
serovar to translocate asymptomatically across the intestine, trigger uptake into and
survival for long periods (i.e., many days) within human macrophages while causing only
slight macrophage cell death may allow this serovar to cross the ileal epithelium silently,
moving stealthily within macrophage vehicles to deep tissues where acute multiplication
in selected tissues (e.g., liver) ultimately results in typhoid fever.

4. Interaction of Salmonella with dendritic cells
Dendritic cells (DCs) are antigen-presenting cells located in the central lymphoid organs
and Peyer’s patches of the intestine. Salmonella can occupy this cell type in the mouse
model of infection (Hopkins et al., 2000), though in vitro studies suggest that replication
in this cell type is limited. DCs have been suggested to play a role in the transport of
Salmonella across the intestinal epithelium in an M-cell independent transport manner
(Rescigno et al., 2001). Interestingly, survival in dendritic cells does not require virulence
factors that are essential for macrophage colonization, suggesting that different effectors
are involved (Niedergang et al., 2000). As in other cells types, the SCV consists of a
single limiting membrane and is distinct from the multilamellar compartments containing
major histocompatibility complex (MHCII) proteins that are characteristic of dendritic
cells. Unlike other cells, the SCV in dendritic cells does not acquire lysosomal
glycoproteins (Garcia-Del Portillo et al., 2000), a feature unique to this cells type. DCs
also produce cytokines, particularly IL-12 (Sousa et al., 1997), upon antigen encounter
and can thus influence the ensuing adaptive immune response. Studies have shown that
this cell type can internalize and process Salmonella for peptide presentation on MHC-II
as well as MHC-I. Dendritic cells have been shown to function as antigen presenting cells
and can serve as co-stimulators of T-lymphocyte activation (Cella et al., 1997; Steinman
et al., 1991). They can also act as bystander antigen presenting cells by presenting
Salmonella antigens after internalizing neighboring cells that have undergone
Salmonella-induced apoptotic cell death (Yrlid and Wick, 2000). Studies with Salmonella
enterica Typhimurium have shown that bacteria can efficiently kill these phagocytes via SipB dependent mechanism involving Caspase-1 (Van der Velden et al., 2003). Salmonella have thus evolved the ability to selectively kill professional antigen presenting cells to combat, exploit or evade immune defense mechanisms.

5. Immune response against Salmonella

Both innate and adaptive immune responses are generated during infection with Salmonella. The innate immune system plays an essential role in the early phase of infection and in most subclinical infections may be enough to control the progression of disease (Lalmanach et al., 1999; Makela et al., 1997). The importance of macrophages and polymorphonuclear neutrophils in the early responses to Salmonella is well documented (O’Brien et al., 1979; Vassiloyanakopoulos et al., 1998). The stimulation of proinflammatory cytokine production (e.g., TNF-α, IL-1, IL-6, IL-8, IL-12 etc.) by agonists of Toll receptors (e.g., LPS, lipoprotein, flagellin etc.) and by specific bacterial effectors delivered by Salmonella through its type III secretion system is also likely to be an important component of this phase of defense response. The polysaccharide portion plays an indispensable role in Salmonella lipopolysaccharide-induced activation of NF-κB through Toll-like receptor-4 (Muroi et al., 2002). Bacterial flagellin can interact with Toll like receptor-5 and evoke IL-8 responses in host cells (Gewirtz, 2001). The stimulation of GTPases by Sop E, an effector protein secreted by Salmonella, leads to the activation of down stream mitogen-activated protein (MAP) kinases, Jnk and p38 (Chen et al., 1996). The activation of the mitogen-activated protein kinase pathways leads to the stimulation of the transcription factors NF-κB and AP-1 (Hobbie, et al., 1997) and to the production of proinflammatory cytokines (Eckmann et al., 1997; Jung et al., 1995).

Although the innate immune system is the primary line of defense against Salmonella infections, it is clear that the acquired immune system is important for clearing the infection as well as providing effective protection to subsequent challenge with related Salmonella strains. There is a general consensus about the importance of T cells in acquired immunity to Salmonella (Makela et al., 1997; Mittrucker et al., 2000). Nude mice and mice deficient in αβ T cells are more susceptible to Salmonella infections. In most cases CD4+ T cells have been shown to be more important than CD8+
T cells, particularly in adoptive transfer experiments (Mastroeni et al., 1992; Nauciel 1990). CD4+ helper T cells (Th) are divided into two types depending on the profile of cytokines they secrete. Th1 cells produce IFN-γ and TNF-α and activate cellular immunity and inflammation, while Th2 cells produce IL-4, IL-5 and IL-13 and induce B cell activation and differentiation. A number of studies have shown that Salmonella infection results in the induction of a Th1 response (Pie et al., 1997; Thatte et al., 1993). It has also been shown that administration of exogenous IFN-γ to mice has bacteriostatic effects and that neutralization of endogenously produced IFN-γ by specific antibodies increases the mortality of mice infected with S. typhimurium (Matsumora et al., 1990; Ramarathinam et al., 1991). These results, in combination with the observation that mice deficient in IFN-γ receptors are highly susceptible to infection, further support a crucial role for CD4+ Th1 cells in Salmonella protection.

Studies have shown that Salmonella could elicit Th1 response in Peyer’s patches and mesenteric lymph nodes and that priming of T cells could occur at other nonmucosal sites but only orally administered Salmonella could direct T cell activity to the lymphatic tissue of the gut (George, 1996). McSorley et al. (2002) have suggested that dendritic cells in T cell areas of Peyer’s patches must be able to rapidly present antigen to nearby T cells without migrating to the nearby lymph nodes, as has been traditionally proposed.

In addition to T cell mediated immunity, the production of antibody has been proposed to be important in mediating immunity to Salmonella and there have been many studies supporting the role of B cells and antibody production in conferring protection (Makela et al., 1997; Mastroeni et al., 1993, 2000). Salmonella infections result in potent antibody responses particularly to LPS. It has been recently shown that mice with a targeted disruption of Igμ gene (Igh-6/−), which are deficient in B cells, showed increased susceptibility to Salmonella infection and an inability to mount a strong immune response (Maestroni et al., 2000). Therefore, it appears that B cells may influence the longevity or quality of the T cell-mediated responses.

6. Limitations of S. typhimurium to study human typhoid

Salmonella typhi is adapted and restricted to the human host. Most of the understanding about S. typhi-host cell interaction comes from S. typhimurium, which causes typhoid like
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disease in mice referred to as murine typhoid. *S.typhi* and *S.typhimurium* serovars share many virulence properties, suggesting that the two bacteria might be employing similar mechanisms to gain access into host tissues and to ensure survival in the host. However, inspite of high degree of similarity, recent studies reveal significant differences in the way these two closely related salmonellae interact with host cells. Each serovar causes a distinctive type of disease in humans. *S.typhimurium* is usually associated with a localized gastroenteritis whereas *S.typhi* is more often associated with systemic infection like typhoid. Studies have suggested that the clinical and pathological sequelae associated with specific serovars of *Salmonella* may be the result of differences in the early steps of pathogenesis. Pascopella et al. (1995) concluded that *S.typhi* invades the murine intestinal epithelium via M cells but that invasion does not destroy the M cells and the organisms are quickly found in phagocytic cell vacuoles beneath the follicle-associated epithelium. Previous studies have demonstrated that *S.typhimurium* also preferentially invades M cells but, more importantly, that invasion results in generalized destruction of the follicle-associated epithelium and is accompanied by replication within the Peyer’s patches (Jones et al., 1996). Moreover, McCormick et al. (1993) demonstrated that *Salmonella* serovars that cause gastroenteritis show transepithelial signaling to neutrophils across polarized human intestinal epithelial monolayers, whereas *Salmonella* serovars that elicit human enteric fever do not elicit this response. Kops et al. (1996) have shown that *S.typhi* migrates through polarized human epithelial cells significantly better than *S.typhimurium*. *S.typhi* induces significantly greater quantities of IL-6 in human small intestinal epithelial cell lines as compared to *S.typhimurium* (Weinstein et al., 1998).

Studies have shown that rough strains of *S.typhi* are deficient in their ability to enter culture mammalian cells (Finlay et al. 1988, Mroczinski-Wildey et al., 1989) while *S.typhimurium* rough strains are not (Kihlstrom et al., 1976, Kihlstrom et al., 1977). In addition, Elsinghorst et al. (1989) cloned a chromosomal region of *S.typhi* that conferred upon *E.coli* HB101, the ability to enter Henle-407 cells. The same chromosomal region of *S.typhimurium* did not confer invasive properties upon *E.coli*, suggesting *S.typhi*-homologous genes are either defective or non-functional in *S.typhimurium* or are not expressed in *E.coli*. Furthermore, the Typhi genome contains fimbrial operons (fimbriae are appendages on bacterial surface that have been implicated in host cell adaptation)
which are unique and restricted to typhoidal *Salmonella* serotype only (Townsend, et al., 2001). *S.typhi* has 12 fimbrial operons of which *tcf* (*Typhi colonization factor*) are unique to bacteria (Townsend et al., 2001). It has been demonstrated that Type IV pili act as intestinal adhesin for *S.typhi* but not for *S.typhimurium* (Zhang et al., 2000). Unlike *S.typhimurium*, *S.typhi* engages Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), a chloride ion channel expressed on many secretory epithelia for entry into intestinal epithelial cells. Cell lines lacking functional CFTR internalize fewer *S.typhi* than do cell lines expressing normal CFTR (Pier et al., 1998). Additionally, transgenic mice heterozygous for the murine AF508 *Cftr* allele (resulting in reduced quantities of cell surface CFTR) translocated 86% fewer *S.typhi* to the submucosa after inoculation into the gastrointestinal lumen compared with wild type mice (Pier et al, 1998). Studies have shown that serovar Typhi LPS core is a ligand that interacts with CFTR on epithelial cells and mediates internalization of bacteria by these cells and that exposure of this ligand on wild-typhi Typhi is induced by the interaction of bacteria with host cells (Lyczak et al, 2001). *S.typhi* induces intestinal epithelial cells to increase membrane CFTR levels, leading to enhanced bacterial ingestion and submucosal translocation (Lyczak et al., 2002). The two serovars also differ in the levels of cytotoxicity and persistence in macrophages (Alpuche-Aranda et al., 1995; Schwan et al., 2000).

Little is known about how *S.typhi* adapt from being gut pathogens to derivatives capable of persistence in deeper tissues. However, the genomes of representative *S.enterica* Typhi (Parkhill, et al., 2000) and *S.enterica* Typhimurium (McClelland, et al., 2001) have been published and comparision reveals some differences. *S.enterica* Typhi and *S.enterica* Typhimurium differ in about 10% of their genes. This difference also includes mutations in over 200 *S.enterica* Typhi genes, 145 of which are apparently intact in *S.enterica* Typhimurium. These *S.enterica* Typhi pseudogenes include mutations in 7 of 12 bacterial attachment factors (fimbrial operons) as well as genes involved in fecal shedding (Kingsley et al., 2002) or modifying the intracellular lifestyle (Hughes et al., 2002) of *Salmonella* (for example, *sopA*, *sopD2*, *sopE2*, *sseJ*, *cigR* and *misL*). Psuedogene accumulation has been associated with other pathogens such as *Yersinia pestis* (Parkhill, et al., 2001) that have moved towards a systemic lifestyle as well as those that have an obligate intracellular lifestyle such as *Mycobacterium leprae* (Cole et
In the case of *S. enterica* Typhi, the loss of multiple adhesive determinants may preferentially target the pathogen to particular cell types such as dendritic cells or CD 18^+^ cells capable of delivering bacteria to the systemic system and avoiding non-specific targeting to epithelial cells, which leads to local gut inflammation (Figure 4). Moreover, studies with *S. typhimurium* have been carried out in mice that are defective in Nramp-1. Because of the increased susceptibility of these mice most
of the studies have been carried out using this background which certainly complicates the extrapolation of these findings to the understanding of the immune response to *Salmonella* during natural infection.

These studies strongly suggest that the conclusions about *S.typhi*-host cell interaction drawn from the mouse model of typhoid need to be revised and that *S.typhi*-host cell interaction needs to be revisited. These studies suggest that one or more molecules not conserved between *S.typhi* and *S.typhimurium* might play a crucial role in host-specific interaction(s). The distinctions between these two closely related salmonellae in their interaction with host cells could be at the level of initial contact with the host cells involving unique cell wall and/or its associated entities leading to differential signaling, or these could be at the level of effector molecules secreted by the organisms following contact with host cells or at both the levels.