CHAPTER 1

Review of Literature
1.1. *Salmonella*

The genus *Salmonella* comprises a group of gram-negative, facultative intracellular bacteria belonging to the family Enterobacteriaceae. These bacteria infect humans as well as other animals; infections are usually acquired by the oral route. These microorganisms can be found in sewage, sea, and river water and can contaminate food. *Salmonella* species cause a variety of diseases ranging from localized gastroenteritis to systemic illnesses. In spite of a very close genetic relationship, these serovars display differing ranges of host specificity. Some are host-restricted whereas others can infect a variety of mammals. *S. typhi* produces a systemic infection, typhoid, exclusively in humans (experimentally typhoid-like clinical manifestation can also be produced in chimpanzees). On the other hand *S. typhimurium* causes only a localized and self-limited gastroenteritis in humans (Benjamin *et al*., 1990; Plant and Glynn, 1979). However, in mice, *S. typhimurium* produces systemic infection analogous to that produced by *S. typhi* in humans; *S. typhi* is avirulent in most animals including mice. *S. typhimurium* infection in mice is therefore widely accepted as an experimental model for typhoid fever in humans.

1.1.1. Typhoid Fever

Enteric fever is a systemic infection, caused in humans by *Salmonella* serotypes which include *Salmonella enterica* serotypes *typhi*, *paratyphi* A, *paratyphi* B, and *paratyphi* C. Typhoid continues to be an important cause of morbidity and mortality in developing countries, with around 22 million new cases annually with a 5% mortality rate (Crump *et al*., 2004; Ivanoff *et al*., 1994). The disease is characterized by the sudden onset of fever, severe headache, nausea, anorexia (loss of appetite), abdominal discomfort, constipation or in some cases diarrhoea (Gupta *et al*., 1994). The fever rises in a stepwise
fashion to reach 40–41°C and is sustained for up to two weeks. Classically, the progression of untreated typhoid fever occurs in four stages (Bhan et al., 2005). In the first week, there is a slowly rising temperature with relative bradycardia, malaise, headache and cough. Epistaxis is seen in a 25% of cases and abdominal pain is also possible. There is leucopenia with eosinopenia and relative lymphocytosis, and blood cultures are positive for *S. typhi*. The classic Widal test, which detects antibodies against the pathogen, is negative in the first week. In the second week of the infection, the patient has high fever plateauing around 40°C, associated with bradycardia and delirium. Rose spots appear in lower chest and abdomen in around 1/3 patients. The abdomen is distended and painful. Diarrhoea can occur at this stage. However, constipation is also frequent. The spleen and liver are enlarged and tender and there is elevation of transaminases. The Widal reaction is strongly positive with anti-O (anti-LPS) and anti-H (anti-flagellin) antibodies. Blood cultures are sometimes still positive in this stage. In the third week of typhoid fever, a number of complications like intestinal perforation, septicemia and hemorrhage can occur (Butler et al., 1991; Bitar and Tarpley, 1985; Butler et al., 1985; Roy et al., 1985). The fever is still very high and oscillates very little through the day. Dehydration ensues and the patient is delirious (typhoid state). By the fourth week, the symptoms of untreated typhoid fever begin to resolve, although relapse occurs in 10% of individuals apparently recovering from the infection (Hornick et al., 1970a). Case-fatality rates of 10%–20% in the absence of treatment can be reduced to less than 1% with appropriate antibiotic therapy. However, antibiotic resistant strains have become prevalent in several areas of the world, leading to an increase in the incidence of severe cases, hospitalization and mortality (Cooke and Wain, 2004).
1.1.2. *Salmonella typhi*

*S. typhi*, taxonomically designated as *Salmonella enterica*, subspecies *enterica*, serovar Typhi, is a gram negative, rod-shaped, non-spore forming bacterium of about 2-4 μm by 0.5 μm in size. This facultative anaerobic and actively motile bacterium has numerous long peritrichous flagella. On primary isolation, almost all the clinical isolates of *S. typhi* are found to be encapsulated. The important antigens of *S. typhi* include:

1.1.2.1. **Somatic antigen**: Lipopolysaccharide (LPS) is a major cell wall component of gram-negative bacteria. LPS is also a potent endotoxin and is generally the most dominant immunostimulant among cell wall components. The O or the somatic antigen forms the outer polysaccharide component of LPS. The presence of characteristic O antigens on the bacterial surface forms the basis for the classification of the genus *Salmonella* into a number of serotypes. For example, *S. typhi* possesses O9 and O12 antigenic determinants on its surface (van der Woude and Baumler, 2004).

1.1.2.2. **Flagellar antigen**: The flagellar antigen (flagellin) is the main protein component of the bacterial flagellum, a highly complex structure extending out from the outer membrane of the bacteria. Flagella are not only important for bacterial motility but also aid in attachment of bacteria to host cells as well as in bacterial invasion, and thereby contributing to the virulence of pathogenic bacteria (Bardy *et al.*, 2003; Josenhans and Suerbaum, 2002). Flagellin consists of a single protein of about 50 – 55 KDa. 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 H1-d (Frankel *et al.*, 1989).
1.1.2.3. **Capsular antigen**: The virulence (Vi) antigen of *S. typhi*, first described by Felix and Pitt in 1934, is a capsular surface antigen consisting of a homopolymer of N-acetyl galactosaminuronic acid with variable O-acetylation at C3 position (Felix and Pitt, 1934a) (Fig. 1). All strains of *S. typhi* and *Salmonella paratyphi* C, as well as some strains of *Citrobacter* are capable of synthesizing Vi antigen. 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 (Qadri et al., 1990). Vi antigen is stably expressed in *S. typhi* and protects the pathogen against the defensive mechanisms of the host by shielding bacteria from the action of antibodies against the O-antigen (Felix and Pitt, 1934b).

![Figure 1](image.png)

**FIG. 1. Repeating structure of the S. typhi (Vi)** is an $\alpha 1,4$-linked N-acetylgalactosaminuronic acid, O-acetylated up to 90% at the C-3 position. NAc, N-acetyl; AcO, O-acetyl.

Adapted from Stone and Szu, 1988.

1.1.3. **S. typhi pathogenesis**

*S. typhi* infects through the oral route, by ingestion of contaminated food or water. After surviving the acidic pH of the stomach, the pathogen is believed to invade M cells of the intestinal epithelium and translocate rapidly from the lumen of the distal ileum through the mucosa to eventually reach the mesenteric lymph nodes. The bacteria multiply here and are released via lymph to the thoracic duct from where they enter the general blood
circulation resulting in a transient primary bacteremia. Macrophages underlying the mesenteric lymph nodes or those in the blood phagocytose the bacteria and aid in dissemination of the bacteria. The pathogen quickly achieves an intracellular haven throughout the organs of the reticulo-endothelial system like spleen, bone marrow and liver. Bacteria multiply in these sites and re-enter the blood. This fairly sustained (albeit low-level) secondary bacteremia marks the onset of clinical disease. *S.typhi* thrives and multiplies in the bile and organisms from the gall bladder re-infect the intestinal mucosa and Peyer’s patches (Everest *et al.*, 2001).

### 1.1.4. Chronic carriers

About 5-6% typhoid patients become chronic carriers and shed bacteria in their stools for a varied period of time ranging from one year to lifetime without any apparent signs of disease (Vogelsang *et al.*, 1948). Moreover, a significant percentage of patients relapse to typhoid disease with the same *S.typhi* strain several months after initial infection (1-5% of antibiotic treated individuals), suggesting the presence of a persistent reservoir of bacteria in these individuals. Colonization of immature immune cells in the bone marrow leads to short-term persistence, whereas infection of the gall bladder from where bacteria can be directly shed into the intestine *via* the bile duct leads to long-term carriage of *S.typhi*. The long-term or chronic carriers are a serious public health hazard because they are the reservoirs for the spread of infection and disease (Hornick, 1985). The fact that *S.typhi* is carried for years even in the presence of a robust immune response (chronic carriers of *S.typhi* have high levels of circulating serum antibodies to the Vi, LPS and flagellar antigens) indicates that the organism has established a privileged niche, well sequestered from the host’s immune defenses (House *et al.*, 2001).
1.1.5. Chemotherapy

*S.typhi* infection is normally resolved within 3-5 days by appropriate antibiotic therapy. Treatment also serves to prevent 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 emergence and prevalence of multidrug resistant strains, this drug has been widely replaced by ampicillin and co-trimazole. More recently, complications arising from increasing resistance to the latter antibiotics, has led to the use of third generation cephalosporins and quinolone derivatives (Kumar and Gupta, 2007).

1.1.6. Prophylaxis

Effective control of typhoid fever can be achieved through preventive strategies such as improved sanitation, hygiene and efficacious vaccines. A number of vaccines have been developed over the years for immunization against typhoid. These include killed *Salmonella*, live attenuated *Salmonella* and a subunit vaccine.

1.1.6.1. Inactivated whole cell vaccines

Heat-killed, phenol preserved whole cell *S.typhi* were used as parenteral vaccines simultaneously in England and Germany as far back as in 1896 (Shandera *et al.*, 1985). Studies conducted in the 1960s showed that the efficacy of vaccines containing inactivated *S.typhi* ranged from 51% to 88% in children and adults and protection lasted for up to 7 years (Ashcroft *et al.*, 1967; Hejfec *et al.*, 1966). Though these vaccines were able to confer protection against *S.typhi*, they were considered unsuitable, because of a tendency to
cause fever in 6-30% of vaccinees, and severe local pain in up to 35% of recipients (Ashcroft et al., 1964). A vaccine comprising of killed S.typhi, S.paratyphi A and S.paratyphi B generated potent antibody responses but poor cell mediated immunity in humans (Bhaskaram et al., 1990; D'Amelio et al., 1988; Kumar et al., 1974). These vaccines have been replaced with a subunit vaccine or the oral attenuated vaccines.

1.1.6.2. Vi vaccine

This subunit vaccine, based on the Vi capsular polysaccharide, was first developed in the 1980s by John Robbins (Robbins and Robbins, 1984). The vaccine is administered as a single intramuscular or subcutaneous dose containing 25 μg of non-denatured Vi antigen. Revaccination is recommended every 2 years. The vaccine is now licensed for use in more than 92 countries worldwide (Hessel et al., 1999). The vaccine elicits serum anti-Vi antibodies in approximately 85-95% of adults and children older than 2 years in endemic and non-endemic areas. Previous exposure to S.typhi seems not to influence the immune response (Girard et al., 2006). In two large-scale clinical trials carried out in South Africa and Nepal in the late 1980s, this vaccine exhibited a protective efficacy of 64% and 72% respectively. (Klugman et al., 1996; Plotkin and Bouveret-Le Cam, 1995; Acharya et al., 1987; Klugman et al., 1987). Seroconversion (i.e.) a four-fold rise in antibody titers was seen 2 weeks after immunization in 82% of vaccinees, indicating the necessity of vaccination at least 2 weeks prior to exposure (Hessel et al., 1999).

An important drawback of the vaccine lies in its inability to stimulate mucosal immunity and the fact that revaccination does not elicit a booster response, as seen in clinical trials (Keitel et al., 1994). This lack of a booster response is due to the fact that the Vi antigen behaves like a T-lymphocyte-independent antigen; therefore generation of
immunological memory is impaired. Moreover these vaccines are not very effective in infants below 2 years of age. The lack of immunogenicity in infants has led to the development of conjugate Vi vaccines, using several protein carriers including the recombinant *Pseudomonas aeruginosa* exotoxin A (rEPA), resulting in the induction of higher and more sustained IgG responses. A recent field trial conducted in Vietnam, showed that a two-dose immunization schedule with the conjugate vaccine resulted in 92% protection in children 2-5 years of age (Lin *et al.*, 2001).

**1.1.6.3. Live attenuated oral vaccines**

Attenuated strains of bacteria administered orally mimic the mucosal and systemic immune response elicited by natural infection (McGhee *et al.*, 1992). Oral vaccination is not only associated with lesser side effects, but administration is also logistically simpler (Levine, 2003). The first human trials using attenuated strains against typhoid fever were made in the 1970s, using a streptomycin dependent mutant of *S.typhi*. Oral immunizations with this vaccine elicited around 80% protection against experimental challenge with *S.typhi* (Levine *et al.*, 1976). The attenuated *S.typhi* strain named Ty21a was generated by chemical mutagenesis of the wild-type strain Ty2 and developed as the first live oral typhoid vaccine. This mutant has a GalE and Vi - phenotype (Germanier and Fuer, 1975). The vaccine generated mucosal IgA and serum IgG antibodies as well as cell-mediated immunity (CMI). The efficacy of Ty21a was assessed in large-scale clinical trials in Egypt and Chile, with an overall protective efficacy of 67-80% over 7 years, after a schedule of 3 doses (Levine *et al.*, 1990; Levine *et al.*, 1987). An important feature of the Ty21a live oral vaccine is generation of herd immunity (Levine *et al.*, 1989). This vaccine has been shown to be extremely safe, with remarkably few side effects. Reversion to virulence has
not been observed \textit{in vitro} or \textit{in vivo} (Gilman et al., 1977). In spite of its efficacy, the need to administer multiple doses to elicit a protective response was seen as a drawback of the Ty21a vaccine. This prompted generation of novel attenuated \textit{S.typhi} strains that would have the ability to provide immunity with one dose. Most of these strains have been generated by mutagenesis of the wild type \textit{S.typhi} Ty2 strain. The strain CVD 908 (Ty2 \textit{aroC aroD}) is one such mutant with deletions in the \textit{aro} genes (Hone et al., 1991). Oral immunization with a single dose of CVD 908 in humans elicited a robust immune response. However, vaccination with high doses of this strain also led to silent vaccinemia in a proportion of subjects (Tacket et al., 1992). Another Ty2 derivative the Ty800, with mutations in the \textit{phoP-phoQ} regulon, was immunogenic in a phase 1 clinical trial, eliciting anti-O IgG and IgA antibodies in young adults (Hohmann et al., 1996). Importantly, none of the live bacterial vaccines developed to date elicit an anti-Vi antibody response. This could be due to the fine-tuned regulation of Vi expression under \textit{in vivo} conditions. Vi expression seems to be down regulated once \textit{Salmonella} have gained access to the phagosomal compartments of professional antigen-presenting cells. Attempts to generate a live vaccine capable of eliciting a strong immune response against the Vi antigen have led to the development of the CVD 909 strain, which is a modified CVD 908 \textit{htrA} strain. In a phase 1 clinical trial CVD 909 was found to elicit strong gut derived IgA responses against Vi, but very low serum derived anti-Vi IgG (Tacket et al., 2004).

\textbf{1.1.7. Virulence mechanisms}

\textit{Salmonella} has evolved extremely intricate mechanisms to sense the host’s environment, engage the innate immune system and modulate a variety of cellular processes in order to establish infection. A number of acid survival strategies allow these
bacteria to tolerate low pH in the stomach (Garcia-del Portillo and Finlay, 1994). Those bacteria that survive this barrier can subsequently colonize the intestine by attaching to the intestinal epithelium and avoid host clearance mechanisms. This is mediated, in part, by the fimbriae (Baumler et al., 1996), iron scavenging systems and other nutrient scavenging mechanisms (Bjarnason et al., 2003). *Salmonella* invade epithelial cells by a morphologically distinct process known as bacterial-mediated endocytosis (Francis et al., 1992). Once across the epithelium, *Salmonella* encounter another obstacle of innate immunity, the macrophage. *Salmonella* enter macrophages by induced macropinocytosis and subsequently activate virulence mechanisms that allow evasion of microbicidal actions of the phagocyte, permitting survival and replication in the intracellular environment (Alpuche-Aranda et al., 1994). Virulence genes encoding effectors that *Salmonella* employs to establish its infection are clustered in regions termed pathogenicity islands (Groisman and Ochman, 1996).

1.1.7.1. *Salmonella* Pathogenicity islands

Pathogenicity islands often contain multiple functionally related genes necessary for a specific virulence trait. These regions are thought to have been acquired by horizontal gene transfer. *Salmonella* pathogenicity islands (SPI) have a GC content lower (between 37 to 47%) than the rest of the chromosome (around 52%) and are often inserted into tRNA genes (Marcus et al., 2000). A total of five pathogenicity islands have been identified so far in *Salmonella*. *Salmonella* pathogenicity island 1 (SPI-1) encodes genes necessary for invasion of intestinal epithelial cells and induction of inflammatory responses (Galyov et al., 1997; Watson et al., 1995). In contrast, *Salmonella* pathogenicity island 2 (SPI-2) encodes genes essential for intracellular replication and establishment of systemic infection
in mice (Cirillo et al., 1998; Hensel et al., 1998). SPIs 3 and 4 are required for growth of bacteria within the host and are manifested in the systemic stage of the disease (Blanc-Potard et al., 1999; Wong et al., 1998). Recently identified virulence factors of SPI5, seem to mediate the inflammation and chloride secretion, which characterize the enteric phase of the disease (Wood et al., 1998). SPI-1 and SPI-2 encode specialized organelles for the delivery of virulence proteins into host cells; these delivery systems have been named type III secretion systems (TTSSs) (Galan, 1999; Galan and Collmer, 1999).

1.1.7.2. Type III Secretion Systems

The translocated bacterial proteins initiate a complex ‘biochemical cross-talk’ between the pathogen and the host and modulate cellular signal transduction, cytoskeletal reorganization, membrane trafficking and cytokine gene expression. TTSSs, which are comprised of more than 20 proteins, are one of the most complex protein secretion systems known in bacteria. These systems are evolutionarily related to the bacterial flagellar export apparatus (Yip et al., 2005) and are highly regulated, both at the transcriptional and post-transcriptional level (Lucas and Lee, 2000). This regulation is essential for the controlled delivery of effector proteins, which then carry out their function in a coordinated manner. TTSS have been reported to be contact dependent in Salmonella, Yersinia and Shigella spp (Lee and Schneewind, 1999; Mounier et al., 1997; Collazo et al., 1995).

Electron microscopy studies of the TTSS show it to be a supra-molecular structure, which spans the inner and outer membranes, resembling needles projecting from the surface of the bacterium, similar to the flagellar hook basal body (Kubori et al., 2000; Kubori et al., 1998). The needle complex is made of two pairs of membrane-localized rings joined by a hollow cylindrical structure that serves as the base for the externally protruding
needle-like structure. Though this structure has been visualized only in Salmonella (Kubori et al., 1998) and Shigella (Blocker et al., 1999), the conservation of its core components in other pathogens, suggests that it is a common attribute of all TTSSs. The base of the Salmonella TTSS is made up of three proteins, InvG which makes up the outer rings, and PrgH and PrgK which form the inner rings and connecting cylindrical substructure (Kimbrough and Miller, 2000; Kubori et al., 2000; Kubori et al., 1998). A single protein, PrgI, forms the needle structure (Kubori et al., 2000).

Proteins destined to be translocated through the TTSS carry multiple signals that route them through the secretion apparatus to the host cell (Cheng and Schneewind, 2000; Cornelis, 2000). The nature of the signal appears to differ among secreted proteins, thus providing the molecular basis for the temporal regulation of secretion of these proteins. Salmonella encodes two TTSSs, which are located in discrete regions. While the SPI-1 encoded TTSS is located at centrisome 63 of the chromosome (Galan, 1999), the SPI-2 encoded system is located at centrisome 31 (Hensel, 2000). Although some cross talk occurs between these systems, their function is largely independent and is exerted at different stages of infection.

1.1.7.2.1. TTSS encoded by SPI1

The SPI-1 encoded TTSS is a well-characterized system, originally identified as being responsible for mediating bacterial entry into non-phagocytic cells (Galan and Curtiss, 1989). It is present in all Salmonella serovars. The SPI-1 TTSS of S. typhimurium secretes at least 20 proteins. Of these, SpaO, InvJ, PrgI and PrgJ are part of the needle complex (Kimbrough and Miller, 2000; Kubori et al., 2000; Kubori et al., 1998; Collazo and Galan, 1996a). SipB, SipC, and SipD, in addition to other functions, are needed for
protein translocation across the host cell membrane (Collazo and Galan, 1997). SopE, which is an exchange factor for Cdc42 and Rac, stimulates actin cytoskeletal rearrangements and nuclear responses (Rudolph et al., 1999), thereby causing membrane ruffling leading to internalization of Salmonella by macropinocytosis (Hardt et al., 1998b). The remaining proteins are termed as effector proteins and carry out effector functions within the host cell. At least one effector protein AvrA is absent from two Salmonella serovars with narrow host range, S.cholerasuis and S.typhi (Hardt and Galan, 1997). While interesting, the significance of this observation is not understood. The SPI-1 TTSS continues to be active after bacterial internalization and intracellular bacteria can deliver type III proteins into the host cytosol through the membrane of the enclosing phagosome (Collazo and Galan, 1997).

1.1.7.2.2 TTSS encoded by SPI 2

The identification of proteins secreted by the SPI-2 TTSS has been hindered by the fact that this system is normally expressed only after entry of Salmonella into host cells (Pfeifer et al., 1999; Cirillo et al., 1998). These proteins contribute to development of systemic disease by promoting growth and survival of S.typhimurium within murine macrophages (Cirillo et al., 1998; Hensel et al., 1998). SpiC and SifA have been implicated in altering the intracellular trafficking route of Salmonella in macrophages (Guy et al., 2000; Uchiya et al., 1999; Stein et al., 1996).

1.1.7.3. Two-component regulator system

Among the genes necessary for survival in the macrophage are constituents of a two-component response regulator termed PhoP/PhoQ (Miller et al., 1989). Two-
component regulators are simple signal transduction systems that often regulate bacterial gene expression in response to environmental cues (Perraud et al., 1999). PhoQ is a membrane-spanning sensor/kinase protein that transfers a phosphate group to the second, cytoplasmic component (PhoP) in response to stimuli. Following phosphorylation, the cytoplasmic component usually serves as a transcriptional regulator, regulating over 40 genes, including components of the SPI-1 TTSS (Pegues et al., 1995).

Salmonellae also express several enzymes that directly inactivate reactive oxygen and nitrogen species produced by the macrophage. Homocysteine, an antagonist of nitric oxide (NO), synthesized by Salmonella mediates resistance to NO and related nitrogen compounds (De Groote et al., 1996). In addition Salmonella are also known to produce at least one, and in some highly virulent strains two, superoxide dismutases that can inactivate reactive oxygen species (Fang et al., 1999).

1.1.7.4. Flagellar secretion system

The bacterial flagellum is an important organelle of motility and in pathogenic strains also plays a key role in virulence (Bardy et al., 2003; Josenhans and Suerbaum, 2002). More than 50 genes are known to be involved in the regulated expression and function of the flagellum, which signifies the importance of motility and chemotaxis in bacterial survival. Flagella contribute to the virulence of pathogenic bacteria through chemotaxis, adhesion to and invasion of host tissues (Macnab, 1992).

It has been demonstrated that flagellated Salmonella strains are more virulent than non-flagellated strains, with enhanced survival within macrophages and increased bacterial numbers in spleens of infected animals (Weinstein et al., 1984). Contrastingly, it has recently been reported that increased virulence of several Salmonella strains could be fully
attributed to a decrease in flagellin expression and that aflagellar *Salmonella* was less inflammatory *in vitro* but more pathogenic *in vivo* (Schmitt *et al.*, 2001; Schmitt *et al.*, 1996). For a long time expression of flagella has been examined as a virulence trait, largely in context of motility rather than immune stimulation. Now it has been established that inflammatory responses are produced through activation of the Toll-like receptor-5 (TLR-5) that recognizes monomeric flagellin, the primary protein component of flagellum (Hayashi *et al.*, 2001). TLR-5 is expressed on many cell types including intestinal epithelial cells, macrophages, dendritic cells and T lymphocytes. Activation of these cells with flagellin monomers triggers a variety of inflammatory and innate immune responses (Gewirtz *et al.*, 2001a; Gewirtz *et al.*, 2001b). Mice deficient in TLR-5 show poor systemic dissemination of *S.typhimurium* highlighting the importance of this host-pathogen interaction in the pathogenesis of the bacterium (Feuillet *et al.*, 2006).

### 1.2. *S.typhimurium* infection in mice as a model for human typhoid

In the absence of a suitable animal model for *S.typhi*, infection of mice with *S.typhimurium* has been used as a model to understand *S.typhi* pathogenesis. Murine typhoid, as it is routinely called, produced by infection with *S.typhimurium* was first described in 1892 by Loefflter. Initial studies showed that the distribution of bacteria in tissue of mice infected with *S.typhimurium* is similar to that seen in typhoid patients. Susceptible strains of mice like Balb/c show signs of disease between 4 to 8 days following oral infection with *S.typhimurium*, and develop a systemic infection characterized by rapid bacterial multiplication in the liver and spleen which results in hepatomegaly and splenomegaly (Shirai *et al.*, 1979). Susceptibility of different strains of mice to *Salmonella* infection is majorly dependent on the *Ity* gene, which codes for the natural-resistance-
associated-macrophage protein 1 (Nramp-1). Mice carrying the dominant \textit{Ity}' allele are very efficient at killing \textit{Salmonella} (Benjamin \textit{et al.}, 1990) compared to the \textit{Salmonella}-sensitive \textit{Ity}' mice, which succumb to sepsis following parenteral injection of very few bacteria (Plant and Glynn, 1979). The mouse model has been very useful in understanding the role of effectors produced by TTSSs and other virulence factors in the pathogenesis of \textit{S.typhimurium} (Hensel \textit{et al.}, 1995; Gulig and Curtiss, 1988). Though these studies have significantly increased our understanding of how pathogenic \textit{Salmonella}, in general, invade intestinal epithelial cells, cause macrophage cytotoxicity etc, they have not shed any light on the host specificity that is exhibited by different \textit{Salmonella} serovars including \textit{S.typhi}.

One obvious limitation of the mouse model is that serotype Typhimurium causes enteritis rather than typhoid fever in humans, mice and humans therefore exhibiting strikingly different host responses to \textit{S.typhimurium} infections. Gastroenteritis is a typical diarrhoeal disease characterized by a massive neutrophil influx in the terminal ileum and colon (McGovern and Slavutin, 1979; Day \textit{et al.}, 1978; Harris \textit{et al.}, 1972). In contrast, typhoid fever is not a typical diarrhoeal disease and the intestinal pathology is characterized by a predominantly mononuclear infiltrate (Nguyen \textit{et al.}, 2004; Kraus \textit{et al.}, 1999; Harris \textit{et al.}, 1972). Recent studies have revealed significant differences between \textit{S.typhi} and \textit{S.typhimurium} in their interaction with host cells, which might have a role to play in the specific disease manifestations the two \textit{Salmonella} species produce in their respective hosts (Fig. 2). It has been demonstrated that \textit{S.typhi} but not \textit{S.typhimurium}, uses cystic fibrosis transmembrane conductance regulator (CFTR) as the receptor for entry into intestinal epithelial cells (Pier \textit{et al.}, 1998). \textit{S.typhi} induces significantly larger quantities of interleukin-6 in human intestinal epithelial cells than \textit{S.typhimurium} and \textit{S.dublin} (Weinstein \textit{et al.}, 1997). \textit{Salmonella} plasmid virulence (spv) operon, which is required for \textit{S.typhimurium}
pathogenesis (Gulig and Curtiss, 1987), is absent in *S.typhi* (Woodward *et al.*, 1989). In mice, *S.typhimurium* entry into M cells is associated with extensive destruction of the epithelial layer with bacteria gaining access to the subepithelial lymphoid tissue, where they encounter macrophages, DCs, lymphocytes and neutrophils. In comparison *S.typhi* enters murine M cells less efficiently, does not destroy the epithelium and is cleared from the Peyer’s patches soon after M cell entry (Pascopella *et al.*, 1995). Based on these, and related studies, it has been suggested that the conclusions about *S.typhi*-host cell interaction drawn from the mouse model of typhoid should be interpreted conservatively.

*S.typhi* and *S.typhimurium* further differ in their abilities to survive, persist and cause cytotoxic damage in human tissue. *S.typhi* is less cytotoxic to both human and murine macrophages than *S.typhimurium*. The ability of *S.typhi* to survive in macrophages without causing significant death may account for its systemic dissemination within macrophages leading to typhoid fever in humans (Schwan *et al.*, 2000). The genome sequence of *S.typhi* (Parkhill *et al.*, 2001), which shows greater than 90% similarity to that of *S.typhimurium* (McClelland *et al.*, 2001), reveals the presence of single mutations or deletions, which appear to generate many pseudogenes; the corresponding open reading frames in *S.typhimurium* are intact. These *S.typhi* pseudogenes include mutations in 7 of 12 bacterial attachment factors. The loss of multiple determinants in *S.typhi* may preferentially target the pathogen to particular cell types such as DCs or CD18+ cells which are capable of delivering the bacteria systemically while avoiding non-specific targeting to intestinal epithelial cells, the latter leading to local gut inflammation (Vazquez-Torres *et al.*, 1999). The inactivation of single genes, as well as the acquisition or loss of single genes or large islands of DNA, may have contributed to host adaptation and restricted specificity of
*S. typhi* (Young *et al.*, 2002). Collectively, these differences between *S. typhi* and *S. typhimurium* suggest that these pathogens cause disease by different mechanisms.

![Diagram of bacterial infection and immune response](image)

**Figure 2. Schematic representation of a model for persistence in human typhoid involving *S. enterica Typhi*.** On the left of the figure, the acute infection (human gastroenteritis) mediated by *S. enterica Typhimurium* involves bacteria replicating freely in the intestinal lumen and using multiple attachment factors (represented by multicolored tips or fimbriae). *S. enterica Typhimurium* targets both enterocytes and M cells for invasion, but is stopped at the mesenteric lymph nodes. Neutrophils are quickly attracted to the invasion site and inflammation follows leading to diarrhoea. *S. enterica Typhi* has dispensed with much attachment and shedding factors and may preferentially target a limited number of host cell types that favor dissemination to deeper tissues. *S. enterica Typhi* can persist in the bone marrow for extended periods and in the gall bladder for life. T, T cell; B, B cell. Adapted from Young, *et al.*, 2002.

### 1.3. Immune responses to *Salmonella*

Numerous studies have demonstrated that humans as well as experimental animal hosts respond to *Salmonella* infection by activating both humoral and cell-mediated immune responses. Most of what is known about the immune response to *Salmonella* comes from studies carried out with a mouse model of infection. While wild-type (*Ity*)
mice are relatively resistant to \textit{S.typhimurium} infection, the \textit{Ity} sup \textsuperscript{+} mice, which lack the natural-resistance-associated-macrophage protein 1 (Nramp-1), are highly susceptible to infection, making them the preferred models (O'Brien \textit{et al.}, 1980).

1.3.1. Innate immune responses

The innate immune response, mediated by macrophages and polymorphonuclear neutrophils, plays an essential role in host defense against \textit{Salmonella} (Vassiloyanakopoulos \textit{et al.}, 1998). Macrophages engulf \textit{Salmonella} by macropinocytosis and this process is enhanced by receptor-mediated uptake after opsonization of \textit{Salmonella} by antibody or complement (Mosser, 1994). The activation of macrophages by cytokines such as Interferon-\textgamma (IFN-\textgamma) or tumor necrosis factor-\textalpha (TNF-\textalpha) appears to be a prerequisite for the killing of \textit{S.typhimurium} in mice (Nauciel and Espinasse-Maes, 1992). These cytokines induce bactericidal mechanisms in macrophages which include, not only production of reactive oxygen and nitrogen intermediates, but also improved functioning of bacteria-containing phagosomes, rendering the bacteria accessible to lytic effector molecules from the lysosomes (Richter-Dahlfors \textit{et al.}, 1997). A critical factor for the bactericidal potential of macrophages is the expression of a functional \textit{Nramp-1} molecule (Vidal \textit{et al.}, 1995). The presence of a non-functional \textit{Nramp-1} gene reduces the efficacy of macrophages to kill \textit{S.typhimurium} (Vidal \textit{et al.}, 1995). The induction of proinflammatory cytokines like TNF-\textalpha, IL-1, IL-6, IL-8 and IL-12 is primarily brought about either by Toll-like receptor agonists like \textit{Salmonella} derived LPS and flagellin, or by specific bacterial effectors delivered by \textit{Salmonella} through its type III secretion system. IFN-\textgamma is also produced by natural killer (NK) cells during early stages of infection with \textit{Salmonella} (Ramarathinam \textit{et al.}, 1993). Cytokines that are important for the enhanced secretion of
IFN- include IL-18 and IL-12 (Mastroeni et al., 1999; Mastroeni et al., 1996), which are secreted mainly by macrophages and dendritic cells (Trinchieri, 1995).

Mucosal DCs play a critical role in regulating the complex interactions between gut microflora, pathogens, and the immune system leading to either tolerance or immunity. Although macrophages are believed to be the predominant cell type infected by *Salmonella*, the pathogen can infect DCs both *in vivo* and *in vitro*, bringing about activation and cytokine secretion from these cells (Svensson et al., 2000; Marriott et al., 1999; Hopkins and Kraehenbuhl, 1997). Both splenic (Yrlid and Wick, 2002; Yrlid et al., 2001) and Peyer’s patch (Hopkins et al., 2000) DCs have been shown to harbor *Salmonella* during infection, suggesting a possible role for these cells in the initiation of an immune response to *Salmonella*. DCs that take up *Salmonella in vitro* can prime bacterium-specific CD4+ and CD8+ T cells following administration into naïve mice (Yrlid et al., 2001). A unique feature of DCs is that these cells can also act as bystander antigen-presenting cells by presenting *Salmonella* antigens after internalizing neighboring cells that have undergone *Salmonella*-induced apoptotic death (Yrlid and Wick, 2000). Although the innate mechanisms of the immune system are highly effective in restricting the initial growth of *S. typhimurium* for several days, these mechanisms fail to eliminate the bacteria from the host tissues. It is seen that, after penetration from the intestine into deeper tissues, *S. typhimurium* successfully adapts to the enormous pressure imposed by the innate immune system, by expressing an array of virulence factors that improve its resistance to bactericidal host mechanisms (Cirillo et al., 1998; Hensel et al., 1998; Miller et al., 1989). Only the generation of a specific lymphocyte response allows eventual effective clearance of *Salmonella* from tissues, and provides increased protection against subsequent encounter to this pathogen.
1.3.2. Acquired immune responses

1.3.2.1. Role of T cells in immunity against Salmonella

The importance of T cells in immunity against *Salmonella* is not debatable. However, the mechanisms by which T cells interfere with infection are poorly understood. Experiments where T cell populations were depleted by antibodies or T cell-enriched fractions from spleen were adoptively transferred demonstrated that T cells were required for recovery from a primary infection with attenuated and virulent strains of *S. typhimurium* (Guilloteau et al., 1993; Mastroeni et al., 1993a; Nauciel, 1990; Chander et al., 1996; Hochadel and Keller, 1977). It was also shown that T cells participate in protective immunity, which develops after vaccination with attenuated strains of *S. typhimurium* (Mastroeni et al., 1992; Nauciel, 1990). Moreover, αβ T cell deficient mice as well as nude mice have been shown to be more susceptible to *Salmonella* infections, further establishing the importance of T cells in acquired immunity against this pathogen (Sinha et al., 1997; Hess et al., 1996). The relative contribution of the different T cell subsets however remains a matter of controversy. Experiments have shown that CD4+ cells are more important than CD8+ cells in clearing infection. Depletion of CD4+ T cells, as opposed to CD8+ T cells, had a more pronounced effect on the control of primary *Salmonella* infection, and on protection induced by vaccination induced with attenuated strains of *S. typhimurium* (Pie et al., 1997; Mastroeni et al., 1992; Nauciel, 1990). Adoptive transfer of CD4+ T cells from vaccinated mice into naïve recipients resulted in higher levels of protection than transfer of CD8+ T cells (Pie et al., 1997; Nauciel, 1990). Consistent with this finding, MHC class II-deficient mice were more susceptible to *Salmonella* infection (Hess et al., 1996).

Conversely, there is also evidence for participation of CD8+ T cells in immune responses against *Salmonella*. Class I deficient β2-microglobulin+ mice were shown to be
more susceptible to infection with *Salmonella*, and mice that had survived primary infection with attenuated *S.typhimurium* suffered from impaired protection against challenge infection with a virulent strain of *S.typhimurium*. These studies suggested that CD8+ T cells also contribute to protection (Lo et al., 1999). Depletion of CD8+ T cells also reduced the ability to transfer protection against virulent *S.typhimurium* (Mastroeni et al., 1992; Nauciel, 1990).

CD4+ helper T (T_H) cells are divided into two types based on the profile of cytokines that they secrete. T_H1 cells produce IFN-γ and TNF-α and activate cellular immunity and inflammation, while T_H2 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 T_H1 response, and that T_H1 and T_H1-inducing cytokines like IFN-γ, IL-12 and IL-18 levels were increased significantly in patients with systemic salmonellosis and in mice infected with *Salmonella* (Pie et al., 1997; Thatte et al., 1993; Matsui and Arai, 1992; Ramarathinam et al., 1991). The importance of IFN-γ in the control of *Salmonella* infections has also been shown in mice deficient in the IFN-γ receptor. These mice were highly susceptible to attenuated *Salmonella* (Lalmanach and Lantier, 1999). In addition, a recent study has shown that IFN-γ neutralization causes reactivation of persistent systemic *S.typhimurium* infection (Monack et al., 2004). The studies described above suggest a critical role for IFN-γ in immunity against *Salmonella*. However, several reports indicate that neutralization of IFN-γ is critical only in the initial phase of *Salmonella* infection and has no consequences in the later stages of infection (Pie et al., 1997; Muotiala and Makela, 1993). Exogenously administered IFN-γ has also been shown to have a bacteriostatic effect (Ramarathinam et al., 1991). It has been speculated that prompt production of IFN-γ by a large number of *Salmonella*-specific memory T cells
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is, at least in part, responsible for protection against a secondary infection. This, coupled with the finding that antibodies that neutralize endogenous IFN-γ, increase the mortality of mice infected with *S.typhimurium,* provides further evidence that CD4⁺ T⁺₁ cells play a crucial role in protection in the initial stages of *Salmonella* infection.

IFN-γ-independent T cell-mediated mechanisms involved in control of *S.typhimurium* could include production of other macrophage-activating cytokines, rendering help for B cells either by direct cell-cell contact or via cytokines, and providing cytotoxicity against infected host cells. Th1 cells can produce large amounts of TNF-α, which is crucial for immunity against *S.typhimurium* because *in vivo* neutralization of TNF-α results in fatal *Salmonella* infection (Gulig *et al*., 1997; Mastroeni *et al*., 1993b; Tite *et al*., 1991). However, since TNF-α is also produced by a variety of other cell types, the relevance of T cell-derived TNF-α in control of *Salmonella* infection is yet to be established. Other T cell-derived cytokines that have been analyzed during *Salmonella* infection are IL-4 and IL-10. IL-10 inhibits macrophage activation, and based on several studies it has been suggested that IL-10 secretion during infection with *Salmonella* might reflect severity of disease (Pie *et al*., 1996). Mice deficient in IL-4 production appeared to be more resistant to *S.typhimurium* than wild-type controls. These mice died later after infection with a wild-type strain of *S.typhimurium* and failed to develop detectable lesions in the liver. Overall, these results suggest that IL-4 production is not protective but rather impairs control of *Salmonella* infection (Everest *et al*., 1997; Pie *et al*., 1997).

CD8⁺ T cells can differentiate into cytolytic T cells (CTLs) and kill antigen-specific target cells (Kaufmann, 1988). Since *S.typhimurium* is a facultative intracellular bacterium, lysis of infected cells by cytolytic T cells (CTL) would release bacteria from their protective habitat, rendering them accessible to activated phagocytes. Several reports have
described the generation of CTL against *S.typhimurium*-infected cells or against proteins expressed by recombinant vaccine strains of *S.typhimurium* (Lo et al., 1999; Pope and Kotlarski, 1994; Pope et al., 1994; Aggarwal et al., 1990). However, the participation of CD8⁺ CTL in the control of *Salmonella* infection still awaits definite clarification and the possibility that CD8⁺ T cells perform other functions that could contribute to protection, such as cytokine production, needs to be considered.

Very little information is available on the role of γδ T cells in immunity to *Salmonella* infection. Susceptible mice deficient in γδ T cells were able to control systemic infection with an attenuated strain of *S.typhimurium* (Hess et al., 1996). Depletion of γδ T cells resulted in increased susceptibility to oral infection with *S.enteritidis*, although the effect was less pronounced than that seen after depletion of αβ T cells (Mixter et al., 1994). Mice deficient in γδ T cells responded equally to *S.dublin* as did wild-type control animals, but mice deficient in both αβ and γδ T cells were more susceptible to *S.dublin* than mice deficient in αβ T cells alone (Weintraub et al., 1997). However, while αβ T cells appear to be far more important for protective immunity against *Salmonella* infection, at sites such as the intestinal epithelium where γδ T cells represent a large fraction of T cells (Mixter et al., 1994), these cells might play a major role in protection.

The antigen specificity of the T-cell response is broad and *Salmonella*-specific T-cell responses are not only directed towards protein antigens but surprisingly also to LPS and the Vi surface polysaccharide. Vaccination of C57BL/6 mice with an attenuated strain of *Salmonella* demonstrated a significant CD4⁺ T cell response to the flagellar antigen FliC, and that these flagellin-specific CD4⁺ T cells were sufficient to protect mice against infection with a virulent *Salmonella* strain (McSorley et al., 2000). In mice infected with
**S.typhimurium** the TTSS protein SipC was seen to elicit SipC specific T cells (Musson et al., 2002).

Studies to identify the components of the human immune response to *S.typhi* are few and have mainly been performed in subjects immunized with candidate vaccines. Volunteers vaccinated with Ty21a had T cell lymphocyte populations in their blood that inhibited the growth of *S.typhi* in an *in vitro* killing assay (Nencioni et al., 1987). T cell response was predominantly T\(_H\)1 type characterized by production of large amounts of IFN-\(\gamma\). CD4\(^+\) as well as CD8\(^+\) cells were found to contribute to the production of IFN-\(\gamma\) (Lundin et al., 2002). These T cells also expressed high levels of \(\alpha_4\beta_7\) integrin which is a gut-homing receptor for T cells. This study suggested that *S.typhi*-specific T cells produced after vaccination might home back to the intestinal mucosa where some of them could persist as memory cells. Immunization with *S.typhi* strain CVD 908-htrA, another oral typhoid vaccine candidate, also elicited *S.typhi* specific CD4\(^+\) and CD8\(^+\) responses (Salerno-Goncalves et al., 2003).

### 1.3.2.2. B cells and antibodies in immunity against Salmonella

In addition to T cell-mediated immunity, B cells have also been shown to be important in mediating immunity to *Salmonella*. Mice with a targeted disruption of the Ig\(\mu\) gene (Igh-6\(^{-}\)), which lack B cells, were unable to mount an effective immune response to *Salmonella* (Mastroeni et al., 2000). CBA/N mice that carry a X-linked defect in Bruton tyrosine kinase (btk) and therefore show impaired B cell functions were more susceptible to infections with virulent *S.typhimurium*, as compared to control mice (Mastroeni et al., 2000; O'Brien et al., 1981). Thus, it appears that B cells may influence the quality of T cell-mediated responses. Recent studies using B-cell deficient mice have shown that B-cells are
needed for the establishment of protective long-lasting Th1 type T-cell immunity to *Salmonella*. In fact, total splenocytes and purified CD4⁺ T-cells obtained from *Igh-6-1-* mice immunized with live attenuated *Salmonella* showed a reduced ability to release the Th1 type cytokines IL2 and IFN-γ upon *in vitro* restimulation (Mastroeni *et al.*, 2000). These mice also failed to control the growth of virulent salmonellae in secondary infections (Mastroeni *et al.*, 2000; McSorley and Jenkins, 2000; Mittrucker *et al.*, 2000).

Infection of mice with *S. typhimurium* results in a profound antibody response not only against a variety of protein antigens like flagellin, porins, outer membrane proteins etc, but also non-proteinaceous antigens like LPS (Brown and Hormaeche, 1989). However, the role of antibodies in the different stages of infection is not yet completely understood. In susceptible mice, killed bacterial vaccines generated only partial protection against challenge with virulent *Salmonella* and protection could not be passively transferred to naïve mice by serum alone (Eisenstein *et al.*, 1984). Vaccination of susceptible mice with live vaccines induced protection against challenge with virulent bacteria (Killar and Eisenstein, 1985), but both serum and T cells were needed for successful transfer of protection to naïve mice (Mastroeni *et al.*, 1993a).

Antibodies can protect in several ways during the different stages of *Salmonella* infection. In the intestinal lumen, antibodies (particularly IgM and IgA) could block penetration of *Salmonella* into deeper tissues. Injection of a B cell hybridoma producing *Salmonella*-specific IgA has been shown to prevent oral infection of mice (Michetti *et al.*, 1994; Michetti *et al.*, 1992). This protection was most likely mediated by inhibiting bacterial adhesion to and infection of epithelial cells (Michetti *et al.*, 1994). Antibodies can also contribute to immunity by enhancing bacterial engulfment *via* Fc-receptor-mediated phagocytosis and by activating complement *via* the classical pathway (Brown, 1991).
Studies carried out in human subjects vaccinated with *S. typhi* strain Ty21a showed significant induction of IgA and IgG antibodies against *S. typhi* LPS (Forrest *et al.*, 1991b; Forrest *et al.*, 1991a). The Vi capsular polysaccharide vaccine is currently one of the vaccines available for use in humans and it provides immunity through antibodies (Acharya *et al.*, 1987).

### 1.4. Modulation of immune cell responses by pathogenic *Salmonella*

For a pathogen to successfully initiate infection, not only does it need to invade and multiply within the host but, also, to overcome the host defense mechanisms. The strategies that pathogens employ to thwart immune surveillance and response, often, decide whether the infection is successful or not. Pathogens must sense changing environments and respond with coordinated programs that provide an adaptive advantage in the host environment. These responses must include adaptation to newly encountered environmental stresses, as well as activation of specific virulence mechanisms that allow the pathogen to evade, resist or even systematically manipulate the effectors of immunity.

#### 1.4.1. *Salmonella*-induced apoptosis as a pathogenic mechanism

Macrophages are an important site of residence for *Salmonella in vivo* (Richter-Dahlfors *et al.*, 1997). *Salmonella* induces rapid cell death (30 min) in mouse liver neutrophils and macrophages *in vivo* (Richter-Dahlfors *et al.*, 1997) and is cytotoxic to cultured macrophages (Chen *et al.*, 1996; Lindgren *et al.*, 1996). Activated macrophages are more cytotoxic after *Salmonella* infection, compared to resting cells (Monack *et al.*, 1996), which suggests that both the host and pathogen contribute to cell death. This cytotoxicity is independent of intracellular bacterial replication, since invasive but non-
replicative *S. typhimurium* strains still induce apoptosis in RAW264.7 macrophages (Monack *et al.*, 1996), but is dependent on *Salmonella* pathogenicity island (SPI)-1 type III secretion (Chen *et al.*, 1996). *Salmonella* reside in intracellular vesicles in macrophages and utilize the TTSS to inject a number of bacterial proteins into the host cytoplasm (Galan, 1996b). One of the secreted proteins is the SPI-1 encoded effector protein SipB, which binds to and activates caspase-1. Caspase-1 activation results in proinflammatory IL-1β secretion and death of the macrophage (Hersh *et al.*, 1999). Although the SipB–caspase 1 interaction results in an inflammatory response that is unlikely to be beneficial to the pathogen, it appears to be essential for the systemic spread of *Salmonella* (Monack *et al.*, 2000). Since SipB induced macrophage cell-death differs from classical apoptosis in that it leads to a proinflammatory response, studies have suggested that it is not apoptosis but caspase-1 dependent necrosis (Brennan and Cookson, 2000; Watson *et al.*, 2000).

It has also been reported that caspase-1 knockout macrophages undergo delayed (4 h) cell death in a SipB dependent manner (Jesenberger *et al.*, 2000). Death in a SipB independent manner, by 12-18 h after infection, has also been reported (Santos *et al.*, 2001; van der Velden *et al.*, 2000). Once *Salmonella* has established a systemic infection, rapid killing of its host cell would be detrimental to the pathogen. During this systemic phase, when bacteria are reliant upon macrophages as a site of intracellular replication, delay in the onset of apoptosis allows the pathogen sufficient time to replicate, escape and invade new macrophages.

Unlike macrophages, infection of intestinal epithelial cells with *Salmonella in vitro* brings about apoptotic cell death 12-18 h post infection (Kim *et al.*, 1998). A *Salmonella* effector encoded within SPI-5 has been shown to activate the prosurvival kinase Akt/protein kinase B in epithelial cells (Steele-Mortimer *et al.*, 2000b). Chronic serovar
Typhi infection in humans has been associated with persistent gall-bladder carriage of *Salmonella* (Sinnott and Teall, 1987) and epithelial cells of the biliary tract have been indicated to serve as a reservoir for *Salmonella* (Runkel et al., 1991). Given that apoptosis is delayed or inhibited in epithelial cells compared to macrophages, it has been suggested that *Salmonella* might have evolved strategies to interfere with the cell death pathway in epithelial cells.

1.4.2. Modulation of macrophage functions by *Salmonella*

Enteric fever causing *Salmonella* serotypes must survive and replicate within the host macrophage to establish systemic infection (Fields et al., 1986). Activation of the two-component response regulatory system PhoP/PhoQ leads to widespread modifications in the protein and lipopolysaccharide components of the bacterial inner and outer membranes. This confers resistance to the activity of antimicrobial peptides, thereby, promoting survival of *Salmonella* inside the phagosome (Guo et al., 1998). Once inside macrophages, *Salmonella* traffics along an unusual endocytic pathway, which results in the establishment of a compartment suitable for its growth called the *Salmonella*-containing vacuole (SCV) (Meresse et al., 2001; Mukherjee et al., 2001; Steele-Mortimer et al., 2000a; Meresse et al., 1999; Steele-Mortimer et al., 1999). The formation of this unusual vesicular compartment is dependent on the function of two effector proteins, SpiC and SifA, which are encoded by the SPI-2 TTSS (Uchiya et al., 1999; Stein et al., 1996). *Salmonella* are also known to actively interfere with antigen processing and presentation in macrophages (Mitchell et al., 2004; Pryjma et al., 1994). *Salmonella*, by expressing enzymes like dismutases and homocysteine, that inactivate reactive oxygen and nitrogen species produced by the macrophage (Fang et al., 1999; De Groote et al., 1996), promotes survival of the pathogen.
within the macrophage and generates an immunosuppressive environment in vivo (al-Ramadi et al., 1992; al-Ramadi et al., 1991).

1.4.3. Interaction of *Salmonella* with dendritic cells

It has been shown that *Salmonella* can infect dendritic cells of diverse origin (Johansson and Wick, 2004; Jantsch et al., 2003; Yrlid and Wick, 2002; Hopkins et al., 2000). As with macrophages, *Salmonella* can also induce apoptosis of dendritic cells by a caspase-1 mediated mechanism (van der Velden et al., 2003) and can interfere with the MHC Class-I antigen presentation pathway by utilizing a bacterial transport system encoded by the *Salmonella yej* operon (Qimron et al., 2004).

1.4.4. Modulation of cell-mediated immune response by *Salmonella*

T lymphocytes have a pivotal role in controlling and clearing infection with intracellular pathogens through cytokine production, direct lysis of infected cells or activation of B cells. Studies with patients suffering from typhoid fever indicate that *S. typhi* effectively compromises cell-mediated immune responses (CMIR). The ratio of T lymphocyte sub-populations was grossly imbalanced in typhoid patients with reduced T<sub>H</sub> cell numbers and increased suppressor T cell numbers, resulting in a significantly lower helper cell / suppressor cell ratio (Rajagopalan et al., 1982a; Rajagopalan et al., 1982b). Another study demonstrated a statistically significant difference in both early and late rosette forming T lymphocytes in enteric versus non-enteric patients (Kumar et al., 1991). Further, *S.typhimurium* infection in mice has been shown to lead to a progressive loss of CD4<sup>+</sup> T<sub>H</sub> cell population and abnormal T cell death by apoptosis (Gupta, 1998). In an adoptive transfer system, following oral *Salmonella* infection, which allowed for
visualization of flagellin-specific CD4⁺ T cells in vivo, it was seen that Salmonella flagellin-specific CD4⁺ T cells migrated inefficiently to the liver and the lamina propria of the small intestine (McSorley et al., 2002). More recently, it has been reported that Salmonella has a direct, contact-dependent inhibitory effect on T cells (van der Velden et al., 2005). This direct Salmonella induced immunosuppressive effect reduced the ability of T cells to proliferate and produce cytokines in response to stimulation. Moreover this effect was seen only with live bacteria and did not require SPI-1, SPI-2, phoP or the Salmonella virulence plasmid.

Salmonella are known to reside and replicate within host cell-derived vacuoles in phagocytes and dendritic cells. However, entry and survival of S.typhimurium has also been reported within immortalized T- and B-cell lines (Verjans et al., 1994). In addition, in experiments directed towards determining the spleen cell populations infected by S.typhi, it was shown that the pathogen infected not only cells from the macrophage/monocyte lineage (CD11 b⁺), but also B (B220⁺) cells and CD4⁺ and CD8⁺ T cells (Salerno-Goncalves et al., 2002). Interestingly, these cells also contained bacterial antigens both intracellularly and expressed on the cell surface. Salmonella enteritidis has been shown to enter and survive inside a chicken T cell line MDCC- MSB-1 (MSB-1) (Kramer et al., 2003). Interestingly, while macrophages were able to internalize high numbers of S.enteritidis, as compared to the T cell line, bacteria survived better inside T cells as compared to macrophages suggesting that macrophages were far more effective at controlling and clearing infection compared to T cells. It has, therefore, been suggested that, like macrophages, migrating T and B cells located in the Peyer's patches may also act as ‘Trojan Horse’ carriers transporting intracellular bacteria throughout the host (Verjans et al., 1994).
Cell free extracts of *Salmonella* have been shown to inhibit T cell mitogen (PHA or Concavalin A) induced proliferation of spleen cells (Matsui and Arai, 1994a). Suppression of T cell proliferation was associated with nitric oxide independent inhibition of the stimulatory activity of protein kinase C (PKC) resulting in inhibition of IL-2 secretion (Matsui and Arai, 1994a; Matsui and Arai, 1994b; Matsui and Arai, 1994c). *Salmonella typhi* has been reported to produce a cytolethal distending toxin (CDT), which is an immunosuppressive protein capable of causing a G₂ arrest in human T cells (Haghjoo and Galan, 2004). The CDT is composed of three subunits, CdtA, CdtB, and CdtC, which form a tripartite complex (Lara-Tejero and Galan, 2002). CdtA and CdtC form a heterologous B subunit that is necessary for the delivery of CdtB, the active or A subunit (Lara-Tejero and Galan, 2001). On delivery into host cells by CdtA and CdtC, the active subunit CdtB is transported to the nucleus where it causes DNA damage (Elwell and Dreyfus, 2000; Lara-Tejero and Galan, 2000). Remarkably, and unlike other pathogens including *Haemophilus ducreyi* (Cope et al., 1997), *Actinobacillus actinomycetemcomitans* (Sugai et al., 1998), *Helicobacter hepaticus* (Young et al., 2000), *Shigella dysenteriae* (Okuda et al., 1995) and *E.coli* (Peres et al., 1997), *S.typhi* does not encode the two essential subunits of this toxin, CdtA and CdtC (Haghjoo and Galan, 2004). It has been suggested that in the absence of CdtA and CdtC, CdtB might be delivered into the cytosol through a TTSS. Pathogenic *Salmonella* also express a protein tyrosine phosphatase, SptP, which can bring about dephosphorylation of key cellular substrates (Lin et al., 2003). In fact, a functionally equivalent molecule YopH from *Y.pseudotuberculosis* has been shown to suppress antigen-specific T cell activation and IL-2 production by inhibiting tyrosine phosphorylation of a key adaptor molecule, SLP-76 in T cells, which is activated following engagement of T cell receptor with a peptide-MHC complex (Gerke et al., 2005).