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Chapter

Immunopathogenesis of Salmonellosis

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Abstract

Salmonella is an intracellular pathogenic, gram-negative, facultative anaerobe and non-spore-forming and usually a motile bacillus that leads to salmonellosis in the host. It is a common food-borne disease that ranges from local gastrointestinal inflammation and diarrhoea to life-threatening typhoid fever and presents usually a serious threat to public health due to its socio-economic value. Inadequate sanitation and impure water help in the propagation of this disease. Despite advancement in the sanitation standards, *Salmonella* enters the food chain and affects communities globally. There is an immediate need to develop improved vaccines to minimise *Salmonella*-related illnesses. Some *Salmonella* serovars infect a wide range of hosts, while others are known to be host restricted. Many different factors determine the adaptability and host specificity of *Salmonella*. The host-pathogen interactions play a unique role in *Salmonella* invasion and progression which needs to be studied in detail. This chapter shall focus on our current understanding of *Salmonella* invasion, pathogenesis and interactions with the host, host specificity and adaptability.

Keywords: *Salmonella*, serovars, adaptability, specificity, invasion, non-typhoidal *Salmonella*, typhoidal *Salmonella*, immune response

1. Introduction

Salmonellae are facultative anaerobes and gram-negative, non-spore-forming and usually motile bacilli. Two species, namely, *Salmonella enterica and Salmonella bongori*, belong to genus *Salmonella*. *Salmonella enterica* is further subdivided into six subspecies that are distinguished by variations in O (somatic) and H (flagellar) antigens with at least 2500 serotypes. *S. enterica* subsp. *enterica* comprises of more than half of the known serotypes [1]. New serotypes are being discovered increasing the serotype complexity. Approximately 99% of the *Salmonella* serotypes that infect humans and other mammals belong to *S. enterica* subspecies. These serovars are mostly the inhabitants of intestinal tract of humans and other organisms that include reptiles, birds and insects. At farm level, sources of bacterial contamination are faecal matter, litter, feed and soil [2]. *Salmonella* most commonly causes food-borne illnesses worldwide; the two commonly associated foods are eggs and poultry

meat [3]. Serovars *S.* Enteritidis, *S.* Typhimurium, *S*. Heidelberg and *S.* Newport are linked to such food-borne diseases, with farm animals being reservoirs for these serotypes [4, 5]. Salmonellosis is a big socio-economic threat worldwide that causes considerable mortality and morbidity in both humans and animals [6]. Most of the human-related diseases are food-borne, and exposure to these bacteria at different places has also been linked to human salmonellosis. The most orthodox mode of bacterial transmission is the faecal-oral route. Once the bacteria are transmitted, the initial site for bacterial infection is the small intestine. Following infection, different manifestations that arise range from gastroenteritis to enteric fever [7].

2. Epidemiology

The extensive investigation of the associated epidemiological risk factors that make an organism a persistent *Salmonella* carrier needs to be carried out. Non-typhoidal *Salmonella* (NTS) infections that cause self-limiting manifestations are the most common to occur globally. In comparison, typhoidal *Salmonella* (TS) causing enteric fever leads to a high rate of mortality and morbidity that predominantly affects the underdeveloped countries [8]. Recent studies conclude that *Salmonella* Paratyphi A incidences have risen especially in South East Asia where approximately half of the TS-infected enteric fever patients are reported to be infected with *S*. Paratyphi A [9]. The food chain can get contaminated at any stage, and most of the transmission can occur by contaminated foods like poultry and dairy-related products. Apart from contaminated food products, NTS transmission can also result from person-to-person contact or from contact with other bacterial reservoirs. After gaining entry into the host, both TS and NTS serovars initially invade the intestinal epithelium of the small intestine.

3. Diseases caused by *Salmonella* infection

Salmonella species cause a varying number of clinical manifestations in the host that can range from self-limiting gastroenteritis typically associated with non-typhoidal *Salmonella* (NTS) to typhoidal or paratyphoidal fevers, which can be life-threatening [6].

3.1 Typhoidal *Salmonella* (TS)

Humans are exclusive hosts for serovars such as *S*. Typhi, *S*. Sendai and *S*. Paratyphi A, B and C. These serovars are known as typhoidal serovars (TS) that can cause enteric fever/typhoidal/paratyphoidal fevers. Enteric fever is a systemic disease that is highly invasive and life threatening and is endemic in the developing world. Within an incubation period of around 2 weeks, bacteraemia occurs, which is marked by fever and malaise. The symptoms that start to appear after a week include fever, malaise, nausea, dry cough and abdominal discomfort. Common symptoms include tender abdomen, coated tongue, splenomegaly and hepatomegaly [10]. As the lack of adequate water and sanitation facilities in the developing countries help in the spread of *Salmonella* through faecal-oral route [11], so to prevent typhoidal *Salmonella* transmission, societal standards need to be improved. The development of improved societal infrastructures is generally cost prohibitive in developing countries, hence, having little significance in reducing the disease frequency. In comparison, the development of effective and safe typhoidal vaccines can have a significant effect on reducing typhoidal cases.

3.2 Non-typhoidal *Salmonella* (NTS)

Many industrialised and underdeveloped countries across the globe face a significant threat of non-typhoidal *Salmonella* (NTS). Worldwide, about 93.8 million gastroenteritis cases arise from *Salmonella* infections leading to 1.5 million deaths annually [7]. In infants, young, aged and immunologically compromised subjects NTS cause invasive bacterial infection [12, 13]. After post infection with NTS, symptoms that arise last for about 10 days that triggers a massive inflammatory response involving the release of pro-inflammatory cytokines and chemokines. The human NTS patients have higher serum levels of different interleukins and cytokines like IL-18, IL-12, IL-10, IL-15, TNF- α and IFN γ [14]. Non-typhoidal *Salmonella* serovars can cause severe extra-intestinal disease in patients with deficiencies in type 1 cytokine pathways such as IFN-γ/IL-12/IL-23 especially IL-12 abnormalities. Effective vaccination against NTS is lacking as there is a greater variance among different serovars. So, for generating effective vaccines against NTS, knowledge regarding different target antigens needs to be studied in detail. To disrupt the bacterial transmission and the incidence, effective preventive measures such as improving sanitation, hygiene and drinking clean water must be taken into consideration. Different host specificities and adaptability are shown by different *Salmonella* serovars that shall be discussed in detail.

4. Host specificity and adaptation

Salmonellosis susceptibly ranges from organism to organism and can occur in almost all animal species, but the clinical severity of this disease varies among the hosts. There are only specific serovars that cause severe clinical manifestations in their specific hosts [15]. Although most of the serovars of *S. enterica* subspecies cause infections which give rise to gastroenteritis that lasts for short durations, some serovars lead to severe systemic illness in humans and animals accompanied by septicaemia, fever and in some cases abortion. Based on the host specificity, these serovars can be grouped into two categories: the first category consists of serovars that are single-host restricted and the second category infecting a broad range of hosts. Different factors can be considered for grouping different serovars under the above-mentioned categories. Also, serovar pathogenicity and host epidemiology define host specificity. Keeping the above factors into account, different serovars have been grouped into three major groups. The first group comprises of serovars that mainly infect cattle and pigs but can also infect other animals including humans in some accidental cases. In this group, *S. choleraesuis* and dublin have been included that cause systemic diseases in the above-mentioned hosts. In humans and other animals, clinical symptoms may not be visible, thus making them asymptomatic. *Salmonella* carriers that can shed the bacteria in the surroundings thus leading to increased risk for susceptible hosts are also known as host-adapted serovars (HA) [16]. The second group infects specific hosts and is collectively known as host-restricted (HR) serovars. The HR serovars cause systemic diseases and can sometimes prove lethal in their hosts that include poultry, humans, sheep, equine and pigs. This group includes *S. gallinarum*, *S.* typhi, *S.* abortus and *S.* abortusequi. They interfere with the environment of their hosts in a way that paves their way for invading the host. This ability to cause mammalian abortions and loss in poultry egg production is due to their remarkable ability to multiply in the foetal tissues [16–18]. The serovars of the third group are known as unrestricted serovars that are of zoonotic, epidemiological importance and impose a great threat to many animals and humans. The serovars of this group that are of much clinical

importance are *S. typhimurium* and *S. enteritidis* [18]. These cause mild symptoms in the adult host, and sometimes the host does not show any visible clinical symptoms despite infection. They severely invade young hosts as compared to adult hosts because the adult hosts have a well-built immune system that hinders the invasion by these serovars [16]. The host specificity and adaptability of different serovars are a complex process and involve many molecular mechanisms. The exact mechanisms are poorly studied, but certain factors have been found to be responsible for determining host specificity and adaptability.

4.1 Factors determining *Salmonella* host specificity and adaptation

Although the exact mechanisms to host specificity have not been fully deciphered, the existing evidence shows that serovars act independently of each other at the various phases of infection. The expression of serovar's pathogenicity is affected by the environmental and genetic factors influencing each host during adaptation [19]. Each HA/HR serovar must overcome the encountered specific and non-specific immune mechanisms. Thus, pathogenicity of HA serovars results from the development of ways helping their survival in a host. Examples of this are serovars of *S. enterica* subsp. *enterica*, which have developed the ability to evade the immune mechanisms of warm-blooded animals. They have, during their evolution, acquired the ability to modify to their favour the physiological functions of their host, such as intracellular engulfment, apoptosis, transfer of antigens by M (microfold) cells, migration of macrophages and lymphocytes in the reticuloendothelial system and others [20]. A well-known serovar *S. typhi* has evolved to survive in human macrophages making it pathogenic to man, but not to mice [21]. Serovars such as the HR *S. typhi*, *S. gallinarum* and *S. abortusovis* show high tropism for the lymphatic organs of their hosts, thereby regulating their natural host's biological environment in their favour [19]. By anchoring to the cells of the bone marrow, PPs and bursa of Fabricius in the development of B cells, thus immune response is affected. The result of such interactions, particularly in adult animals, helps in the establishment of chronic or subclinical infection and thus prolonged subclinical excretion, but they do not help in the development of severe gastroenteritis [22]. Serovars not fully adapted to evade the mature immune system lack specificity, causing deadly systemic disease in adult animals by invading their defence mechanisms compared to HR serovars [20]. HR serovars are mildly enteropathogenic compared to the unrestricted serovars; thus, they do not cause intestinal inflammation [23]. It has also been shown that the ability of a serovar to metabolise a wide range of amino acids adds to its virulence and is thought to be closely related [24, 25]; however, HR or HA serovars have most likely evolved independently. On the other hand, the heterogenicity of serovars in relation to different metabolic profiles facilitates either the completion of the pathway to infection [26] or, when lacking specificity, it is favouring host adaptation [19]. The process of *Salmonella* host adaptation is believed to be involving either the loss of genes or the acquisition of novel genetic elements that encode specific virulence factors, and thus an inconvenience is observed frequently in the pathogenic strains. Best examples of host specificity dependent on gene deletions are, perhaps, of *S. enteritidis*, Typhimurium, Choleraesuis, Gallinarum, Abortusovis, Pullorum and Paratyphi C [27]. Genome sequencing of HA/HR serovars, such as Typhi, Gallinarum, Choleraesuis and the newly emerging in sub-Saharan Africa invasive strains of *S.* Typhi, has divulged that these have encountered extensive gene deletions and truncation [28, 29]. In systemically noninvasive *Salmonella*, the majority of lost genes have functional orthologues, which play a key role in intestinal colonisation, thus resulting in the loss of an intestinal multiplication cycle for narrowly host-adapted *Salmonellae*

followed by a concurrent acquisition of mechanisms helping the microorganism to survive in a systemic niche [30]. Point mutations, horizontal gene transfer, positive selection and genome degradation could be responsible for a differential pathoadaptive evolution of some *Salmonella* serovars [31]. It appears from the analysis of the mannose-sensitive fimbrial adhesin FimH that even single amino acid replacement, resulting in specific structural mutations in FimH variants of HA serovars, plays an important role in the differential adaptive evolution of *Salmonella* spp. Thus, activation or inactivation of mannose-specific adhesive properties in different systemically invasive serovars reflects the dynamic trajectories of adaptation to the biological environment of a specific host. Furthermore, phylogenetic analysis has indicated that these mutations are, most likely, of a convergent nature (common pathogenic traits incorporated into different genetic backgrounds) and occur under strong positive selection, illustrative of the role of point amino acid changes for HA *Salmonella*. Certainly, deep study for the molecular composition of flagella, chemotaxis genes [32], fimbriae and bacteriophages and the presence of virulence plasmids and subtypes of each specific serovar is needed, to understand mechanisms of pathogenicity and host specificity [19]. Correlation between some phage types of *S*. Typhi with their hosts has shown considerable host specificity [33]. However, the majority of phage types studied had a broad host range, perhaps, suggesting a phage transfer of virulent genes between hosts, leading eventually to host specificity. The unrestricted serovar Typhimurium may comprise a spectrum of variants differing in regard to virulence, reflecting a summation of the spatial and/or temporal selective pressures within a particular host [34]. *Salmonella* Typhimurium strains derived from animal cases were also virulent in mice, whereas many strains derived from a clinically ill man lacked this ability. Of interest was that many derived from human gastroenteritis lacked the *Salmonella* virulence plasmid, present in all animal strains and strains isolated from human bacteraemia. Furthermore, some strains harbouring the virulence plasmid isolated from the man were avirulent in mice, and the opposite was observed with those derived from animals. Altogether, isolates of a specific bacterial serovar obtained from human salmonellosis are different from those isolated from animals. This means that selective pressure within a specific host gives rise to bacterial strain variants that exhibit different pathogenicity determinants, thus varying degree of pathogenicity. Similarly, serovars of *S. enterica* subsp. *enterica*, associated with disease in mammals and birds, show different degrees of adaptability. Pathogenicity determinants, such as the FimH adhesins, play an important role. Type 1 FimH adhesins are expressed by serovars of *S. enterica* isolated from mammalian and avian hosts, while type 2 FimH is expressed exclusively by the avian-adapted serovar Gallinarum. Allelic variation of the *S. enterica* FimH adhesin directs host-cell-specific recognition, thus selectively binding to mammalian or avian receptors [35]. The distribution of SPIs, fimbriae operons and virulence plasmids has shown that various combinations of virulent determinants formed during the evolution of the microorganism are needed for a variant to become pathogenic in a particular range of host species. Mutations horizontally transmitted could have helped the development of host specificity by helping *Salmonella* serovars to harbour unique virulence factors [36]. Molecular and phylogenetic analyses of the SPI genes showed that these encode for translocon proteins (SipD, SseC and SseD) present on both *Salmonella* pathogenicity islands SPI-1 and SPI-2 and also encode an effector protein that inhibits the MAPK pathway of the host cells [37]. In addition, they encode effector proteins (SseF and SifA) important in placing the *Salmonella*-containing vacuole (SCV) in a juxtanuclear position. The products of SPI genes interact directly with the host and modulate its functions, thus favouring host specificity. Another study of the SPI genes has shown the close evolutionary relatedness between serovars Gallinarum

and Enteritidis [38], although the former is highly adapted (restricted) to poultry and is the only known non-motile serovar, while serovar Enteritidis is unrestricted. Analysis of the functions of genes associated to SPI-1 showed that virulence genes might have evolved under positive selection imposed by a serovar's respective host(s) contributing to the different host specificity observed between different serovars. This has displayed that a close similarity of core regions exists within as well as among different serovar genomes [39]. In particular, genomic comparisons of HR and HA serovars show that genomic degradation is a common evolutionary mechanism for host adaptation and increased pathogenicity [39, 40]. Others have shown that host restriction and change of ecological niche are associated with the accumulation of pseudogenes and an overall reduction in genome size [28]. For example, *S*. Typhi and Paratyphi A are restricted to the man and cause a similar systemic disease. Genome sequence similarity between Typhi and Paratyphi A serovars and their different pathogenicity when compared to the unrestricted serovars of *S. enterica* have been attributed to a relatively recent recombination of a quarter of their genomes, making the aggregation of pseudogenes a key feature of convergent evolution for these and other HA pathogens [31]. Another example supporting the role of convergent evolution is serovar Paratyphi C, which has diverged from the same ancestor as serovar Choleraesuis, by accumulating genomic novelty during its adaptation process to man. The genomic analysis of these two *Salmonella* serovars has revealed a highly similar genomic construction between the two and their distinct pathogenic features, making them excellent models for studying *Salmonella's* host adaptation and pathogenic divergence [39]. Hence, *Salmonella* adaptation to a particular host species is a complex phenomenon, which depends, apparently, on a large number of gene products. The prowess of understanding host-pathogen interactions requires analysis of the physiological associations between various animal species and genetic composition.

5. *Salmonella* invasion

After ingestion of S*almonella* by the host organism, it travels from the stomach and invades intestinal epithelial cells. Bacterial recognition generates an inflammatory response following the recruitment of a variety of bone-marrow-derived phagocytes [41]. The ability of *Salmonella* to access intestinal epithelium (M cells) is due to the presence of virulence genes encoded by *Salmonella* pathogenicity island 1 (SPI-1). Proteins that are encoded by SPI-1 form a needle-like Type III secretion system which allows the transport of several bacterial proteins into the host cell cytosol. These proteins induce changes in the host cells such as the rearrangement of the cytoskeleton and cell membrane and disconnection of epithelial cell junctions, facilitating bacterial invasion [42]. The primary site of *Salmonella* infection occurs at specialised microfold, or M cells, that are dispersed among the enterocytes, covering the follicle-associated epithelium (FAE) of the Peyer's patch (PP) [43] (**Figure 1**). *Salmonella* is considered to preferentially invade PPs in the distal ileum, but in practice, all intestinal PPs will harbour bacteria after moderate-to-high-dose infection. Once *Salmonella* is penetrated, it initiates destruction of M cell which disrupts the mucosal barrier and allows additional entry of bacteria through neighbouring enterocytes [43]. This process is extremely efficient, with M-cell penetration followed by M-cell destruction. Once access to PP via FAE is gained, invading bacteria enter the lymphatic system where they interact with professional killing cells (phagocytes) that ultimately determine the fate of the infection. Phagocytes are involved in both oxygen-dependent and oxygen-independent killing of the engulfed bacteria. During intestinal NTS infection, the release of reactive oxygen

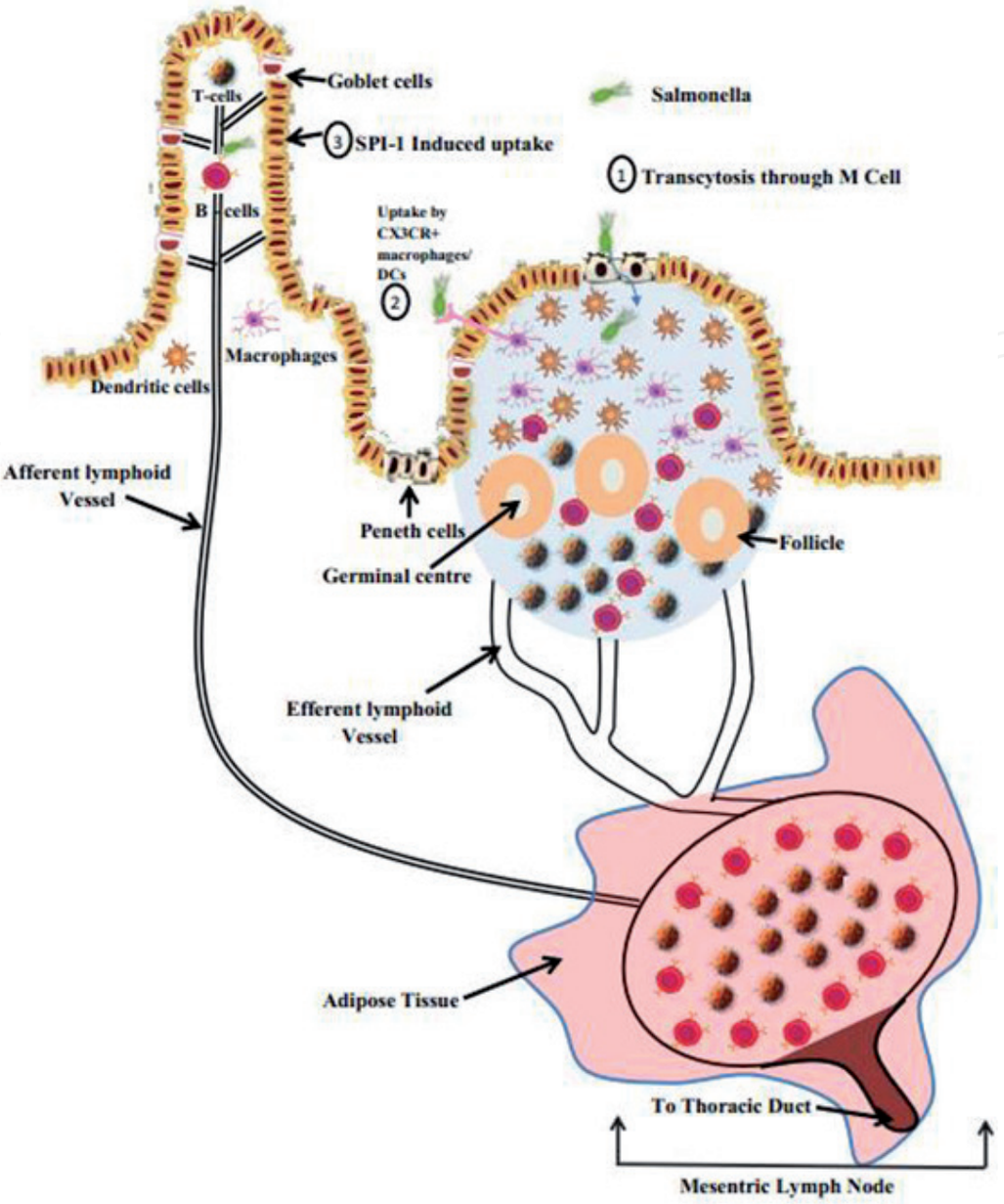

Figure 1.
Salmonella entry in intestinal epithelial cells. SPI-1 facilitates uptake and destruction of M cells, SILTs. After invasion of under tissues, Salmonella is taken by phagocytes and transported to mesenteric lymph nodes.

and nitrogen species creates a highly oxidative environment which is not permissive for the growth of bacteria. The subepithelial dome also contains dendritic cell (DC) subsets apart from macrophage populations, each of which can phagocytise the bacteria and then undergo apoptosis through a caspase-1-dependent mechanism [44]. Consequently, during *Salmonella* infection, the number of obligate anaerobes decline in the gut. Also, host-signalling environment is highly crucial for the disease development initiated by contact between microbe and host cells in various tissues, largely mediated by cytokine signalling. These cytokines aid in initiating and regulating the innate and adaptive branches of the immune response against *Salmonella*. In order to avoid damage to the host, the release of pro- and anti-inflammatory cytokines must be balanced [45]. M cells are not only abundant in PPs; they also predominate in other intestinal locations and so can, therefore, mediate infection of non-PP intestinal tissues (**Figure 1**). The most likely non-PP entry route is through the bacterial invasion of solitary intestinal lymphoid tissues (SILTs), which are heterogeneous intestinal lymphoid aggregates found in mice and humans that contain

certain features of PPs, including the presence of FAE-containing M cells [46, 47]. These SILTs are invaded by bacteria in a much similar manner as described above for PPs [48]. SILTs can be important in humans since in a study of typhoid patients, both PPs and SILTs showed inflammation. It has also been reported that intravillous M cells, which are sparsely located along the intestinal tract, may serve as a portal of entry for invasive *Salmonella* bacteria [49, 50] (**Figure 1**).

5.1 Alternative route for invasion

The main entry route described above involve, bacterial interactions with M cells, the possibility is that it can invade the host by an alternative route that does not involve M cells. A population of phagocytes in the lamina propria capture bacteria directly from luminal contents which also allow bacterial entry [51, 52]. This is for those bacteria that lack SPI-1 genes as this route does not involve M cell-mediated uptake. These cells might have been referred to as DCs, but as this is not clear [53, 54], they will be referred to as lamina propria phagocytes in this chapter. Although this pathway has now become an alternative to our general understanding of bacterial entry through M cells, the physiological importance of this route to systemic salmonellosis is poorly defined. The compelling evidence for a non-M-cell pathway is largely derived from microbiological and immunological investigations. Recent interest was stimulated by demonstrating that strains lacking SPI-1 and the fimbrial lpfC gene that did not normally infect mice retained the ability to infect mice in a CD18-dependent manner and were rapidly detected in the blood after oral inoculation [55, 56]. This extremely rapid dissemination to the blood and lack of serovar specificity might be due to bacterial entry in the bloodstream of the host through abrasions caused during gavage. Many cervical lymph node infection cases that attributed to the entry through mucosal abrasions during gavage were revealed through bacterial imaging system [57]. Expression of the SPI-2 type-III secretion system effector protein (SrfH) of bacteria was required for very early dissemination of bacteria to the blood and spleen [58]. This finding supports the idea that rapid entry through an alternative pathway involves active processes, so, therefore, it is important to examine this route from a microbiological perspective. In vitro studies demonstrated that DCs could capture bacteria by extending processes between the tight junctions of a monolayer and the apical surface of epithelial cells [59]. Subsequently, a similar process was directly visualised in vivo when CX3CR1-expressing phagocytes were detected extending transepithelial dendrites in the lamina propria, and the number of dendrites increased in the terminal ileum after infection [60]. So, these studies suggested an alternative entry model, whereby *Salmonella* might commonly access the intestinal lamina propria by cell sampling, as large numbers of bacteria were detected within the lamina propria [60]. However, *Salmonella* is not normally recoverable in large numbers from the lamina propria unless the bacterial flora is first depleted before infection [48]. Also, the formation of transepithelial dendrites is dispensable for the uptake of other pathogenic microorganisms [61]. More importantly, it has been demonstrated that CX3CR1+ lamina propria cells are unlikely to migrate to the mesenteric lymph nodes (MLN) and have poor immunostimulatory capacity [53]. Thus, CX3CR1+ cells most likely represent a population of non-migrating phagocytes that provide innate immune defence against infection within the lamina propria. Surprisingly, the role of cell-mediated uptake has not been examined carefully in PPs or in SILTs, but still, phagocytic cells are often found in association with the epithelium of tissues [48, 62]. In summary, a prominent role for M cell-mediated intestinal entry by *Salmonella* is played both in the PPs and SILTs, whereas *Salmonella* entry of the lamina propria and the mechanisms like immune activation and bacterial dissemination associated with this pathway of entry remain largely speculative.

6. *Salmonella* infection of mesenteric lymph nodes (MLNs) and systemic tissues

After initial invasion through PPs, the ultimate fate of infection is decided in the lymphatic system. The indication for the bacterial migration is based on our understanding of the lymph and the conjectural finding that bacteria are detected initially in PPs, followed by the MLN and finally the liver and spleen [63, 64]. *Salmonella* after getting access to efferent lymphatics reaches the systemic tissues via the thoracic duct and blood after reaching the MLN [65, 66]. The immune cell population that aids in the transport of bacteria to the blood and other tissues is not well known; however, intestinal DCs are usually considered as a possibility. The majority of bacteria were found free in the lymph or were associated with non-DC phagocytes [67], but it is not clear whether this also occurs during exit from the MLN. Disseminated bacteria show a tropism of tissues that contain a high number of phagocytic cells, and in most circumstances, this involves the spleen, liver, and bone marrow [65, 68]. Disruption of erythropoiesis and splenomegaly by *Salmonella* can be explained majorly by the expansion of immature erythrocytes in the spleen in an erythropoietin-dependent manner. Cancer studies have demonstrated that bacteria preferentially accumulate in primary and metastatic tumours [69, 70], suggesting that it does not have a precise organ tropism but finds tissues that contain a sufficient number of cells that support bacterial replication. The large size of the spleen, liver, and bone marrow means that these tissues gradually comprise the major sites of bacterial replication [71, 72]. Thus, *Salmonella* causes systemic infection that uses intestinal lymphoid tissues as a portal of entry. Also that bacteria clearance from the host and resistance to secondary infection requires the coordinated action of both systemic and mucosal immunity.

7. Host innate immune response to *Salmonella*

After phagocytosis by macrophages, *Salmonella* can survive and replicate within modified intracellular vesicles, termed as *Salmonella*-containing vacuoles (SCV) [73, 74]. The ability of *Salmonella* to survive within the phagosome is mediated by SPI-2, which prevents movement of RNS and ROS into the phagosome where the bacteria reside [75, 76]. In addition, *Salmonella* phoP/phoQ regulon inhibits fusion of the SCV with toxic lysosomes and endosomes [77]. The natural resistance-associated macrophage protein encoding gene, which enables macrophages to transport ions into the SCV, provides resistance/susceptibility to infection [78]. Survival of bacteria intracellularly within tissue phagocytes is a prerequisite to the bacterial virulence, and bacterial mutants that cannot survive and replicate within macrophages are attenuated for virulence [79]. The initial invasion induces a massive inflammatory response, characterised by recruitment of neutrophils, DCs, inflammatory monocytes and macrophages [48, 80]. Neutrophils follow the chemokine gradient to the gut and extravagate into the mucosa. As they encounter and eliminate the bacteria by mechanisms that are not yet fully elucidated [81], neutrophils are believed to be important in preventing dissemination of the bacteria from the intestine to systemic tissues, so the patients with low neutrophil levels have a high risk of bacteraemia during infection with NTS strains [82]. Also, that depletion of neutrophils allows extracellular growth of bacteria, suggesting that neutrophils confine and reduce bacterial replication immediately after entry. Inflammatory monocytes are an important source of antimicrobial factors, such as TNFs and inducible NO synthase, during the initial stages of infection [80]. Myd88-dependent chemokine production within the PPs drives the recruitment of these

inflammatory cells [81]. Indeed, *Salmonella* expresses several pathogen-associated molecular patterns (PAMPs), including lipopolysaccharide (LPS) and flagellin, which can be detected by Toll-like receptors (TLRs) expressed by enterocytes and phagocytes [83]. Also, macrophages after sensing cytosolic flagellin through NLRC4 (also known as Ipaf) activate caspase-1 and induce the production of IL-18 (pro-inflammatory) [84, 85]. Dendritic cells are professional antigen-presenting cells and increase the expression of MHC class II and the co-stimulatory molecules CD86, CD80 and CD40 by responding to the recognition of *Salmonella* LPS or flagellin [86, 87]. DCs present antigen to naive $CD4^+$ T cells, thus providing a vital link between innate immune responses and the induction of adaptive immunity. In the PPs, flagellin also induces the secretion of the inflammatory chemokine CCL20, which is an important ligand for CCR6 [88]. This response activates an early process whereby CCR6-expressing DCs are recruited to the FAE, for efficient activation of $CD4^+$ T cells [89].

8. Host-adaptive immune response to *Salmonella*

Adaptive immune response to *Salmonella* can be mediated via early $CD4^+$ T-cell activation. Due to the small size of intestinal lymphoid tissues and low frequency of naive $CD4^+$ T cells specific for any given antigen [90], detecting initial bacterial specific T-cell activation in these tissues is challenging. However, studies with T-cell receptor transgenic mice visualised the processes of bacterial specific $CD4^+$ T cells responding to oral infection [64, 91]. An artificially elevated naive precursor frequency of $CD4^+$ T cells at a high dose of infection provides the most accurate assessment of *Salmonella*-specific $CD4^+$ T-cell activation [92]. The earliest *Salmonella*-specific $CD4^+$ T-cell activation occurs within the MLN after oral infection but usually peaks few hours after that in the PPs [91]. At very early time points, $CD4^+$ T cells were not found to be activated in any other secondary lymphoid tissues [89], suggesting that whatever the explanation for early bacterial dissemination to blood as discussed above, no early adaptive immune response is initiated outside the gut-associated lymphoid tissue. Interestingly, the T-cell receptor transgenic model used recognises a bacterial peptide from the carboxy-terminal region of flagellin [93] which is also a ligand for TLR5 [94]. Generally, the early activation of flagellin-specific $CD4^+$ T cells in the PPs is representative of the naive $CD4^+$ response to other bacterial antigens [95, 96]. However, this is difficult to demonstrate conclusively as very few, naturally occurring bacterial specific MHC class-II peptides are known [97]. Interestingly, activation of $CD4^+$ T cells in the MLN is also dependent on $CD11c^+$ DCs and CCR6, indicating that T-cell activation in the MLN and PP has similar requirements. The evidence clearly suggests that the MLNs are an important site for immune protection during the course of *Salmonella* infection. Indeed, after MLNs were surgically removed, there was an elevated bacterial load and severe immunopathology in the liver [98]. The importance was also highlighted using a relapsing model of murine typhoid in which primary infection returns after apparent antibiotic clearance [57]. Although MLN is often considered a potential site of bacterial accumulation [99, 100], it acts as a protective firewall, preventing bacterial dissemination and relapsing *Salmonella* infection.

9. Host effector responses against *Salmonella*

The development of robust protective immunity against *Salmonella* infection requires the coordination of B and T cells. One hundred and sixteen $CD4^+$ T

cells have a critical role in clearing the primary infection and are also required for acquired resistance to secondary infection [100]. In contrast, B cells are dispensable for resolving primary infection but are required for protection against secondary challenge [101]. There is a massive expansion of *Salmonella*-specific $CD4^+$ T cells and rapid acquisition of Th1 effector functions, i.e. the enhanced ability to secrete INF-γ, TNF α and IL-2 upon restimulation [102] (**Figure 2**). These activated Th1 cells can be clearly detected a week after infection, which is consistent with the rapid tempo of $CD4^+$ T-cell activation. Optimal expansion of Th1 cells have been shown to require expression of both programmed death ligand-1 (PD-L1) and the TNF receptor family members, OX40 and CD30. Appropriately, expansion of activated Th1 cells eventually comprises ~50% of all $CD4^+$ T cells few weeks after infection [103]. Furthermore, Th1 cells are capable of responding to innate signals such as *Salmonella* LPS by secreting cytokines [104]. This innate response is unexpected as effector Th1 cells are normally stimulated only after recognition of cognate peptide and MHCs [105]. This innate immune responsiveness suggests a means by which the host can rapidly produce INF-γ to activate macrophages within an infected tissue, even if bacteria are capable of inhibiting antigen presentation by infected phagocytes [106]. Despite the rapid and efficient development of Th1 effector cells, there is actually little evidence that suggests their contribution to bacterial clearance during primary infection. Thus, it was found that when $CD4^+$ or $CD4^+$ Th1 cells were completely absent, bacterial growth enhanced a few weeks after infection which indicates that Th1 cells contribute little to regulate bacterial growth before this point [107]. Many in vitro studies point to an inhibitory effect of *Salmonella* on antigen presentation to naive T cells in vitro [108], but in vivo, there is no effect on *Salmonella*-specific $CD4^+$ expansion [109]. In contrast, the gradual loss of effector $CD4^+$ T cells detected in the process of *Salmonella* infection that required the presence of live bacteria and the expression of SPI-2 genes indicated that the effector function of cells is specifically

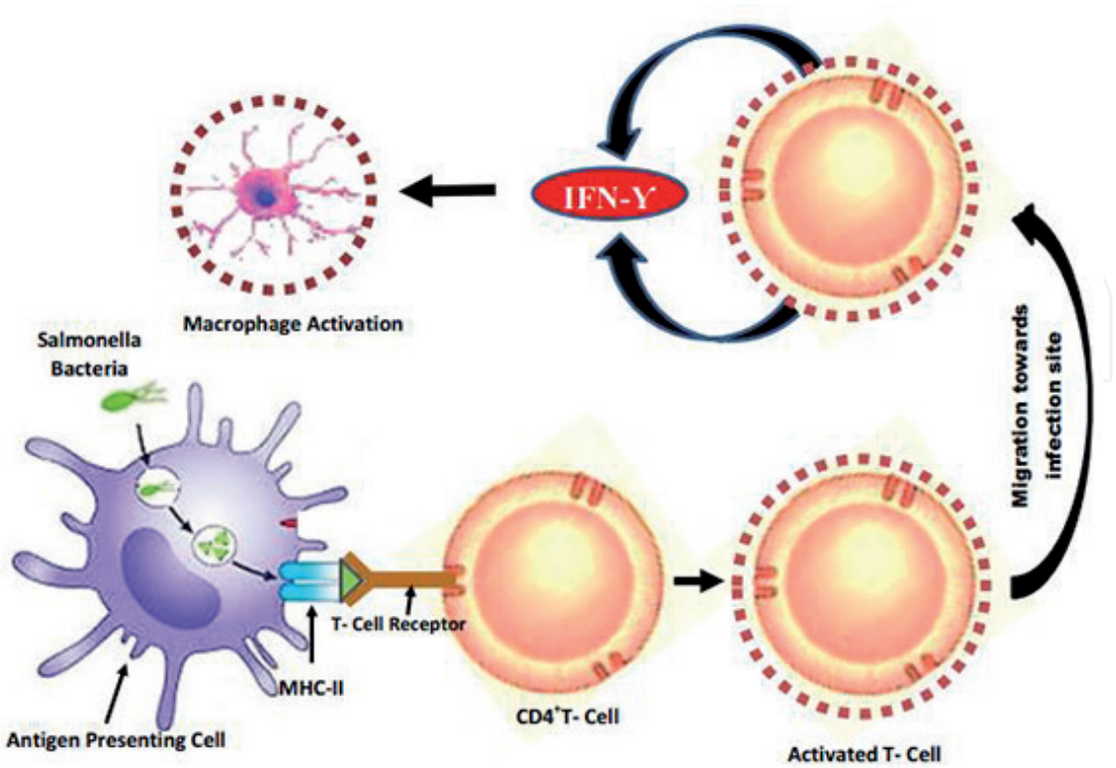

Figure 2.
Induction of IFN γ production by Salmonella-specific $CD4^+$T cells. Expansion of activated $CD4^+$T cells in secondary lymphoid tissues which in turn produces IFNγ at infection sites. Production of IFNγ finally activates macrophages.

inhibited by actively replicating bacteria [110]. Effector Th1 cells are effective in providing immunity to salmonellosis [111]; however, effector $CD4^+$ subsets including regulatory T cells (Tregs) and Th17 cells are also known to contribute. Tregs arise from the thymus or develop after naive T-cell activation in the presence of TNF β which suppresses effector T-cell responses [112]. In contrast, Th17 cells arise from naive $CD4^+$ T-cell stimulation in the presence of IL-6 and TNF-β and are important in mediating immunity against extracellular bacterial infections [113, 114]. During the development of Th1 cells and Tregs after infection, it was found that changes in the cogency of Tregs reduced the efficacy of Th1 responses and increased bacterial growth [102]. After oral infection with *Salmonella*, cytokines associated with Th17 cells, IL-17 and IL-22 are rapidly produced within the intestinal mucosa [115], and the production is induced by innate responses to infection rather than Th17 cells, however, still indicating the potential for Th17 cytokines to participate in intestinal defence against bacteria. In vivo, production of IL-22 dependent on IL12B, rather than IL-17, contributed to bacterial clearance [116]. Taken together, it is suggested that Th17 cells contribute additionally to protection against *Salmonella* infection by not only initiating or enhancing neutrophil infiltration to intestinal tissues but by the production of antimicrobial peptides by the epithelium which is effective against luminal bacteria as well [117]. In summary, it has been suggested that Th17 cells have an additional role in defence against *Salmonella* in the intestine and a role for Tregs in modulating the potency of *Salmonella*-specific Th1 cells in vivo.

10. Host antibody (Ab) response against *Salmonella*

Salmonella-specific B-cell responses contribute to bacterial clearance in the hosts [39, 120, 121]. Although the bacteria are generally found within SCV in phagocytic cells, there is a short period during the infection cycle when bacteria are expected to be extracellular. *Salmonella* is not only tightly associated with mononuclear phagocytes in vivo [118] but also induces these infected cells to undergo apoptosis [44]. After cell death, bacteria are presumably found in the extracellular compartment before infecting a neighbouring phagocyte. Thus, antibody might have direct access to the bacteria during this short period of time and prevent cell-to-cell transmission [92]. Bacterial colonisation obstructs the bacterial opsonization with *Salmonella*-specific Ab [119]. The Ab also plays a role in amplifying the processing and presentation of antigens to $CD4^+$ T cells, thus affecting the Th1 response [120]. B-cell innate immune response to TLR-specific ligands is necessary for the development of Th1 responses in vivo [121]. New findings also suggest the suppression of protective immunity through B-cell MyD88 pathway during infection [122]. Therefore, innate immune signalling in B cells contributes to an important regulatory function but requires further analysis. The presence of *Salmonella*-specific Ab IgA in the intestinal mucosa may also prevent or reduce bacterial penetration of the intestinal barrier [123]. However, which of these mechanisms makes the greatest contribution to protective immunity is yet to be deciphered, but an important role for Ab is also suggested from human studies [124]. Although the specificity of Ab responses is undefined, Abs specific for the LPS O-antigen, flagellin, Vi capsular polysaccharide (ViCPS) antigen and outer membrane porin protein (OmpD) are all believed to be protective [125].

11. Conclusion

Members belonging to genus *Salmonella* are the major intestinal pathogens of human beings and animals. The increased food production and growth in human

populations have led to the increase in dissemination potential of these ubiquitous microorganisms. Due to the systemic nature of some infections, where many tissues get involved to display immunity to specific infection, salmonellosis and the immune response that results are pliable. Deciphering the pathogenesis of invasive salmonellosis may hopefully lead to potential therapeutic treatment strategies that are urgently required in light of propagating antimicrobial resistance. Future studies must focus on the identification of molecular targets of *Salmonella* virulence factors during intracellular life in immune cells and designate the molecular mechanisms of interference. This would impart a novel perception into the cell biology of DCs and other immune cells. Furthermore, understanding the intracellular life of *Salmonella* may lead to new advancement in generating reliable vaccines against infections, to wield *Salmonella* strains as live carriers for recombinant vaccines and to evolve novel forms of treatment that target the function of specific virulence factors. Further explorations to clarify the contribution of genes differently represented/expressed in the genomes of various *Salmonella* serotypes during infection are required.

Abbreviations

NTS	non-typhoidal *Salmonella*
TS	typhoidal *Salmonella*
HA	host-adapted serovars
HR	host restricted
FAE	follicle-associated epithelium
PP	Peyer's patch
DC	dendritic cells
MHC	major histocompatibility complex
SPI	*Salmonella* pathogenicity island
SCV	*Salmonella*-containing vacuoles
MLN	mesenteric lymph nodes
PAMPs	pathogen-associated molecular patterns
LPS	lipopolysaccharide
Tregs	regulatory T cells
PDL	programmed death ligand
IL	interleukin
IFN	interferon
DC	dendritic cell
Myd	myeloid differentiation primary response
Fim	fimbrin
TNF	tumour necrosis factor
CD	cluster of differentiation
MAPK	mitogen-activated protein kinase
SILTs	solitary intestinal lymphoid tissues
ViCPS	Vi capsular polysaccharide
TLRs	Toll-like receptors
OmpD	outer membrane porin protein

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