

Review

Host–Pathogen Interaction in Invasive Salmonellosis

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Abstract: *Salmonella enterica* infections result in diverse clinical manifestations. Typhoid fever, caused by *S. enterica* serovar Typhi (*S. Typhi*) and *S. Paratyphi A*, is a bacteremic illness but whose clinical features differ from other Gram-negative bacteremias. Non-typhoidal *Salmonella* (NTS) serovars cause self-limiting diarrhea with occasional secondary bacteremia. Primary NTS bacteremia can occur in the immunocompromised host and infants in sub-Saharan Africa. Recent studies on host–pathogen interactions in Salmonellosis using genome sequencing, murine models, and patient studies have provided new insights. The full genome sequences of numerous *S. enterica* serovars have been determined. The *S. Typhi* genome, compared to that of *S. Typhimurium*, harbors many inactivated or disrupted genes. This can partly explain the different immune responses both serovars induce upon entering their host. Similar genome degradation is also observed in the ST313 *S. Typhimurium* strain implicated in invasive infection in sub-Saharan Africa. Virulence factors, most notably, type III secretion systems, Vi antigen, lipopolysaccharide and other surface polysaccharides, flagella, and various factors essential for the intracellular life cycle of *S. enterica* have been characterized. Genes for these factors are commonly carried on *Salmonella* Pathogenicity Islands (SPIs). Plasmids also carry putative virulence-associated genes as well as those responsible for antimicrobial resistance. The interaction of *Salmonella* pathogen-associated molecular patterns (PAMPs) with Toll-like receptors (TLRs) and NOD-like receptors (NLRs) leads to inflammasome formation, activation, and recruitment of neutrophils and macrophages and the production of pro-inflammatory cytokines, most notably interleukin (IL)-6, IL-1 β , tumor necrosis factor (TNF)- α , and interferon-gamma (IFN)- γ . The gut microbiome may be an important modulator of this immune response. *S. Typhimurium* usually causes a local intestinal immune response, whereas *S. Typhi*, by preventing neutrophil attraction resulting from activation of TLRs, evades the local response and causes systemic infection. Potential new therapeutic strategies may lead from an increased understanding of infection pathogenesis.

disease [2]. *S. Typhi* is an exclusively human pathogen causing a bacteremic disease that, unlike many other Gram-negative bacteremias, does not typically manifest with neutrophilia or septic shock [3]. The widespread appearance of antimicrobial-resistant strains has limited treatment options [4,5]. Relapse and chronic asymptomatic fecal carriage may complicate the illness (Figure 1) [6,7]. Mortality usually results from intestinal perforation and peritonitis or from a severe toxic encephalopathy associated with myocarditis and hemodynamic shock [8].

Infections with non-typhoidal *Salmonella* (NTS) serovars, such as *S. enterica* serovar Typhimurium and *S. Enteritidis*, also cause a significant disease burden, with an estimated 93.8 million cases worldwide and 155,000 deaths each year (see [9] for review) [10]. NTS serovars usually cause self-limiting diarrhea with secondary bacteremia occurring in less than 10% of patients. The host range of non-typhoidal *Salmonella* serovars is broad, including poultry and cattle, and NTS infection is commonly due to food poisoning in developed countries. NTS serovars cause high rates of bacteremia in the immunocompromised and, in sub-Saharan Africa, in children below 5 years old and those with HIV infection [9,11]. Antimicrobial resistance is widespread [12].

The variations in the clinical features of infection with this intracellular pathogen relate to differences in the interaction between different *Salmonella* serovars and the host. This review summarizes new and significant insights concerning the virulence factors of both typhoid and non-typhoidal *Salmonellae*, their difference at the genome level, novel mechanisms employed by these intruders to circumvent the host defense, and their interactions with both host microbiome and the innate immune system.

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Introduction

Typhoid fever is a global problem, with more than 27 million cases worldwide each year resulting in an estimated 217,000 deaths [1]. *Salmonella enterica* serovar Typhi (*S. Typhi*) and *S. Paratyphi A* are the Gram-negative bacteria that cause this debilitating condition. It is most common among children, especially in areas of Asia and Africa that lack clean water and adequate sanitation, and is also an important travel-associated

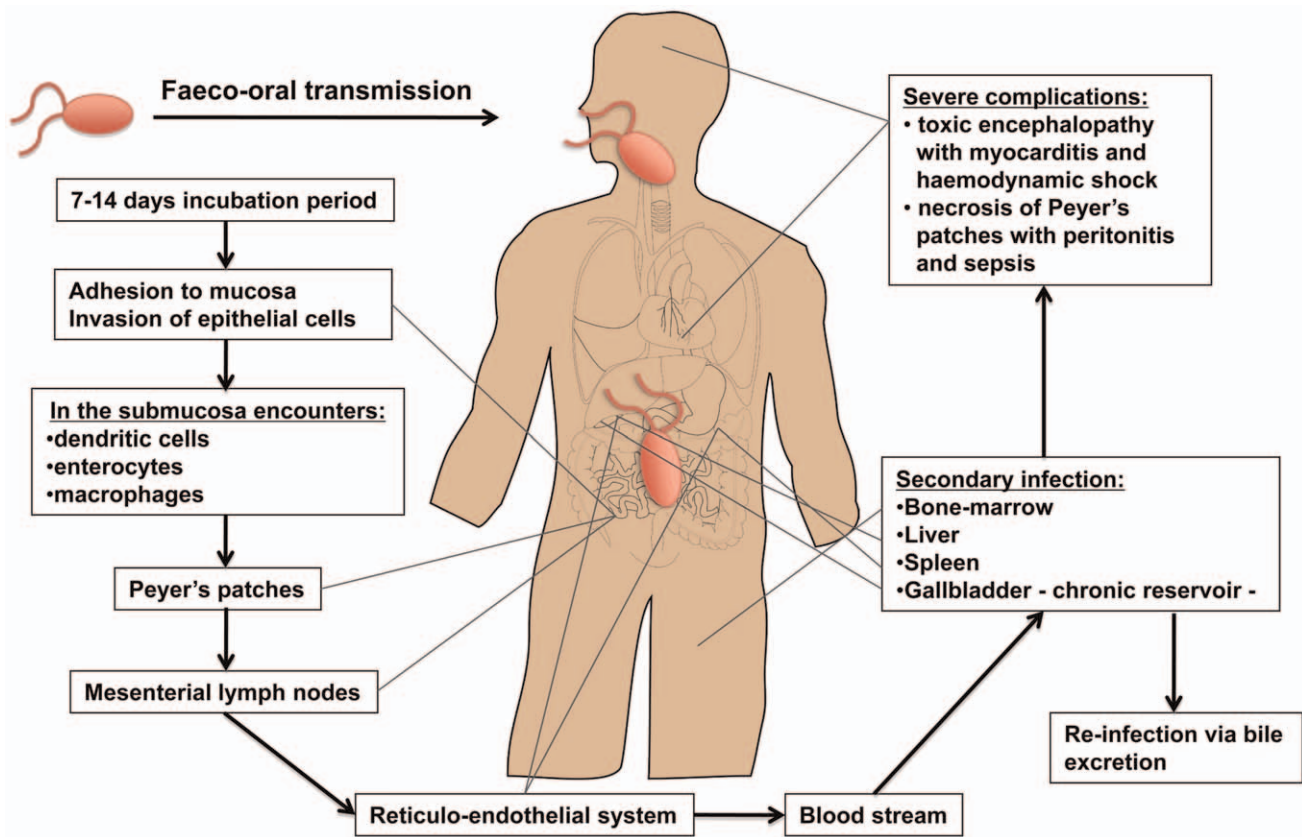


Figure 1. Dissemination of *S. Typhi* during systemic infection. Typhoid is usually contracted by ingestion of food or water contaminated by fecal or urinary carriers excreting *S. Typhi*. The incubation period is usually 7 to 14 d. In the small intestine the bacteria adhere to the mucosa and then invade the epithelial cells. The Peyer's patches, which are aggregated lymphoid nodules of the terminal ileum, play an important role in the transport to the underlying lymphoid tissue. Specialized epithelial cells such as M cells overlying these Peyer's patches are probably the site of internalization of *S. Typhi*. Once the bacteria have penetrated the mucosal barrier, the invading organism translocates to the intestinal lymphoid follicles and the draining mesenteric lymph nodes, and some pass on to the reticuloendothelial cells of the liver and spleen. During the bacteremic phase, the bacteria are widely disseminated throughout the body. Secondary infection can occur with liver, spleen, bone-marrow, gallbladder, and Peyer's patches as the most preferred sites. The gallbladder is the main reservoir during a chronic infection with *S. Typhi* and invasion occurs either directly from the blood or by retrograde spread from the bile. Of interest, the ability of *Salmonella* to form biofilms on gallstones is likely to be a critical factor in establishment of chronic carriage and shedding of *S. Typhi* [88]. The bacteria that are excreted in the bile can then reinvade the intestinal wall by the mechanism previously described or are excreted by feces. Typical clinical symptoms are fever, malaise, and abdominal discomfort. Clinical features such as a tender abdomen, hepatomegaly, splenomegaly, and a relative bradycardia are common. Rose spots, the classical skin lesions associated with typhoid fever, are relatively uncommon and occur in 5%–30% of cases. The most severe manifestations of typhoid leading to sepsis and death are either necrosis of the Peyer's patches resulting in gut perforation and peritonitis or a toxic encephalopathy associated with myocarditis and haemodynamic shock [8,89].
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The Bacteria

Taxonomy and Genomics of *Salmonella*

The genus *Salmonella* is composed of two distinct species: *Salmonella bongori* and *Salmonella enterica*, the latter being divided into six subspecies. These subspecies are classified into more than 50 serogroups based on the O (somatic) antigen, and divided into >2,400 serovars based on the H (flagellar) antigen. Complete genome sequence from multiple *Salmonella* strains are available [13]. For example, the *S. Typhi* type strain Ty2, the multidrug-resistant (MDR) isolate CT18, and the *S. Typhimurium* strain LT2 are composed of 4.79 (Ty2), 4.86 (CT18), and 4.81 (LT2) megabases, respectively [14–16]. The core genomes of *Escherichia coli* and *S. enterica* differ by only 10% in their DNA sequences and suggest that the two species derived from a common ancestor about 100 million years ago. Comparison of different *S. Typhi* isolates show that they are highly related (clonal) and have arisen from a single point of origin approximately 30,000–50,000 years

ago [17]. The sequence-based technique of MLST (multilocus sequence typing) provides a more accurate indication of the genomic relationship between different *Salmonella* isolates and may supersede serotyping in the future [18]. Of the ~4,000 *S. Typhi* genes, more than 200 are functionally disrupted or inactive, while most of these homologs are still fully functional in *S. Typhimurium*. This could in part explain the restricted host range of *S. Typhi* [16]. Although it has been suggested that the different clinical outcomes of infection between typhoid and NTS serovars may be explained by differences in genome expression leading to differences in host-pathogen recognition, one should also consider the opposite possibility; that is, differences in host-pathogen interaction may make certain genes dispensable, resulting in the accumulation of pseudogenes [3,19,20].

Recent analysis of *S. Typhimurium* isolates from the unusually invasive infections seen in sub-Saharan Africa have shown dominance of a particular MLST type, ST313, distinct from the usual *S. Typhimurium* sequence type, ST19, associated in other

parts of the world with diarrhea. In the D23580 invasive *S. Typhimurium* isolate from Malawi, there is loss of gene function, including genes previously implicated in the virulence of *S. Typhimurium* in the murine model of infection, such as *sseI* (encoding a type III-secreted effector protein) and *ratB* (encoding a secreted protein associated with intestinal persistence), and of the 44 novel pseudogenes or deletions in the strain relative to LT2, 26 are also pseudogenes or deletions in *S. Typhi* or *S. Paratyphi A* [21]. These observations suggest that a similar process of adaption to the human host may be occurring in African *S. Typhimurium* as has been observed in *S. Typhi*.

Virulence Factors

About 90% of the genes in *S. Typhi* and *S. Typhimurium* serovars are identical [16]. The 10% of genes that differ include virulence factors, which determine their pathogenic potential (Figure 2) [20]. The virulence factors of the *Salmonella* serovars are mostly encoded on the *Salmonella* pathogenicity islands (SPI).

Plasmids and prophages. Integrated bacteriophages, phage remnants, or plasmids are single- or double-stranded DNA molecules that can be exchanged between bacteria by horizontal gene transfer. They give bacteria the opportunity to pass on or receive selected genes that may enhance virulence or result in antimicrobial resistance [17,22]. Certain *Salmonella* spp. have a self-transmissible virulence plasmid called pSLT, which harbors

the *spv* genes. The *spvB* enzyme, which acts as an intracellular ADP-ribosylating toxin causing host cytotoxicity, is necessary for intra-macrophage survival but is absent in *S. Typhi* and *S. Paratyphi A* [20,23]. Putative virulence-associated plasmid has only recently been identified in *S. Typhi*. The chimeric plasmid pR(ST98) carries genes that are involved in drug resistance and apoptosis induction in macrophages [24,25]. Additionally, a linear plasmid in *S. Typhi* strains originating from Indonesia, called pBSSB1, carries the *fljB⁶⁶* gene, which encodes a flagellin antigen known as H:z66 [26]. Whether the presence of these particular plasmids has an impact on the virulence of *S. Typhi* is not known.

The role of plasmids carrying antimicrobial resistance genes, such as *cat*, *dhfr7*, *dhfr14*, *sul1*, and *bla_{TEM-1}*, in the transfer and spread of antimicrobial resistance has been well described [20,27]. *S. Typhi* is able to exchange multidrug resistance R-plasmids with *E. coli* and other enteric bacteria [28,29]. The self-transmissible incompatibility group (Inc)HI1 plasmids almost exclusively confer the phenotype of MDR *S. Typhi*. The presence of the MDR phenotype has been suggested to be associated with the development of severe or fatal disease [30,31]. The presence of a composite genetic element encoding multiple antimicrobial resistance genes on the virulence-associated plasmid in the ST313 serovar *Typhimurium* isolates causing invasive disease in Africa may provide an explanation for a linkage between drug resistance and an invasive phenotype [21]. Prophages and phage remnants

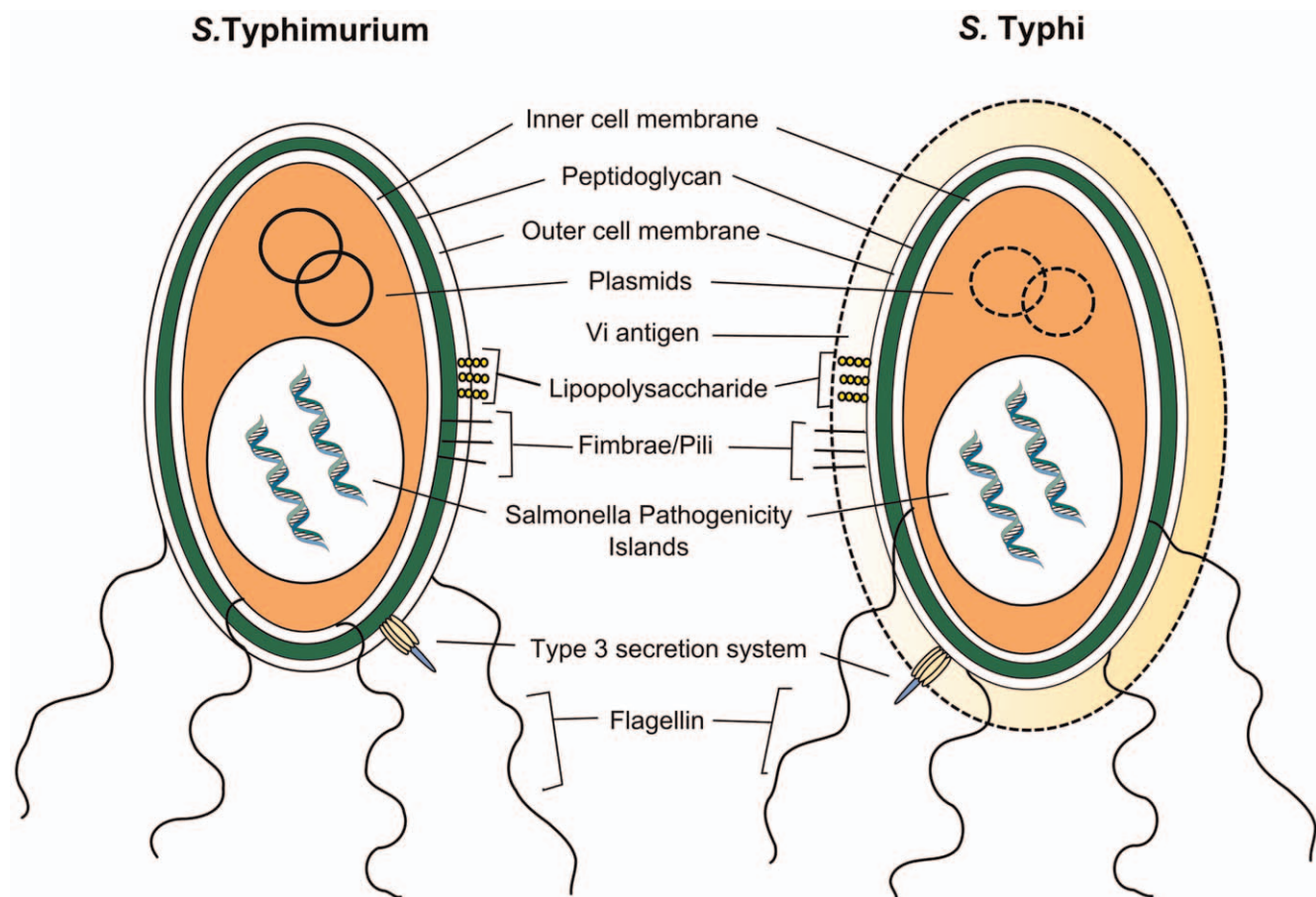


Figure 2. Virulence of *S. Typhimurium* and *S. Typhi*. *S. Typhimurium* and *S. Typhi* possess partly overlapping and a partly distinct repertoire of virulence factors. Both serovars express the type III secretion system, lipopolysaccharide, and other surface polysaccharides, fimbriae, flagellin, and bacterial DNA. The Vi antigen is exclusively expressed by *S. Typhi* and is able to circumvent the innate immune response by repressing flagellin and LPS expression. SPI, *Salmonella* pathogenicity islands. doi:10.1371/journal.ppat.1002933.g002

can carry non-essential “cargo” genes involved in bacterial virulence including several type III secretion system effectors, which play an important role in *Salmonella* virulence [20,32].

Type III secretion system and outer membrane vesicles. *Salmonella enterica* spp. contains two type III secretion system (T3SS) gene clusters encoding a secretion apparatus that functions like a molecular syringe. The T3SS secretes effector proteins into the target-cell cytosol, which manipulate host-cell signaling cascades. These effector proteins are suggested to have multiple activities within host cells; for example, SopB is involved in invasion and Akt activation, which causes fluid secretion and *Salmonella* containing vacuole (SCV) formation (Figure 3a) [33–36]. The majority of genes encoding for these virulence-associated effector molecules are located on the SPIs. Other effector proteins such as SopE, SspH1, SseI, SodC-1, and SopE2 are encoded by phages or phage remnants [22,37,38].

Of the 21 SPIs known to date, SPI-1 and SPI-2 are the most studied. *S. Typhimurium* and *S. Typhi* genomes share 11 common SPIs; four are specific to *S. Typhi* (SPI-7, 15, 17, and 18) and only one (SPI-14) for *S. Typhimurium* [20]. SPI-1 harbors the genes for T3SS1, which is crucial for the invasion of non-phagocytic cells such as M cells in the gut lumen and activation of pro-

inflammatory responses [39]. However, *S. Typhimurium* mutants deficient in SPI-1 can disseminate and cause systemic infection from the gastrointestinal tract by CD-18 expressing phagocytic cells such as dendritic cells (DCs) without disrupting the epithelial barrier [40,41]. Therefore, this alternative pathway via CD-18 immune cells facilitates the development of a systemic infection for intracellular-adapted bacteria like *Salmonella*. T3SS2, encoded on SPI-2, plays a crucial role during the second phase of invasion, intracellular survival in macrophages [42]. Within the phagocyte, T3SS2 prevents trafficking from the phagocyte NADPH oxidase (nicotinamide adenine dinucleotide phosphate-oxidase) towards the SCV, thereby preventing a phagocytic burst [43,44]. Although the importance of these systems for the virulence of *S. Typhimurium* is clear, limited data are available concerning the role of T3SS in *S. Typhi*. Of note, the SPI-2 T3SS of *S. Typhi* is not required for survival in human macrophages but may be used during infection of other cell types, such as DCs or natural killer cells, leading to the notion that the SPI-2 T3SS may be required to modulate the host immune system to establish long-term asymptomatic infection [45].

Bacterial outer membrane vesicles (OMV) have been recently identified as another method used by *Salmonella* to transfer its

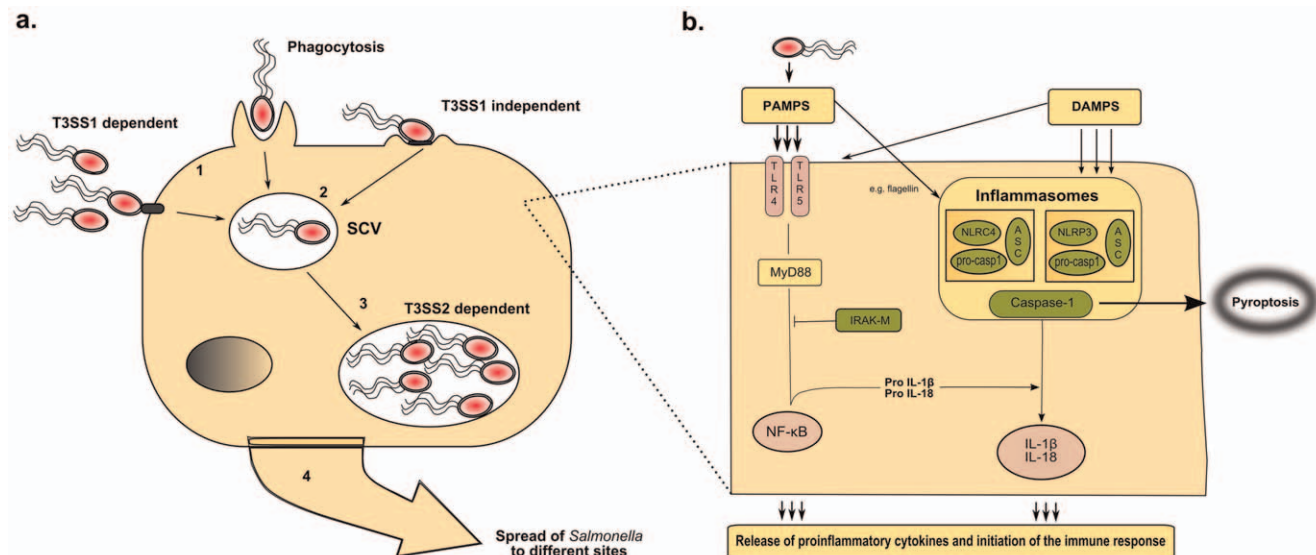


Figure 3. *Salmonella* and its first encounter with the host. (a) The intracellular life of *Salmonella*. Invasion of phagocytic and non-phagocytic cells. *Salmonella* is a facultative intracellular pathogen that can be found in a variety of phagocytic and non-phagocytic cells, in which it is able to survive and replicate. To establish this intracellular niche, the T3SS1 and -2 play a predominant role; key virulence factors are involved in accessing and utilizing these cells [36]. After ingestion, intestinal colonization follows and *Salmonella* enters enterocytes and dendritic cells in the intestinal epithelium [36]. Subsequently, *Salmonella* that reach the submucosa can be internalized by resident macrophages via different mechanisms: by phagocytosis, active invasion using the T3SS1 or T3SS1-independent invasion using fimbriae or other adhesins on the bacterial surface. (1) *Salmonella* survives and replicates within the SCV, where it is able to avoid host antimicrobial effector mechanisms. The T3SS2 is required for systemic virulence in the mouse and survival within macrophages. (2) Replication within the SCV. *Salmonella* survives and replicates within the SCV, where it is able to avoid host antimicrobial effector mechanisms. The T3SS2 is required for systemic virulence in the mouse and survival within macrophages. (3) Transport of *Salmonella* to distant sites. After penetration of the M cells, the invading microorganisms translocate to the intestinal lymphoid follicles and the draining mesenteric lymph nodes, and some pass on to the reticuloendothelial cells of the liver and spleen. *Salmonella* organisms are able to survive and multiply within the mononuclear phagocytic cells of the lymphoid follicles, liver, and spleen [36]. (b) Host–pathogen interaction in typhoid and non-typhoid *Salmonella*. Simplified scheme of the first encounter between *Salmonella* spp. and the immune system. Specified cells such as neutrophils, macrophages, dendritic, phagocytic, and epithelial cells recognize specific pathogen associated molecular patterns (PAMPs) and danger-associated-molecular patterns (DAMPs), thereby eliciting an immune response. PAMPs such as LPS, Flagella, and bacterial DNA can trigger TLR4, TLR5, and TLR9, respectively. TLR-induced activation of NF-κB is essential for the production of pro-IL-1β, pro-IL-18, which can be negatively regulated by IRAK-M [90]. The NLRs are situated in the cytosol and can also recognize PAMPs. However, NLRP3 is triggered by a different, yet unknown, mechanism, although DAMPs are thought to play a crucial role. TLR, toll-like receptors; LPS, lipopolysaccharide; NF-κB, regulated nuclear factor kappa-light-chain-enhancer of activated B cells; IRAK-M, IL-1R-associated kinase-M; IL, Interleukin; ASC, apoptotic speck protein containing a caspase recruitment domain; NLR, NOD-like receptors (including NLRP3 and NLRC4); MyD88, myeloid differentiation primary response gene [88]. doi:10.1371/journal.ppat.1002933.g003

virulence factors into the cytoplasm of the host cell [46]. For example, *S. Typhi* uses the OMV to enclose ClyA, a pore-forming cytotoxin, and subsequently release this virulence factor extracellularly [47]. Moreover, it has also been shown that OMVs can stimulate responses important for the activation of DCs, priming *Salmonella*-specific T and B cells, and possess pro-inflammatory and antigenic function, which makes them therefore attractive as vaccine candidates [48].

Fimbriae and flagella. Fimbriae or pili are found on the bacterial surface and are thought to be mainly important for biofilm formation, colonization, and initial attachment to the host cells, although little is known about their true virulence potential [20,36]. Each *Salmonella* serovar harbors a unique combination of fimbrial operons. Flagella are long helical filaments attached to rotary motors embedded within the membrane that enable *Salmonella* species to travel to the epithelial barrier after ingestion. In vitro, flagellin causes upregulation of pro-inflammatory cytokines in tissue culture models [49]. However, in vivo data showed that the role of flagella in virulence can be dispensable and model-dependent [50–52].

Polysaccharides and other putative virulence factors. The polysaccharidic capsule Vi antigen is of key importance for *S. Typhi* virulence, but notably absent in *S. Typhimurium*, *S. Paratyphi A*, and most other *Salmonella* serovars. Its presence increases infectivity of *S. Typhi* and disease severity, and natural infection is usually associated with the expression of Vi antigen in isolated *S. Typhi* [53]. However, Vi negative mutants are still able to cause a typhoid-like illness in human volunteers [54]. Two widely separated chromosomal regions, *ViaA* together with *ViaB*, located on SPI-7, are needed for Vi synthesis [55]. Recently it has been hypothesized that the Vi capsule can prevent host-pathogen recognition by preventing lipopolysaccharide (LPS) recognition by pattern recognition receptors (PRRs) [56]. In this way *S. Typhi* does not elicit a neutrophil influx in the small bowel but is able to disseminate systemically and lead to a persistent bacterial infection [3]. Other well-documented virulence factors include ion transporters and superoxide-dismutases [36].

The Host Defense

PRRs, most notably the Toll-like receptors (TLR) and NOD-like receptors (NLR), are the first component of the immune system to detect host invasion by pathogens, initiate immune responses, and form the crucial link between innate and adaptive immunity [57,58]. PRRs recognize conserved motifs on pathogens termed “pathogen-associated-molecular-patterns” (PAMPs) and are also able to recognize endogenous danger signals or “danger-associated molecular-patterns” (DAMPs). During invasive *Salmonella* infection, PAMPs and DAMPs initiate the innate immune system leading to activation and recruitment of neutrophils and macrophages and the production of pro-inflammatory cytokines, most notably Interleukin (IL)-6, IL-1 β , tumor necrosis factor (TNF)- α , and interferon-gamma (IFN)- γ (Figure 3b) [59–65]. IFN- γ plays a central role in the control of persistent infection by affecting the extent of macrophage activation [64]. IL-18 is important for IFN- γ release and early host resistance to *Salmonella* infections [66].

During severe bacterial infection uncontrolled activation of the innate immune response can lead to detrimental systemic inflammation, intravascular coagulation, tissue injury, and eventually death [67,68]. This hyper pro-inflammatory response is only seen to a limited extent in patients with typhoid fever. Coagulation abnormalities do not become clinically apparent and serum levels of TNF- α and IL-1 β are low compared to the levels measured in

patients with systemic infections caused by other Gram-negative bacteria [60,62]. Patients with typhoid fever demonstrate a distinct and highly reproducible signature in the peripheral blood, shown by micro-arrays and transcriptional profiling, that changes during treatment and convalescence, returning in the majority of cases to a normal profile as measured in healthy uninfected controls [63]. Patients who do return to a normal profile may be genetically or temporarily incapable of developing an effective immune response and may be more susceptible to re-infection, relapse, or the establishment of a carrier state [63]. In this respect it is of importance to note that antimicrobial treatment—which can lead to depletion of the gut microbiome—is associated with prolonged deleterious effects on intestinal *Salmonella* colonization resistance, which can result in increased fecal shedding and carrier status (Box 1) [69]. In contrast to NTS, which is an important cause of morbidity and mortality in patients with an inherited or acquired immunodeficiency syndrome such as HIV infection, IL-12R deficiency, or chronic granulomatous disease, typhoid fever has not been associated with any primary or acquired immunodeficiency or underlying disease [9,70,71]. Such a difference can potentially be ascribed to a difference in signaling through PRRs where production of interleukin-17-producing T cells and their associated family cytokines (IL-17, -21, -22, -26) play an important role in the dissemination of NTS but not *S. Typhi* [9].

Pattern Recognition Receptors

Salmonella spp. expresses multiple PAMPs, most notably T3SS, flagella, fimbriae, LPS (Vi antigen), and bacterial DNA, which are recognized by PRRs (Figure 2). Not surprisingly, TLR4 plays an important role in invasive Salmonellosis. TLR4-deficient mice

Box 1. *Salmonella* and the Gut Microbiome

In recent years it has become clear that the intestinal microbiome, consisting of more bacteria than the total number of cells in the human body, can be seen as an exteriorised organ that exerts numerous functions in the host response against Salmonellosis. The gut hosts $\sim 1 \times 10^{14}$ bacteria from 500–1,000 different species of which three bacterial divisions—the *Firmicutes* (Gram-positive), *Bacteroides* (Gram-negative), and *Actinobacteria* (Gram-positive)—dominate. The healthy gut microbiome can protect against epithelial cell injury by producing toxic metabolites known to repress *Salmonella* virulence gene expression, optimizes host immune systems, and mediates pathogen clearance from the gut lumen after non-typhoidal *Salmonella* diarrhea [69,91]. Of importance, antimicrobial treatment depletes the gut microbiome and is associated with prolonged deleterious effects on intestinal *Salmonella* colonization resistance, which can result in increased fecal shedding and carrier status induction [69,92,93]. Ingeniously, *S. Typhimurium* is able to exploit a specific microbiome-derived nutrient, named ethanolamine, in order to acquire a significant growth advantage in the lumen of inflamed intestine over other potential pathogens [94]. Similarly, *S. Typhimurium* virulence factors have been found to induce host-driven production of a new electron acceptor that allows the pathogen to use respiration to compete with fermenting gut microbes [95]. The potential of microbiota-based therapies for curing *Salmonella*-infected patients has opened a whole new area of research [69].

Box 2. Mouse Models for *S. Typhi* Infection

S. Typhi infects humans exclusively. The consequent lack of animal models has hampered the study of host–pathogen interactions in typhoid fever. To overcome this problem experimental murine *S. Typhimurium* infection has been used extensively as a model for typhoid fever. The intestinal pathology and inflammatory response seen in this model resembles the changes observed in patients with typhoid fever [96]. Current murine models include both oral and systemic (intravenous or intraperitoneal) inoculation with or without streptomycin pretreatment [91]. Infection of susceptible mouse strains that carry a mutation in the gene encoding for a metal transporter present on the SCV membrane named *Nramp1* (*Slc11a1*), such as CL57/BL6 or BALB/C mice, produces a disease that resembles typhoid fever upon inoculation with *S. Typhimurium* [20]. To study chronic and persistent infection such as can be seen in *S. Typhi* carriers, strains of mice possessing the *Nramp* +/+ allele, which are consequently resistant to the infection with *S. Typhimurium*, are used [64]. Of note, no allelic association was identified in humans between the *Nramp* alleles and typhoid susceptibility, and as *S. Typhimurium* causes a different disease in humans than *S. Typhi*, conclusions regarding typhoid fever pathogenesis derived from animal experiments must be interpreted carefully [97]. An ingenious mouse model for *S. Typhi* was proposed by making use of immunodeficient *Rag2*^{−/−} *yc*^{−/−} mice engrafted with human fetal liver hematopoietic stem and progenitor cells creating humanized mice susceptible to *S. Typhi* [98]. Although these humanized mice were able to support *S. Typhi* replication and persistent infection, it did not lead to an acute lethal infection [98]. Most recently, another murine lethal *S. Typhi* model resembling characteristic features of human typhoid fever was created by making use of humanized nonobese diabetic-*scid* *IL2r γ* ^{null} mice, which are engrafted with human hematopoietic stem cells (hu-SRC-SCID mice). This model, which has already been proven to be useful for detecting new virulence determinants, could also be useful to study host–pathogen interactions and evaluate vaccine candidates [99].

have increased susceptibility to *Salmonella* infection, and stimulation of TLR4 by LPS has an important role in the development of septic shock during *S. Typhimurium* infection [56,72–74]. *Salmonella* flagellin leads to TLR5 activation [49]. Intriguingly, the Vi capsule expressed in mutated *S. Typhimurium* prevents both in vitro and in vivo recognition of *Salmonella* LPS by TLR4 [50,56]. The presence of the Vi antigen on the cell surface leads to capsule formation, which ultimately prevents this recognition. A crucial role is ascribed to the TviA regulatory protein, encoded on the *S. Typhi*-specific *SPI-7* (*viaB* locus), which downregulates flagellin production and enhances Vi antigen attachment to the cell surface [75]. The TviA protein therefore serves as a regulatory switch affecting the ability of the host to recognize *S. Typhi* as an intruder at crucial stages of the spread in humans.

The inflammasomes, which are intracellular complexes consisting of caspase-1, NLRs (e.g., NLRP3- or NLRC4), and the adaptor molecule ASC (apoptosis-associated speck-like protein containing a CARD), play a central role in the innate immune defense against *S. Typhimurium* [76]. Mice deficient in caspase-1 or the end product of inflammasome activation, namely IL-1 β and IL-18, have higher bacterial load and succumb earlier upon infection with *S. Typhimurium* [77]. In vitro experiments have

Box 3. Questions for Future Research

Genomics

- Can bacterial genotype be linked to the clinical disease phenotype in humans?
- Is there a true association between bacterial genotype and/or the presence of a multidrug resistant plasmid and disease severity? If there is an association, what is the mechanism?

Host-response

- Is there a difference between *S. Typhi*- and *S. Paratyphi*-induced enteric fever in the host gene expression pathways?
- What is the role of DAMPs during severe typhoid fever? Are these danger-associated molecular patterns causing the damage that occurs in the gut, or are they mere bystanders triggering the NLRP3 inflammasome?
- Is a change in gut flora the reason that typhoid patients still have an altered immune profile nine months after infection? Moreover, do these patients have an increased risk for re-infection with other invasive *Salmonellae*?

Treatment

- Can we exploit a better understanding of disease pathogenesis to lead to new therapeutic approaches? Potential immunomodulating treatment strategies for invasive Salmonellosis could target the pathogen directly (e.g., based on drugs targeting T3SS or flagella) or target key host response proteins (e.g., TLR4, NLRC4, or IL-1 β) depending on the phase of the immune response.
- Are steroids beneficial in severe typhoid fever as has previously been suggested [100]? And if so, what is the mechanism behind these observations?

shown that *S. Typhimurium* induces caspase-1 and NLRC4-dependent IL-1 β release and cell death [78]. NLRC4 is able to recognize bacterial flagellin that—as has been hypothesized by different authors—can be injected accidentally into the cytoplasm by the T3SS causing cytosolic perturbations [65,79–81]. However, during experimental infection mice deficient in NLRC4 are able to clear *S. Typhimurium* just as efficiently as control mice, suggesting the involvement of other inflammasomes [78]. Indeed, Broz et al. demonstrated that both NLRP3 and NLRC4 activate caspase-1 in response to *S. Typhimurium* infection, and mice lacking both *NLRP3* and *NLRC4* genes show increased susceptibility to infection. In response to a bacterial trigger, NLRP3 and NLRC4 will recruit ASC and caspase-1 into a single cytoplasmic focus, which subsequently serves as the site for pro-IL-1 β processing [82]. Recent work provides evidence that the recognition of bacterial flagellin by the NLRC4 inflammasome in splenic dendritic cells triggers rapid release of IL-18, which leads to IFN- γ production by memory CD8⁺ T cells [65]. NLRC4-mediated release of IL-1 β has also been shown to be flagellin dependent, while the bacterial trigger for NLRP3 remains unclear. However, NLRP3 is able to recognize *SPI-2* T3SS mutants, which lack the capacity to replicate intracellularly; therefore, the receptor is thought to play a role in the detection of persistent bacteria [83]. Although these studies underscore the central role of the inflammasome during *S. Typhimurium* infection, further research is needed to define its role in typhoid fever [83].

Apoptosis and Pyroptosis

Apoptosis or programmed cell death is regarded as a protective mechanism of host defense by preventing further release of pro-inflammatory cellular mediators [84]. Serovars of *S. enterica* are able to employ different mechanisms to induce macrophage cell death [85,86]. Recently it has been proposed that activation of caspase-1 can also trigger a form of pro-inflammatory cell death called “pyroptosis” [87]. Caspase-1, which is triggered via the flagellin-detection of the NLR4-inflammasome, is able to cause clearance of *S. Typhimurium* independent of IL-1 β or IL-18 by pyroptotic macrophage death [76]. In infected cells, caspase-1-induced lysis of macrophages can result in the release of bacteria into the extracellular space, which will enable efficient reactive oxygen species (ROS) mediated killing by neutrophils. During the systemic phase of infection, *S. Typhimurium* is able to completely suppress flagellin expression, which results in evasion of NLR4 detection and subsequent pyroptosis [76]. Interestingly selected *Salmonella*-infected caspase-1-deficient macrophages do not undergo pyroptosis but display a form of delayed cell death with features of autophagy [86].

Conclusion

Significant progress has been made in our understanding of host–pathogen interactions in invasive Salmonellosis. Mouse models using *S. Typhimurium* have been instrumental in unraveling complex pathways and have shed new light on the

role of key virulence factors such as Vi antigen and T3SS (Box 2). Studies of genome differences between *S. Typhi*, *S. Paratyphi A*, *S. Typhimurium*, and other *Salmonella* serovars have begun to explain some of the variation in disease manifestations. The important yet undefined roles of DAMPs and NLR-recognition in typhoid fever remain to be clarified, and these may be major players in the severe gut ulceration that is an important cause of the mortality. Despite these major advances, large gaps remain in our understanding of the pathogenesis of the disease in humans. Unraveling of the pathogenesis of invasive Salmonellosis hopefully leads to new therapeutic treatment strategies, urgently needed in the light of growing antimicrobial resistance. Box 3 summarizes some important questions for future research on invasive *Salmonella* pathogenesis research.

Search Strategy and Selection Criteria

Data for this review were identified by searches of PubMed, with the search terms “Typhoid Fever” in combination with “epidemiology,” “clinical features,” “therapy,” and “origin.” “*Salmonella Typhi*” or “*Salmonella Typhimurium*” in combination with “genome,” “virulence factors,” “toll-like receptors,” “NOD-like receptors,” and “inflammasome.” The references of identified articles were manually searched for further relevant papers, and we also searched our own reference databases. English and French papers were reviewed.

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