Salmonella enterica Serovar Typhi and the Pathogenesis of Typhoid Fever

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Abstract

Salmonella enterica serovar Typhi, the cause of typhoid, is host restricted to humans. *S.* Typhi has a monophyletic population structure, indicating that typhoid in humans is a relatively new disease. Antimicrobial usage is reshaping the current *S.* Typhi global population and may be driving the emergence of a specific haplotype, H58, that is well adapted to transmission in modern settings and is able to resist antimicrobial killing more efficiently than other *S.* Typhi. Evidence gathered through genomics and functional studies using the mouse and in vitro cell systems, together with clinical investigations, has provided insight into the mechanisms that underpin the pathogenesis of human typhoid and host restriction. Here we review the latest scientific advances in typhoid research and discuss how these novel approaches are changing our understanding of the disease.

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INTRODUCTION

Typhoid is a common infection in regions with poor economic development and limited public health infrastructure (24, 96, 101). Global incidence is difficult to estimate because the current gold standard for diagnosis is culture of *Salmonella enterica* serovar Typhi from a clinical sample (103). Global estimates of 200,000 deaths a year have been made, but morbidity is significantly higher, with >20 million new cases per year (12, 20, 21, 97). This review focuses on *S.* Typhi, with occasional reference to *S.* Paratyphi A, which is also a common cause of enteric fever, particularly in parts of Asia (98, 149).

TYPHOID IN HUMANS

Human infection with *S.* Typhi normally occurs through the consumption of contaminated food or water (71). The infectious dose has been determined in human challenge studies to be around 10,000 organisms; this likely varies between individuals and different settings, and recent studies indicate that it may in fact be lower (47, 139). The bacteria can invade the intestinal mucosa potentially through microfold (M) cells and establish an initially clinically undetectable infection involving significant systemic dissemination and a transient primary bacteremia (118) (**Figure 1**). Thus, the pathogen is invasive but does not normally trigger a rapid inflammatory or diarrheal response. This lack of a mucosal inflammatory response is a key feature of *S.* Typhi disease and is distinct from most disease caused by nontyphoidal *Salmonella* (NTS) serovars. After infection, the incubation period may not always be followed by clinical symptoms. Those who go on to develop typhoid become fatigued, and the fever begins to rise in a classical stepwise manner (**Figure 2**). If left untreated, the temperature will remain high (>39°C), and associated symptoms generally include coughing, vomiting, headache, and a rapid pulse (100). Typhoid can be difficult to distinguish clinically from other causes of fever, such as malaria. However, a well-trained clinician with knowledge of other febrile diseases may observe particular clinical patterns, including a spiking temperature or rose spots on the chest, suggesting the underlying etiology (46). Typhoid is generally regarded as a disease of children, adolescents, and elderly people. Infections of very

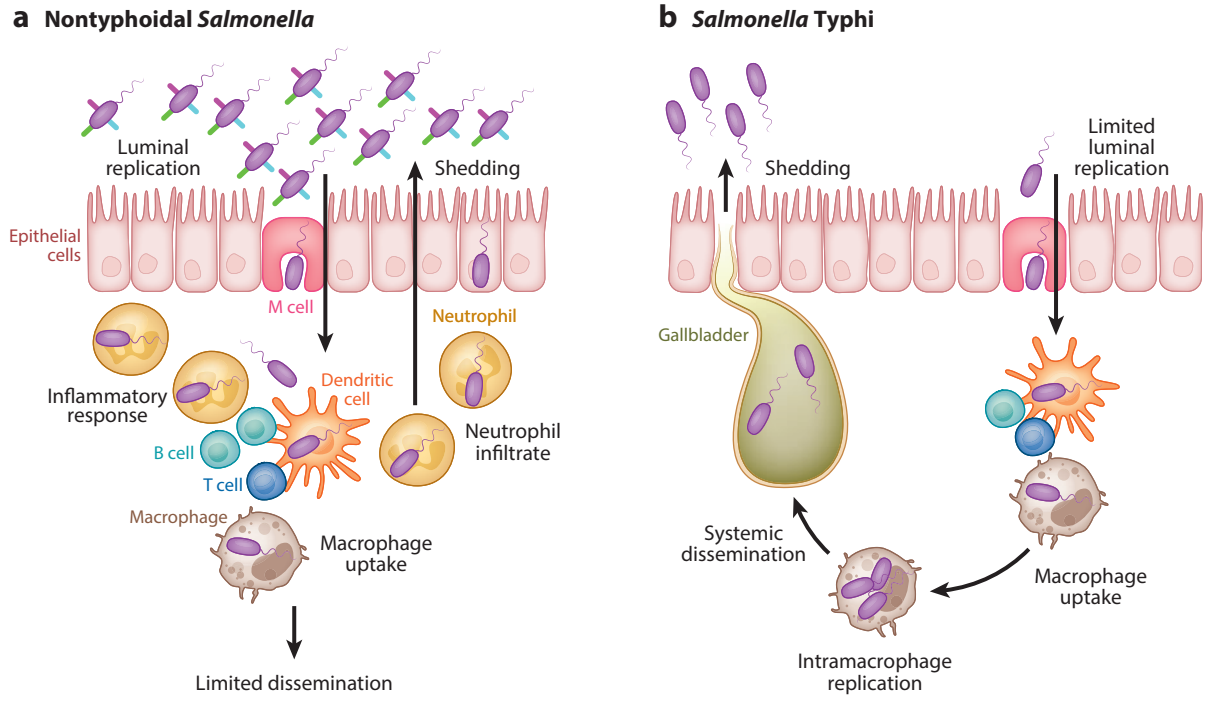

Figure 1

Typhoidal and nontyphoidal *Salmonella* infections. A generalized comparison of infection associated with (*a*) gastroenteritis (nontyphoidal *Salmonella*) and (*b*) typhoid (*S.* Typhi). The multicolored fingers for nontyphoidal bacteria represent a repertoire of adhesion molecules. Nontyphoidal strains may consequently have more options in terms of their route of infection within humans. Vi expression and other factors such as modified regulation of gene expression and effector repertoire may influence mucosal inflammatory responses to *S.* Typhi.

young children are generally relatively rare, although there are consistent reports of such infections (87). It is not known why very young children display atypical responses to *S.* Typhi infection.

One of the complications of typhoid is intestinal perforation (93). Histological examinations of perforation sites have identified a combination of acute and chronic inflammation close to the perforation, rather like a hole has been punched through the intestine. Indeed, the intestinal surface adjacent to the primary lesion can be relatively healthy. Interestingly, culturable *S.* Typhi are not normally present at the sites of perforation, although *S.* Typhi DNA can frequently be detected (95). $CD68^+$ macrophages are the predominant immune cell type at perforation sites, although other immune cells such as B and T cells are also present. Thus, typhoid-associated perforation may be similar to Shwartzman and Koch reactions, where there is presensitization (35). Neurological complications of typhoid are rare but do occur and have been reported in Africa and Asia (79, 84).

Salmonella Typhi

S. Typhi is a highly conserved serovar within subspecies 1 of the broader species *S. enterica* (130). The Kaufmann-White scheme classifies *S.* Typhi as Group D with O-antigen type O9-12, phase 1 flagellin type H:*d*, and Vi capsule positivity. Therefore, *S.* Typhi is normally monophasic. Most

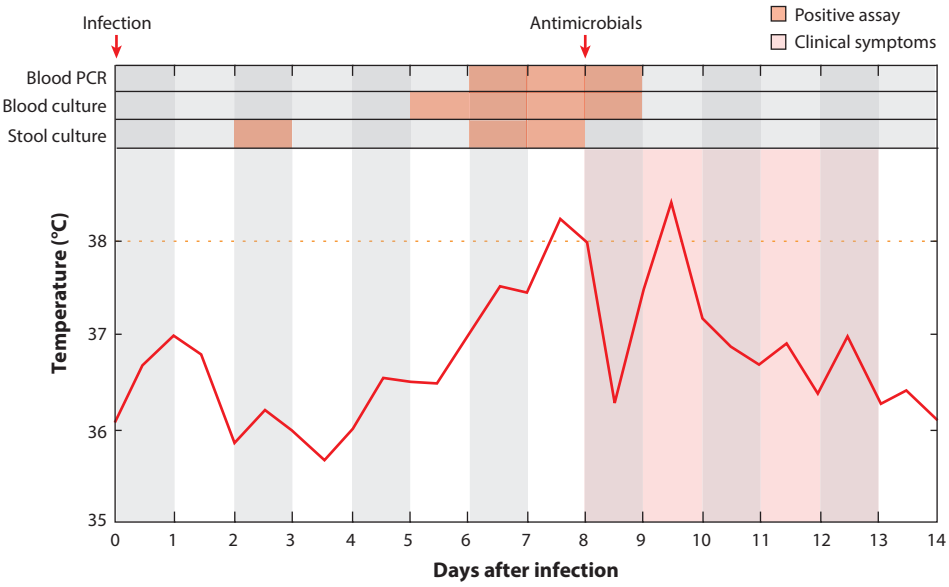

Figure 2

The clinical progression of typhoid fever. Chart depicting the clinical progression of symptomatic typhoid in a volunteer treated with antimicrobials when febrile. The red boxes at the top represent times positive for the outlined assays. Alternate days after challenge are shaded in dark gray, whereas the red-shaded days represent the presence of clinical symptoms. The temperature changes are depicted as twice-daily temperature points. See Reference 139. Abbreviation: PCR, polymerase chain reaction.

S. Typhi fall within the Kaufmann-White classification, but rare isolates are Vi negative. Additionally, some isolates from Indonesia express alternative phase 1 flagellin that type serologically as H:*j* (6). H:*j* flagella harbor a 261-base-pair in-frame deletion of a flexible polypeptide domain, which determines the flagella type (40). The flg locus, which encodes phase 2 flagella in other *S. enterica*, is absent from *S.* Typhi (63). However, Indonesian isolates of *S.* Typhi can express an alternative phase 2 flagellin from the linear plasmid pBSSB1 (4). pBSSB1 also encodes a repressor, FlgB, that represses *fliC*, ensuring strains harboring this plasmid express z66 and not H:*d* (7). A further variant of *S.* Typhi flagella, Ind, has also recently been identified in Indonesian isolates (55).

Multiple-locus enzyme electrophoresis (115) and, more recently, genome sequencing of globally representative *S.* Typhi have generated parsimonious phylogenetic trees onto which all current *S.* Typhi isolates can be mapped (58) (**Figure 3**). Such trees predict that all extant *S.* Typhi evolved from a single organism and that *S.* Typhi is highly monomorphic, likely entering the human population once, relatively recently (112). Indeed, only a few thousand single-nucleotide polymorphisms (SNPs) distinguish large (~2,000) collections of *S.* Typhi isolates sequenced at a whole-genome level (our unpublished data; 58). Recombination is remarkably infrequent, suggesting a genetically isolated population. Although plasmids and phage sequences can move through this population, this is relatively rare (133). SNPs are broadly distributed throughout the genome, and only a few genes have accumulated multiple nonsynonymous mutations. Thus, there is little evidence of antigenic variation in *S.* Typhi, suggesting infection by stealth or immune modulation. Up to a quarter of the *S.* Typhi and *S.* Paratyphi A genomes may originate from a common ancestor, a factor that may be linked to the evolution of host restriction (26).

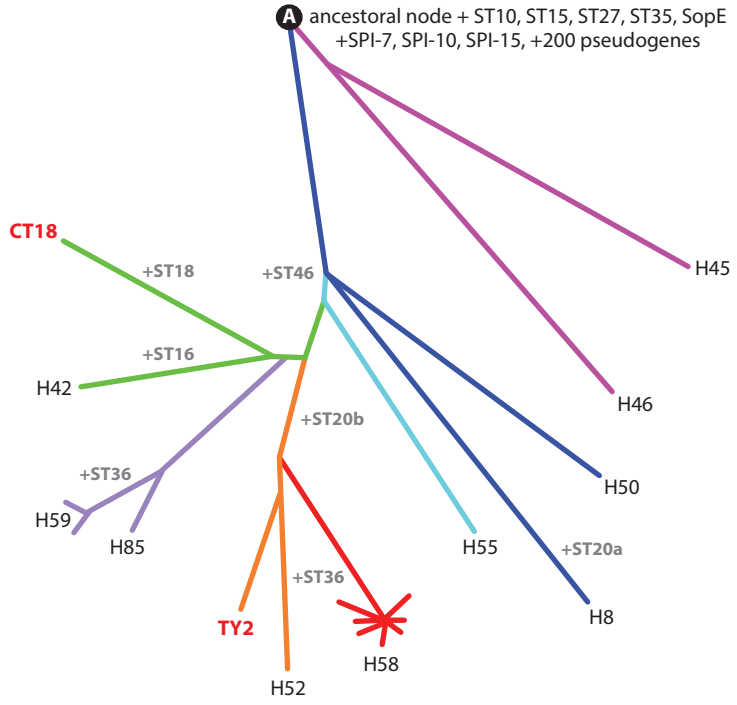

Figure 3

The population structure of *Salmonella* Typhi. A simplified depiction of the *S.* Typhi phylogenetic tree determined based on single-nucleotide polymorphisms (SNPs) present in nonrepetitive regions in the *S.* Typhi genome. The different colors represent distinct lineages. The ancestral node is shown along with genetic signatures present at this point. SNPs and pseudogenes accumulate as the lineages move away from the node. The entry points in the phylogenies of particular phages are marked by their corresponding ST numbers in gray text. Haplotypes are designated at the terminus of each lineage. Haplotype 58 (H58) can be seen on the red lineage as an expanding node. The reference stains CT18 and Ty2 are shown in red text. Other abbreviations: SPI, *Salmonella* pathogenicity islands; ST, specific *Salmonella* Typhi bacteriophage.

Multidrug resistance (MDR) in *S.* Typhi is almost exclusively associated with IncH1 plasmids that have apparently become adapted to this serovar (59). The MDR phenotype has emerged on several occasions at different locations in the phylogenetic tree, but most of these lineages are short lived or geographically restricted. However, one lineage, haplotype 58 (H58), has become dominant, causing an ongoing epidemic that has spread across Asia and into Africa (34, 57, 69). The H58 lineage appears to be able to outcompete other *S.* Typhi, particularly in the face of antimicrobial exposure (83). Fluoroquinolones are a treatment of choice for typhoid, but resistance has emerged (71), mediated in part by mutations within *gyrA*, encoding the DNA gyrase A subunit, a primary target of these drugs (15, 101, 102). *S.* Typhi harboring *gyrA* mutations can be fitter than wild-type equivalent *S.* Typhi in some experimental settings, indicating that such strains may have continued selective advantage even if exposure to fluoroquinolones is removed (2).

A striking feature of the genomes of *S.* Typhi and *S.* Paratyphi A is the accumulation of so-called pseudogenes (24, 60, 90, 99) (**Figure 3**). Genome degradation is regarded as a signature of adaptation and is present in other host-restricted pathogens such as *Yersinia pestis*, *Bordetella pertussis*, and *Mycobacterium leprae*. Pseudogene formation is still ongoing within *S.* Typhi, raising

the possibility that different pathovars are emerging. In the case of typhoid, efficient colonization of the intestine has been lost in favor of privileged systemic sites such as bone marrow and gallbladder. Many of the approximately 200 pseudogenes of *S.* Typhi fall into functional classes, such as those contributing to intestinal persistence (e.g., *ratA* and *shdA*), metabolism (e.g., *rhaD* and *araH*), adhesion (e.g., *pilN* and *sefD*), and the secretome (e.g., *sopA* and *wcaK*) (60).

THE PATHOGENESIS OF TYPHOID FEVER

Much recent work on *Salmonella* infection has focused on *S. enterica* that can infect mice or other tractable animal species. This is because *S.* Typhi is host restricted to humans, although some primates have been experimentally infected (33, 44). Some serovars, such as *S.* Gallinarum and *S.* Pullorum, exhibit host restriction to other animals (in these cases to birds), and comparative studies with typhoid in humans are of value (38). Murine studies using promiscuous *S. enterica* such as *S.* Typhimurium have informed the general molecular mechanisms associated with invasive *Salmonella* pathogenesis but have, arguably, been less enlightening for typhoid disease in humans.

Approximately 90% of the genome of *S.* Typhi is related to sequences present in other *Salmonella* serovars (13, 25, 91). Thus, there is a *Salmonella* core genome that likely facilitates colonization, infection, and transmission. The *S.* Typhi–specific or accessory genome comprises 300–400 genes, many of which are associated with specific phage or *Salmonella* pathogenicity islands (SPIs) (113). For example, *S.* Typhi has four relatively unique SPIs, SPI-7, -15, -17, and -18. SPI-7 is a large mobilizable element that encodes the Via locus (expression of the Vi capsule), a SopE prophage ST44, and a Type IVB pili implicated in cell attachment (107, 137). SPI-18 is a 2.3-kb sequence that encodes the cytolysin ClyA/HlyE (42, 43). *S.* Typhi also encodes a relatively conserved repertoire of phage elements and prophage (133) and multiple SNPs and indels distinct from other *Salmonella* serovars. The potential upshot of this genome variation is that *S.* Typhi has a distinct genetic hardwiring, a view supported by transcriptome and proteomic studies conducted on *S.* Typhi growing in vitro or within eukaryotic cells (1, 36, 105).

Volunteer studies have shown that fecal shedding of *S.* Typhi is relatively intermittent during the subclinical and early clinical stages of typhoid (47). Shedding can become more persistent if patients are untreated, and typhoid carriers can shed high numbers of *S.* Typhi, but here the organisms are likely re-entering the intestine via the bile duct. When volunteers were challenged with either *S.* Typhi or *S.* Typhimurium harboring attenuating mutations in *aroC* (chorismate pathway) and *ssaV* (a component of SPI-2) (56), those challenged with *S.* Typhi Δ*aroC* Δ*ssaV* rapidly cleared the challenge strain, whereas those challenged with *S.* Typhimurium Δ*aroC* Δ*ssaV* shed the bacteria for several weeks and had to be cleared with antimicrobials. This phenomenon may correlate with the presence of mutations in *S.* Typhi genes associated in other serovars with intestinal persistence, e.g., *sbdA*, *ratB*, and *sinH* from the CS54 pathogenicity island (74).

S. Typhi expresses a comparatively unique repertoire of products that influence attachment to, and invasion of, human cells. The Vi capsule, a homopolymer of α(1→4)-D-GalpANAc variably acetylated at the C-3 position, influences attachment, potentially by shielding surface components of *S.* Typhi from host receptors and complement components in vivo (**Figure 4**). A Type IVB pilus has been implicated in attachment to human cells through the cystic fibrosis conductance regulator (108, 138), although others have not found this association (10). The 27-kDa outer membrane protein T2544 of *S.* Typhi targets laminin and has been implicated in virulence (45). Gene loss may also influence attachment to host cells. In *S.* Typhi, genes within many fimbrial loci harbor likely null mutations (76), suggesting that the fimbrial repertoire may be compromised (9, 114, 134). *S.* Typhi also harbors mutations within the mannose-specific type 1 fimbrial adhesin FimH, which may be associated with host adaption (76).

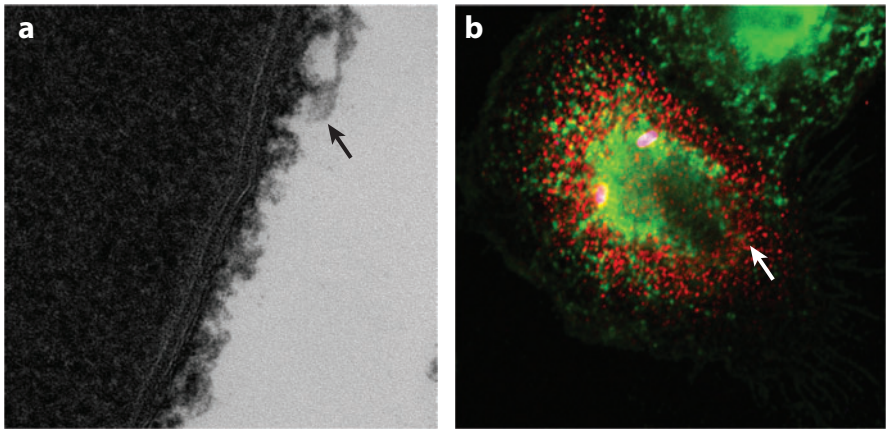

Figure 4

The Vi antigen of *Salmonella* Typhi. (*a*) Negatively stained transmission electron micrograph of *S.* Typhi showing the Vi capsule (*black arrow*) covering the bacterial surface. (*b*) *S.* Typhi (stained in *pink*) resident within a human primary macrophage. Extracellular Vi (*white arrow*) is stained in red.

A GENERAL LACK OF INFLAMMATION AND DIARRHEA DURING ACUTE TYPHOID

S. Typhi has the ability to pass through immunological checkpoints including lymph nodes that constrain other *Salmonella*. Further, *S.* Typhi bypasses these without stimulating a rapid clinical response. Indeed, individuals in typhoid endemic regions who have never reported having typhoid can have elevated anti-Vi antibody levels, suggesting that subclinical infections occur (70, 110). Importantly, the number of *S.* Typhi passing into the bloodstream is probably small (**Figure 1**); again, this highlights that typhoid is not a classical bacterial sepsis. It is noteworthy that the numbers of *S.* Typhi recoverable from the blood and bone marrow of patients with typhoid are remarkably low, with a median of 1 cfu/mL of blood and 10 cfu/mL of bone marrow (94, 140, 141).

Intestinal inflammation is not generally regarded as a feature of typhoid. Local inflammation may enhance the infections by NTS by disrupting the microbiota and reducing interbacterial competition (123). Promiscuous *Salmonella* can manipulate the metabolic environment within the intestine, providing a growth advantage over competitors (131). For example, the influx of neutrophils during gastroenteritis can generate enhanced levels of reactive oxygen with the potential to oxidize endogenous thiosulphate to form tetrathionate, an alternative electron acceptor available to the pathogen (147). Genes conferring the ability to exploit tetrathionate are present in most *Salmonella*, but *S.* Typhi harbors mutations in some of these genes, e.g., *ttrS*, potentially further affecting the ability of *S.* Typhi to colonize the intestine.

S. Typhi clearly has a modified metabolic and adhesive potential and may enter human tissue via a specialized route with limited intestinal replication. We do not know the precise mode of entry of *S.* Typhi into human tissues during typhoid, but it may involve targeting of M cells (**Figure 1**) (65). Dendritic cells in the intestinal mucosa also play a key role in mouse typhoid, but whether they play any role in human typhoid is less clear (111, 125). *S.* Typhi harbors a number of pattern-associated molecular patterns (PAMPs), including lipopolysaccharide (LPS), flagella, and peptidoglycan (51, 61). Flagella expression may be downregulated when *S.* Typhi interacts with human cells, potentially diminishing the inflammatory response (146). Further, the expression of flagella is coregulated in *S.* Typhi with components of SPI-1 and the Vi polysaccharide (53). The Vi

capsule has anti-inflammatory properties, limiting the deposition of complement component CR3 onto the bacterial cell surface, restricting immune activation, and increasing resistance to serum killing (**Figure 4**). Vi may also modulate interactions between LPS and TLR4 and increase the local concentrations of the immunoregulatory cytokine interleukin 10 (IL-10) in the infected tissues (64, 145). The expression of Vi is subjected to a remarkable level of transcriptional regulation (106), and the Vi capsule is also rapidly released from the surface of *S.* Typhi once the bacteria are within human cells (**Figure 4**). Furthermore, *S.* Typhi harbors a mutation in *fepE*, which encodes a regulator of O-antigen chains, and this genotype may limit the exposure of O side chains to immune cells (19). While we consider the role of Vi in *S.* Typhi, it is important to note that *S.* Paratyphi A does not express Vi yet causes a typhoid-like disease (88). Thus, multiple interwoven mechanisms are likely associated with the lack of obvious early-onset inflammation in typhoid.

SALMONELLA TYPHI GENE PRODUCTS

S. Typhi produces a repertoire of products that contribute to pathogenesis, many of which are shared with other *Salmonella* serovars. However, *S.* Typhi does produce products, such as the Vi capsule, that are classically associated with this serovar. *S.* Typhi and *S.* Paratyphi A also express typhoid toxin, which harbors three polypeptides assembled in an A_2B_5 structure (119). Typhoid toxin targets eukaryotic polysaccharides via the B subunit encoded by *pltB*. This B subunit is linked to an ADP ribosylating protein PltA, which in turn is linked via a disulphide bond to CdtB, a cytolethal distending toxin-like component (52). Typhoid toxin is highly immunogenic, and its expression is significantly upregulated when *S.* Typhi enters human cells (122). The purified toxin is highly potent in mice, reproducing some of the symptoms associated with typhoid, although it is not significantly pyrogenic (119). PltA harbors homology to other ADP ribosylating toxins, including cholera and pertussis toxin (122), which are key proteins for both pathogenicity and the induction of protective immunity. Thus, it is likely that typhoid toxin plays a key role in the interplay of *S.* Typhi with the human host; currently we can only speculate what this function may be, but this is clearly an area that warrants further investigation.

Ex vivo experiments using gentamicin protection assays have provided evidence for *S.* Typhi exploiting an intracellular niche, likely predominantly within monocytes and macrophages. However, anti-Vi antibody responses are protective in humans, so an extracellular growth phase is also likely critical. *S.* Typhi harbors functional SPI-1 and SPI-2 systems, although some have argued that SPI-2 may not be required for certain aspects of its pathogenicity (39). The repertoire of functional SPI-1 and SPI-2 effector proteins is distinct from that of other *Salmonella* serovars, and some effectors found in other *Salmonella* serovars are encoded as pseudogenes in *S.* Typhi and/or Paratyphi A. For example, the SPI-1 effector protein SopA, with activity that mimics mammalian HECT E3 ubiquitin ligases, is a pseudogene in both *S.* Typhi and *S.* Paratyphi A (151). *S.* Typhimurium expressing inactivated SopA mutant proteins exhibits a reduced ability to promote polymorphonuclear leukocytes' transepithelial migration through monolayers, and others have predicted a role for the SopA protein of *S.* Dublin in the induction of enteritis (148, 151). Thus, the inactivation of SopA in *S.* Typhi may have implications for the induction of diarrhea and inflammation in humans. The gene encoding SopE2 is also a pseudogene in *S.* Typhi, as is the gene encoding the SPI-2 effector SseJ, which exhibits amino acid similarity to GDSL lipases associated with glycerophospholipid:cholesterol acyltransferase (GCAT) activity (77, 78). The impact of the inactivation of these effectors in *S.* Typhi on pathogenicity in humans is unknown, but this could limit pathogenic potential in other hosts. For example, if a functional *S.* Typhimurium *sseJ* gene is expressed in *S.* Typhi the recombinant bacteria exhibit altered proliferation and cytotoxicity in human cell lines (136).

Other studies have used non–*S.* Typhi effectors to provide insight into host cell restriction of *S.* Typhi. *S.* Typhimurium (but not *S.* Typhi) expresses the effector protein GtgE, which targets the degradation of the Rab32 GTPase (121). Engineered *S.* Typhi expressing a functional GtgE exhibits increased survival in macrophages derived from mouse bone marrow and in the tissues of mice. The impact of GtgE is mediated through the targeting of Rab32 rather than Rab29, which influences traffic to the *Salmonella*-associated vacuole (SAV) in conjunction with components of the biogenesis of lysosome-related organelle complexes (BLOCs). The manipulation of SAV within human tissues could clearly affect the balance between host survival and susceptibility and the repertoire of susceptible host species.

THE CARRIER STATE

One of the classical features of typhoid is the carrier state. Human carriers, such as "Typhoid" Mary Mallone, are a potential source of typhoid, as they can shed high levels of *S.* Typhi while being outwardly asymptomatic and leading a normal life (49). The molecular mechanisms involved in establishing the carrier state are poorly understood, but the colonization of the gallbladder is key to *S.* Typhi being maintained for long periods in the human host (48). The precise risk factors for becoming a persistent carrier and the organisms causing carriage are not well described, as this is a very challenging population to investigate (150). Various studies have found that between 2% and 4% of individuals in endemic regions can be carriers. Carriers can harbor abnormally high anti-Vi antibody titers (14, 96), presumably because they are boosted by persistent expression of the T cell–independent antigen Vi. Recent work has indicated that the levels of antibodies to a range of *S.* Typhi antigens can be enhanced during carriage (14). Gallbladder damage and gallstones can potentially contribute to carriage (18), and other gram-negative bacteria, most notably *S.* Paratyphi A, can also colonize the gallbladder (27, 72). The precise role of gallstones, the immunological mechanisms maintaining carriage, and the location of *S.* Typhi within the gallbladder are questions that currently remain unanswered, although there is evidence that biofilm formation may be important (17).

IMMUNITY TO *SALMONELLA* TYPHI

Most research on immunity to *Salmonella* is conducted in mice using nontyphoidal strains, so the relevance of such studies to human typhoid is difficult to gauge. The symptoms of human typhoid are associated with classical immune pathology, and many clinical studies have outlined elevated cellular responses and cytokine expression during acute typhoid (62). There is a drop in systemic zinc concentration and the levels of circulating platelets and lymphocytes during typhoid, and anemia is common (104, 144). Some signatures of liver involvement and damage can also be detected in the blood during the acute stage; for example, there is a rise in the levels of α1-antitrypsin and in high-molecular-weight kininogen clotting activity (16). Also, historical observations on pathological specimens have identified granuloma-like lesions in the liver.

Few immunological studies on human typhoid have exploited defined *S.* Typhi antigens for measuring specific response. Significant interferon γ (IFN-γ) responses were detected in cells stimulated with various antigens including fimbriae and outer membrane proteins using polymorphonuclear cells from the blood of patients with typhoid (117). The majority of IFN-γ-expressing T cells were $CD4^+$, although antigen-specific CD8 responses were also detected. When whole blood from typhoid patients was stimulated with LPS, the levels of tumor necrosis factor α (TNF-α) release were lower during acute typhoid fever than after antimicrobial treatment, providing some evidence of immune suppression. Antigen arrays and other probing

techniques have been used to interrogate the antibody response during early infection (14, 54, 81, 116), detecting IgG and IgM responses to a range of antigens that can distinguish typhoid patients from asymptomatic controls (81). These data may suggest new approaches to diagnosis (3).

Another approach to investigating the host response during typhoid has been to perform transcriptome studies on blood during acute disease and convalescence (73, 132). Typhoid patients have a blood transcriptome signature distinct from other patients with fever or from those suffering from malaria or dengue. The blood transcriptome indicates a systemic activation of neutrophils, even though the concentration of neutrophils in the blood remains relatively stable. The data are generally concordant with the transient decrease in platelets and lymphocytes associated with short-lived anemia. Many immune signatures are disrupted during the acute disease, with a strong IFN-γ signature present. Interestingly, although the transcript profile of most patients returns to baseline, some individuals maintain a distinct convalescent signature for many months, and these could still be carrying *S.* Typhi, although this is unproven. An alternative RNA capture and amplification technique known as selective capture of transcribed sequences was used to characterize the *S.* Typhi genes expressed in blood (22, 116), identifying a repertoire of genes that are expressed in vivo.

There is evidence that *S.* Typhi infection is not solidly protective and that people can be reinfected within weeks or months of a primary episode. Relapse may be a consequence of either recrudescence by the same strain from within tissues or reinfection from an environment source, sometimes with a different haplotype of *S.* Typhi (142). Typhoid vaccination studies have shown that protection can be induced by a variety of different vaccines, including those based on live attenuated bacteria (40, 127–129, 135) and Vi (8, 86, 124). Interestingly, live vaccines that do not express Vi (e.g., Ty21a) can induce protection against *S.* Typhi (67, 80), indicating that there are both Vi-dependent and Vi-independent protective mechanisms. Immune responses to Vi are clearly protective and can direct killing by complement and direct opsonic killing mechanisms but the exact protective correlate remains elusive.

A number of live oral vaccines have been developed from attenuated mutants of *S.* Typhi. Ty21a has been licensed for many years and can induce significant protection against *S.* Typhi if administered in multiple doses (65, 66, 80). Other experimental vaccines are in various stages of development but are still some way from being licensed. These can induce a significant antibody response as well as cellular responses in volunteers (41, 143), and several genes have been shown to be essential for virulence in humans. Such attenuating genes include *aroC/D* (aromatic metabolism, chorismate pathway), *htrA* (degradation of unfolded proteins), *crp/cya* (cyclic AMP metabolism), *phoP* (Pho regulon), and *ssaV* (SPI-2 secretion system). *S.* Typhi engineered to be defective in the chorismate pathway (*aroC*, *aroD*) can be detected for short periods in the bloodstream, whereas derivatives harboring these *aro* mutations alongside mutations in either *htrA* or *ssaV* survive less well in blood (128). Highly sophisticated studies have dissected the immune response to typhoid vaccines, providing important insight into the immunity induced by vaccination (41, 66, 67, 126).

DEVELOPING A PHYSIOLOGICALLY RELEVANT MOUSE MODEL OF TYPHOID

S. Typhi is attenuated in mice, even in inbred lines that are naturally hypersusceptible to *S.* Typhimurium and other promiscuous *Salmonella* serovars. For many years, impure reagents such as hog gastric mucin were used to potentiate the infectivity of *S.* Typhi in mice, but such approaches were deemed to be too nonspecific to be of real experimental value (109). Many highly immunocompromised transgenic mouse lines are also not readily infected by *S.* Typhi, suggesting that there are complex mechanisms behind the inability of *S.* Typhi to infect mice

(the authors' own observations). However, several groups have recently demonstrated that humanized mice can be used to establish *S.* Typhi infections in vivo (11, 92). These models exploit immunocompromised mice lacking the ability to make antibody or T cells and use human stem cells obtained from different tissues to complement these missing components. Nonobese diabetic *scid IL2rγ^{null}* mice grafted with human hematopoietic stem cells can be colonized if challenged parenterally with *S.* Typhi, with the inoculum undergoing several rounds of replication (~20) (82). These *S.* Typhi–infected mice develop pathological lesions, including granulomas, and some of the challenged mice succumb to infection. Rag2$^{-/-}$ $\gamma^{-/-}$ mice engrafted with human fetal liver cells also become infected, although the pathology and symptoms appear to be less severe (120). Finally, Rag2$^{-/-}$ $\gamma^{-/-}$ mice engrafted with CD34^{+} human blood stem cells can be infected with *S.* Typhi, exhibiting significant clinical pathology and some mortality (92). *S.* Typhi replication in these mice is dependent on the presence of the engrafted cells, and human cellular responses can be detected. Further, these models have the potential to be used to discriminate between *S.* Typhi and mutant derivatives in terms of pathogenicity and virulence. It will be important to further develop these models, including the ability to cause infection by the oral route.

Intriguingly, mice lacking a key immune surveillance receptor, TLR11, are hypersusceptible to *S.* Typhi infection by the oral route (89). Indeed, such mice can succumb to infection with relatively low challenge doses even though they harbor relatively low levels of *S.* Typhi in their livers. TLR11 is a pattern recognition receptor that, in common with TLR5, recognizes bacterial flagellin. An equivalent of TLR11 does not appear to be expressed in human cells, and the TLR11 gene is absent from the human genome. TLR11 is expressed in the mouse intestine and is hyperexpressed in certain cells if TLR5 is absent. Notably, TLR11$^{-/-}$ mice are hypersusceptible to both *S.* Typhimurium and *S.* Typhi following both oral and parenteral challenge. *S.* Typhi harboring mutations that prevent flagella production, unlike aflagellated *S.* Typhimurium, are attenuated in these TLR11-defective mice. These mice could also be protected against *S.* Typhi challenge using an inactivated whole cell vaccine or immune sera, extending their potential value as a model. The mechanisms by which TLR11 mediates restriction of *S.* Typhi growth are not clear, but further investigations in these mutant mice are warranted.

GENETIC SUSCEPTIBILITY TO *SALMONELLA* TYPHI IN HUMANS

Few studies have attempted to elucidate human genetic susceptibility to typhoid. A number of rare genetic diseases have been linked to general susceptibility to *Salmonella*; for example, individuals harboring mutations affecting the IL-12 or IFN-γ pathways are hypersusceptible to NTS, but such mutations have not been associated with susceptibility to typhoid (28, 85). The major histocompatibility complex class II and class III loci have been implicated in typhoid susceptibility (32). *HLA-DRB1*0301/6/8*, *HLA-DQB1*0201-3*, and polymorphisms in TNF-α were associated with susceptibility to typhoid, whereas *HLA-DRB1*04*, *HLA-DQB1*0401/2*, and other TNF-α alleles were associated with protection against disease. An additional study identified other SNPs in the TNF locus association with typhoid (31). Further studies on both TLR5 (29) and NRAMP (30) did not find an association with human typhoid. Additional investigations have identified an influence of polymorphisms in the TLR4 locus in humans on typhoid, but in reality much larger cohorts or new approaches may be required to bring sufficient power to these data.

SOME THOUGHTS FOR THE FUTURE

We have made enormous progress in our understanding of the molecular pathogenesis, evolution, epidemiology, and immunology of *Salmonella* infections in recent decades (23). Despite relatively

little work being focused on human typhoid and *S.* Typhi there have been key advances, some arguably spectacular. *S.* Typhi, as a human-restricted pathogen that survives poorly in the environment, is trapped in the human population. Thus, improvements in public health and the management of water supplies could lead to the control and even the local elimination of this disease. However, such improvements are not likely to reach all areas where typhoid is endemic in the near future. Even though we may be able to develop improved murine models, perhaps based on mice engineered to express a more humanlike immune system, such models will likely have a relatively limited impact on typhoid control. What is really needed is a diagnostic that can work in the field and that can identify individuals in real time who are infected with *S.* Typhi or are asymptomatic carriers (3). With ever-improving technology this is, perhaps, a tractable short-term goal, and research in this area should be encouraged. A new generation of typhoid vaccines using Vi antigen conjugated to carriers can potentially improve efficacy, and they are likely to be licensed with World Health Organization prequalification in the future. Such vaccines may induce significant herd immunity if used appropriately, and targeted campaigns could be used to reduce the geographical range and incidence of typhoid. Further, improved genetic analysis employing haplotyping of *S.* Typhi isolates could identify transmission routes, further facilitating control (5).

CONCLUSIONS

Although *S.* Typhi is clonal, the organism is undergoing continuous evolution in terms of genome degradation and adaptation to antimicrobial therapies. The emergence of antimicrobial-resistant isolates is a threat, particularly as clones such as H58 appear to have an ability to cause epidemic disease and spread to areas where typhoid was previously rare. There is also a need to be aware about the potential emergence of other *Salmonella* serovars with invasive potential. The spread of untreated HIV disease and the uncontrolled use of antimicrobials may have facilitated the emergence of a specific *S.* Typhimurium clade (ST313) that has the propensity to cause invasive salmonellosis in humans (37). The genome of ST313 *S.* Typhimurium harbors some signatures of genome degradation that in part resemble those in the *S.* Typhi genome (75). Thus, ST313 *S.* Typhimurium may be adapting to the human host, although it generally retains the ability to infect other hosts. There is currently no obvious zoonotic source for the ST313 *S.* Typhimurium in parts of Africa, and there is some evidence of transmission between humans without an intermediate host (68). Thankfully, although untreated HIV disease is predisposing to invasive disease by NTS, that does not appear to be the case for typhoid. Indeed, some have postulated that HIV may actually be protective for the expression of clinical typhoid (50). This is another area that merits further research, and clinical microbiologists need to remain vigilant for the appearance of novel *Salmonella* strains causing invasive disease in immunocompetent and immunocompromised individuals.

Finally, this review has focused on *S.* Typhi, but *S.* Paratyphi A (and Paratyphi B and C) can cause typhoid-like disease in humans. *S.* Paratyphi A is common in parts of Asia, particularly in China and Nepal, where the disease incidence can outnumber that of *S.* Typhi. Very little is known about the pathogenesis of paratyphoid disease, and there is currently no vaccine. It is particularly interesting that *S.* Paratyphi A does not express the Vi antigen, so obvious questions remain about how this pathogen survives in the gastrointestinal tract, invades, and moderates inflammation in humans.

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