Review Article

Clinical pathogenesis of typhoid fever

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Abstract

Human infections with *Salmonella enterica* results in two major groups of diseases: gastroenteritis and typhoid fever. Clinical observations suggest that gastroenteritis, caused by non-typhoidal *Salmonella* serovars, is characterized by a massive neutrophil influx, which keeps the infection localized to the intestinal mucosa. In contrast, the absence of neutrophilic intestinal infiltrates in the acute phase of typhoid fever suggests a propensity for typhoidal *Salmonella* serovars (*S.* Typhi, *S.* Paratyphi A, *S.* Paratyphi B and *S.* Paratyphi C) to evade aspects of the innate immune response and cause a systemic infection. The fact that there are no virulence genes shared by typhoidal *Salmonella* serovars that are absent from non-typhoidal *Salmonella* serovars, suggests that this innate immune evasion is mediated by different mechanisms in different typhoidal serovars. This review discusses what is known about the clinical pathogenesis of typhoid fever.

Key Words: *Salmonella*, Typhi, pathogenesis

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Introduction

Typhoid fever, one of the major bacterial infections worldwide, is caused by the human-adapted *S. enterica* serovar Typhi [1]. For every ten cases of *S.* Typhi infection, there are one or two cases of paratyphoid fever, caused by the human-adapted *S. enterica* serovars Paratyphi A, Paratyphi B and Paratyphi C [1]. Since paratyphoid fever is indistinguishable from typhoid fever in its clinical course, *S. enterica* serovars Typhi, Paratyphi A, Paratyphi B and Paratyphi C are collectively referred to as typhoidal *Salmonella* serovars. For unknown reasons, the frequency of infection with serovar Paratyphi A is increasing in some areas of the world [2]. The highest incidence rates of typhoid fever can be found in Asia, particularly in the South-central and South-eastern regions, where an estimated 100 cases/100,000 population occur each year [1]. The improvement in sanitary conditions and health care is thought to be a key factor in reducing the incidence of typhoid fever. However, sanitation cannot explain why in Africa there is a lower incidence of typhoid fever when compared to Asia (10 cases/100,000 per year) [1].

Clinical differences between typhoid fever and gastroenteritis

Typhoid fever is transmitted by ingestion of food or water contaminated with the typhoid bacillus. This route of infection is shared with non-typhoidal *Salmonella* serovars (NTS), the causative agents of gastroenteritis. However, the diseases caused by these two distinct groups of bacteria are very different from a clinical perspective. While gastroenteritis caused by NTS is characterised by a rapid onset after a short incubation period (12-72h) and a brief duration (<10 days), typhoid fever has a considerably longer incubation period (median of 5 to 9 days) and longer duration of symptoms (fever persists for approximately three weeks). Also, gastroenteritis is an infection that remains localized to the intestine and mesenteric lymph nodes in immunocompetent patients, while typhoid fever is a systemic infection during which *S.* Typhi colonizes the liver, spleen and bone marrow in addition to the intestine and the mesenteric lymph nodes [3,4]. The short clinical course of gastroenteritis suggests that the onset of an adaptive immune response results in clearance of the infection. On the contrary, *S.* Typhi can persist in human tissue for long periods

of time, indicating that the organism has a greater propensity to evade immune responses than non-typhoidal *Salmonella* serovars.

S. Typhi overcomes the mucosal barrier in immunocompetent individuals

NTS are unable to overcome defence mechanisms that limit bacterial dissemination from the intestinal mucosa to systemic sites of infection. However, NTS bacteraemia may occur in patients with impaired immunity [5,6]. Perhaps the most important group of patients that are at high risk for NTS bacteraemia are individuals affected with acquired immunodeficiency syndrome (AIDS). Because of the high prevalence of AIDS in sub-Saharan Africa, NTS have become a leading cause of bacteraemia [7-11]. Although AIDS patients develop NTS bacteraemia at a considerably higher frequency than healthy individuals, the frequency of typhoid fever does not differ [12,13]. These epidemiological observations suggest that some components of the immune response, which are defective in AIDS patients, are required to prevent systemic dissemination of NTS. In contrast, *S.* Typhi does not require an immunodeficient host to cause a systemic infection, as shown by its ability to cause typhoid fever in both healthy individuals and AIDS patients with similar efficiency. Thus, unlike NTS, it is likely that *S.* Typhi possesses unique virulence traits that allow it to overcome mucosal barrier functions in the immunocompetent host.

One aspect of the immune response that is important to control infection with NTS but not with *S.* Typhi is the interleukin (IL)-12/interferon (IFN)-γ axis. Mutations in genes that encode components of the IL-12/IFN-γ axis increase patients' susceptibility to infections with NTS, but not to infections with *S.* Typhi [14,15]. In agreement with these clinical observations, a recent study showed that polymorphisms in *IFNG*, *IL-12B* and *IFNGR1* do not appear to contribute to increased susceptibility to typhoid fever [16]. A possible explanation for this observation is that *S.* Typhi (but not NTS) may possess virulence mechanisms that allow it to evade the TLR-dependent IL-12/IFN-γ axis. The IL-12/IFN-γ axis is a major immunoregulatory system that bridges innate and adaptive immunity and is induced by stimulation of Toll-like receptors (TLRs) in macrophages and dendritic cells [17]. IL-12 and IFN-γ are important for the control of *S.* Typhimurium infection in mice [18-20] and evasion of the IL-12/IFN-γ axis by *S.* Typhi may help explain its greater propensity to evade immune responses encountered in tissue.

NTS trigger a stereotypical host response that results in inflammatory diarrhoea

An important difference between typhoid fever and gastroenteritis is the host response elicited in the intestinal mucosa. Gastroenteritis caused by NTS is a typical diarrhoeal disease characterised by exudative inflammation, with neutrophil recruitment in the terminal ileum and colon and the predominance of neutrophils in stool samples of patients [21-23]. Invasion of the intestinal mucosa by NTS is detected by the innate immune system of the host, which responds by recruiting neutrophils to the site of infection [24]. The innate immune system can distinguish between self and microbial intruders by recognizing molecular patterns exclusively found in microorganisms through pathogen recognition receptors (PRRs), including the membrane localized TLRs and the cytosolic Nod-like receptors (NLRs). Expression of PRRs at the basolateral (but not the apical) pole of intestinal epithelial cells and on cells in the lamina propria (e.g. macrophages and dendritic cells) enable the innate immune system to distinguish lumenal from invasive microbes. This allows for an appropriate response to invasive enteric pathogens, such as NTS, by triggering exudative inflammation [25, 26]. Because a small number of PRRs triggers the bulk of transcriptional changes through activation of the transcription factors NF-κB, AP-1 and IRF3 in response to bacterial invasion, this host response is not specific to a pathogen but rather to a group of enteroinvasive organisms, including *Shigella* spp., *Campylobacter* spp. and NTS [21-23]. The resulting neutrophil influx and inflammatory diarrhoea are a stereotypic host response to bacterial invasion of the intestinal mucosa [27].

Clinical data suggest that *S.* Typhi may evade innate immune responses

In contrast to gastroenteritis, typhoid fever is not a typical diarrhoeal disease and the intestinal pathology is characterised by interstitial inflammation with predominantly mononuclear infiltrates, while neutrophils are scarce [23, 28-31].

Diarrhoea, which may occur after the onset of fever in approximately one third of typhoid fever patients, is associated with faecal leukocyte populations that are dominated by mononuclear cells [23,32,33]. The fact that typhoid fever is a very invasive infection raises the question why penetration of the human intestinal mucosa by *S.* Typhi does not trigger the stereotypic host response (i.e. neutrophil influx) that is typically observed during infection with other enteroinvasive pathogens. Several tissue culture studies support the concept that *S.* Typhi infection results in reduced inflammatory responses when compared to NTS, such as *S.* Typhimurium. Gene expression analysis in intestinal epithelial cells shows that unlike *S.* Typhimurium, *S.* Typhi does not trigger a pro-inflammatory response through TLR5 stimulation [34]. Also, while *S.* Typhimurium triggers neutrophil transmigration across a monolayer of polarized colonic epithelial cells, *S.* Typhi does not [35]. Furthermore, infection of macrophage-like cells with *S.* Typhi results in markedly reduced production of the neutrophil chemoattractant interleukin (IL)-8 compared to infection with *S.* Typhimurium [36]. Collectively, these observations raise the possibility that *S.* Typhi expresses virulence mechanisms allowing it to down-regulate a PRR-mediated host response in the intestinal mucosa that results in the absence of neutrophil infiltration and inflammatory diarrhoea.

The hypothesis that *S.* Typhi can evade PRR-mediated host responses is consistent with two additional clinical observations. First, pyrogenic cytokines, like tumor necrosis factor (TNF)-α and IL-1β, are elevated in sera of typhoid fever patients compared to healthy individuals [37, 38] but to a lesser extent than in sera of patients with sepsis [39-41]. Also, higher concentrations of bacteria in the blood would predict a more severe prognosis in patients with Gram-negative sepsis but not in typhoid fever patients [42-45]. An attenuation of PRR-mediated host responses may be one of the reasons septic shock appears not to contribute to mortality during typhoid fever. Although polymorphisms in the tumor necrosis factor alpha promoter region (*TNFA-308*) were found to be associated either with increased susceptibility to typhoid fever or with its severity [46,47], most polymorphisms in pro-inflammatory genes (*TNFA-238*, *IL1A*, *IL1B*, *TNFRSF1A*, *CASP1* and *CRP*) do not seem to contribute to increased susceptibility to typhoid fever [16]. These epidemiological findings seem to provide support for clinical observations that septic shock does not occur in typhoid fever patients because *S.* Typhi does not trigger a strong pro-inflammatory response through PRR signalling.

The second clinical observation is the following: patients with chronic granulomatous disease (CGD), a primary immunodeficiency preventing the generation of phagocyte oxidative burst, are more susceptible to infection with NTS and frequently develop bacteraemia [48]. However, there is no positive correlation between the frequency or severity of typhoid fever and CGD. The induction of an oxidative burst in professional phagocytes depends on TLR signalling, since activation of NADPH oxidase by MAP kinases depends on the TLR adaptor protein MyD88 [49, 50]. During a systemic bacterial infection, leukocytes generally produce more reactive oxygen intermediates, as indicated by an increased ability to reduce nitroblue tetrazoleum (NBT). Based on this observation, it has been proposed that an NBT blood test can be used clinically to differentiate bacterial sepsis from other fevers (i.e. from local enteric diseases, viral infection, plasmodium infection or fever not caused by an infectious agent) [51]. Interestingly, this study found that typhoid fever (but not bacteraemia with NTS) is associated with a false negative NBT blood test, indicating a markedly reduced oxidative response in blood of patients infected with *S.* Typhi [51]. Moreover, neutrophils isolated from human volunteers exhibit low oxygen consumption when infected with virulent *S.* Typhi expressing a capsule [52, 53]. These data show that one TLR-dependent host response evaded by *S.* Typhi is the generation of an oxidative burst during interaction with phagocytes. The above review of clinical observations suggests that many differences between typhoid fever and gastroenteritis may be explained by assuming that *S.* Typhi modulates aspects of the innate immune response.

Relatively few studies directly investigate the pathogenesis of typhoid fever, but instead use a surrogate host (i.e. mice instead of humans) and a surrogate pathogen (i.e. *S.* Typhimurium instead of *S.* Typhi) to model the infection. This approach has been highly successful in identifying and

characterizing some major virulence mechanisms common to enteric fever and NTS. However, an important limitation of this approach is that NTS cause a typhoid-like disease solely in the mouse, but not in humans, where they cause a localized gastroenteritis. Thus, enteric fever serovars must possess virulence mechanisms that are absent from NTS and are responsible for the ability to cause typhoid or paratyphoid fever in humans. Interestingly, virulence traits that contribute to the host response are common to all NTS serovars. These include (i) the type three secretion system encoded on pathogenicity island 1 (T3SS-1), which mediates invasion of the intestinal epithelium; (ii) the type three secretion system encoded on pathogenicity island 2 (T3SS-2), which is required for survival within macrophages; (iii) expression of strong agonists of innate pattern recognition receptors (LPS and flagellin), which are important for triggering a TLR-mediated inflammatory response. These observations suggest that *S.* Typhi must have acquired additional factors that further modulate the host response during infection. For example, a reduction of TLR-mediated responses by *S.* Typhi could help explain the scarcity of neutrophils in intestinal infiltrates, the relatively low levels of TNF-α during bacteraemia, and the inhibition of an oxidative burst in phagocytes. Reduction of TLR signalling may interfere with the induction of the IL-12/IFN-γ axis, which may be one of the mechanisms by which *S.* Typhi evades adaptive immunity and persists in the host.

The *viaB* locus enables *S.* Typhi to evade recognition through TLR4 and TLR5

One of the virulence determinants that allows *S.* Typhi to modulate host responses during infection is the *viaB* locus encoded on *Salmonella* Pathogenicity Island 7 (SPI7) [36,54,55], which is the largest of the genomic regions present in *S.* Typhi but absent in *S.* Typhimurium [56]. The presence of the *viaB* locus allows *S.* Typhi to reduce the production of IL-8 in intestinal epithelial cells, TNF–α in macrophages and neutrophil recruitment into the intestinal mucosa *in vivo* [36, 54, 55, 57-59]. This DNA locus contains genes involved in the regulation (*tviA*), biosynthesis (*tviBCDE*) and export (*vexABCDE*) of *S.* Typhi's Virulence (Vi) capsule [60]. Recent analysis of the *viaB* locus has begun to shed light on the mechanisms by which *S.* Typhi evades aspects of the innate immune surveillance system.

IL-8 production in intestinal epithelial cells can be induced by bacterial flagellin, which stimulates TLR5, a pathogen recognition receptor expressed basolaterally on polarized monolayers [26]. The regulator TviA, which is the activator of the biosynthesis genes of the *viaB* operon, is a repressor of genes outside the *viaB* locus, including the T3SS-1 and the flagella regulon [54, 61]. *In vitro* studies show that under conditions of high osmolarity, expression of TviA, and therefore of the Vi capsule, are repressed, while the T3SS-1 and flagella are expressed [61]. Under conditions of low osmolarity, TviA is expressed, resulting in expression of the Vi capsule and repression of the T3SS-1 as well as the flagella regulon [61]. Because a high osmolarity environment mimics the intestinal lumen, and low osmolarity environment mimics the salt concentration in blood or tissue, a plausible explanation is that *S.* Typhi expresses flagella and T3SS-1 in the intestinal lumen, to achieve maximal colonization. However, once in the lamina propria, *S.* Typhi activates expression of the *viaB* locus (including the regulator TviA) and down-regulates flagella expression to avoid TLR recognition. TviA represses expression and secretion of FliC, the ligand for TLR5, which results in reduced IL-8 secretion by intestinal epithelial cells [54]. Furthermore, introduction of the *tviA* gene into *S.* Typhimurium results in repression of flagellin secretion and IL-8 expression in epithelial cells [54]. Thus, TviA-mediated repression of flagellin explains the low propensity of *S.* Typhi to elicit IL-8 expression in intestinal epithelial cells [34].

Recognition of LPS by TLR4 expressed on macrophages and monocytes during bacteraemia is an important source of cytokine production, most importantly TNF-α. Purified *S.* Typhi LPS is a potent TLR4 agonist, thus raising the question why bacteraemia during typhoid fever is associated with only moderately increased levels of TNF-α. A role of the Vi capsule in evading TLR4 signalling became evident by comparison of TNF-α production in the sepsis mouse model after infection with either *S.* Typhimurium wild-type or an *S.* Typhimurium strain carrying the *viaB* operon [55]. The capsulated strain elicited less TNF-α expression in the liver than the non-capsulated strain in wild-type mice, while no difference in

TNF-α expression elicited by both strains was observed in TLR4$^{-/-}$ mice. Thus, expression of the Vi capsular antigen is required for evading TLR4 dependent responses, suggesting that the Vi capsule is able to mask *S.* Typhi's LPS [55].

Conclusions

The picture emerging from these studies is that the *viaB*-locus allows *S.* Typhi to modulate host responses by evading innate immune surveillance through TLR5 and TLR4. These recent insights into typhoid fever pathogenesis suggest a possible mechanism for numerous clinical observations that hint at the ability of *S.* Typhi to evade aspects of the innate immune system. The fact that *S.* Typhi, *S.* Paratyphi A, *S.* Paratyphi B and *S.* Paratyphi C do not share virulence genes that are absent from non-typhoidal *Salmonella* serovars suggests that innate immune evasion is mediated by different mechanisms by these pathogens. Additional research is needed to identify these virulence mechanisms in typhoidal *Salmonella* serovars lacking the *viaB* region.

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