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Minireview

MINIREVIEW

# Disseminated infections with antibiotic-resistant non-typhoidal *Salmonella* strains: contributions of host and pathogen factors

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**One sentence summary:** Non-typhoidal *Salmonella* species normally cause a self-resolving gastroenteritis, but in individuals with concomitant HIV or malaria infection or certain comorbidities, these pathogens can cause disseminated infections; currently, they are a leading cause of childhood bloodstream infection in sub-Saharan Africa.

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## ABSTRACT

Non-typhoidal *Salmonella enterica* serovars (NTS) are generally associated with gastroenteritis; however, the very young and elderly, as well as individuals with compromised immunity, are at risk of developing disseminated infection that can manifest as bacteremia or focal infections at systemic sites. Disseminated NTS infections can be fatal and are responsible for over 600 000 deaths annually. Most of these deaths are in sub-Saharan Africa, where multidrug-resistant NTS clones are currently circulating in a population with a high proportion of individuals that are susceptible to disseminated disease. This review considers how genome degradation observed in African NTS isolates has resulted in phenotypic differences in traits related to environmental persistence and host–pathogen interactions. Further, it discusses host mechanisms promoting susceptibility to invasive infection with NTS in individuals with immunocompromising conditions. We conclude that mechanistic knowledge of how risk factors compromise immunity to disseminated NTS infection will be important for the design of interventions to protect against systemic disease.

**Keywords:** *Salmonella*; co-infection; malaria; bacteremia

## INTRODUCTION

*Salmonella enterica* serovars are associated with three major disease syndromes: typhoid fever, gastroenteritis and bacteremia. Typhoid fever, a disseminated infection caused by typhoidal *S. enterica* serovars in immunocompetent individuals and characterized by fever and abdominal pain, is associated with ∼433 000 deaths annually (Crump, Luby and Mintz 2004; Crump *et al.* 2008). In immunocompetent individuals, non-typhoidal *S. enterica* serovars (NTS) are associated with gastroenteritis, a localized infection of the terminal ileum and colon manifesting as diarrhea, vomiting and intestinal cramping, which is associated with an estimated 155 000 deaths annually (Majowicz *et al.* 2010). In immunocompromised individuals, including the very young, individuals undergoing cancer chemotherapy and those with inborn or acquired immunodeficiencies, NTS are associated with bacteremia, an extraintestinal infection responsible for ∼681 000 deaths annually (Ao *et al.* 2015). The clinical presentation of NTS bacteremia is characterized by a non-specific

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fever, while diarrhea is commonly absent (De Wit *et al.* 1988). These disseminated infections have also been referred to in the clinical/epidemiologic literature as 'invasive NTS (iNTS) disease' (Gordon 2011).

With a combined annual global death toll of 1.24 million, *S. enterica* serovars are one of the leading causes of human mortality worldwide. More than half of these deaths are attributable to NTS bacteremia, which is most prevalent in sub-Saharan Africa. Epidemiologic studies identified the principal underlying factors for NTS bacteremia in sub-Saharan Africa as very young age, malaria and malnutrition in children, and human immunodeficiency virus (HIV) infection in adults (summarized in Feasey *et al.* 2012). Notably, the age distribution for NTS bacteremia cases in children is quite different from that of typhoid, with NTS bacteremia affecting younger children (<5 years of age), and typhoid cases being identified in older children (Feasey *et al.* 2010). In this review, we will consider features of *S. enterica* serovar Typhimurium (*S.* Typhimurium) currently circulating in sub-Saharan Africa where disseminated NTS infections are highly prevalent. In addition, we will discuss insights into the pathogenesis of NTS bacteremia gleaned from clinical epidemiologic studies, from elegant whole genome analyses of epidemic clones, and from animal models investigating how underlying comorbidities compromise containment of NTS infection.

## PHENOTYPIC CHARACTERISTICS OF THE ST313 STRAINS

Sequence analysis of *Salmonella* Typhimurium blood isolates shows that NTS bacteremia in sub-Saharan Africa is associated with a clone, designated by multilocus sequence typing as sequence type (ST)313, which is rarely reported outside of Africa (Kingsley *et al.* 2009). In contrast, the most common sequence type reported in the rest of the world (including North America and Europe) is ST19. Whole genome sequencing of ST313 isolates identified a number of features that differentiate them from ST19 strains, including distinct repertoires of prophage elements and insertion sequences. In addition, evidence of genome degradation was noted, including pseudogene formation and chromosomal deletions, relative to reference genomes such as LT2 and SL1344 (Kingsley *et al.* 2009; Okoro *et al.* 2015). A more detailed characterization of degraded genome content in the ST313 isolates has revealed some notable differences to strains currently circulating outside sub-Saharan Africa, including predicted loss of functions associated with surface and exported proteins (Okoro *et al.* 2015), suggesting alterations in functions associated with host interaction. In addition, functions associated with utilization of two substrates available in the mammalian intestinal tract have been lost in ST313 strains: tartrate and allantoin (Okoro *et al.* 2015). Tartrate can replenish intermediates in the glyoxylate cycle (Nuccio and Baumler 2014). Allantoin is produced by mammalian gut bacteria via 5-hydroxyisourate hydrolases from uric acid, a product of mammalian purine metabolism (Lim *et al.* 2014). *Enterobacteriaceae* are able to utilize allantoin as a source of nitrogen and carbon (Cusa *et al.* 1999). Interestingly, increased intestinal allantoin content was reported to be a marker of intestinal inflammation in patients with inflammatory bowel disease (Schicho *et al.* 2012), suggesting that allantoin may provide a nutrient source for growth of *Enterobacteriaceae* in the inflamed intestine. Conversely, loss of allantoin metabolism in the ST313 strains may reduce their fitness in the inflamed intestine, as was observed for a set of ST313 strains during infection of chickens (Parsons *et al.* 2013) (Table 1).

Two *in vitro* phenotypes observed for ST313 strains are related to multicellular behavior. One is the loss of the RDAR (red, dry and rough) colony phenotype on Congo red agar that is associated with biofilm formation (Simm *et al.* 2014). This phenotype is caused by defective cellulose production resulting from a nonsense mutation in the biosynthesis gene *bcsG* (Singletary *et al.* 2016), and was shown to be shared by a large panel of ST313 strains (Ramachandran *et al.* 2016). The second phenotype is loss of the stationary-phase catalase KatE, which is protective in high-density bacterial cultures. Together these features of ST313 strains suggest a reduced ability to survive in extreme environments or at high density (Singletary *et al.* 2016).

A further characteristic of the ST313 strains is reduced motility, which correlates with their reduced expression of flagellin compared to ST19 strains (Carden *et al.* 2015; Ramachandran *et al.* 2015). This is of particular interest, since invasive serovars of *Salmonella* such as *S.* Typhi, the causative agent of typhoid fever, downregulate flagellin rapidly on invasion of the epithelium to evade detection by TLR5 and NLRC4, as well as by flagellin-specific CD4 T cells (Winter *et al.* 2010, 2015; Atif *et al.* 2014). A similar pattern of reduced flagellin expression and motility was also observed for *S.* Paratyphi A, which causes a typhoid fever-like disease in humans (Elhadad *et al.* 2015). Further, loss of flagellin expression by *S.* Gallinarum, a poultry-adapted serovar causing fowl typhoid, is part of its adaptation to extraintestinal infection in chickens (de Freitas Neto *et al.* 2013). Consistent with the idea that reduced flagellin expression can promote invasive disease via immune evasion, ST313 strains were less inflammatory in cultured macrophages and induced less NLRC4-dependent pyroptotic cell death; these features may therefore reflect adaptation to disseminated infection (Carden *et al.* 2015).

Comparative studies on host responses to ST19 and ST313 strains in animal models have yielded some conflicting results, which may be attributed to the different animal models utilized and different ST19 strains (with different degrees of invasiveness) used for comparison. However, despite these differences an overall theme emerging from this work is that the ST313 strains are capable of invading the intestinal mucosa and eliciting intestinal inflammation in chicken, mouse, bovine and non-human primate models, findings that are consistent with isolation of ST313 strains from a subset of patients in sub-Saharan Africa with diarrheal disease (Paglietti *et al.* 2013; Kariuki and Onsare 2015). Therefore, while hospital-based studies have identified the ST313 strains as frequent causes of invasive disease, they have not lost their ability to cause intestinal inflammation and diarrhea—the absence of diarrhea in cases of invasive disease is likely to result from host factors that blunt intestinal inflammation (see below Raffatellu *et al.* 2008; Mooney *et al.* 2014). This being said, the ST313 strains appear to have a slightly reduced ability to cause intestinal inflammation in streptomycin-treated mice and bovine ligated ileal loops, compared to ST19 (Okoro *et al.* 2015). While the genetic basis for this phenotype is unknown, it is consistent with the reduced expression in ST313 strains of both flagellin and the Type III effector *sopE*, two proteins that contribute to intestinal inflammation in animal models (Hapfelmeier *et al.* 2004; Winter *et al.* 2009; Carden *et al.* 2015). It is not yet clear how this reduced inflammation observed in animal models with the ST313 strains relates to severity of diarrheal disease from salmonellosis in humans.

**Table 1.** Phenotypes distinguishing ST313 from ST19 strains.

| Phenotype | Genetic difference | Reference |
|---|---|---|
| *In vitro phenotypes* | | |
| Increased utilization of M-Tartrate and tricarbalyllic acid | *ttdA* (tartrate dehydratase) pseudogene in ST313 strains | Okoro *et al.* (2015) |
| Decreased metabolism of L-Tartrate and dihydroxyacetone | *ttdA* pseudogene in ST313 strains | Okoro *et al.* (2015) |
| Decreased stationary-phase catalase production | KatE E117G mutation in D23580 | Singletary *et al.* (2016) |
| Loss of RDAR colony formation | Nonsense mutation in *bcsG* in D23580 | Ramachandran *et al.* (2016); Singletary *et al.* (2016) |
| Increased resistance to low pH | Unknown | Yang *et al.* (2015) |
| Decreased resistance to desiccation | Unknown | Ramachandran *et al.* (2016) |
| Reduced motility in agar | Unknown | Ramachandran *et al.* (2015) |
| Reduced expression of flagellin | Unknown | Carden *et al.* (2015); Ramachandran *et al.* (2015) |
| Acetylation of O antigen | Possibly insertion of BTP1 prophage | Micoli *et al.* (2014); Kintz *et al.* (2015); Onsare *et al.* (2015) |
| *Interactions in cellular infection models* | | |
| Increased survival within macrophages | Unknown | Ramachandran *et al.* (2015) |
| Reduced NLRC4 inflammasome activation | Reduced *fliC* expression | Carden *et al.* (2015) |
| Reduced death of infected macrophages | Reduced *fliC* expression | Carden *et al.* (2015); Ramachandran *et al.* (2015) |
| Reduced invasiveness for non-phagocytic cells | Reduced *sopE* expression | Carden *et al.* (2015); Okoro *et al.* (2015) |
| *Interactions in animal models* | | |
| Increased invasiveness in chickens | Unknown | Parsons *et al.* (2013) |
| Reduced inflammatory changes in bovine ligated ileal loops and streptomycin-treated mice | Unknown | Okoro *et al.* (2015) |
| Equivalent levels of inflammation in rhesus macaque ligated ileal loops and streptomycin-treated mice | Unknown | Singletary *et al.* (2016) |
| More rapid dissemination to mesenteric lymph nodes in mice | Unknown | Singletary *et al.* (2016) |
| Increased colonization of liver and spleen in mice | Unknown | Yang *et al.* (2015); Singletary *et al.* (2016) |

## ARE THE AFRICAN NTS ISOLATES MORE INVASIVE?

Epidemiologic evidence suggests that worldwide most NTS isolated from humans are able to cause bacteremia in a small fraction of individuals who are susceptible as a result of very young age or underlying immunodeficiency (Threlfall, Hall and Rowe 1992). *Salmonella* Typhimurium and S. Enteritidis are the serovars associated most frequently with human gastroenteritis (summarized in Rabsch, Tschape and Baumler 2001), and therefore, these pathogens are also the most common NTS cultured from blood of patients with bacteremia in England and Wales (Threlfall, Hall and Rowe 1992). Similarly, the pathogens most commonly associated with NTS bacteremia in Africa are S. Typhimurium and S. Enteritidis (reviewed in Reddy, Shaw and Crump 2010). However, while NTS bacteremia is rare in high-income countries, the large number of individuals in sub-Saharan Africa that are at risk for this complication due to advanced HIV disease, malnutrition or severe malarial anemia makes NTS bacteremia a major public health challenge in this region.

The finding that African ST313 isolates exhibit accelerated dissemination from the intestine to internal organs in chickens and in mouse infection models suggests that this lineage represents a distinct pathovariant of S. Typhimurium that has evolved, perhaps via genome degradation, to become more invasive (Parsons *et al.* 2013; Yang *et al.* 2015; Singletary *et al.* 2016). Increased pseudogene formation is also evident in genomes of the S. Typhimurium clone DT2, which is associated with extraintestinal disease in pigeons (Kingsley *et al.* 2013). Increased accumulation of pseudogenes in *S. enterica* serovars that are exclusively associated with extraintestinal infections is likely a marker indicating a switch to invasive rather than enteric disease; however, it does not indicate an association with a specific host species (Nuccio and Baumler 2014; Langridge *et al.* 2015; Matthews *et al.* 2015). Therefore, while it has been hypothesized that pseudogene formation in ST313 occurred in a reservoir of immunocompromised humans, the possibility that this property was selected in a wild animal reservoir in which the pathogen causes invasive disease seems equally likely, and cannot be ruled out currently (Wain *et al.* 2013). However, it should be emphasized that in contrast to invasive serovars such as S. Typhi and S. Paratyphi A, the ability of the ST313 to cause enteric infection appears to be intact, because S. Typhimurium isolates from African children with diarrhea do not differ in their genotype from those associated with bacteremia (Kariuki *et al.* 2006; Kariuki and Onsare 2015), and ST313 strains have been isolated from cases of gastroenteritis in immunocompetent individuals (Paglietti *et al.* 2013).

The dominance of ST313 in sub-Saharan Africa raises the question of what selective forces were responsible for its epidemiologic success. An important factor contributing to the rise of epidemic S. Typhimurium clones in Europe and North

America is the acquisition of genes conferring resistance against antibiotics (reviewed in Rabsch, Tschape and Baumler 2001). The first recorded outbreak of drug-resistant S. Typhimurium affected cattle and humans in England and Wales in the early 1960s (Anderson 1968). The epidemic S. Typhimurium clone causing this outbreak was designated DT29 and carried resistance to ampicillin, tetracycline and streptomycin on conjugative plasmids (R-plasmids) (Anderson and Lewis 1965). Antibiotic resistance first emerged within African S. Typhimurium isolates around 1960, but this was only detected by a retrospective analysis performed more than 50 years later. Here, the emergence of antibiotic resistance involved insertion of a Tn*21*-like transposon element into the virulence plasmid (pSLT) of an S. Typhimurium clone designated as ST313 lineage I (Okoro *et al.* 2012). This Tn*21*-like element carries multidrug resistance determinants, suggesting that antibiotic resistance was likely an important factor in the subsequent epidemic spread of ST313 within sub-Saharan Africa.

Epidemiologic surveillance in Europe shows that after dominating in the 1960s, DT29 was replaced by a new multidrug-resistant S. Typhimurium clone, termed DT204, in the 1970s and 80s, which in turn was supplanted by S. Typhimurium clone DT104 in the 1990s (summarized in Rabsch, Tschape and Baumler 2001). Retrospective analysis suggests that a similar succession of epidemic multidrug-resistant S. Typhimurium clones also unfolded in sub-Saharan Africa. After its emergence in the 1960s, ST313 lineage I was initially the dominant S. Typhimurium clone in sub-Saharan Africa. However, a new clone, termed ST313 lineage II, emerged around 1975 and by 2006 it had completely replaced the preceding clone, ST313 lineage I (Okoro *et al.* 2012). The ST313 lineage II carries a chloramphenicol resistance determinant, which is absent from ST313 lineage I (Okoro *et al.* 2012). Since chloramphenicol was the drug of choice to treat severe bacterial infections in Africa at the time, it is likely that this resistance determinant drove replacement of ST313 lineage I by ST313 lineage II.

How does the current ST313 epidemic in sub-Saharan Africa affect the human population at risk for NTS bacteremia? The most important factor is likely the resistance of ST313 strains to multiple antibiotics, including ampicillin, chloramphenicol, streptomycin, sulfonamides, streptomycin and trimethoprim (Kingsley *et al.* 2009). More recent surveillance of antimicrobial resistance profiles of iNTS isolates in the Democratic Republic of the Congo and Kenya found these strains to have, in addition, decreased susceptibility to ciprofloxacin and third-generation cephalosporins (Lunguya *et al.* 2013; Kariuki *et al.* 2015); further spread of these strains will therefore curtail the already limited treatment options for disseminated infections with NTS. On the other hand, increased invasiveness of ST313 observed in animal models (Parsons *et al.* 2013; Yang *et al.* 2015; Singletary *et al.* 2016) is likely to be of minor importance in immunocompromised individuals. *Salmonella* Typhimurium clones circulating in Europe and North America were a cause of NTS bacteremia in patients with advanced HIV disease in the 1980s, before the advent of highly active antiretroviral therapy (Bottone, Wormser and Duncanson 1984; Jacobs *et al.* 1985; Nadelman *et al.* 1985; Fischl *et al.* 1986). Increases in the incidence of NTS bacteremia were noted on each of these continents during the first decade of the HIV pandemic (Levine *et al.* 1991; Pedro-Botet *et al.* 2002). These data show that S. Typhimurium isolates other than ST313 are fully capable of causing NTS bacteremia in immunocompromised individuals. Hence, factors compromising the host immune system are the main drivers of NTS bacteremia in individual patients, while differences in the virulence and other phenotypic characteristics of different S. Typhimurium clones are likely to play a less prominent role in the overall outcome of the host–pathogen interaction. However, these phenotypic differences are of interest, because they shed light on the role of the degraded genome content in infection outcome.

## RISK FACTORS FOR DISSEMINATED NTS INFECTION

The very young and the elderly are at particular risk of developing bacteremic infection with NTS (Mandal and Brennand 1988). Further, NTS have long been recognized as a frequent cause of disseminated infection in individuals with compromised immune function (Table 2). In chronic granulomatous disease (CGD), a rare primary immunodeficiency caused by mutations in subunits of the NADPH oxidase, NTS is the most common cause of bacteremia (Winkelstein *et al.* 2000). In addition, individuals with genetic deficiencies in the IL-12/IFN-$\gamma$ pathways, a group of conditions known as 'Mendelian susceptibility to mycobacterial disease (MSMD)', are at increased risk of recurrent bacteremias with NTS (reviewed in Bustamante *et al.* 2014). A further set of genetic conditions that result in structural changes to hemoglobin, including sickle cell disease and the thalassemias, can lead to recurrent episodes of hemolytic anemia (Hook *et al.* 1957; Wanachiwanawin 2000; Williams *et al.* 2009; Richards, Howard and Klein 2011). For a more comprehensive review of genetic risk factors underlying NTS bacteremia, the reader is referred to an excellent recent article by Gilchrist and colleagues (Gilchrist, MacLennan and Hill 2015). Acquired immunodeficiencies can also predispose to invasive infections with NTS. These include HIV and immunosuppression secondary to corticosteroid treatment, as would occur with transplantation or autoinflammatory conditions. In fact, during the first years of the AIDS epidemic, NTS bacteremia was considered to be an early indicator of HIV infection (Bottone, Wormser and Duncanson 1984); however, with the availability of highly active antiretroviral therapy, it has become less common in North America.

In contrast to the situation in North America and Europe, disseminated NTS infections in sub-Saharan Africa have increased in prevalence in recent years, especially in the setting of HIV (Gordon *et al.* 2001, 2002). While HIV is the most common factor in adults that predisposes to NTS bacteremia in sub-Saharan Africa, in children the predisposing factors are very young age (<2 years old), *Plasmodium falciparum* malaria and malnutrition (Reddy, Shaw and Crump 2010). In Malawian children with exposure to NTS, an interesting correlation has been observed between acquisition of antibody responses around 2 years of age and declining incidence of invasive disease that suggests a role for antibodies in protection in this setting (Nyirenda *et al.* 2014). Epidemiological studies have noted a seasonal pattern in the frequency of bloodstream NTS infections in children, with maximal incidence following the rainy season, when the rates of both malaria and severe malnutrition are also greatest (O'Dempsey *et al.* 1994; Graham *et al.* 2000; Brent *et al.* 2006; Gordon *et al.* 2008). Several studies have found a strong association between severe childhood malnutrition and bloodstream infections with NTS (Bachou *et al.* 2006; Brent *et al.* 2006; Sigauque *et al.* 2009). However, despite substantial evidence linking malnutrition with extraintestinal NTS infection, it remains unclear how malnutrition compromises control of NTS infection. Furthermore, while a protein-deficient diet has been linked to increased severity of disease in mice (Peck, Babcock and Alexander 1992), other

**Table 2.** Epidemiologic factors associated with disseminated NTS infection.

| Factor | References | Mechanism(s) | References |
|---|---|---|---|
| Young age | Muthumbi *et al.* (2015) | Possibly lack of specific antibodies | Nyirenda *et al.* (2014) |
| *Acquired immunodeficiencies* | | | |
| HIV infection | Bottone, Wormser and Duncanson (1984); Roberts *et al.* (1984); Israel and Plaisance (1991); Pitrak (1999); Feasey *et al.* (2012) | Th1/Th17 cell deficiency | Raffatellu *et al.* (2008) |
| Malnutrition | Brent *et al.* (2006); Feasey *et al.* (2015); Muthumbi *et al.* (2015); Shahunja *et al.* (2015) | Protein restriction | Peck, Babcock and Alexander (1992) |
| Neoplasia | Noriega *et al.* (1994); Beebe and Koneman (1995) | Unknown | – |
| Malaria | Park *et al.* (2016) | Deficiency in neutrophils | Cunnington *et al.* (2012b) |
| *Genetic immunodeficiencies* | | | |
| Sickle cell disease | Hook *et al.* (1957); Williams *et al.* (2009); Richards, Howard and Klein (2011) | Functional deficiency of neutrophils | Evans *et al.* (2015) |
| E-beta thalassemia | Ampel *et al.* (1989); Wanachiwanawin (2000) | Unknown | – |
| CGD | Winkelstein *et al.* (2000) | NADPH oxidase deficiency | Mastroeni *et al.* (2000); Vazquez-Torres *et al.* (2000) |
| Interferon-$\gamma$/IL-12/IL-23 pathways (MSMD) | Bustamante *et al.* (2014) | Deficiency of Th1 cell and macrophage function | Nauciel and Espinasse-Maes (1992); Mastroeni *et al.* (1996) |

micronutrient deficiencies that impair host defense against NTS are not known. Although our knowledge of causal relationships for any of these susceptibility factors is limited, studies in animal infection models (discussed below) are starting to unearth potential mechanisms.

## UNDERLYING IMMUNOLOGIC DEFICITS PREDISPOSE TO DISSEMINATED INFECTION

Clinical/epidemiologic associations illustrate that disruption of discrete immune functions can lead to NTS bacteremia, but the precise mechanisms by which these immune functions prevent bacterial dissemination has long remained elusive. Recent studies in animal models have begun to shed light on possible mechanisms underlying susceptibility to disseminated NTS infection in the context of HIV infection and *Plasmodium falciparum* malaria. A common mechanism that may impact both comorbidities is a reduced mucosal response to invasion of the epithelium by NTS. In a simian immunodeficiency virus (SIV) model of HIV/*Salmonella* Typhimurium co-infection utilizing rhesus macaques, SIV infection led to depletion of Th17 cells (a population of CD4 T cells) from the intestinal mucosa (Raffatellu *et al.* 2008), which corresponded with reduced mucosal inflammation in *S.* Typhimurium-infected ligated ileal loops and increased bacterial dissemination from the intestine to systemic sites. Th17 cells were subsequently shown in mouse models to be important for eliciting the neutrophil influx into the gut mucosa that is important for limiting systemic dissemination of NTS (Raffatellu *et al.* 2008). Reduced *Il17* expression and attenuated neutrophil infiltration into the ileal mucosa were also observed in a rhesus macaque model of malaria/NTS co-infection using *P. fragile* (Mooney *et al.* 2014). Follow-up studies in a mouse model of malaria/NTS co-infection showed that reduced intestinal inflammation in response to *S.* Typhimurium invasion of the mucosa resulted from local induction of the immunoregulatory cytokine IL-10 by malaria parasites (Mooney *et al.* 2014). Hence, both HIV and malaria attenuate mucosal responses to *S.* Typhimurium invasion, but by different mechanisms—depletion of Th17 cells in the case of HIV vs. production of immunoregulatory cytokines in the case of malaria. Reduced intestinal inflammation observed during NTS co-infection with SIV or malaria in animal models may explain why diarrhea is commonly absent in patients with NTS bacteremia (De Wit *et al.* 1988).

In clinical malaria studies, deficits in both absorptive and barrier functions of the intestinal mucosa have been observed. Patients in the acute phase of malaria showed evidence of malabsorption and loss of epithelial tight junction integrity, which correlated with alterations to the intestinal architecture, including edema, mononuclear infiltrates and shortening and widening of the villi (Karney and Tong 1972; Molyneux *et al.* 1989). Similar pathologic features in the intestinal tract were also observed in murine malaria models (*P. berghei* and *P. yoelii*) in association with alterations to the colonic microbiota, most strikingly a reduction of members of the phylum Firmicutes (Mooney *et al.* 2015b; Taniguchi *et al.* 2015). Shifts in the microbiota composition of *P. yoelii*-infected mice promoted their colonization by *S.* Typhimurium and *Escherichia coli* (Mooney *et al.* 2015b). Notably, *P. yoelii* infection markedly reduced the implantation dose for *S.* Typhimurium in mice via the intragastric route, a finding that may be of significance during the initial exposure of children with malaria to contaminated food and water (Mooney *et al.* 2015b).

Loss of epithelial tight junction integrity during malaria was recapitulated in the *P. yoelii* murine malaria model, where it was linked to an increase in the abundance of mucosal mast cells—a feature that was also observed during *P. fragile* infection of rhesus macaques (Potts *et al.* 2016). While the precise role of mast cells in the intestinal pathology of malaria is not yet understood, blockade of histamine, a product of activated mast cells, in *P. yoelii*-infected mice increased detection of tight junction

complexes in the ileal epithelium, suggesting that histamine release from mast cells may contribute to increased intestinal permeability during malaria. Together, these studies suggest that multiple effects of HIV and malaria parasite infection on the gut mucosa may weaken intestinal barrier functions that limit systemic dissemination of NTS.

While effects of malaria and HIV on the intestinal mucosa may promote dissemination of NTS, it is important to note that NTS are capable of invading the intestinal epithelium and disseminating systemically in the absence of any underlying condition. However, once bacteria have disseminated from the intestinal tract, cells of the mononuclear phagocyte system—most importantly macrophages and neutrophils—limit the infection. In the case of malaria co-infection, murine models have provided insights into how underlying malaria can compromise control of systemic NTS infection by phagocytic cells. A study by Cunnington and colleagues demonstrated that malaria, via hemolysis of red blood cells and release of heme, impairs granulocyte maturation and oxidative burst capacity via activation of heme oxygenase (Cunnington *et al.* 2012a). As a consequence, immature granulocytes are released into the circulation of infected mice. These circulating immature cells harbor *S.* Typhimurium, suggesting that they may provide an intracellular niche in the bloodstream. Thus, heme oxygenase, which helps the host cope with elevated free heme during malaria, suppresses neutrophil development, exerting a detrimental effect on control of *S.* Typhimurium. Reduced neutrophil function secondary to hemolysis could also be an important determinant of susceptibility to disseminated NTS infection in the context of other hemolytic anemias such as sickle cell disease and the thalassemias. Taken together, data from mouse models suggest that malaria impacts neutrophil function in two ways: indirectly by blunting expression of inflammatory chemokines that recruit neutrophils to sites of epithelial invasion, and directly by reducing their maturation state and antibacterial activity. Considering that some genetic predispositions to disseminated NTS infection in humans, including sickle cell disease, and CGD involve a reduced function of neutrophils, these effects of malaria on neutrophil function are likely to be important in the context of human co-infection.

An additional immunocompromising consequence of malaria is suppression of macrophage function. During malaria, immunoregulatory responses such as IL-10 are induced to limit inflammatory pathology caused by the parasite infection. However, this response has negative consequences for the host response to NTS: by suppressing the ability of macrophages to respond to *S.* Typhimurium with proinflammatory cytokine production, IL-10 production blocks recruitment of neutrophils to sites of systemic infection, such as the liver (Lokken *et al.* 2014). Further, IL-10 produced by tissue macrophages in the liver acts locally on neighboring macrophages to drive development of an alternatively activated phenotype with reduced bactericidal function, and consequently, an inability to control bacteria that have disseminated from the intestine (Lokken *et al.* 2014). Another mechanism that may compromise macrophage function during malaria is phagocytosis of infected and uninfected red blood cells, a process that leads to malarial anemia (Facer and Brown 1981). Hemophagocytic macrophages also exhibit an alternatively activated phenotype, with reduction in both inflammatory responses to *S.* Typhimurium and bactericidal activity (Nix *et al.* 2007; McCoy, Moreland and Detweiler 2012). Since hemophagocytic macrophages and other alternatively activated macrophage populations are targeted by *S.* Typhimurium infection in mouse models, it is possible that these cells may also harbor NTS in malaria patients, providing an intracellular niche for systemic infection (Nix *et al.* 2007; McCoy, Moreland and Detweiler 2012; Eisele *et al.* 2013). As a whole, these studies suggest that multiple components of the immune response to malaria, that are beneficial in the context of limiting parasite infection and its resulting immunopathology, serve to blunt responses that are critical to control of invasive bacterial infection.

## HOW CAN WE PREVENT DISSEMINATED INFECTION?

Given the devastating effects of disseminated NTS infections in sub-Saharan Africa, it is critical to develop strategies for reducing the burden of this disease. Epidemiologic evidence suggests that strategies for control of malaria impact NTS bacteremia by reducing the number of children with underlying malaria, as the prevalence of bacteremia cases has been shown to decline with decreasing malaria transmission (Scott *et al.* 2011; Verani *et al.* 2015). Another approach to prevention is the development of vaccines for use in susceptible populations (MacLennan, Martin and Micoli 2014). However, strategies will need to be targeted to the populations at risk, with consideration to the immunocompromised status of many of these individuals. Studies in animal models have shown that malaria causes transient suppression of both CD4 T cell responses and B cell responses conferred by vaccination against other infections (Ng *et al.* 2014; Mooney *et al.* 2015a), and to be effective at clearing NTS, circulating antibodies generated by subunit vaccines rely on functional neutrophils and macrophages, cells whose function is compromised during severe malaria. Suppression of existing adaptive immunity by acquired immune deficiencies may also explain in part the occurrence of disseminated NTS infection in a population in which immune responses to NTS are already detected in early childhood (Nyirenda *et al.* 2014). Therefore, incorporating these immunologic deficits found in the very young and in children with malnutrition, malaria and HIV into preclinical studies may aid in development of more efficacious vaccination strategies by identifying functional components of immunity that can be targeted by vaccines to enhance resistance to disseminated infection.

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