REVIEW

Malaria, anemia, and invasive bacterial disease:
A neutrophil problem?

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Abstract
Invasive bacterial disease is well described in immunocompromised hosts, including those with malaria infection. One bacterial infection frequently observed in children with Plasmodium falciparum infection is nontyphoidal salmonella (NTS) infection, in which a typically intestinal infection becomes systemic with serious, often fatal, consequences. In this review, we consider the role of malaria-induced immunoregulatory responses in tipping the balance from tissue homeostasis during malaria infection to risk of invasive NTS. Also, neutrophils are crucial in the clearance of NTS but their ability to mount an oxidative burst and kill intracellular Salmonella is severely compromised during, and for some time after, an acute malaria infection. Here, we summarize the evidence linking malaria and invasive NTS infections; describe the role of neutrophils in clearing NTS infections; review evidence for neutrophil dysfunction in malaria infections; and explore roles of heme oxygenase-1, IL-10, and complement in mediating this dysfunction. Finally, given the epidemiological evidence that low density, subclinical malaria infections pose a risk for invasive NTS infections, we consider whether the high prevalence of such infections might underlie the very high incidence of invasive bacterial disease across much of sub-Saharan Africa.

KEYWORDS
malaria, salmonella, anemia, sepsis, neutrophil, heme oxygenase-1, IL-10

1 | BACTEREMIA AND MALARIA

Bloodstream bacterial infections remain a global health concern, with high case fatality rates and the potential for long-term, life-changing sequelae. Life-threatening organ dysfunction resulting from systemic bacterial infection, or more commonly sepsis,1 is mediated by a systemic inflammatory response2,3 wherein septic shock leads to severe tissue damage and death.4,5 Sepsis is one of the most challenging and most costly conditions to treat in hospital—amassing a bill of $24 billion in the United States for 2013 alone.6

In developed economies, the organisms most frequently isolated from blood include Staphylococcus aureus and Escherichia coli7 (each accounting for ~20% of cases). Methicillin-resistant S. aureus8 and highly pathogenic E. coli are emerging as major causes of nosocomial infections.9 In contrast, developing nations in Africa see a much greater incidence of community-acquired bacteremia with Salmonella enterica (often nontyphoidal Salmonella [NTS]) and Streptococcus pneumoniae as the most commonly isolated organisms.10 Laboratory diagnosis for microbiological pathogens in Africa remains poor, with insufficient infrastructure and related funding. Despite challenges in detection, Ao et al. have estimated that NTS causes 3.4 million cases of bacteremia globally each year, of which the majority (1.9 million cases and 380,000 deaths) are in children and young adults in sub-Saharan Africa.11 In Kenya, 70% of these deaths occur within 2 days of admission to hospital,12 providing a very narrow window for effective intervention. Further, multiple drug-resistant NTS serotypes have been reported in East and Southern Africa, with sequence type 313 (ST313) seen as a distinct lineage associated with septicemia.13,14 Increasingly, lack of access to effective and affordable antibiotics may lead to even higher morbidity and mortality in low-income settings.

NTS thrives in the intestinal environment where, in otherwise healthy hosts, localized gastroenteritis allows NTS to outcompete the microbiota, causing diarrhea and promoting transmission.15 However, the infection can "escape" the gut and invade other tissues, eventually becoming systemic, particularly when the host is immunocompromised. One well-documented risk factor for invasive NTS is Plasmodium...
INTESTINAL AND INVASIVE NTS INFECTIONS

Salmonella can infect a broad host range (e.g., pigs, cattle, chickens, and humans) causing varying levels of damage, from enteric fever to severe gastroenteritis to asymptomatic carriage, depending on the particular serovar, typically defined by expression of LPS, flagellar, and capsular Vi antigens.26 With over 2500 known serovars,27 sterile immunity through natural infection or vaccination remains elusive.28,29 The human-restricted typhoidal serovars (Salmonella typhi and Salmonella paratyphi) are associated with systemic infection and carriage in the gallbladder30 but, intriguingly, these are not the serovars that are associated with malaria infections. Rather, malaria is associated with invasive disease caused by nontyphoidal serovars that can infect a broad range of different host species and are normally restricted to the intestine.17 Invasion of NTS through the intestinal mucosa can occur via their uptake by dendritic cells extruding dendrites between enterocytes into the intestinal lumen (paracellular uptake), via direct invasion of enterocytes or by passage through M cells of the Peyers Patches31 (Fig. 1), and this is dependent on a degree of inflammation.32,33 Bacterial invasion triggers the IL-23/IL-18 inflammatory axis leading to T cell activation and production of inflammatory cytokines (including IFN-γ, IL-17, and IL-22) and chemokines (CXCL1 and Mip2),34–36 eventually resulting in edema and infiltration of monocytes and neutrophils into the lamina propria,37 which are hallmarks of NTS pathophysiology.

Although phagocytes, such as neutrophils and macrophages, are efficient in their uptake of NTS, the bacteria can disable the antibacterial machinery of macrophages to create Salmonella containing vacuoles (SCVs) within which they can persist and replicate.38 Proteins encoded within Salmonella pathogenicity island-2 block lysosomal fusion allowing evasion of ROS-mediated killing.39 Ultimately, clearance of bacteria from phagocytes is mediated by IFN-γ, which induces breakdown of the SCV,40 releasing bacteria into the cytosol. Bacterial products now present in the cytosol can induce pyroptosis, a form of cell death involving both canonical and noncanonical inflammatory signaling with caspase-1 and NLRP3 or caspase-11 (in mice) and caspase-4 and caspase-5 (in humans), which lead to activation of IL-1 and IL-18.41–43 Bacteria released from disintegrating macrophages are cleared by neutrophils, a process that has been termed “phagocyte roulette.”44 Neutrophils, also called polymorphonuclear cells (PMNs), are terminally differentiated leukocytes with distinctive lobulated nuclei and contain antimicrobial cytoplasmic granules. PMNs are the most abundant white blood cell, with 1 × 1011 new cells emerging from the bone marrow daily. They are typically thought to have a very short lifespan in blood (7–24 hours45), although infection may delay apoptosis and increase lifespan.46–48 During infection and inflammation, PMNs are quickly mobilized to sites of injury. In the blood vessel, activated PMNs adhere to endothelium, extravasate and migrate along chemokine gradients to infectious foci.

PMNs are professional phagocytes, which use receptor-mediated phagocytosis to internalize pathogens and debris into phagolysosomes.49 Intracytoplasmic granules containing cathepsins, elastases, and myeloperoxidases fuse with the phagolysosome to digest internalized pathogens, in a process known as degranulation; leakage of granules or their contents into the extracellular milieu can be a significant cause of tissue damage during infection.49,50 Release of reactive oxygen species (ROS), produced via an NADPH oxidase-dependent process, into the phagolysosome is an additional, very important, bactericidal mechanism. PMNs can also kill extracellular pathogens by degranulation, secretion of ROS, or the release of neutrophil extracellular traps (NETs). NETs consist of externalized decondensed chromatin decorated with granular proteins and histones to prevent the dissemination of pathogens.51,52 Serine proteases and histones provide antimicrobial activity against trapped pathogens. NETs also permit subsequent phagocytosis by proximate phagocytes (Fig. 2).

Although macrophages can be permissive to NTS growth, inflammatory monocytes and neutrophils are efficient in killing Salmonella through oxidative stress.53 In the intestine, both mucosa-associated and luminal neutrophils engulf Salmonella.54 However, infiltrating PMNs also promote intestinal inflammation,55 thereby increasing the risk of bacterial invasion, and produce ROS, which can transform carbon sources such as thiosulfate in the intestinal lumen into tetrathionate and the microbial fermentation product 1,2-propanediol, allowing NTS to outgrow the competing microbiota.32,33 PMNs also release calprotectin into the intestinal milieu where it sequesters zinc, further restricting the growth of the intestinal microbiota.56 Therefore, although PMNs can limit bacterial growth and prevent overwhelming infection, NTS can exploit neutrophil-mediated inflammation in the intestine to ensure its survival and eventual transmission.53,57
FIGURE 1  NTS intestinal immune response. NTS is a fecal-oral pathogen, which thrives in the inflamed intestine. Tissue invasion in the distal intestine can be via direct invasion, uptake by M cells, or through paracellular spaces. Uptake by dendritic cells can initiate inflammation through the IL-23/IL-17 axis. Th17 cells promote neutrophil influx via the induction of neutrophil chemokines. NTS is able to persist within the Salmonella-containing vacuole of macrophages, whereas neutrophils are efficient at NTS clearance. Systemic dissemination to draining lymph nodes is through CD18+ phagocytes. During experimental malaria, NTS colonization resistance is lowered, although it is unclear if there is an increase in tissue invasion. Regardless, inflammation (with reduced PMN influx) is reduced due to increased IL-10 concentrations. However, the role of IL-10, and potentially HO-1, on intestinal neutrophil function and role for increased systemic dissemination are unclear.

3 | PATHOPHYSIOLOGY OF NTS–MALARIA COINFECTIONS

Clinically, NTS infections in patients in Africa are not associated with overt diarrheal disease, suggesting that underlying coinfection may ameliorate the intestinal inflammation typically associated with NTS. Evidence from coinfection models supports this idea (Table 1): intestinal inflammation is markedly reduced in both mice and macaques coinfected with malaria and NTS compared to animals infected with NTS alone and this is associated with reduced neutrophil influx and lower levels of IFN-γ and IL-17 (Fig. 1). In mice, this reduction in intestinal inflammation is mediated by IL-10, a potent anti-inflammatory cytokine, which is essential for minimizing tissue damage during malaria infections (discussed in detail below). Although reducing intestinal inflammation might be expected to reduce the likelihood of invasion of NTS into the lamina propria and subsequent systemic dissemination, malaria infection has other, less beneficial consequences for the gut. In humans, acute malaria infection, in the apparent absence of gastrointestinal pathogens, is commonly associated with mild to moderate diarrhea, perhaps indicative of dysbiosis.
In mice, malaria infection includes: maturation, extravasation, migration, degranulation, and NETosis. PMNs maintain the ability to phagocytose NTS; however, PMNs display impaired ROS production. Mechanisms still to be investigated include: maturation, extravasation, migration, degranulation, and NETosis.

Whether increased NTS colonization or lymph node dissemination directly increases the risk of systemic spread is not yet clear but major defects in control of systemic NTS are evident in malaria coinfection: bacterial loads in blood, liver, spleen, and bone marrow after intraperitoneal injection of Salmonella Typhimurium (bypassing any intestinal contribution) were 1000–10,000-fold higher in Plasmodium yoelii-infected mice than in malaria-uninfected mice. Increased colonization, taken together with increased gut permeability and reduced availability of neutrophils to control the bacterial infection, may explain why, 48 h after challenge with NTS, bacterial loads in the draining mesenteric lymph nodes are 100-fold higher in malaria-coinfected mice than in mice without malaria.

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Hemolytic crisis in patients with sickle cell anemia, which gives rise to periodic hemolytic crises, are also highly susceptible to invasive NTS disease75,76; similarly, induction of acute hemolysis by antibody-mediated RBC lysis77 or phenylhydrazine treatment63 also renders mice highly susceptible to NTS.
**TABLE 1** Animal models of malaria and salmonella coinfection

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animal (strain)</th>
<th>Plasmodium spp.</th>
<th>NTS strain (serovar)</th>
<th>Route of NTS challenge</th>
<th>NTS challenge after Plasmodium</th>
<th>Endpoint</th>
<th>NTS-related outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roux et al.77</td>
<td>Mus (CBA/J)</td>
<td><em>P. yoelii nigeriensis</em></td>
<td>IR715 (ATCC 14028)</td>
<td>Intragastric</td>
<td>Day 0 Day 5</td>
<td>Increased CFU in Spleen, liver, and Peyer’s patch.</td>
<td></td>
</tr>
<tr>
<td>Cunnington et al.63</td>
<td>Mus (C57BL/6)</td>
<td><em>P. yoelii 17XNL</em></td>
<td>12023-GFP</td>
<td>Intraperitoneal</td>
<td>Day 15 18 h</td>
<td>Increased CFU in blood, spleen, and liver.</td>
<td></td>
</tr>
<tr>
<td>Chau et al.124</td>
<td>Mus (CBA/J)</td>
<td><em>P. yoelii 17XNL</em></td>
<td>IR715 (ATCC 14028)</td>
<td>Intragastric</td>
<td>Day 10 Day 14</td>
<td>L-Arginine &amp; L-Citruline supplementation during Py. reduces NTS burden in mesLN</td>
<td></td>
</tr>
<tr>
<td>Lokken et al.64</td>
<td>Mus (CBA/J)</td>
<td><em>P. yoelii nigeriensis</em></td>
<td>IR715 (ATCC 14028)</td>
<td>Intragastric</td>
<td>Day 10 Day 12 14</td>
<td>Increased CFU in liver (day 12/14), blood (day 14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mus (CBA/J)</td>
<td></td>
<td></td>
<td>Intraperitoneal</td>
<td>Day 10 Day 12 13</td>
<td>Increased CFU in liver (day 12/13), blood (day 13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mus (C57BL/6J)</td>
<td></td>
<td></td>
<td>Intragastric</td>
<td>Day 10</td>
<td>No increase in CFU, reduced liver PMN chemokines</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mus (C57BL/6J)</td>
<td></td>
<td></td>
<td>Intraperitoneal</td>
<td>Day 10</td>
<td>No increase in CFU</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mus (C57BL/6J)</td>
<td></td>
<td></td>
<td>Intragastric</td>
<td>Day 10</td>
<td>Reduces CFU in single &amp; co-infected, restored liver PMN chemokines</td>
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<td></td>
<td>Mus (C57BL/6J il10 Rflx:LysMcre)</td>
<td></td>
<td></td>
<td>Intraperitoneal</td>
<td>Day 10 Day 12</td>
<td>IL-10R–/– myeloid cells; loss in increased liver, blood CFU</td>
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<tr>
<td></td>
<td>Mus (C57BL/6J il10flx:LysMcre)</td>
<td></td>
<td></td>
<td>Intraperitoneal</td>
<td>Day 10 Day 12</td>
<td>IL-10–/– myeloid cells; loss in increased blood CFU</td>
<td></td>
</tr>
<tr>
<td>Mooney et al.59</td>
<td>Macaca mulatta</td>
<td><em>P. fragile</em></td>
<td>IR715 (ATCC 14028)</td>
<td>Ligated ileal loops</td>
<td>Day 14, 15 8 h</td>
<td>Reduced intestinal inflammation to NTS</td>
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<td></td>
<td>Mus (CBA/J)</td>
<td><em>P. yoelii nigeriensis</em></td>
<td></td>
<td>Intrastragric</td>
<td>Day 10</td>
<td>Reduced intestinal inflammation to NTS, increased mesLN CFU</td>
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<td></td>
<td>Mus (C57BL/6J)</td>
<td></td>
<td></td>
<td>Day 10</td>
<td>Reduced intestinal inflammation &amp; PMN influx</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Mus (C57BL/6J il10–/–)</td>
<td></td>
<td></td>
<td>Day 10</td>
<td>Restored intestinal inflammation &amp; PMN influx in IL-10–/–</td>
<td></td>
<td></td>
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<tr>
<td>Mooney et al.125</td>
<td>Mus (C57BL/6)</td>
<td><em>P. yoelii 17XNL</em></td>
<td>BRD509</td>
<td>Intravenous</td>
<td>Day 14, 28 Day 17, 31</td>
<td>Increased CFU in liver</td>
<td></td>
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<tr>
<td></td>
<td>Mus (C57BL/6J-Slc11a1 +/+)</td>
<td></td>
<td></td>
<td>Intravenous</td>
<td>Day 14</td>
<td>No increase in CFU</td>
<td></td>
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<tr>
<td></td>
<td>Mus (CBA/J)</td>
<td></td>
<td></td>
<td>Intragastric</td>
<td>Day 14</td>
<td>No increase in CFU</td>
<td></td>
</tr>
<tr>
<td>Mooney et al.62</td>
<td>Mus (C57BL/6J)</td>
<td><em>P. yoelii nigeriensis</em></td>
<td>IR715 (ATCC 14028)</td>
<td>Intragastric</td>
<td>Day 10</td>
<td>Increased NTS colonization in feces</td>
<td></td>
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<tr>
<td></td>
<td>Mus (C57BL/6J - Germ Free)</td>
<td></td>
<td></td>
<td>Day 10</td>
<td>Increased NTS colonization in feces with fecal donation from P. yoelii-infected mice into germ-free mice</td>
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<tr>
<td>Lokken et al.79</td>
<td>Mus (CBA/J)</td>
<td><em>P. yoelii nigeriensis</em></td>
<td>IR715 (ATCC 14028)</td>
<td>Intraperitoneal</td>
<td>Day 10 Day 12, 14</td>
<td>Increased CFU in liver (day 14 only)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mus (C57BL/6J il10–/–)</td>
<td></td>
<td></td>
<td>Intraperitoneal</td>
<td>Day 10</td>
<td>Reduced CFU in liver in IL-10–/–</td>
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</table>

The first indication that hemolysis has a negative impact on neutrophil function, and thus that neutrophil dysfunction might underlie increased risk of invasive bacterial disease in people with malaria or other hemolytic diseases, came from mouse coinfection studies where circulating neutrophils from malaria-infected and phenylhydrazine-treated mice were shown to efficiently phagocytose *S. Typhimurium* but were unable to kill; viable bacteria persisted and, indeed, replicated inside neutrophils, which were severely deficient in ROS production.63 Of note, in vitro, heme pretreatment reduced phagocytosis of *E. coli* by human and murine neutrophils78 and high circulating heme during *P. falciparum* infection can reduce in vitro phagocytosis of *Salmonella* by neutrophils.72 The observation that treatment with a synthetic heme polymer, hemin, induced similar neutrophil defects and that these defects can be reversed by competitive inhibition of HO-1,
then provided a link among malaria, hemolysis, HO-1, and neutrophil dysfunction. ROS-defective neutrophils have also been observed in children with acute malaria; importantly, these defects persist for up to 8 weeks after treatment, perhaps explaining why children with recent (past) malaria infection remain susceptible to invasive NTS. Plasmodium infection also reduces neutrophil mobilization into infected tissues including blood and liver; infiltration of inflammatory monocytes into the liver is also impaired. Although this may be due in part to the anti-inflammatory effects of IL-10 (as described below), there are also cell-intrinsic effects within developing neutrophils. Neutrophil precursors in the bone marrow (i.e., granulocyte macrophage progenitor cells) of malaria-infected mice express HO-1 and have unusual surface phenotypes (F4/80 and Gr-1 expression). It has been shown that HO-1 reduces neutrophil influx into the inflamed lung suggesting a causal relationship between HO-1 and reduced neutrophil migration but much more work is needed to fully characterize neutrophil maturation and function during malaria infection and to determine the extent to which the altered phenotype is mediated by the heme/HO-1 pathway.

5 THE ROLE OF IL-10 IN MALARIA NTS COINFECTION

IL-10 is a potent anti-inflammatory cytokine and an important regulator of inflammation-induced pathology, it is therefore no surprise that systemic IL-10 concentrations are elevated in highly inflammatory diseases such as sepsis and malaria. More surprisingly, however, circulating IL-10 is also elevated during mild/uncomplicated and asymptomatic/subclinical malaria infections; indeed these cases may represent successful balancing of inflammation-mediated parasite control and effective regulation of inflammation by IL-10. In malaria, IL-10 can come from both innate and adaptive immune cells, including Th1-derived regulatory T cells that coproduce IFN-γ and IL-10, and plays an essential role in both adaptive humoral immunity (promoting differentiation of T-bet+ germinal center B cells) and limiting tissue damage.

The anti-inflammatory properties of IL-10 include rendering phagocytes refractory to activation and/or directing macrophage polarization to a regulatory "M2" phenotype. The impact of IL-10 on neutrophil function is well described, reducing recruitment and migration in response to anaphylatoxins and ultimately bacterial clearance. Further, neutrophils themselves can be a source of IL-10, induced by regulatory T cells. During malaria NTS coinfection in mice, LysM-expressing cells are a significant source of IL-10 and its ablation reduces NTS bacteremia. IL-10 suppresses neutrophil function through the activation of STAT3 and suppressor of cytokine signaling 3 leading to the down-regulation of IFN regulatory factor and NF-κB family transcription factor. In addition, HO-1 can be directly induced by IL-10 (Fig. 3). One hypothesis, therefore, is that malaria-induced neutrophil dysfunction results from hemolysis and IL-10-driven HO-1 induction. In support of this hypothesis, in a recent study of persistent malaria infections, we have found that heme drives inflammation, that parasite density and inflammation then drives IL-10 production, and that heme and IL-10 both then induce HO-1.

6 COMPLEMENT DEPLETION DURING MALARIA

Complement proteins play an essential role in orchestrating inflammation and pathogen clearance, including during Salmonella infection. In brief, the classical pathway is activated by
CONCLUDING REMARKS

We have provided a rational for considering that hemolysis and inflammation, leading to induction of HO-1 and IL-10 and activation of complement, during malaria infections might all contribute to the increased susceptibility to bacterial coinfection. It is likely that these pathways synergize to increase risk of invasive NTS: in malaria-infected mice, both exogenous IL-10 and anemia were required for increased bacteremia; HO-1 is induced both by heme and by IL-10; and the carbon monoxide generated from heme catabolism can induce both HO-1 and IL-10. Ultimately, it is important to define how these factors may converge to alter neutrophil biology. Through this, we may begin to understand if targeted treatment, or even prophylactic anti-malarial treatment, can improve neutrophil function and so reduce the burden of invasive bacterial disease in malaria endemic populations. Given the importance of neutrophils in clearance of NTS, work is now needed to better describe the impact of malaria on this innate immune cell.

The risk of invasive NTS during malaria in sub-Saharan Africa is well defined for acute malaria infection. However, the majority of malaria infections in the world are asymptomatic, with chronic, low-density infections. As recent and low-density malaria infections are a risk factor for NTS bacteremia, it is also important to understand if hemolysis, and resulting induction of HO-1 and IL-10, seen during these "asymptomatic" infections reaches the threshold needed for neutrophil dysfunction. In other words, in addition to defining the pathways leading to neutrophil dysfunction, we also need to identify the point at which the balance tips from these being host protective to increasing the risk to invasive NTS. Importantly, these pathways may contribute to severe bacterial disease even in the absence of malaria infections: in sepsis patients, we have observed that raised concentrations of heme, HO-1, and IL-10 are positively correlated with primed through the interaction of C5a and its receptor (C5aR and CD88). Indeed, the E. coli-induced oxidative burst in phagocytes is almost entirely dependent on CD88 signaling. Opsonization and killing of invasive NTS strains by neutrophils isolated from Malawian children require both antibodies and complement.

Complement activation on the surface of uninfected RBCs presumably increases the rate of turnover of RBCs during malaria infection, possibly due to reduced availability of complement regulatory proteins such as CD55. Complement-mediated red cell lysis not only exacerbates the anemia associated with malaria, but will also increase circulating heme and therefore HO-1 concentrations and reduce the availability of complement components, which may impair ability to control invasive bacterial infection (Fig. 4). Hypothetically, excess C5a in plasma may reduce CD88 on PMNs, and thus reduce the oxidative burst. Further, depletion of complement proteins (with concomitant reduction in expression of endothelial selectin) and reduced generation of anaphylatoxins could reduce neutrophil migration into infected tissues. Further studies are required to determine the significance of complement activation and complement consumption during malaria and risk of invasive NTS.

FIGURE 4 Complement depletion. During malaria infection, systemic complement activation occurs due to deposition on both infected and uninfected RBCs. This results in reduced concentrations of circulating C3 and C5 and generation of anaphylatoxins (C3a and C5a). The consequences that may impact PMN function are 2-fold; (1) a reduction in C3 available for deposition on extracellular NTS leading to opsonization and migration and/or (2) excessive C5a reducing CD88 (C5aR) on neutrophils leading to reduced ROS and cytokine production. Additional work is needed to clarify the impact of complement activation and subsequent depletion during malaria on risk to NTS bacteremia.
disease severity and mortality.82 Also, while “invasive” NTS is seen in immunocompromised hosts (such as those with malaria infection), it remains unclear if this is due to increased intestinal invasion, increased dissemination from draining lymph nodes, failure to control systemic bacterial replication, or a combination of any of these. Moreover, the extent to which this is primarily a neutrophil defect requires further exploration.

AUTHORSHIP
J.P.M. wrote the first draft of the manuscript. J.P.M., L.G., and E.M.R. contributed to the writing of the manuscript. J.P.M., L.G., and E.M.R. agreed with the manuscript’s conclusions. E.M.R. conceived the idea. E.M.R. presented the topic at the Lorne Infection and Immunity 2018 meeting. Review solicited by the Journal of Leukocyte Biology editorial board.

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DISCLOSURES
The authors declare no conflicts of interest.

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