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The iron link between malaria and invasive non-typhoid *Salmonella* infections

Susanne van Santen[1,2], **Quirijn de Mast**[1], **Dorine W. Swinkels**[2], and **André J.A.M. van der Ven**[1]

[1]Department of General Internal Medicine, Nijmegen Institute for International Health (456)
[2]Department of Laboratory Medicine, Laboratory of Genetic, Endocrine and Metabolic diseases (830); Radboud University Medical Center, Geert Grooteplein Zuid 8, PO Box 9101, 6500 HB Nijmegen, the Netherlands

Abstract

Epidemiological studies have demonstrated an association between malaria and invasive non-typhoid *Salmonella* (NTS) infections, especially in children. We explore the role of iron as a possible co-factor in this association. Malarial disease, among others, is associated with enhanced erythrophagocytosis and inflammation, which increases the iron content of macrophages and thereby also the survival of *Salmonellae spp* within macrophages. Whether iron supplementation programs augment the risk of invasive NTS infections in malaria endemic regions is an important global health issue that still needs to be determined.

Keywords

malaria; *Plasmodium falciparum*; *Salmonella*; iron; co-infection

An introduction to the association between malaria, *Salmonella* and iron

Malaria and invasive non-typhoidal *Salmonella* (NTS) infections are both prominent causes of severe illness and death in sub-Saharan Africa, particularly in children. While infections with NTS usually cause a self-limiting diarrheal illness in high-income countries, these micro-organisms have emerged as a leading cause of bloodstream infections with subsequent increased mortality in sub-Saharan Africa [1, 2]. In these areas, malaria is predominantly caused by *Plasmodium falciparum*, and many people are estimated to be at high transmission risk [3].

Corresponding author: Prof Dr A. J.A.M. van der Ven, Radboud University Medical Center, Department of General Internal Medicine, Nijmegen Institute for International Health (456), Geert Grooteplein Zuid 8, PO Box 9101, 6500 HB Nijmegen, the Netherlands., T +31 24 3616980, F +31 24 356 63 36., A.vanderVen@aig.umcn.nl.

Iron deficiency is very common in areas which suffer from a high burden of malaria and invasive NTS disease [4]. Iron supplementation was a common intervention until a large clinical trial in preschool children on the Tanzanian island Pemba, which is an area of holo-endemic malaria transmission, showed that routine iron and folic acid supplementation was associated with an increased risk for hospital admission and death [5]. In a sub-analysis of this trial in children whose baseline iron status was assessed, adverse events of iron and folic acid supplementation were more common in children without iron deficiency. In response, the World Health Organization (WHO) recommended to restrict iron supplementation to those children with proven iron deficiency [6]. The debate on benefits and possible harm of iron supplementation and fortification in malaria endemic areas continues [7]. For example, recent Cochrane reviews concluded that iron supplementation and fortification in malaria endemic areas are probably safe, in the case that access to health care facilities are present [8-10]. The mechanisms underlying the possible adverse outcome of iron interventions remain poorly understood. One of the ways that iron may exert its harmful effects in malaria endemic areas is through predisposing individuals to malaria and NTS co-infection. In this review, we first focus on the epidemiologic evidence of the co-occurrence of *Salmonella* bacteremia and *P. falciparum* malaria, followed by a discussion of how malaria influences macrophageal iron homeostasis. Subsequently, we address the role of iron in the individual pathogenesis of *Salmonella* and malaria infections and, finally, explore the iron-related pathways that may be involved in the co-occurrence of malaria and invasive NTS disease.

Epidemiologic evidence for malaria and *Salmonella* co-infections

The association between malaria and *Salmonella* dates back to the 19th century, when ‘typhomalarial fever’ was a common diagnosis of army surgeons [11]. In addition, a physician reported in 1929: “The epidemiological relation of paratyphoid C to malaria in British Guiana is interesting. Not only does the disease become much more prevalent in coincidence with malarial outbreaks, but its virulence increases tenfold” [12]. Many decades later, in 1987, Mabey and coworkers renewed attention to this topic. They described the co-occurrence of NTS bacteremia and malaria parasitemia in Gambian children [13].

Since then, several prospective studies have reported similar findings. A study in 166 Kenyan children with NTS bacteremia reported that around three quarters of them had concurrent malaria parasitemia or recent malaria [14]. There was a clear seasonal trend in NTS bacteremia with the highest incidence during the rainy season when malaria rates peaked. In a study in children in Malawi with severe malaria, nearly 5% had positive blood cultures, of which NTS was the most common isolate [15]. Children with severe malarial anemia had the highest risk for NTS bacteremia. A similar outcome was found in two Kenyan studies and one Tanzanian study, in which NTS was the most common bloodstream isolate in children with parasitemia [16, 17], while *Streptococcus pneumoniae* and *Haemophilus influenza* were common isolates in aparasitemic children [17, 18]. In Kenya, NTS bacteremia was also significantly more common in rural areas with intense malaria transmission, in contrast to urban sites with less malaria burden where *Salmonella typhi* and *S. pneumoniae* were more prevalent [19].

More evidence came from a systematic review and meta-analysis of community acquired bloodstream infections in Africa [20]. This meta-analysis included 58 296 patients with febrile illness of whom almost three quarters were children. A total number of 5578 (9.6%) bacterial or fungal bloodstream infections were diagnosed, of which NTS was the most common isolate, accounting for 29.1% of the isolates recovered overall and 42.3% of pathogenic isolates in adults. Malaria parasitemia was documented in 11 814 of the cases, and 769 (6.5%) of these cases had concurrent bacterial or fungal bloodstream infection. Additional support for the existence of co-infection was derived from an observation in the Gambia and Kenya where the reduction in malaria infections was associated with a concurrent decline in NTS, while the incidence of pneumococcal bacteremia remained stable [21, 22].

Together, these studies show that invasive NTS disease and malaria are among the most common causes of fever in sub-Saharan Africa and, even though blood cultures are often not available or can be falsely negative, the data presented above suggest that co-infection of malaria and invasive NTS are common. This is in contrast to findings from Northern Africa and Asia, where invasive NTS disease appears to be relatively rare, and enteric fever caused by *S. typhi* and *S. paratyphi* are more common causes of fever [23]. The lower infection burden of both malaria and HIV in these areas and the fact that parasite densities are considerably lower in *vivax* malaria most likely contribute to these regional differences in NTS epidemiology.

How can this apparent association of malaria and invasive NTS disease in sub-Saharan Africa be explained? While there is no doubt that environmental factors (e.g., rainy season, humidity [1]) are important, several observations also support the importance of host factors. For example, prevalence rates of *Salmonella*-positive stools remain relatively stable year round, while NTS-bacteremia has a seasonal variation with a peak in the number of cases when malaria rates are also highest [13, 14]. The well-described link between invasive NTS disease, HIV infection, malnutrition, and (severe) malarial anemia also suggests host specific modulations [14-16]. These modulations involve several immune and non-immune mechanisms, and we suggest to include malaria-induced changes in the host iron homeostasis as one of the possible factors that predispose to invasive NTS, as described below.

Malaria affects macrophage iron homeostasis

In humans, the *Plasmodium* parasite first grows and multiplies in hepatocytes, followed by a blood stage characterized by a cycle of red blood cell (RBC) invasion, intra-erythrocytic parasite multiplication, and RBC burst, which is responsible for the characteristic inflammatory storm that is a feature of clinical malaria. Both infected and uninfected RBCs are cleared from the bloodstream by macrophages of the reticuloendothelial system taken up in phagosomes (Figure 1a). Iron is liberated from heme by the enzyme heme oxygenase (HO-1) and exported to the cytosol via the divalent metal transporter-1 (DMT-1) and natural resistance-associated macrophage protein-1 (Nramp-1), which are localized predominantly to early and late phagosomes, respectively [24, 25]. In the cytosol, the excess iron is incorporated in ferritin molecules, which is the main iron storage protein, or exported back

to the plasma via the sole cellular iron exporter ferroportin. To deal with the excessive supply of heme and free iron in conditions with increased erythrophagocytosis, these proteins are all upregulated to maximize iron recycling by macrophages (Figure 1,Table 1) [26]. Malaria is also associated with intra- and extravascular hemolysis [27]. Free circulating hemoglobin and heme are bound by haptoglobin and hemopexin, respectively, taken up by macrophages via specific receptors (CD163 and CD91, respectively) and then processed as described above via the HO-1 system [28].

Besides erythrophagocytosis and hemolysis, malaria is also associated with a pronounced inflammatory response. Inflammation is well known to have a major impact on iron homeostasis, predominantly mediated by the iron regulatory hormone hepcidin. Human iron homeostasis is tightly regulated via hepcidin, produced in the liver by pro-inflammatory cytokines like interleukin (IL)-6 and suppressed in conditions of iron deficiency or increased erythropoietic activity [29]. Hepcidin acts by binding and subsequent degradation of the sole cellular iron exporter ferroportin, mainly situated on duodenal enterocytes and macrophages [29]. As a consequence, hepcidin simultaneously prevents iron absorption from the diet and the recycling of iron after erythrophagocytosis in macrophages. This hepcidin-mediated, macrophage iron withholding is nowadays regarded as an ancient host defense mechanism against extracellular pathogens by depriving these pathogens of iron [30]. Previous work has shown that febrile malaria is associated with a strong increase in systemic hepcidin levels and pronounced disturbances in iron distribution, characterized by hypoferremia and increased ferritin levels [31-33]. Studies in Indonesian schoolchildren [34] and Beninese women [35] with asymptomatic *P. falciparum* parasitemia and a study in volunteers participating in an experimental malaria infection [33] showed that disturbances in iron homeostasis already occur with mild inflammation.

Macrophages also express hepcidin upon stimulation with an array of infectious and inflammatory stimuli including parasitized RBCs [36-38], which may lead to autocrine ferroportin degradation and iron withholding. Besides the effects of hepcidin, inflammation also leads to increased uptake of both transferrin-bound and non-transferrin-bound iron via modulation of transferrin receptors and DMT-1 [39, 40]. A schematic description of systemic iron metabolism during malaria infections is given in Figure 2. The role of iron in malaria infections is discussed in Box 1.

In summary, during malaria infections macrophageal iron contents increase as a result of these putative effects of enhanced erythrophagocytosis, cytokine activation and hepcidin upregulation.

Iron is an essential micronutrient for *Salmonella*

To establish a systemic infection, *Salmonellae spp* have to invade the epithelial wall of the intestine, after which the bacteria are ingested by immune effector cells and transported to lymph nodes, the spleen, and other organs [41, 42]. *Salmonellae spp* reside within modified phagosomes in macrophages where replication is promoted and killing evaded [41, 42]. Iron is an essential micronutrient for replication, and *Salmonellae spp* (like other intracellular pathogens) harbor various iron acquisition systems, such as the siderophores enterobactin

and salmochelin [43]. *Salmonellae spp* can also upregulate HO-1 expression in macrophages, resulting in increased iron supply from heme [44]. Several studies have directly related macrophage iron content to *Salmonella* pathogenesis. These studies showed that iron loading is associated with increased survival of *Salmonellae spp* within macrophages, while cellular iron deprivation results in the opposite [44, 45]. In response to a *Salmonella* infection, macrophages try to reduce the availability of intracellular iron both by increasing the incorporation of free iron into ferritin and cellular iron release via ferroportin (Table 1) [44, 46, 47]. Another iron transporter that appears crucial in the control of *Salmonella* is Nramp-1. As mentioned above, Nramp-1 is localized to late phagosomes in which *Salmonellae spp* reside, where it depletes the phagosomes of iron (Figure 1c). This limits growth of intracellular pathogens [25], and its expression was required to control *Salmonella* growth [46]. Apart from Nramp-1, ferroportin and DMT-1 are also located in phagosomal and endosomal membranes and to mycobacterium-containing phagosomes [48]. Although little is known about the functional role of ferroportin and DMT-1 in these endosomal and bacteria-containing phagosomes, it is conceivable that they are important in modulating endosomal iron flows. Hepcidin peptide was also detected intracellularly in mycobacterium-containing phagosomes [49], where it may control local ferroportin expression [36], and in high concentrations exert direct antibacterial activity [50].

In addition, the interaction with iron also occurs in the gut lumen, the main starting point of invasive NTS disease. For example, oral iron supplementation influenced the colonization of *Salmonellae spp* in the intestines of African children [51]. The number of fecal enterobacteriae, predominantly *Salmonellae spp*, significantly increased at the cost of non-iron dependent, non-pathogenic lactobacilli, and this correlated with a fecal marker (calprotectin) of gut inflammation, although no overt clinical disease was observed [51]. Recent *in vitro* data also demonstrated that iron increased the virulence of prevalent enteric pathogens, including *Salmonellae* [52]. Indeed, gut microbiota differed in iron-depleted versus iron replete rats, and iron supplementation increased neutrophilic infiltration of the colonic mucosa [53]. This agreed with observations suggesting that the development and function of the gut immune system is iron-dependent [54].

Taken together, the interaction between the host and *Salmonellae spp* includes the modulation of intracellular iron availability. On the one hand, *Salmonellae spp* utilize various iron acquisition systems to access iron; on the other hand, the host response aims to deprive phagosomes of iron, via the endosomal iron exporter Nramp-1, as well as the cytosol by way of upregulation of ferroportin. It is conceivable, but speculative, that *P. falciparum*, or *Salmonellae* in phagosomal compartments, modulate these iron regulatory proteins to increase their iron availability, while the host aims to deplete the phagosome of iron.

How malaria leads to more *Salmonella* infections: the link with iron

Despite the strong epidemiological evidence for a link between malaria and invasive NTS disease, the biological mechanisms underlying this link are still incompletely understood. Several mechanisms have been implicated, including malaria-associated impairments of immune function, antigen presentation, cytokine dysregulation, bacterial seeding of the

bloodstream because of microvascular parasite sequestration in gut mucosa, and the effects of iron, heme, and the malaria pigment hemozoin [22]. The relative importance of iron, heme and hemozoin and the exact pathways are unknown. In any case, the following partly overlapping pathways are potentially involved.

Macrophage iron retention in malaria favors NTS multiplication

As described above, malaria leads to intramacrophageal iron sequestration, which is of nutritional benefit to the intracellularly growing *Salmonellae*. Apart from the systemic effects of hepcidin and cytokines on macrophageal iron homeostasis, the redistribution to specific locations of macrophages also seems important [30]. The observation that malaria parasites and S*almonellae spp* frequently reside in close proximity is therefore of special interest: malaria-infected RBCs are retained in the slow microcirculation of the red pulp zone in the spleen [55], while *Salmonellae spp* reside predominantly in phagocytic macrophages of the red pulp and marginal zone of the spleen [56]. Another study in which mice were co-infected with malaria and NTS also found a marked increase in macrophages containing hemozoin and neutrophil infiltrates of *Salmonellae* in the red pulp of the spleen [57]. The latter is also exemplified by the preferential presence of *Salmonellae spp* in (iron-rich) hemophagocytic macrophages [41].

Modulation of the host immune response by iron

Besides a direct influence on *Salmonella* growth, iron status also influences the immune effector functions of macrophages, and disturbances in the iron load of the body have been linked to increased susceptibility to infections [30, 58]. An interesting example is the finding that iron loading of macrophages inhibits their interferon gamma (IFNγ) expression [43]. IFNγ is an essential part of the immune response against intracellular pathogens and crucially involved in protection against both malaria [59] and *Salmonella* [41]. The suggestion that hemophagocytic cells provide a place for *Salmonellae spp* to establish a chronic infection as a consequence of iron overload that interferes with immune function is in line with this hypothesis [41]. Conversely, iron deficiency may negatively affect innate and cellular immunity [60], both processes important for *Salmonella* host defense [30].

Iron-dependent effects of erythrophagocytosis, hemozoin and hemolysis on macrophage function

The vast proportion of iron in the human body is incorporated in hemoglobin. During the intra-erythrocytic stage, *Plasmodium* digests large amounts of hemoglobin into heme, which is highly deleterious for both the parasite and the host and therefore polymerized by the parasite into inert hemozoin [61]. Macrophages clear both infected and uninfected RBCs and hemozoin, which impairs macrophage function [57, 61, 62]. Hemolysis is another component that may explain the increased susceptibility to *Salmonellae spp* during malaria infection [57, 63]. Host defense strategies to prevent heme toxicity include the binding of heme to hemopexin and albumin, the internalization of these heme protein complexes by macrophages and, ultimately, the degradation of heme into iron via upregulation of HO-1 [28]. A recent study showed that malaria NTS co-infection of mice causes acute, fatal bacteremia with increased bacterial load; features reproduced by phenylhydrazine hemolysis

or hemin administration [63]. In this study, heme and upregulation of HO-1 were associated with dysfunctional maturation and mobilization of granulocytes upon NTS infection, which resulted in an impairment of their oxidative burst and bacterial killing ability. In addition, pharmacotherapeutic inhibition of HO-1 prolonged survival of NTS and malaria co-infected mice. However, HO-1 was also reported to confer protection against non-cerebral forms of severe malaria [64] and may mediate the well known protection of sickle cell anemia against malaria [65]. This suggests a dual role for heme and HO-1 and suggests that chronic hemolysis facilitates NTS infections as a result of modulation of host immune responses in combination with the increased iron availability induced by HO-1 activity [63].

The three mechanisms described above for the connection between malarial infection and invasive NTS may also explain the reported link between severe malarial anemia and invasive NTS. The pathogenesis of malarial anemia is multifactorial and includes hemolysis, erythrophagocytosis of infected and uninfected erythrocytes, and iron delocalization due to the effects of hepcidin, all processes that favor invasive NTS, as delineated above and illustrated in the Figures 1 and 2.

Concluding remarks and future directions

The interplay between iron, malaria and invasive NTS infections is intriguing and complex to unravel. The epidemiological evidence supporting an association between these infections is convincing. Also, the specific features of malaria on one hand, whereby parasites reside in erythrocytes and hemolysis, erythrophagocytosis and inflammation are important, and the widely present and intracellular growing NTS on the other hand, makes iron a logical candidate to explain this association. Moreover, NTS, more than other bacteria, especially benefits in this scenario from its sophisticated iron acquisition systems. However, the host iron status also influences the risk for other intracellular infections such as *Mycobacterium tuberculosis* [66, 67].

Can these observations also explain the debated findings of an increased risk for malaria-associated morbidity and mortality after iron supplementation [5]? An increased incidence of invasive NTS may contribute to the increased morbidity and mortality after iron supplementation in malaria-endemic regions. Diagnostic facilities for invasive NTS disease are not widely available in many malaria-endemic countries, and, especially in areas where asymptomatic parasitemia is common, severe febrile disease may wrongly be attributed to malaria. Therefore, we suggest that future trials assessing the safety of iron supplementation should specifically include diagnostics for invasive NTS. Moreover, molecular studies exploring the interaction between *P. falciparum* and macrophage iron homeostasis are needed to understand the role of iron in the interplay with host immune responses. Modulation of macrophage iron regulatory proteins and iron distribution may also provide an explanation for NTS bacteremia during malaria infections that warrant further studies. These insights may have important implications in nutritional public health programs in developing countries.

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Box 1

The importance of iron in malaria infections

The *Plasmodium* parasite is dependent on iron and other nutrients for its development. However, it is currently unclear what the iron source for the parasite is, since neither activity of HO-1 [68], nor functional iron transport or active iron acquisition systems have been detected in *P. falciparum* [69]. Possible sources are residual intra-erythrocytic ferritin, hemeiron or extracellular (transferrin) iron [69].

Several explanations for the possible harmful effects of iron supplementation on clinical outcome in malaria have been postulated [8, 9]. The increased availability of serum non-transferrin bound iron after iron supplementation could facilitate hepatic parasite growth, or result in an increased expression of endothelial adhesion molecules and, as such, influence malaria morbidity [70, 71]. However, evidence is scarce. In line with a harmful interaction between iron and malaria are observations of community studies in Tanzania and Malawi that young infants with iron deficiency were protected against *P. falciparum* infection [72, 73].

The hypothesis that this harmful effect is caused by the iron need of *Plasmodium* spp may be oversimplified as the host iron status influences immune effector functions as well. For instance, mice fed an iron deficient diet had lower levels of parasitemia, which related to increased clearance of RBCs and not to lower proliferation of erythrocytic parasites [74]. On the contrary, iron chelators were reported to inhibit the intra-erythrocytic parasite growth in association with a decrease in the intracellular iron pool of the infected erythrocytes and reticulocytes [71]. This observation could argue for iron-dependency, or to the generation of nitric oxide after iron chelation, an important component of the host immune response against malaria [75].

Interestingly, recent studies in mice showed that hepatic hepcidin gene expression was increased during an infection with the plasmodial mouse strain *Plasmodium berghei* and resulted in impaired hepatic proliferation of the parasite and lower rates of parasitemia [76], while treatment of these mice with anti-hepcidin antibodies deteriorated their survival upon malaria infection [77]. These studies suggest that increased hepcidin levels redistribute iron away from the hepatocyte, resulting in impaired growth of secondary liver stage malaria parasites during bloodstream malaria infection and protection against malarial superinfections [76, 77]. However, the question remains as to what the consequence is of this redistribution of iron toward macrophages and whether these findings can be translated to humans.

Nevertheless, it seems likely that iron-deficiency impairs malaria infection, and both nutritional and immunological aspects should be considered to play a role in which hepcidin might function as an arm of immunity linking malaria with iron.

Highlights

- Malaria and non-typhoid *Salmonella* infections frequently co-occur
- We speculate that iron may play a central role in this association
- Malaria leads to iron sequestration in macrophages
- Iron sequestration in macrophages increases survival of *Salmonellae spp*
- This might also contribute to the adverse effects of iron therapy in malaria areas

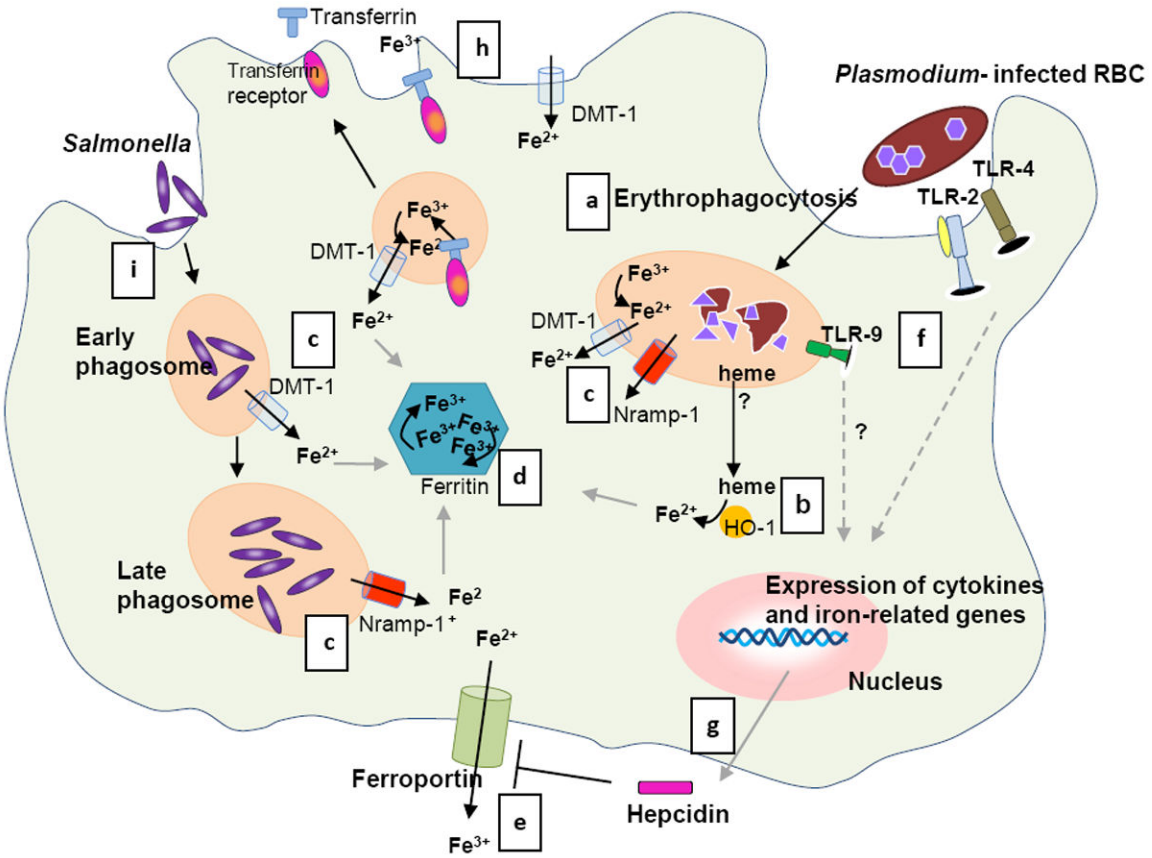

Figure 1. Interaction of malaria and *Salmonella* with macrophage iron

Phagocytosis of both uninfected and infected RBCs **(a)** is increased during the blood stage malaria infection [83], which results in degradation of the RBCs by proteolytic enzymes into heme. **(b)** HO-1 converts heme into iron (and carbon monoxide and biliverdin). **(c)** Excess iron is transported to the cytosol via phagosomal transporters DMT-1 and Nramp-1 [24] and further processed: (i) stored in ferritin **(d)**, and (ii) used in metabolic processes or released from the cell via ferroportin **(e)** [26, 79]. **(f)** Meanwhile, parasite products activate the innate immune system via Toll Like receptors (TLR) 2, 4 and 9 [84]. This systemic response during malaria induces hepatic hepcidin production; **(e)** hepcidin functions by blocking ferroportin [29, 78]. In addition, monocytes and macrophages also express hepcidin upon stimulation with various pro-inflammatory cytokines and parasitized RBCs [36-38], **(g)** which may result in autocrine ferroportin blocking. **(h)** In addition, inflammatory stimuli inhibit ferroportin and modulate cellular iron uptake by DMT-1 and transferrin receptor [36, 39]. As a consequence of these processes iron is sequestered in macrophages. **(i)** *Salmonella* enters the cell via endocytosis and proliferates in phagosomes. Nramp-1 expression is required to control *Salmonella* growth by depleting the phagosome of iron **(c)** [46]. In a co-infection, *Salmonellae spp.* may benefit from the increased cellular iron induced by a malaria infection and establish an infection. Whether both pathogens reside in the same macrophage during invasive NTS infection and malaria as depicted in the figure is unknown. Illustration by A. Kartikasari.

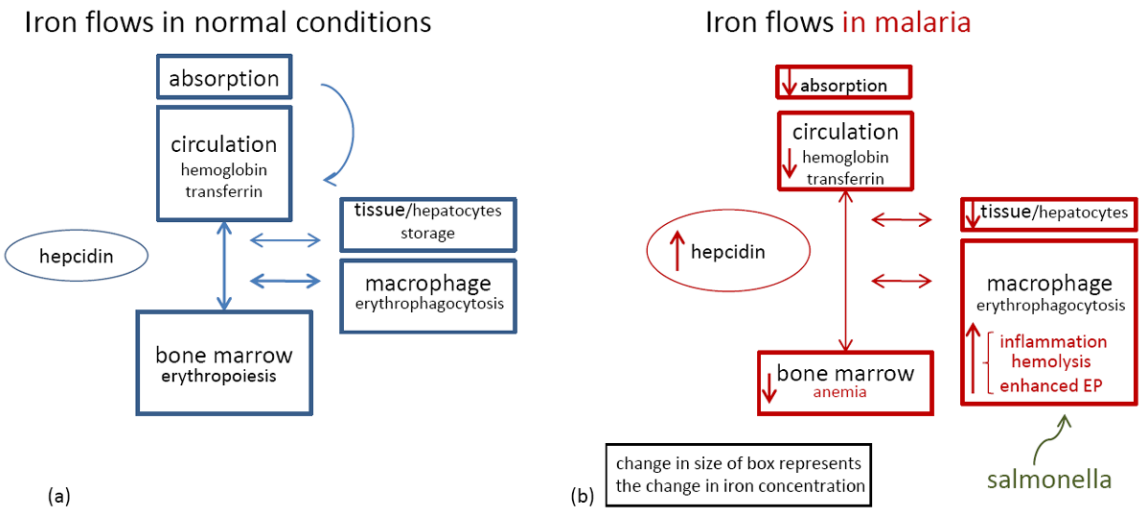

Figure 2. Systemic effects of malaria on body iron stores

(a) A schematic presentation of iron flows in normal circumstances. Hepcidin controls the amount of iron absorbed from the diet and the release of iron from macrophages from the reticulo-endothelial system [29]. There is a steady state of iron recycling from senescent RBCs that are degraded in macrophages into iron. This iron is transported via transferrin in the circulation towards the bone marrow where iron is essential for erythropoiesis. Body iron losses are minimal and not regulated. **(b)** Iron flows in malaria. During malaria infection the body iron homeostasis changes, but total amount of body iron remains similar as is visualized by the change in the size of boxes. Inflammatory factors increase hepcidin release [30, 33, 76, 77]. As a consequence, absorption of iron from the diet is impaired, iron is redistributed to macrophages, less iron is bound to transferrin and iron stores become depleted. Finally, the erythropoiesis is impaired, due to hepcidin-mediated iron restriction, in addition to malaria-specific inhibitory factors (e.g., cytokines, hemozoin) [71]. Also, a blood stage malaria infection is hallmarked by hemolysis and increased phagocytosis of parasitized and non infected RBCs, which also augments the macrophage iron content. As suggested, the increased iron availability in malaria could facilitate the growth and replication of *Salmonellae spp*. Abbreviation: EP, erythrophagocytosis.

Table 1

Regulation of iron regulatory proteins in macrophages[a]

stimuli	iron		heme		inflammation		pRBCs		*Salmonella*	Refs
hepcidin	no effect	[36]	n.a.		↑ transcriptional e.g., IL-6, LPS	[36, 37]	↑ transcriptional	[38, 76]	↑ transcriptional	[47]
ferroportin	↑ post-transcriptional ↑ transcriptional	[78] [36]	↑ transcriptional	[26, 79]	↓posttranslational by hepcidin ↓ transcriptional LPS, IFNγ, TNFα	[36, 39]	n.a.		↑ transcriptional /no effect	[44, 47]
H- ferritin	↑ post-transcriptional	[78]	↑ transcriptional	[26]	↑transcriptional?		n.a.		↑ transcriptional /no effect	[44, 47]
HO-1	n.a.		↑ transcriptional	[26, 79]	↑ IL- 10, not LPS	[80]	↑ transcriptional	[63]	↑ transcriptional	[44, 47]
DMT-1	↓posttranslational ↑ transcriptional?	[78] [36]	no effect	[81]	↑ transcriptional LPS, IFNγ, TNFα	[39]	n.a.		↑ transcriptional	[44, 47]
Nramp-1	n.a.		↑ transcriptional	[81]	↑ transcriptional LPS	[82]	n.a.		n.a.	

[a]Overview of how iron regulatory proteins of macrophages (vertical column) are affected by various components related to malaria infection (iron, heme, inflammatory stimuli, pRBCs) as well as by *Salmonella*. The consequence of these alterations is discussed in Figure 1.

Abbreviations: LPS, lipopolysaccharide; TNFα, tumor necrosis factor α; IFNγ, interferon γ; n.a., no reports available.