Published in final edited form as:
Curr Opin Hematol. 2014 May ; 21(3): 193–200. doi:10.1097/MOH.0000000000000039.

Malaria parasites and red cell variants: when a house is not a home

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Abstract

Purpose of review—Multiple red cell variants are known to confer protection from malaria. Here we review advances in identifying new variants that modulate malaria risk and in defining molecular mechanisms that mediate malaria protection.

Recent findings—New red cell variants, including an innate variant in the red cell's major Ca^{2+} pump and the acquired state of iron deficiency, have been associated with protection from clinical falciparum malaria. The hemoglobin (Hb) mutants HbC and HbS – known to protect carriers from severe falciparum malaria – enhance parasite passage to mosquitoes and may promote malaria transmission. At the molecular level, substantial advances have been made in understanding the impact of HbS and HbC upon the interactions between host microRNAs and *Plasmodium falciparum* protein translation; remodeling of red cell cytoskeletal components and transport of parasite proteins to the red cell surface; and chronic activation of the human innate immune system which induces tolerance to blood-stage parasites. Several polymorphisms have now been associated with protection from clinical vivax malaria or reduced *P. vivax* density, including Southeast Asian ovalocytosis and two common forms of glucose-6-phosphate dehydrogenase deficiency.

Summary—Red cell variants that modulate malaria risk can serve as models to identify clinically relevant mechanisms of pathogenesis, and thus define parasite and host targets for next-generation therapies.

Keywords

malaria; hemoglobinopathy; enzymopathy; ovalocytosis; PfEMP1

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Conflicts of interest
There are no conflicts of interest.

INTRODUCTION

In 1949, J.B.S. Haldane suggested that β-thalassemia trait may protect red cells from *Plasmodium* infection and thus humans from clinical malaria (1). This general hypothesis – that red cell variants modulate susceptibility to malaria – has since been investigated for hemoglobin (Hb) mutants, enzymopathies, ABO blood groups, and acquired iron deficiency. Specifically, studies have confirmed that protection from severe falciparum malaria is conferred in African children by heterozygosity for HbA and HbS (HbAS, sickle-cell trait), hetero- and homozygosity for HbC (HbAC, HbCC) [reviewed in (2)], hemizygosity for glucose-6-phosphate dehydrogenase (G6PD) deficiency (3), and lack of blood group antigens A and B (i.e., type O) (4) (Table (3–18)). HbAS and HbCC seem to provide substantial protection from severe disease (91% and 73%, respectively) (2), but little or no protection from parasitemia, suggesting that these traits interfere specifically with the pathogenic mechanisms governing transitions from parasitization to severe and fatal infections. From a biological point-of-view, these insights highlight the co-evolution of host and parasite and serve as a model of balancing selection. From a clinical perspective, these relationships represent a naturally occurring model of protection from severe, life threatening malaria, which can be used to isolate the mechanisms of parasite pathogenesis. By preventing malaria parasites from causing disease, these red cell variants can help us discover clinically significant mechanisms of pathogenesis and investigating them as targets for future therapeutics.

Plasmodium species

Plasmodium spp. parasites are vector-borne pathogens that infect the red cells of reptiles, birds, and primates. Five species of *Plasmodium* (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*) parasitize and cause malaria in humans. *P. falciparum* and *P. vivax* cause most episodes of malaria and are therefore the focus of this review. *P. falciparum* is endemic in tropical areas worldwide but is transmitted most intensely in sub-Saharan Africa, where it kills more than 500,000 children each year (19). *P. vivax* is endemic in South and Southeast Asia, South America, and the Western Pacific, and is a major cause of morbidity in these regions.

All five *Plasmodium* species are transmitted by female *Anopheles* spp. mosquitoes during a blood meal on humans. After injection into the skin, sporozoites migrate to the liver, develop within hepatocytes for 7–10 days, and emerge as a large population of merozoites capable of invading red cells. This new blood stage of asexual parasite development produces the clinical syndrome of malaria, and is marked by repeated cycles of merozoite invasion of red cells, parasite maturation and multiplication within red cells, and merozoite egress from red cells. During this process, a small fraction of parasites differentiates into sexual gametocytes, which are ingested by another female anopheline mosquito. In this vector, gametocytes undergo sexual recombination and eventually develop into sporozoites, which migrate to the mosquito's salivary glands and await inoculation into the next human host.

Impact of red cell variants on malaria

Malaria is the clinical syndrome that results from infection with *Plasmodium* spp. parasites. The symptoms of malaria are notoriously nonspecific, but fever is a cardinal finding in nearly all clinically significant infections. Additionally, the clinical outcomes of blood-stage infections vary widely in severity from mild, uncomplicated malaria to severe, life threatening malaria, which encompasses the often non-overlapping syndromes of cerebral malaria, severe anemia, and respiratory distress. An estimated 451 million clinical cases of falciparum malaria occurred in 2007, largely in sub-Saharan Africa, and nearly 3 billion people are at annual risk of infection with *P. vivax*, largely in Central Asia (20, 21). As noted earlier, the interactions between red cell variants and *P. falciparum* parasites have generated longstanding interest, and recent studies have begun to define the mechanisms of these relationships. Even today, there exist few data on the impact of red cell variants upon other *Plasmodium* species and the malaria syndromes they cause.

P. falciparum malaria

Recent studies of *P. falciparum* in West Africa have underscored the influence of red cell variants on human malaria cases. In extending an earlier study of the influence of known Hb mutants on severe falciparum malaria (5), Timmann *et al.* (22) performed a genome-wide association study (GWAS) of severe malaria in Ghanaian children to identify novel polymorphisms that modulate susceptibility to this disease. In this study, four loci achieved genome-wide significance: known variants at the β-globin and ABO blood group loci, a locus near the *MARVELD3* gene which encodes an endothelial tight-junction protein, and a locus in the *ATP2B4* gene which encodes a Ca^{2+} pump. Although this Ca^{2+} pump is expressed in numerous tissues, it serves as the principal pump in red cells, in which *P. falciparum* growth may conceivably be disrupted by alterations in Ca^{2+} homeostasis. This finding was replicated by the authors in a separate GWAS study in Gambian children, and awaits functional exploration as a component of yet another mechanism by which red cell variants may attenuate the virulence of *P. falciparum*.

A study of *P. falciparum* transmission in Burkina Faso elegantly demonstrated that human β-globin mutations influence the transmissibility of *P. falciparum* parasites. Gouagna *et al*. (23) employed several study designs to test whether HbC and HbS children infected with *P. falciparum* transmit parasites more efficiently to anopheline mosquitoes. In cross-sectional surveys, they noted a higher prevalence and greater density of gametocytes in HbC children, and using *in-vivo* and *ex-vivo* blood feeding experiments they reported that HbC or HbS (compared with HbAA) red cells were associated with two- to four-fold higher transmission of parasites to mosquitoes. Therefore, these prevalent β-globin mutations seem to protect individual carriers from life-threatening disease states, while at the same time promoting transmission of parasites through mosquito vectors and into human populations. These data highlight how malaria parasites and humans have co-evolved: while *P. falciparum* parasites drive the continued inheritance of HbS and HbC in human generations, these mutations enhance the continued transmission of these parasites into successive mosquito populations.

In Uganda, Gong *et al*. (24) conducted a longitudinal study of HbAA and HbAS children to better characterize the natural history of *P. falciparum* infections and the patterns of

protection from asymptomatic parasitemia and symptomatic malaria. In a mesoendemic setting, 601 children aged 1–10 years were followed frequently over 18 months for parasite genotypes, patent parasitemia, and malaria episodes. Compared to HbAA children, HbAS children had a lower "molecular force of infection" (i.e., the number of new parasite strains in a person per year), suggesting that *P. falciparum* established fewer blood-stage infections in HbAS children. Once parasitemic, HbAS children had lower parasite densities and less likelihood to develop malaria symptoms. These three measures of protection were more pronounced with increasing age, suggesting that HbAS protects in part through an acquired mechanism. HbAS children were also less likely to have hyperparasitemia, but protection from this life-threatening phenotype was greater in younger than in older children, suggesting it is mediated by an innate mechanism. Identifying specific innate and acquired immune responses that enhance HbAS-mediate protection may inform vaccine design. Further investigations should also explore potential roles of the HbAS red cell itself in augmenting immune responses, for example, by acting as an adjuvant.

Most studies of red cell variants and malaria have investigated innate disorders, but a recent study by Gwamaka *et al.* (25) explored the impact of the acquired state of iron deficiency upon the risk of malaria. In a longitudinal study of Tanzanian children with frequent, active follow-up visits, iron deficiency at routine, well-child visits reduced the risk of subsequent parasitemia, severe malaria, and malaria-attributable mortality. Because diagnosing iron deficiency in such settings is challenging, the authors employed multiple definitions of iron deficiency using various cut-off values for serum levels of ferritin, C-reactive protein, and transferrin receptor – all of which were associated with similar risk reductions. These data suggest that iron deficiency protects children from severe and fatal falciparum malaria, though it remains unclear if the mechanisms of this effect occur principally in the red cells or sera of these children.

P. vivax malaria

Southeast Asian ovalocytosis (SAO, conferred by the band 3 deletion SLC4AΔ27) is lethal in the homozygous state but achieves high prevalences in the heterozygous state in Papua New Guinea (PNG), suggesting balancing selection conferred by some survival advantage in this geographic area. SAO has previously been associated with reduced risk of cerebral *P. falciparum* malaria in PNG (18), and Rosanas-Urgell and colleagues (26) hypothesized that it may also confer protection from *P. vivax* malaria. In three complementary clinical studies in PNG, SAO was associated with a 43% reduced incidence of vivax malaria in a prospective cohort of children, and possibly with reduced risk of severe vivax malaria as well. SAO was not associated with other protective red cell phenotypes, such as low expression of the Duffy antigen receptor for chemokines (DARC, an invasion receptor for *P. vivax*) or the presence of α-thalassemia. Additional field- and laboratory-based studies are needed to confirm and elucidate the mechanism of this newly discovered protection.

Two recent studies have explored the influence of G6PD deficiency on *P. vivax* infection. Louicharoen *et al.* (27) investigated the G6PD-Mahidol variant, which reduces enzyme function by 5 to 32% and is prevalent in several ethnic groups in malarious areas of Southeast Asia. Population genetics analyses indicated significant, recent positive selection

of G6PD-Mahidol in human populations, suggesting it confers a clinical benefit to carriers. In partial support of this possibility, clinical studies found an allele-dependent reduction in *P. vivax* density in both males and females; however, this variant was not associated with the frequency of malaria episodes or the prevalence of *P. falciparum* parasitemia.

In complementary work, Leslie *et al.* (28) conducted a case-control study of *P. vivax* malaria and its relationship with G6PD deficiency on the Afghanistan-Pakistan border. Phenotypic G6PD deficiency was significantly reduced among the cases of vivax malaria, and genotypic deficiency (G6PD-Mediterranean) was reduced in both males and females, providing further evidence that this class II–III variant protects carriers from vivax malaria. Taken together, these studies establish an evidence base for exploring the impact of red cell variants upon vivax malaria, and suggest that this disease has historically been severe enough to impair fitness and to naturally select red cell variants that are potentially deleterious.

Mechanisms of protection against malaria

The mechanisms by which red cell variants confer protection from malaria have not been definitively elucidated (Fig. 1) [reviewed in (29) and (30)]. While recent studies have elucidated remarkable cellular, molecular, and immunological impacts of HbS upon red cell parasitization and malaria risk, further efforts are required to more finely characterize these phenomena and exploit them in the development of clinical interventions.

Effects on parasite pathogenicity

Two studies have highlighted the dialogue between red cells and *P. falciparum* and explored how these interactions are impacted by β-globin mutations. HbCC and HbAS red cells reduce the expression and impair the function of *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) variants (31, 32), which principally mediate the binding of infected red cells to extracellular host receptors. Using electron tomography of infected HbAA, HbCC, and HbSC red cells, Cyrklaff *et al.* (33) investigated the intraerythrocytic machinery that *P. falciparum* establishes *de novo* and utilizes to traffic its PfEMP1 proteins to the red cell surface. In HbAA red cells, parasites mined their host cell actin and used it to construct an intracellular network for transporting parasite proteins – including PfEMP1 – to the red cell surface. In HbCC and HbSC red cells, parasites developed normally but the cytoskeletal network was aberrant in appearance, and the transport of parasite-derived proteins to the red cell surface was impaired. Because these parasite-derived surface proteins mediate the pathogenicity and immunogenicity of infected red cells, these findings suggest new avenues by which their expression may be modified for clinical benefit.

In another study, LaMonte *et al.* (34) explored the interactions of human red cell microRNAs (miRNAs) upon *P. falciparum* development and how this relationship may be modified by HbS. The premises of their experiments were that *P. falciparum* development is attenuated in HbAS and HbSS red cells under low oxygen tension (35, 36), and that these cells manifest altered miRNA profiles (37). Remarkably, the investigators demonstrated that host miRNAs are transported into the parasite, where they can fuse with parasite mRNA to produce chimeric host-parasite RNAs which demonstrate aberrant translation and function. Furthermore, transfection of infected HbAA red cells with host miRNAs that are

overexpressed in HbAS and HbSS red cells impaired parasite development, modulated parasite metabolic pathways, and increased parasite differentiation into gametocytes. Given recent developments in the therapeutic targeting of human miRNAs as a treatment for other infections (38), these novel miRNA activities in the host-parasite relationship may represent another pathway for attenuating the virulence of *P. falciparum*.

Effects on antiparasitic immunity

The mechanisms of innate and adaptive immunity to human malaria remain largely obscure. Because epidemiologic data suggest that enhanced immunity contributes to protection conferred by hemoglobinopathies (24, 39), red cell variants may offer a model for identifying clinically protective, antimalarial immune responses and recapitulating them with new therapeutics and vaccines. Humoral immunity was explored by Tan *et al.* (40) in a longitudinal study of antibody responses to *P. falciparum* antigens in HbAS and HbAC children in Mali. By interrogating plasma before and after the malaria transmission season with a printed array of nearly 500 parasite proteins, the authors demonstrated that these β-globin variants did not enhance the acquisition of seroreactivity to any of the investigated antigens. Based on these comprehensive data, it seems unlikely that HbAS or HbAC generally enhance the development of IgG responses to parasite proteins. It remains possible, however, that these β-globin variants specifically promote the acquisition of IgG responses to PfEMP1 and other variant surface antigens that were not well represented on the array.

A recent study using murine models of HbS and experimental cerebral malaria explored the interactions between HbS, *Plasmodium* spp., and the innate immune system. Ferreira *et al.* (41) infected mice with the *P. berghei* ANKA strain and demonstrated that, similar to humans with HbAS, mice with a sickle-cell trait phenotype did not develop cerebral malaria. These mice expressed higher levels of heme oxygenase-1 (HO-1), and deletion of the *hmox1* gene abrogated the protection afforded by the murine sickle-cell trait. Furthermore, induction of HO-1 attenuated the elaboration of chemokines implicated in the pathogenesis of cerebral malaria. Heme itself was protective against murine cerebral malaria when present in small amounts prior to infection, but deleterious when administered in large amounts after infection. Together, this series of experiments suggests that HbAS, by keeping free heme levels chronically elevated, constitutively induces the production of HO-1 which mitigates heme toxicity after *Plasmodium* infection and suppresses chemokine levels that normally cause cerebral malaria. By exploring similar or unrelated mechanisms that enable HbAS children to “tolerate” *Plasmodium* infection (Fig. 2), we may discover strategies to prevent the development of severe and fatal malaria syndromes.

CONCLUSION

Recent studies have successfully identified red cell variants that confer protection from falciparum and vivax malaria, and have used some of these variants as models to explore the pathogenesis of falciparum malaria. These latter studies have uncovered novel mechanisms of disease in which: parasite mRNA and host microRNA fuse to constitute chimeric mRNA with altered translation; *P. falciparum* parasites mine host red cell actin in order to remodel

the cytoskeleton and enable trafficking of parasite proteins to the red cell surface; and chronic activation of HO-1 and alteration of chemokine profiles mitigates murine cerebral malaria. These findings reflect promising opportunities to develop future preventive and therapeutic interventions for malaria.

Acknowledgments

Dr. Taylor is supported by an award from the NIAID, K08AI100924. Dr. Fairhurst is supported by the Intramural Research Program of the NIAID, NIH.

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KEY POINTS

- Southeast Asian ovalocytosis and the “Mahidol” and “Mediterranean” variants of G6PD deficiency protect against vivax malaria, reduce *P. vivax* parasite density, or both.
- β-globin variants HbS and HbC confer substantial protection from severe falciparum malaria but enhance the passage of *Plasmodium falciparum* to mosquitoes, which may contribute to onward transmission in human populations.
- Aberrant host microRNA profiles in HbS-containing red cells may impair *P. falciparum* development by forming chimeric fusion mRNA with parasite transcripts and adversely affecting protein translation.
- HbS and HbC interfere with parasite-dependent remodeling of red cell cytoskeletal actin and transit of parasite proteins to the red cell surface.
- The chronic release of heme from HbS red cells activates heme oxygenase-1 which mediates protection from cerebral malaria in a murine model, suggesting a role for the innate immune system in the malaria protective effects of sickle-cell trait in humans.

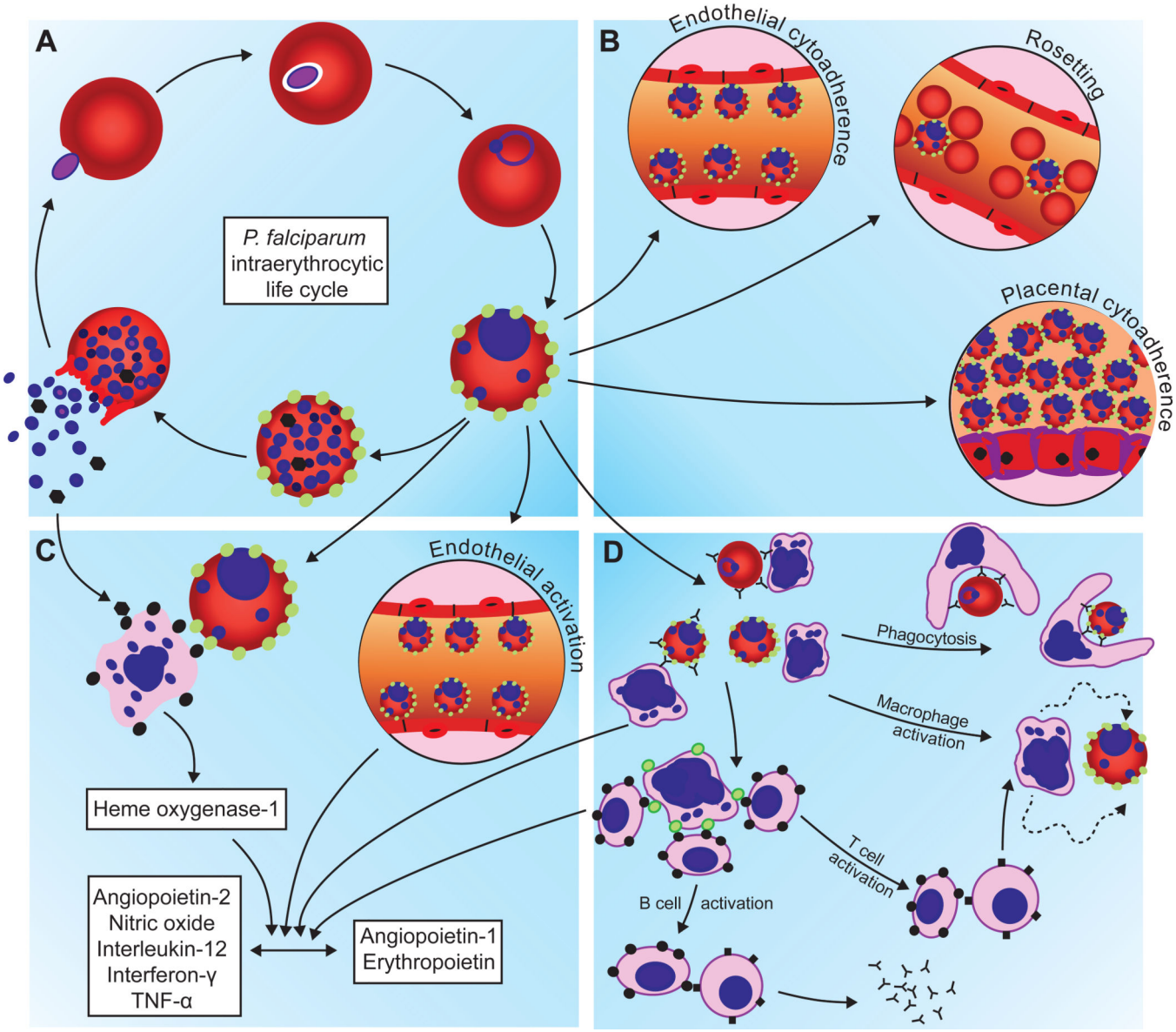

Figure 1. Diverse mechanisms by which red cell variants may influence malaria pathogenesis

A. *Plasmodium* spp. parasites invade, mature, and replicate in red cells, and these events may be disrupted by a variety of mechanisms including the influence of host microRNAs on parasite protein translation, as described by LaMonte *et al.* (34). **B.** *P. falciparum*-infected red cells cause disease by binding to extracellular receptors on the surface of microvascular endothelial cells, uninfected red cells, and placental syncytiotrophoblasts via the parasite-derived surface antigen PfEMP1; trafficking of PfEMP1 to the red cell surface is impaired in HbS- and HbC-containing cells owing to a disruption of the parasite's remodeling of the cell's actin cytoskeleton, as described by Cyrklaff *et al.* (33). **C.** Activation of the innate immune system by infected red cells, parasite DNA, and free heme can alter the balance of pro- and anti-inflammatory cytokines to influence pathogenesis, as described by Ferreira *et al.* (41). These investigators propose that the chronic release of free heme from HbS-

containing red cells activates heme oxygenase-1, which then mediates tolerance of *Plasmodium* parasites in a murine model of cerebral malaria. **D.** *Plasmodium* spp. parasites may be cleared more efficiently by the adaptive immune system when infecting red cell variants, though Tan *et al.* (40) report that children with β-globin variants did not acquire antibodies to *P. falciparum* proteins more rapidly or efficiently than children with normal β-globins. Originally published in Taylor SM, Cerami C, Fairhurst RM. Hemoglobinopathies: Slicing the Gordian knot of *Plasmodium falciparum* malaria pathogenesis. *PLoS Pathogens* 2013;9:e1003327. Made available under the Creative Commons Attribution CCBY license.

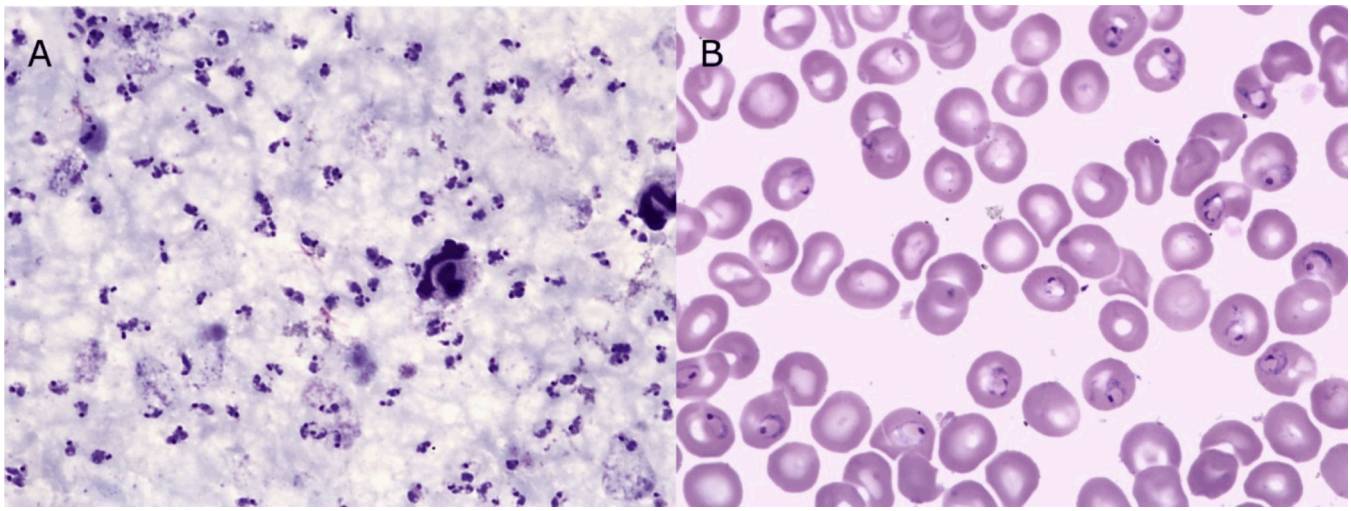

Figure 2. Tolerance of *Plasmodium falciparum* hyperparasitemia

A 2-year-old Malian boy presented with a history of fever and malaise. Thick and thin blood films stained with Giemsa showed 273,450 parasites per μL of whole blood (**A**) and 25.7% parasitemia (**B**), respectively. At this time, he had a normal temperature (36.9°C) and moderate anemia (Hb level 7.8 g/dL). The child received artesunate and amodiaquine over 3 days, never developed symptoms or signs of severe malaria, and was later found to carry the sickle-cell trait. This case was originally published in *Blood*. Beaudry JT, Fairhurst RM. Microvascular sequestration of *Plasmodium falciparum. Blood*. 2011;117:6410. © the American Society of Hematology.

Table

Interactions between innate red cell variants and *Plasmodium* spp. parasites

Disorder	Effects	References
β-globin variants		
HbS	>90% reduction in risk of severe falciparum malaria by HbAS; >30% reduction in risk of uncomplicated falciparum malaria by HbAS	(5, 6, 7)
HbC	73–86% reduction in risk of severe falciparum malaria by HbCC	(5, 8)
HbE	Possible protection from severe falciparum malaria by HbAE and HbEE	(9)
HbF	Reduced *P. falciparum* growth in red cells with high HbF content; reduced cytoadherence of infected red cells with high HbF content	(10, 11)
β-thalassemia	Reduced risk of uncomplicated falciparum malaria	(12)
α-globin variants		
α^{+} trait	Small reduction in risk of severe falciparum malaria	(5)
α^{0} trait	38% reduction in risk of severe falciparum malaria	(13)
Surface receptor polymorphisms		
ABO blood group	66% reduction in risk of severe falciparum malaria by blood group O	(4)
Duffy antigen receptor for chemokines (DARC)	Reduced invasion of DARC-negative, *FyFy*, red cells by *P. vivax in vitro*	(14)
Complement receptor 1	Reduced risk of severe falciparum malaria with HL allele	(15)
Enzymopathies		
G6PD deficiency	Reduced risk of severe falciparum malaria in hemizygous males and homozygous females	(3)
Pyruvate kinase deficiency	Reduced *P. falciparum* invasion of deficient red cells and enhanced clearance of infected red cells*in vitro*	(16)
Cytoskeletal abnormalities		
Hereditary spherocytosis	Reduced *P. falciparum* growth *in vitro*	(17)
Hereditary elliptocytosis	Reduced *P. falciparum* growth *in vitro*	(17)
Southeast Asian ovalocytosis	Protection from cerebral malaria due to *P. falciparum*	(18)