Published in final edited form as:
Med Microbiol Immunol. 2012 November ; 201(4): 593–598. doi:10.1007/s00430-012-0272-z.

Malaria and Human Red Blood Cells

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Abstract

Invasion by the malaria parasite, *P. falciparum* brings about extensive changes in the host red cells. These include loss of the normal discoid shape, increased rigidity of the membrane, elevated permeability to a wide variety of ionic and other species, and increased adhesiveness, most notably to endothelial surfaces. These effects facilitate survival of the parasite within the host cell and tend to increase the virulence of disease that include cerebral malaria and anemia. Numerous proteins secreted by the internalized parasite and interaction with red cell membrane proteins are responsible for the changes occurring to the host cell. Anemia a serious clinical manifestation of malaria is due to increased destruction of both infected and uninfected red cells due to membrane alterations, as well as ineffective erythropoiesis. There is very good evidence that various red cell disorders including hemoglobinopathies and hereditary ovalocytosis decrease the virulence of disease following parasite infection. A number of mechanism(s) are likely responsible for the protective effect of various red cell abnormalities including decreased invasion, impaired intraerythrocytic development of the parasites and altered interaction between exported parasite proteins and the red cell membrane skeleton.

Keywords

Red cells; Membrane Biology; Plasmodium falciparum; Malaria

Introduction

As a result of survival advantage against malaria, inherited red cell disorders are the most common monogenic diseases affecting over a billion people globally. Each year, more than 500 million people are infected with malaria parasites and half a million (predominantly infants and young children) die as a consequence of the infection [1]. Malaria is one of the most serious and widespread parasitic disease of humans. The clinical symptoms of malaria are manifested when parasites invade and multiply inside human red cells. A large number of interactions occur between various red cell membrane and parasite-expressed proteins during all facets of the parasite life cycle, starting at the initial stages of invasion and continuing through 48 hours of intra-erythrocytic development during which over 400 parasite-encoded proteins are exported into red cell cytoplasm. Intracellular development of the parasite is accompanied by a number of striking structural, biochemical and functional changes in red cells. These induced membrane and cellular changes are responsible for the clinical symptoms and pathologies associated with malaria including severe anemia and cerebral malaria. Alterations in the adhesive and rheological properties of red cells are of particular importance since these traits are directly linked to increased destruction of red

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cells leading to anemia and to the sequestration of parasitised red cells in the microvasculature resulting in cerebral malaria. A human disease of this severity operating over the course of thousands of years of evolution has selected for a number of red cell genotypes that offer some protection against severe forms of the disease in the heterozygous state including sickle cell disease, thalassaemias, G6PD deficiency, blood group polymorphisms and hereditary ovalocytosis. It is very important to note that none of these inherited red cell disorders offer complete protection against parasite infection; they only reduce the morbidity and mortality. The potential mechanisms responsible for the protective effect of various red cell disorders against severe forms of malaria will be discussed. We also summarize our current understanding of the mechanistic basis for red cell membrane and cellular changes induced by the parasite and their potential contribution to various clinical manifestations.

Red blood cell membrane surface proteins and parasite invasion

Parasite invasion of red cell occurs when the extracellular form of the parasite, the merozoite, released following rupture of an infected red cell attaches to the surface of an uninfected red cell. In a relatively short period of 30 to 90 seconds the invasion process is complete [2]. While the initial contact between the merozoite and the red cell is random, for successful invasion, the merozoite actively re-orients itself using actin-myosin motors, to bring its apical end in contact with the red cell membrane [3]. A junctional zone at the site of apposition is formed and specialized organelles in the apical end of the polar merozoite, called rhoptries and micronemes, discharge their contents which include proteases, phospholipases and lipids. These discharged components induce significant structural changes in the red cell membrane including membrane invagination and phosphorylation of membrane skeletal components. Entry of the merozoite into this specialized region follows, again by the action of the parasite actin-myosin motors. Material of both host and parasite origin is recruited into the membrane of the parasitophorous vacuole that surrounds the developing parasite and it is here that the parasite resides for the remainder of its life span of 48 hours within the red blood cell [3].

The various parasite ligands that are involved in the invasion process are erythrocyte binding antigen (EBA) family of proteins that contain one or more Duffy-binding ligand domains, regions of sequence characterised by the presence of 12 conserved cysteine residues within tryptophan and tyrosine-rich sequences [4, 5]. A large number of such parasite proteins have been identified in the merozoite of *P. falciparum*, including EBA175, EBL-1 and EBA-140, for which cognate receptors on red cells are glycophorin A, glycophorin B and glycophorin C, respectively. The other major group of parasite ligands are a family of proteins designated as the reticulocyte binding proteins (Rh family) for which cognate receptors on the host cell include complement receptor-1 and basigin [6]. Finally, the red cell anion transporter protein, band 3, has also been implicated in the initial interaction between the merozoite and the red cell [7]. There are undoubtedly further complexities to be unraveled in ligands and receptors involved in the invasion process.

In terms of natural selection of red cell variants that confer protection against malaria by decreasing invasion efficiency, there exist a number of examples with mutations in genes encoding red cell membrane proteins that are receptors for merozoite surface proteins (Table 1). For example, there is high incidence up to 30% of Gerbich variant of glycophorin C in endemic areas of Papua New Guinea, a large number of glycophorin A and glycophorin B variants in endemic areas of Brazil and a high incidence of Band 3 variant (10 to 30%) with deletion of 9 amino acids in the cytoplasmic domain in Melanesia and other regions in Far East Asia.

Apart from the involvement of individual red cell surface proteins in the invasion process, it is becoming clear that the overall arrangement of these surface molecules in the membrane is also important. A sub-class of membrane anchored proteins associate with cholesterol-rich sub-domains within the cell membrane commonly referred to as lipid "rafts" [8]. A number of raft-associated proteins of both red cell and parasite origin have been identified in the parasitophorous vacuolar membrane suggesting that lipid rafts play a role in the transport of macromolecules into malaria-infected cells [8–10]. Experiments with cholesterol-depleted red cells demonstrated that following depletion the red cells become resistant to invasion by malaria parasites, implicating a role for lipid rafts in parasite invasion. As lipid rafts have been shown to regulate cell signaling in various cell types, there findings imply a potential role for red cell signaling in parasite invasion. In fact, an important role for G-protein-coupled receptor signalling mechanism involving the red blood cell β2-adrenergic receptor and the Gαs G-protein sub-unit have been shown to play a critical role in parasite invasion both in vivo and in vitro [11, 12]. It is likely that genetic polymorphic variants of β2-adrenergic receptor may play a role in protection against malaria.

Red cell volume regulation and parasite invasion

A large number of membrane transporters enable the human red cells to tightly regulate their cell volume and thus their state of cell hydration enabling them to maintain cell hemoglobin concentration between narrow limits of 29 to 37 g/dL with a mean cell hemoglobin concentration of 33 g/dL. An important role for state of red cell hydration in parasite invasion has been documented [13]. Dehydration of red cells due to deregulation of membrane transport function resulting in cell haemoglobin concentration >37 g/dL leads to decreased efficiency of invasion and at a cell haemoglobin concentration >41 g/dL the red cells are resistant to invasion. The mechanistic basis for the effects of state of cell dehydration on parasite invasion has yet to be defined.

Cell dehydration is a feature of red cells in hemoglobinopathies including HbAS, HbSS, HbAC, HbSC and HbCC which are highly prevalent in Africa, as well as in hereditary xerocytosis, a less prevalent red cell membrane disorder. In these red cell disorders, 5 to 30 % of the red cells have cell haemoglobin concentration > 37 g/dL, while in normal individuals <1% of circulating red cells have cell hemoglobin concentration > 37 g/dL, The presence of such a large fraction of dehydrated red cells in these red cell disorders markedly reduces the risk of development of high degree of parasitaemia and hence decrease the severity of disease (Table 1).

Membrane and Cellular Alterations in Infected Red Cells during Intraerythrocytic Parasite Development

Once inside the red blood cell, the malaria parasite residing within a vacuole increases in size and over the duration of its 48 hour life cycle, digesting 70% of hemoglobin obtained from the red cell cytoplasm generating amino acids needed for protein synthesis. The undigested heme residue is deposited as a polymerised pigment material called haemozoin. A number of parasite derived proteases that digest hemoglobin including the cysteine-protease falcipains and the aspartic-protease plasmepsins have been characterized [14]. Members of these protease families also play a role in rupture of the red cell to allow merozoite release at the end of the parasite life cycle. Maturation of the malaria parasite causes striking structural and morphological changes in the infected red cell including perturbations in the rheological and adhesive properties of the cell [15]. The red cell becomes more spherical and its surface becomes punctuated by up to 10,000 distinct electron-dense elevations called knobs that are associated with altered adhesive properties of infected red cells.

During this phase of development, over 400 proteins produced by maturing parasites are exported into the red cell cytoplasm and a number of these exported proteins interact with membrane skeleton [16]. The function of a subset of these proteins has begun to emerge; however, most remain without an identified function. The best studied of parasite proteins that associate with the red cell membrane are RESA, MESA, PfEMP-3, KAHRP and PfEMP1. While RESA, PfEMP-3 and MESA are distributed evenly around the membrane of infected red cell, KAHRP and PfEMP1 tend to cluster together in higher density beneath membrane knobs. In early maturing parasites, a number of these proteins, and others such as PfSBP1 and MAHRP appear to be associated with discrete membrane bound structures, known as Maurers' clefts, which are scattered throughout the cytoplasm of the infected red cell. It has been documented that PfSBP1 which associates with Maurers' clefts plays a critical role in assembly of the various parasite proteins at the red cell membrane [17].

Some of these exported proteins are critically important for the normal growth and pathogenicity of malaria parasites. For example, targeted deletion of the *kahrp* gene results in a failure to form knobs on the surface of the infected red cell and abrogates their ability to adhere to vascular endothelial cells under conditions of flow. Similarly, disruption of the *Pfemp3* gene can affect the trafficking of PfEMP1 to the red cell surface with a consequential decrease in their ability to cytoadhere. Further, although the precise function of MESA remains to be defined, its failure to bind to protein 4.1 in the membrane skeleton results in intracellular parasite death.

Significant progress is being made in identifying the binding domains in both parasite proteins and red cell proteins that mediate protein-protein interactions (Table 2) and the functional sequelae of these interactions [18–21]. For example, RESA, expressed at the ring stage of parasite development binds to repeat 16 of beta-spectrin, thereby stabilising the spectrin dimer-dimer interaction and increasing membrane mechanical stability (Figure 1). This stabilisation is likely to be important in enabling the parasite to continue to develop without loss of the structural integrity of the red cell. KAHRP binds to repeat 4 of alpha-spectrin (Figure 1) and also to the cytoplasmic tail of PfEMP1, the parasite ligand expressed on the surface of the infected red cell that mediates all of the adhesive interaction of infected red cells. KAHRP and PfEMP1 are part of electron dense knob structures and play a key role in modulating the avidity of the adhesive interactions. PfEMP3, expressed at the late stages of parasite development binds to C-terminus of alpha-spectrin, thereby destabilising the spectrin-actin-protein 4.1R junctional complex and decreasing membrane mechanical stability (Figure 1). This destabilisation is likely to be important in enabling the release of merozoites from infected red cells.

At the conclusion of the asexual cycle, the red cell is ruptured to release merozoites for a fresh round of red cell invasion. While the details of the cell rupture are still being elucidated, significant progress is being made [22, 23]. Release of merozoites into red cell cytoplasm involves disruption of the internal membrane that surrounds the parasite, the parasitophorous vacuole membrane. At the next stage red cell membrane is disrupted facilitating the release of merozoites into circulation. Development of inhibitors of haemoglobin degradation and of the red cell membrane disruption could be a valuable new therapeutic option for treatment of malaria.

Adhesive interactions of infected red blood cells

Red cells infected by mature forms of *P. falciparum* become adhesive for a number of different cell types including vascular endothelial cells (cytoadherence), platelets and other infected or non-infected red cells [24–26]. From the parasites' standpoint, imparting an adhesive phenotype on the red blood cells in which they reside is the key to both its survival

and its pathogenicity, preventing destruction of infected red cells in the spleen and allowing the microaerophilic parasites to sequester and mature in a relatively hypoxic environment within the deep microvasculature of a variety of organs. For the infected human, however, the consequences of sequestration are often extremely detrimental, causing obstruction of blood flow particularly in small diameter vessels of the microcirculation. All of these interactions are mediated by parasite ligands expressed on the surface of the infected red cell, *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family of proteins.

Cytoadherence has been studied in a number of *in vitro* and *ex vivo* systems and infected red blood cells have been demonstrated to be capable of adhering to a number of different receptors that are expressed on the surface of vascular endothelial cells or on syncytiotrophoblasts that line the placenta. The repertoires of receptors to which infected red cells can bind are diverse and include members of the immunoglobulin super family, integrins and glycosamino- and proteoglycans. These include CD36, ICAM1, VCAM1, E-selectin, PECAM1, chondroitin sulphate A and hyaluronic acid. The development of cerebral malaria, possibly the worst and lethal complication of falciparum malaria infection is related to sequestration of infected red cells in the vasculature of the brain. It is now clear that the ability of PfEMP1 to mediate adhesion also depends on its interaction with other exported parasite proteins that are located inside infected red cells and that interact specifically with the red cell membrane skeleton. For example, if the cytoplasmic domain of surface-expressed PfEMP1 does not bind to the histidine-rich parasite-produced protein KAHRP, that clusters PfEMP1 at knobs on the red cell surface, then the infected red cells are incapable of binding to the vascular endothelium under normal circulatory flow.

A recent ultra structural study revealed fewer and smaller knobs at the membrane skeleton of *P. falciparum*-infected HbCC red cells and Hb AS red cells which may affect the amount or distribution of PfEMP1 or other antigens expressed on the red cell surface which could affect their ability to cytoadhere [27]. This is a very interesting hypothesis and could offer a possible additional mechanism for protection against severe disease in HbCC and sickle cell disease (Table 1).

Anemia

Normochromic and normocytic anemia is a common and frequently severe complication of malaria, particularly in young children and pregnant women. In some endemic areas it can account for more than 50% of malaria-associated mortality. The pathogenesis of severe anemia (defined as Hb <5g/dL) during malaria infection is not fully defined [28]. Both increased destruction of infected red cells and decreased production of red cells in response to anaemia due to dyserythropoiesis appear to play a role. There is very good evidence of accelerated destruction of uninfected red cells but the mechanism by which uninfected red blood cells are destroyed has not been elucidated. Marked splenomegaly during acute infection reflects extensive sequestration of red cells by the spleen resulting in anemia [29]. The pathophysiology of severe malarial anaemia remains an important yet relatively neglected area of research.

Recent Progress

The availability of the complete sequence of the malaria parasite genomes and the establishment of a transfection system for the red blood cell stages of *P. falciparum* are enabling development of our improved understanding of the function of parasite proteins in the altered properties of infected red blood cells. It is anticipated these advances in combination with significant advances in our understanding of red cell membrane structure and function will offer opportunities for the discovery of new and urgently needed therapeutic targets for the treatment of malaria.

Acknowledgments

We would like to thank our colleagues and long-time collaborators, Ross Coppel and Kasturi Haldar, for teaching us about malaria and for their sustained and productive collaborative efforts with us over many years.

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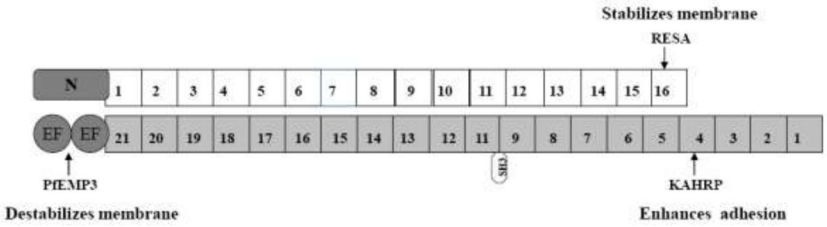

Figure 1.
Interaction of malarial parasite proteins, RESA, KAHRP and pfEMP3, with specific regions of spectrin of the red cell membrane skeleton.

Table 1

RBC variants and protection against malaria

Rd cell phenotype	Potential mechanism(s) of protection against severe forms of malaria
Glycophorin A variants or deficiency	Reduced invasion efficiency due to altered red cell receptor
Glycophorin B variants or deficiency	Reduced invasion efficiency due to altered red cell receptor
Glycophorin C variants or deficiency	Reduced invasion efficiency due to altered red cell receptor
Hereditary ovalocytosis due to mutant band 3	Reduced invasion efficiency due to altered red cell receptor and/or as consequence of increased membrane rigidity
Hemoglobinopathies: HbAS, HbSS, HbSC, HbAC and Hb CC	Reduced invasion efficiency due to red cell dehydration and decreased ability of infected red cells to adhere to endothelial cells due to reduced expression of adhesive ligands on infected red cells.

Table 2

Binding of RBC Membrane Skeleton Proteins to Malaria Proteins

Host	Parasite
Spectrin	RESA, PfEMP1, KAHRP, PfEMP3
Actin	PfEMP1, KAHRP, PfEMP3
Ankyrin	KAHRP
Protein 4.1	PfEMP1, PfEMP3, MESA