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Review

Pathogenesis of *Plasmodium falciparum* malaria: the roles of parasite adhesion and antigenic variation

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Abstract. Malaria results in up to 2.5 million deaths annually, with young children and pregnant women at greatest risk. The great majority of severe disease is caused by *Plasmodium falciparum*. A characteristic feature of infection with *P. falciparum* is the accumulation or sequestration of parasite-infected red blood cells (RBCs) in various organs, such as the brain, lung and placenta, and together with other factors is important in the pathogenesis of severe forms of malaria. Sequestration results from adhesive interactions between parasite-derived proteins expressed on the surface of infected RBCs and a number of host molecules on the surface of endothelial cells, placental cells and uninfected RBCs. Some receptors for parasite adhesion have been implicated in particular malaria syndromes, such as intercellular adhesion molecule 1 in cerebral malaria and chondroitin sulfate A and hyaluronic acid in placental infection. The principal parasite ligand and antigen on the RBC surface, *P. falciparum* erythrocyte membrane protein 1 encoded by a multigene family termed *var*, is clonally variant, enabling evasion of specific immune responses. An understanding of these host-parasite interactions in the context of clinical disease and immunity may reveal potential targets to prevent or treat severe forms of malaria.

Key words. Malaria; pathogenesis; adhesion; antigenic variation; cerebral malaria; placenta; *var* genes.

Introduction

Around 40% of the world's population lives in malaria-endemic areas, distributed across 100 countries in tropical and subtropical regions [1]. Malaria infection results in 300–500 million clinical cases and 1.5–2.7 million deaths annually, with ~1 million deaths in children under 5 years of age [1]. About 90% of cases and most deaths occur in tropical Africa.

Plasmodium falciparum is responsible for the majority of severe clinical disease, most affecting young children, nonimmune adults and pregnant women, and is the focus of this review. It is the predominant species in tropical Africa, eastern Asia, Oceania and the Amazon basin of South America. *Plasmodium vivax* also causes a substantial amount of clinical malaria and is widely distributed geographically; however, severe clinical disease is rarely seen with *P. vivax* infections, or with *Plasmodium ovale* and *Plasmodium malariae*.

The life cycle of *P. falciparum* (and other *Plasmodium* species) involves several stages in both human and mosquito hosts. Following the bite of an infected female *Anopheles* mosquito, sporozoites injected from the insect's salivary glands enter the bloodstream and travel quickly to the liver. Invasion of hepatocytes ensues, and an 8- to 12-day incubation period enables asexual replication to generate many daughter merozoites. Infected hepatocytes then rupture, releasing merozoites into the circulation, thus commencing the blood stage of the infection during which clinical malaria may develop.

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After invasion of red blood cells (RBCs) by merozoites, parasites mature over an ~48-h period through ring stage to pigmented trophozoites and finally divide into daughter merozoites at the schizont stage. Rupture of the infected RBCs releases merozoites into the circulation to continue further cycles of asexual replication. Some parasites at the ring stage develop into male and female gametocytes, which may be taken up by a feeding mosquito where sexual reproduction occurs. Developmental time in the mosquito varies according to ambient temperatures, but is typically in the order of 7–14 days.

Clinical features of *P. falciparum* infection

Most episodes of infection with *P. falciparum* in malaria-endemic areas lead to mild clinical symptoms, such as fever, malaise, headache, and lethargy followed by spontaneous recovery, which may occur even without drug therapy. In a proportion of cases, severe disease results. Following repeated exposure to malaria, immune responses eventually develop that protect against serious disease but do not convey sterile immunity; low-grade infections occur with few or no clinical symptoms.

Clinical manifestations of severe *P. falciparum* infection are variable in nature and severity, and it can be difficult to clearly define syndromes in many cases [2]. A cerebral malaria syndrome appears to account for the majority of malaria deaths and is characterised by unrousable coma often with convulsions, but any degree of impaired consciousness may indicate cerebral involvement [2]. Severe normocytic anaemia is probably the second most common presentation of severe *P. falciparum* infection and probably results from increased RBC destruction and reduced erythropoiesis [3]. Respiratory distress carries a very poor prognosis and may be a consequence of fluid retention, but has been observed in individuals with a normal or negative fluid balance status, and metabolic acidosis [2, 4]. Renal dysfunction or failure, hypoglycaemia, circulatory collapse and shock, disseminated intravascular coagulation and spontaneous bleeding, and acidosis can also occur [2]. Severe haemoglobinuria, or 'black water fever', as a consequence of severe intravascular haemolysis, is sometimes observed and can result from immune lysis of quinine-sensitised erythrocytes, or from antimalarial therapy in individuals with glucose-6-phosphate dehydrogenase deficiency [2].

Among malaria-exposed adults, pregnant women are particularly susceptible to malaria, despite substantial immunity prior to pregnancy, and the risk is highest in first pregnancies [5, 6]. The major complications of infection are maternal anaemia, which in turn increases maternal deaths, and reduced infant birthweight from a combination of intrauterine growth retardation and premature delivery leading to excess infant mortality [5–7]. In some settings maternal malaria may also cause spontaneous abortion or stillbirth [6].

Histopathological findings in severe *P. falciparum* infection: parasite sequestration

Postmortem examination of individuals who die of cerebral malaria typically reveals gross swelling of the brain with ring or petechial haemorrhages associated with the sequestration or accumulation of parasite-infected RBCs in small blood vessels on histopathology [8–10] (fig. 1A). Several studies have shown that in cases defined with cerebral malaria on clinical grounds, parasite sequestration at postmortem examination is greater in the

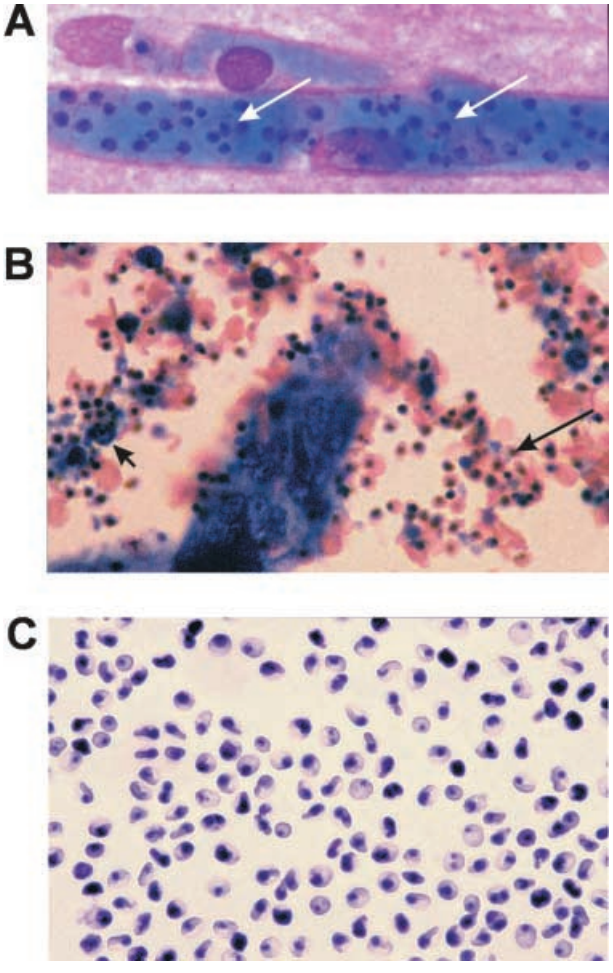

Figure 1. (*A*) Histological section of tissue collected post-mortem from a Malawian child. RBCs infected with mature stages of *P. falciparum* (arrows) are packed tightly within a small vessel in the brain. (Image courtesy of Dr Terrie Taylor, Wellcome Trust Research Laboratories, College of Medicine, Blantyre, Malawi.) (*B*) Large numbers of infected RBCs (long arrow) and inflammatory cells (short arrow) in the vascular spaces of an infected placenta from a Malawian woman. (*C*) RBCs infected with mature stages of *P. falciparum* adherent to HA, a receptor for parasite adhesion, immobilised on a plastic surface.

brain than other organs, and parasite density in cerebral vessels is higher than in the peripheral blood [8–12]. However, studies linking sequestration in vessels with cerebral malaria have faced a number of practical difficulties such as clinical case definition and the delay between death and autopsy, which may influence the finding of sequestered parasites given the cyclical nature of the development of *P. falciparum* blood stages. In some individuals believed to have died from cerebral malaria, sequestered parasites have not been found. Parasite sequestration is also observed in many other organs and vascular beds such as the lung, liver, intestine and skin [9, 13]. However, most interest has focussed on sequestration in the brain in regard to understanding the pathology and pathogenesis of cerebral malaria.

Parasite-infected RBCs also concentrate in large numbers in the placenta (fig. 1B) during pregnancy [14, 15], in some cases associated with prominent infiltrates of monocytes and macrophages [16], and are deleterious to foetal development [5, 6]. The placenta provides a unique opportunity to study sequestration, because viable parasites can be isolated relatively easily from infected tissue following delivery rather than relying on examination of tissue post-mortem. Recent studies on placental malaria have given us important insights into how organ-specific parasite sequestration might occur and is discussed later. The link between parasite sequestration and other forms of severe malaria is not clear.

Role of sequestration in the pathogenesis of severe disease

The observation of large numbers of parasites accumulated in specific organs, such as the brain and the placenta, associated with adverse clinical outcomes does suggest that organ-specific accumulation of parasites is important in the pathogenesis of malarial disease. The downstream effects of sequestration might include mechanical obstruction of blood flow [17], leading to hypoxia, the focal release of parasite toxins and inflammatory mediators, and/or cerebral oedema or raised intracranial pressure [18] (fig. 2). Persistent focal neurological deficits similar to those following a stroke, such as hemiparesis and ataxia, have been recorded in about 10% of children recovering from cerebral malaria [19, 20], and more subtle effects may be more common [21].

Toxic mediators, inflammatory responses and metabolic disturbances

Individuals with profound coma can regain consciousness very rapidly, and may show no neurological sequelae, suggesting that the pathology of cerebral malaria has a metabolic component [22]. Furthermore, organ-specific sequestration of parasites does not account for all features of severe malaria, such as severe malarial anaemia, which may result from parasite and immune-mediated RBC destruction and reduced eythropoiesis associated with increased turmour necrosis factor α (TNF-α) and reduced interleukin (IL)-10 levels [3]. Sequestration of parasites may concentrate the metabolic disturbances and inflammatory responses triggered by malaria parasites to a particular organ, such as the brain, contributing to the development of severe clinical disease. The role of inflammatory and metabolic disturbances to severe malarial disease is only briefly reviewed here, but has been extensively studied.

Studies in animals and humans suggest an important role for cytokines in the development of severe malaria. TNF-α appears to be involved in the development of severe murine malaria [23], and higher TNF-α levels were recorded in children that died of severe malaria in two African populations [24, 25]. Nitric oxide (NO) has also been proposed to be important in cerebral malaria, and the expression of inducible NO synthase (iNOS) is increased by proinflammatory cytokines and hypoxia [26]. However, studies of nitrate levels as a marker for NO production in the peripheral blood and urine of infected individuals have revealed a negative association between nitrate levels and severe malaria in some studies [27, 28], and positive associations in others [29]. Studies of NO production and iNOS expression in postmortem tissue from the brain and other organs may help clarify the role of NO.

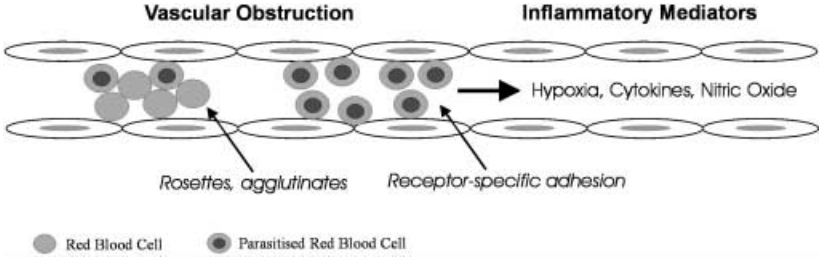

Figure 2. Model of proposed mechanisms involved in the pathogenesis of severe malarial syndromes. *P. falciparum*-infected RBCs may sequester in small blood vessels though a combination of direct adhesion to endothelial cells and the formation of rosettes or clumps comprising infected and uninfected RBCs. The resultant sequestration may obstruct blood flow in vessels, leading to focal hypoxia, and trigger inflammatory responses and metabolic disturbances.

The existence of a malaria toxin that leads to severe disease in the host is an attractive hypothesis that has led to the identification of *P. falciparum* glycosylphosphatidylinositol (GPI) as a candidate toxin [30]. GPI purified from cultured parasites was shown to induce cytokine release, fever and hypoglycaemia in mice, or death in primed mice, effects that are inhibited by specific antibodies. GPI also stimulates TNF-α production and iNOS expression by macrophages, and its effects on endothelial cells include upregulation of parasite adhesion molecules, intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1 and E-selectin [31, 32].
Acidosis and hypoglycaemia have been associated with worse outcomes in individuals with severe malaria infections [33–35]. Acidosis is largely metabolic, attributable to increased lactate production by the host and parasite biomass [33, 36]. Hypoglycaemia may arise from reduced hepatic gluconeogenesis, increased glucose consumption by a large parasite biomass [36] or triggered by parasite toxins such as GPI [30].

Parasite biomass

Some investigators favour the proposal that the total parasite biomass is important in the development of severe malaria, which may manifest in a variety of syndromes [36, 37]. The total biomass of parasites within the body may influence concentrations of parasite toxins and inflammatory mediators and result in metabolic and biochemical disturbances contributing to severe disease. Sequestration of parasites, at any site, would contribute to a greater biomass of parasites. Using a combination of mathematical models and clinical findings, associations have been found between the estimated or predicted mass of infected RBCs in an infected individual and severe disease [37, 38].

Cytoadherence of *P. falciparum*-infected RBCs

The observed sequestration of infected RBCs in the microvasculature of various organs, and its association with clinical disease, has led to intense investigation of the adhesive interactions between infected RBCs and host endothelial cells. These phenomena form the focus of this review. Mature-stage infected RBCs can adhere to endothelial cells in vitro [39], and a number of host molecules have been identified that can act as receptors for the adhesion of parasitised erythrocytes in vitro (table 1). Examination of postmortem tissue by electron microscopy suggests infected RBCs directly adhere to endothelial cells via electron-dense knobs on the RBC surface [8, 10].
It is predominantly mature-stage parasites that can adhere at substantial levels in vitro, consistent with the observation that the great majority of sequestered parasites are trophozoites or schizonts [10, 12] (fig. 1). These forms are typically not seen in the peripheral blood of infected individuals [38]. As circulating parasites mature, antigens expressed on the RBC surface enable adhesion to endothelial cells and sequestration in vascular beds [40].

Table 1. Host cell adhesion molecules for *P. falciparum*-infected RBCs and their possible clinical significance.

Receptor	Clinical significance	Comments	References
CD36	uncomplicated malaria severe malaria	– nearly all isolates from nonpregnant individuals can adhere – little expression in cerebral vessels – synergy with ICAM-1 to augment adhesion – no clear association between adhesion to CD36 and disease severity	60, 61, 67 13 62
ICAM-1	cerebral malaria	– cerebral ICAM-1 expression and parasite sequestration colocalised – both positive and negative associations reported between adhesion of clinical isolates and disease severity	13 60, 61
Rosetting receptors	severe malaria	– rosette formation associated with complicated malaria in some studies but not others	43–48
CSA	placental infection other forms of disease	– adhesion to CSA is a feature of placentally sequestered parasites – CSA-dependent adhesion to brain and lung endothelial cells reported	55, 56 78
HA	placental infection	– adhesion to HA is a feature of placentally sequestered parasites	58
E-selectin	?	– little or no adhesion of clinical isolates in two studies – little expression in the cerebral vasculature, but increased in cerebral malaria	60, 104 13
VCAM-1	?	– little adhesion of clinical isolates in two studies	60, 104
PECAM/CD31	?	– low levels of adhesion of clinical isolates in one large study – cerebral CD31 expression and parasite sequestration not colocalised	60 13
P-selectin	?	– no studies of adhesion in the context of clinical disease yet published	

Those infected RBCs failing to express adhesive phenotypes are thought to be cleared from the circulation by the spleen. Recently, it has been demonstrated that the early ring forms of infected RBCs can adhere to host cells at low levels in vitro, and this may contribute to parasite sequestration [41].

Mature-stage infected RBCs can also adhere to uninfected RBCs to form spontaneous rosettes in vitro. Apparent rosettes have also been observed in vivo by histological examination of infected tissue collected postmortem [9, 42], and this property is thought to contribute to microvascular obstruction and severe disease. Some studies [43–45], but not all [46–48], have found a positive association between rosette formation of peripheral blood isolates, or negative association with rosette-disrupting ability of serum, and severe clinical disease.

Further adhesive phenotypes of infected RBCs have been defined in vitro. Autoagglutination or clumping is the term used to describe the adhesion of infected RBCs to each other [49, 50] and a recent African study found an association between autoagglutination of peripheral blood isolates and severity of clinical disease [51]. Parasitised RBCs may also adhere to leucocytes [52].

As will be discussed below, infected RBCs are antigenically diverse and clonally variant, enabling repeat and recrudescent infections. Changes in antigenic phenotype are associated with changes in adhesive properties (fig. 3), and these properties may vary greatly from one individual to the next, or from one infection to the next. Furthermore, the antigenic and adhesive properties of circulating parasites may be quite different to those sequestered at a particular site. These features have made it difficult to reliably identify parasite virulence factors associated with specific clinical syndromes.

Intraerythrocytic development of parasites markedly reduces the deformability of the RBC membrane, which may also contribute to the sequestration of mature parasite stages [53, 54], particularly in combination with adhesive processes. The early stages or ring forms of infected RBCs show reduced cellular deformability, but by the mature stage infected cells are very rigid, having lost the properties that normally enable RBCs to pass through the narrow lumen of capillaries [53]. However, histological studies of postmortem tissue suggest that most infected RBCs appear to be sequestered in postcapillary venules [10] rather than arterioles and capillaries, suggesting that changes in RBC deformability do not account for the bulk of parasite sequestration.

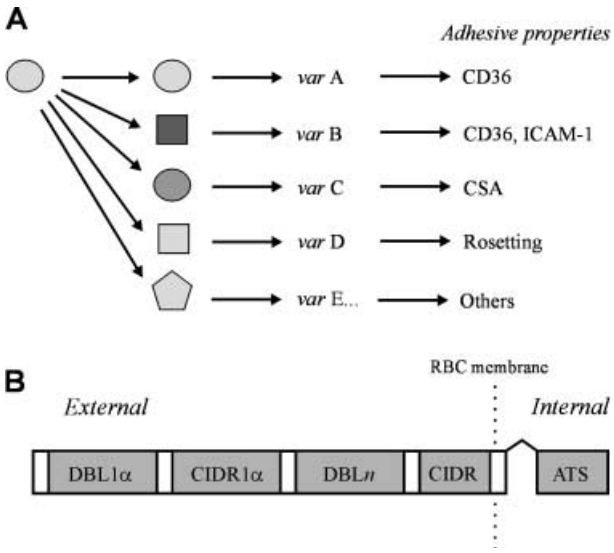

Figure 3. (*A*) Schematic representation of clonal antigenic variation of *P. falciparum* infected-RBCs. A single infected RBC may undergo spontaneous antigenic variation by switching between the expression of different *var* genes, encoding PfEMP1, conveying different antigenic and adhesive properties to the cell. The names of *var* genes used are examples only. (*B*) Predicted domain organisation of PfEMP1 encoded by *var* genes. All PfEMP1 species are thought to have a head structure comprised by a DBL1α and CIDR1α domain, followed by a variable number of additional DBL domains, or less commonly, additional CIDRs. Domains are named in numerical order from the N-terminus and according to sequence homology with other known *var* genes classified as α, β, γ, δ and ε types. DBL, Duffy-binding-like; CIDR, cysteine-rich interdomain region; ATS, acidic terminal sequence.

Receptors for parasite adhesion

Numerous host molecules have been identified that can act as receptors for the adhesion of infected RBCs (table 1), but the role of each of these in the pathogenesis of malarial disease remains largely unclear. Several studies have implicated adhesion to chondroitin sulfate A (CSA) in placental malaria [55–57], and more recently hyaluronic acid (HA) has also been identified as a receptor that is probably involved in this process [58, 59]. Most parasite isolates can adhere to CD36 and ICAM-1 [60, 61], which are both widely distributed in vascular beds and likely to be important in infection and disease.

Sequestration in an organ probably involves multiple receptors, and different combinations of specific receptors for adhesion may determine the site at which parasites adhere and accumulate. For example, the phenotypes of parasites sequestered in the placenta are quite different from parasites infecting nonpregnant adults and children [55, 56, 59]. Synergistic effects that augment adhesion have been observed when multiple receptors are expressed on a cell surface [62]. Sequestration is probably a multistep process involving initial attachment and rolling before tethering occurs, akin to adhesion of leukocytes. This process has recently been directly observed in vivo in subcutaneous vessels of human tissue grafted onto SCID mice [63].

CD36

Parasitised RBCs can bind to CD36 in vitro [64–66], and it appears that most clinical *P. falciparum* isolates can bind CD36 [60, 61, 67], but the role of this receptor in disease pathogenesis is not established. In two large African studies, there was no association between high levels of adhesion to CD36 and severe malarial syndromes [60, 61]. CD36 does not appear to be prominent in cerebral vessels, but is expressed in other organs such as lung, kidney, liver and muscle, and no association has been found between parasite sequestration and CD36 immunolabelling in postmortem tissue of brains from individuals who died of severe malaria [13]. However, CD36 does act synergistically with other receptors to augment adhesion [62]. One African study found that mutations in the CD36 gene were associated with increased risk of severe malaria [68], whereas another African study found the opposite association [69].

Infected RBCs can also adhere to CD36 on platelets to form clumps [50], and to CD36 on monocytes [70] and dendritic cells [71]. Parasite interaction with CD36 on monocytes appears important in phagocytosis and clearance of parasitised RBCs [70], whereas the adhesion of parasites to dendritic cells appears to have immunosuppressive effects [71].

ICAM-1

ICAM-1 is a member of the immunoglobulin superfamily and supports parasite adhesion in vitro and in vivo [63, 72]. It is widely expressed in vascular beds in vivo, such as brain, liver, kidney and lung, and expression is increased in malaria [13]. The parasite binding region on ICAM-1 has been mapped to the junction of the first and second immunoglobulin-like domains [73]. ICAM-1 can synergise with CD36 to augment adhesion when the two receptors are coexpressed on the surface of endothelial cells [62].

Some evidence supports a role for ICAM-1 in the development of severe malarial disease, particularly cerebral malaria. Studies of postmortem tissue found significant levels of ICAM-1 expression in cerebral vessels and parasite sequestration colocalised with ICAM-1 immunolabelling [13]. A large study conducted in Kenya, using isolates from the peripheral blood of children with well-defined clinical syndromes, found some association between severe malaria in children and parasite binding to ICAM-1 in vitro [60]. However, a similar large study in Malawian children found a negative association between severe disease and ICAM-1 adhesion [61]. Interestingly, a specific mutation in the ICAM-1 gene was associated with increased disease severity in an East African population [74], but in West Africa the opposite association was found [75].

CSA

Studies in several populations in Africa have implicated CSA as a key receptor for adhesion of infected RBCs in the placenta, discussed in more detail below. Parasites from infected placentas typically adhere to CSA, and infected RBCs can adhere to placental tissue in a CSA-dependent manner [55–57]. CSA can act as a cell adhesion molecule for infected RBCs in static and flow-based assays [76–78] and parasite adhesion is strongly dependent on 4-*O* sulfation of the saccharide chains [76, 79, 80]. The CS proteoglycan thrombomodulin, present on a range of vascular surfaces, can support parasite adhesion and may be an important receptor in vivo [81, 82]. Although CSA and thrombomodulin have been detected in vascular beds such as the brain [83], there is no consistent association between adhesion to CSA and severe clinical disease in children and nonpregnant adults [61, 84, 85].

HA

HA has only recently been identified as a receptor for parasite adhesion [58] and appears to be important for sequestration in the placenta, discussed in more detail later in this review. Parasite isolates from infected placentas typically bind to HA, in addition to CSA [58]. Findings suggest that most infected RBCs demonstrate dual specificity of binding to HA and CSA [58], both of which are expressed on the placental lining [86–88]. The specificity of adhesion to the two GAGs was established by the use of defined oligosaccharide fragments and enzymatic degradation of the GAG receptors and parasite ligand [58, 89]. HA is also expressed on microvascular endothelial cells [90] and may therefore act as a receptor for sequestration in a number of organs. However, parasite isolates from children with and without severe malaria adhere to HA less frequently and at lower levels than to receptors such as CD36 and ICAM-1 [58].

Rosetting receptors

Rosettes of infected and uninfected RBCs are observed with some, but not all, *P. falciparum* isolates, and appear to involve several RBC molecules. Heparan sulfate (HS) proteoglycans on the surface of uninfected RBCs can act as receptors for the adhesion of infected RBCs in the formation of rosettes [91], and with many isolates, rosettes can be disrupted by heparin or HS [92–94]. HS is thought to be present on many, if not all, cell surfaces and has been identified on endothelial cells. It remains an untested possibility that HS proteoglycans in the microvasculature might act as receptors for the sequestration of infected RBCs in vivo.

Rosette formation may also involve complement receptor 1 (CR1) on the surface of RBCs. Red cells deficient in

CR1 do not form rosettes, and soluble CR1 or antibodies can inhibit rosette formation in a range of isolates [95, 96]. Furthermore, blood group antigens A and B can also act as coreceptors in rosette formation, and isolates show different rosetting rates and rosette sizes when cultured with erythrocytes of different blood groups [97, 98].

Other receptors

A number of other host molecules have been identified as potential receptors for sequestration of infected RBCs in vivo, including VCAM-1, E-selectin, P-selectin, CD31/platelet endothelial cell adhesion molecule (PECAM), thrombospondin and $\alpha_v\beta_3$ integrin [99–103]. Infected RBCs have also been shown to bind normal immunoglobulins and may play a role in rosette formation and/or parasite sequestration [42]

Available data do not generally support a role for VCAM-1 and E-selectin in disease pathogenesis. Newbold et al. reported that adhesion to E-selectin and VCAM-1 was uncommon and generally occurred at low levels in vitro, and was not associated with clinical syndromes among clinical isolates from African donors [60]. Similarly, a Thai study reported no adhesion of 19 clinical isolates to VCAM-1 and little binding to E-selectin [104]. The expression of E-selectin in the cerebral vasculature is sparse, but expression is increased in malaria and was associated with parasite sequestration in one study [13]. The role of P-selectin in pathogenesis remains unknown, but adhesion of clinical isolates has been reported [100].

Parasite adhesion molecules

P. falciparum erythrocyte membrane protein 1 (PfEMP1)

PfEMP1 appears to be the principal adhesive ligand of infected RBCs, having recently been shown by several independent groups to mediate adhesion to a range of candidate host receptors, including CD36, ICAM-1, HS, CSA and CR1 [91, 95, 105–107]. It is a clonally variant protein encoded by *var* genes, it is important in antigenic variation and immune evasion, and is clustered on the surface of infected RBCs in knoblike structures comprising of KAHRP (knob-associated histidine-rich protein) and other proteins. Targeted disruption of the KAHRP gene abolishes knob formation [108], and knob-negative infected RBCs show reduced adhesion to receptors under conditions of physiologically relevant flow [108].

Var genes encoding PfEMP1 comprise a variable number of cysteine-rich extracellular domains (fig. 3) that have homology to the *P. falciparum* molecule EBA-175, which binds glycophorin A during invasion of erythrocytes by merozoites, and the Duffy blood group antigen-binding protein of *P. vivax* [109]. The intracellular region, termed the acidic terminal sequence (ATS), is highly conserved. The first Duffy binding-like (DBL) domain and the adjacent cysteine-rich interdomain region form a head structure, which is relatively conserved between different *var* genes. Recent findings have implicated the head region in multiple adhesive properties [110]. A variable number of additional DBL domains follow downstream, having low degrees of homology with one another or between different *var* genes. There is also substantial sequence diversity in the interdomain regions. In many *var* genes there is an additional CIDR domain upstream of the proposed transmembrane sequence.

When *var* genes were first sequenced, predicted DBL domains were named according to their sequential position from the 5′ end (i.e., DBL1, DBL2, DBL3 and so on) and characteristic signature sequences [109]. Subsequently, many more *var* genes have been sequenced from different isolates which has enabled a reclassification of DBL domains in to five categories on the basis of sequence homology (α, β, γ, δ and ε) and CIDR domains into three clusters (α, β, γ) [111]. Both types of nomenclature are used here.

Various domains of PfEMP1 from a number of parasite isolates have been implicated in adhesion to several receptors (table 2). Adhesion to CD36 appears to be mediated by the CIDR1α domain [105, 112–114], and a region of this domain that shows substantial sequence homology between different parasite isolates that bind CD36 has been implicated in adhesion [105]. However, the CIDR1α domain does not appear to bind CD36 exclusively, as recent studies have suggested a role for this region in adhesion to CSA, CD31 and HS in some parasite isolates [106, 110, 115, 116]. Adhesion to CSA appears to be mediated through DBL3 or γ-type domains [106, 117], but in some isolates adhesion may also involve the CIDR1α domain [106, 115, 116].

Table 2. Parasite ligands implicated in adhesion to host receptors

Receptor	PfEMP1 domain	Other ligands	References
CD36	CIDR1α	band 3	105, 114, 122
		clag9	125
		sequestrin	121
ICAM-1	DBL2/β type	no	107
CSA	DBL3/γ type	no	106, 117
	CIDR1α	no	106, 115, 116
HS	DBL1α	no	91
CR1	DBL1α	no	95
CD31/ PECAM	CIDR1α	no	110
	DBL2/δ type	no	110
TSP	unknown	band 3	118, 123

Note: The binding properties of the PfEMP1 domains vary from one isolate to the next. Therefore any single domain from a particular PfEMP1 species may not bind to all of the receptors listed.

PfEMP1extracted from whole infected RBCs has been shown to directly bind ICAM-1 and may be mediated by DBL2 or β-type domains [107, 118]. The DBL1α domain of PfEMP1 has been shown to bind to the surface of uninfected RBCs, mediating rosetting, through adhesion to HS [91, 119], or CR1 [95].

Currently it is unclear how the presence of consensus motifs in DBL and CIDR domains relates to parasite biology, such as immune recognition and cytoadhesion. For example, although it appears that all PfEMP1 species or *var* genes possess DBL1α-type domains, not all parasite isolates form rosettes or adhere to HS. Similarly, not all isolates expressing *var* genes with a CIDR1α domain bind to CD36, and this domain has been linked to other adhesive properties. The presence of a DBL γ-type domain does not necessarily indicate an ability to bind CSA. Recently, it was shown that a change of only three amino acid residues in the prosed active region of the CIDR1α domain can be sufficient ablate binding to CD36 [120], suggesting that a detailed knowledge of binding interactions is needed before it is possible to predict adhesive properties on the basis of known sequence.

Other ligands

Other molecules have been proposed to act as ligands for cellular adhesion. Their role remains somewhat unclear in light of the substantial evidence implicating PfEMP1 in adhesion, they may act in association with PfEMP1. The use of anti-idiotype antibodies against a CD36 monoclonal antibody led to the identification of a large molecular weight protein associated with adhesion to CD36, termed sequestrin [121]. To date, a direct interaction between sequestrin and CD36 has not been reported, nor has sequestrin been implicated in adhesion to other parasite receptors.

Peptides derived from parasite-modified band 3, a red cell protein, have been shown to inhibit adhesion to CD36 [122], and antibodies targeting two putative exofacial loops of the protein inhibited adhesion to melanoma cells expressing CD36. Band 3 has also been suggested to bind TSP [123], but involvement in adhesion to other receptors has not been described. The relationship to PfEMP1-mediated CD36 adhesion is unknown.

P. falciparum maintained in vitro may spontaneously lose its ability to adhere due to a deletion in chromosome 9 [124], and this activity was mapped to a region termed *clag9* (cytoadherence-linked asexual gene) [125]. Targeted disruption of *clag9* by transfection resulted in loss of adhesion to CD36. *clag9* may encode a surface protein that is directly involved in mediating adhesion to CD36, as antibodies against *clag9* protein were found to inhibit adhesion to CD36 and labelled the surface of infected RBCs [125].

Antigenic variation of *P. falciparum*-infected RBCs

P. falciparum-infected RBCs have been shown to undergo clonal antigenic variation in vitro and can potentially switch to different antigenic phenotypes at rates of up to 2% per generation [49, 126]. Although direct evidence for antigenic variation in vivo is lacking for *P. falciparum*, it has been demonstrated with nonhuman malarias such as *Plasmodium knowlesi* [127] and *Plasmodium fragile* [128] in monkeys, and *Plasmodium chabaudi* in mice [129]. An interesting aspect of studies of antigenic variation in *P. falciparum* was the finding that switching of antigenic types was associated with changes in adhesive properties. When cloned isolates were selected for adhesion to endothelial cells, changes in were observed in the antigenic type as detected by agglutination assays [49, 130]. The observed comodulation of antigenic type and parasite adhesion was subsequently explained by the identification and sequencing of *var* genes encoding the immunodominant and adhesive protein PfEMP1 on the erythrocyte surface.

Clonally variant antigens on the RBC surface: PfEMP1, Rif and Stevor

Prior to the identification of *var* genes, substantial evidence suggested the involvement of PfEMP1 in antigenic variation and adhesion of infected RBCs. PfEMP1 was first defined as a high molecular weight protein that could be labelled on intact infected RBCs, or degraded by trypsin cleavage, suggesting a cell surface location [131]. PfEMP1 can be immunoprecipitated by human hyperimmune serum consistent with its location on the red cell surface where it would be exposed to host immune responses. Switching antigenic types of clonal isolates in vitro was associated with size changes in PfEMP1 and the immunoprecipitation of specific PfEMP1 types by agglutinating sera [126]. Supporting a role for PfEMP1 in cytoadherence, selection of infected RBCs for increasing levels of adhesion or for different adhesive phenotypes resulted in variations in protein size, and trypsin cleavage of PfEMP1 was associated with loss of cytoadherence [49, 130, 132].

The molecular basis for antigenic variation and adhesion in *P. falciparum* was revealed by the identification of *var* genes, a large family of up to 50 genes distributed throughout the genome [109, 112, 133] (fig. 3). Antisera raised against *var*-derived proteins reacted with PfEMP1 on Western blots or by immunoprecipitation and labelled the surface of infected RBCs in a variant-specific manner. Analysis of a series of cloned isolates with different adhesive and antigenic properties showed *var* expression to be specific for the different variants [133]. Other investigators have also demonstrated that changes in antigenic and/or adhesive phenotypes are correlated with the expression of specific *var* genes [106, 134, 135].

Recently, additional multigene families, termed *stevor* (sub-telomeric open reading frames) and *rif* (repetitive interspersed family), have been identified [136–138], but their possible roles in antigenic variation or adhesion are not known. The *rif* family comprises about 200 genes encoding proteins of 30–40 kDa that can be labelled on the surface of infected RBCs and do appear to be targeted by host antibodies [137, 138].

Variant-specific immunity

Observations in human studies support an important role for variant-specific immunity in protection from infection and clinical disease. In several settings, it has been shown that convalescent serum, collected after acute *P. falciparum* infection in children, can agglutinate the infecting parasite isolate, but generally not other isolates. This suggests that specific antibodies develop following infections that target variant antigens on the infected RBC surface [139–142]. Sera from adults in endemic areas generally demonstrate a large repertoire of variant-specific agglutinating antibodies, whereas children, who are more susceptible, possess antibodies that agglutinate a limited number of different variants. By association, this suggests that variant-specific agglutinating antibodies, which appear to target PfEMP1, may be involved in protection from clinical disease.

Several African studies have directly tested this hypothesis [142–144]. In the Gambia, a number of potential indicators of immunity to blood-stage antigens were measured, but only agglutinating antibodies were predictive of protection from clinical malaria in children [143]. A more recent large study in Kenya also found a significant association between agglutinating antibodies targeting antigens on the surface of infected red cells and protection from clinical disease [142]. Children were generally infected with variant types against which they did not possess antibodies prior to infection.

An unresolved question is to what extent immunity is variant specific and achieved by exposure to a finite set of antigenic variants, or reliant on the development of immunity to conserved, and possibly poorly immunogenic, epitopes on the infected erythrocyte surface. Support for the latter comes from the report of pan-agglutinating antibodies able to agglutinate virtually all variant types or isolates present in some sera [139], although other studies have not reported this phenomenon [140, 141]. Interestingly, sera collected from one geographic area are able to agglutinate some isolates from quite separate regions [145], suggesting that there may be conserved epitopes among different variants or that some antigenic variants may have a wide distribution. It was recently shown that monoclonal antibodies to PfEMP1 can demonstrate cross-reactivity to a number of different variants, suggesting the presence of conserved epitopes that may be suitable vaccine targets [146].

Malaria during pregnancy

The pathogenesis of maternal malaria is worth special mention, as recent studies have provided significant insights that illustrate how adhesion and antigenic variation of *P. falciparum*-infected RBCs are important in the development of specific forms of malaria disease, and is reviewed in detail elsewhere [59]. Two observations are particularly striking about malaria during pregnancy. First, infection typically leads to the accumulation of vast numbers of infected RBCs in the placental blood spaces (fig. 1B), at much higher concentrations than seen in the peripheral blood and, second, pregnant women are much more susceptible to malaria than their nonpregnant counterparts, suffering more frequent and severe malaria infections.

Substantial findings now point to the importance of specific receptor-mediated adhesion in the sequestration of infected RBCs in the placental blood spaces, although other mechanisms may also be involved [59, 147]. Several African studies have shown that parasites accumulated in the placenta typically adhere to CSA, which is expressed on the placental vascular surface, and infected RBCs can adhere to placental tissue in a CSA-dependent manner [55–57, 148]. By contrast, infected RBCs isolated from nonpregnant adults or children typically adhere to other receptors such as CD36 and ICAM-1 [55, 56]. Accumulation of parasites in the placenta also appears to involve adhesion to HA. A recent African study showed that placentally sequestered infected RBCs typically bound to HA, as well as CSA, rather than other receptors [58]. Similar to observations with CSA, adhesion to HA was not typical of parasites collected from children, and was less common among circulating parasites than those sequestered in the placenta. Therefore, circulating parasites that express an ability to adhere to CSA and/or HA, and possibly other receptors yet to be identified, would preferentially accumulate in the placental blood spaces. The ability of parasites to adhere to multiple receptors may augment adhesion and sequestration, or may be essential in determining precisely in which vascular beds parasites accumulate. It should be noted that not all parasites sequestered in the placenta adhere to CSA, HA or syncytiotrophoblasts using in vitro assays [58, 148], suggesting that other mechanisms may be involved.

Of further interest is that these placental-binding parasite types appear to be antigenically different from serotypes or variants that typically infect nonpregnant adults and children [55, 149]. As discussed earlier, variant-specific antibodies form an important component of the naturally acquired protective response to malaria infections.

Adults, including pregnant women, have a broad repertoire of variant-specific antibodies to parasite variants infecting children and nonpregnant adults [55, 139, 140]. However, antibodies to parasites infecting pregnant women are uncommon or rare among men or women who are in their first pregnancy [55, 149]. Following several pregnancies, many women develop antibodies specifically against placental parasite isolates [55, 149], or parasites selected for adhesion to CSA [150]. These variant specific antibodies appear to primarily target PfEMP1 [59]. It appears that a switch to a placental-binding phenotype is associated with switching to novel antigenic phenotypes to which individuals are not exposed prior to pregnancy. Thus, at first pregnancy a woman is most susceptible, but following exposure to these variants during pregnancy, immune responses may develop that protect in subsequent pregnancies. Aside from the development of variant-specific antibodies, women also develop antibodies that can block the adhesion of parasites to CSA, and this was associated with protection from malaria in one study [149]. As specific domains of PfEMP1 have been shown to be responsible for mediating the adhesion of infected RBCs to CSA, there may be opportunities to develop therapeutic or preventative strategies, such as vaccination, targeting the adhesion and subsequent accumulation of parasites in the placenta.

Conclusion

Infection with *P. falciparum* can lead to a diverse range of clinical syndromes, and the pathogenesis of disease appears to involve a combination of host and parasite factors. Substantial evidence points to the importance of receptor-specific adhesion and resultant accumulation of infected RBCs in the vasculature of organs such as the brain and placenta. Clinical and laboratory studies have identified a number of potential host receptors, such as CSA and HA implicated in placental malaria, ICAM-1 implicated in cerebral malaria and CD36. Further investigation is needed to clarify the role of other known adhesion receptors in the development of disease and whether adhesion to specific receptor combinations is an important determinant of organ-specific sequestration and disease. A greater knowledge of these events in vivo and the identification of parasite ligands and specific adhesive motifs may lead to opportunities for interventions to treat severe malaria and its complications, or to prevent malarial disease through vaccination.

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