J Mol Med (1998) 76:162–171 

REVIEW

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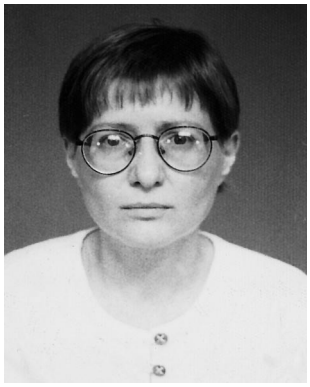
# Maternal malaria and parasite adhesion

Received: 8 April 1997 / Accepted: 19 June 1997

**Abstract** Malaria during pregnancy continues to be a major health problem in endemic countries, with clinical consequences, including death, for both mother and child. Just as cerebral malaria results from parasite sequestration in the brain, maternal malaria results from parasite sequestration in the placenta, and a distinct subpopulation of parasites which bind chondroitin sulfate A but not CD36 causes the syndrome. Women have little or no immunological experience with this parasite prior to first pregnancy, making primigravid women particularly vulnerable to infection. Parasites adhere to the surface of trophoblastic villi, eliciting the accumulation of inflammatory leukocytes in the intervillous space, and the necrosis of adjacent placental tissue. Maternal malaria results in poor pregnancy outcomes, although the responsible mechanisms have not been defined. In holoendemic areas both placental infection and poor outcome decrease in frequency with successive pregnancies; protection may result from control of parasite adhesion, suggesting an attractive target for new therapies.

**Key words** Maternal malaria · Sequestration · Cytoadherence · Chondroitin sulfate A · *Plasmodium falciparum*

**Abbreviations** *CS* Chondroitin sulfate · *ELAM-1* E-selectin · *ICAM-1* Intercellular adhesion molecule-1 · *IRBC* Infected red blood cell · *LBW* Low birthweight · *TSP* Thrombospondin · *VCAM-1* Vascular cell adhesion molecule-1



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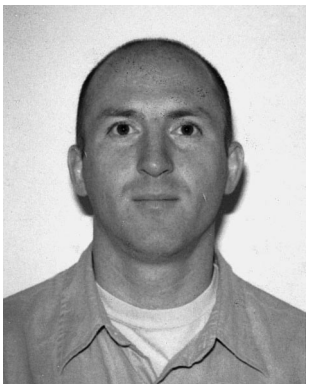

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## Introduction

Malaria continues to be a global epidemic with devastating consequences. Each year *Plasmodium falciparum*, the most important malaria protozoan, infects between 200 and 400 million persons, causing 1–4 million deaths. Human malaria may be caused by any of four *Plasmodium* species: *P. falciparum, P. vivax, P. malariae,* and *P. ovale. P. falciparum* is distinguished by the absence of mature forms in the peripheral circulation. Instead, by adhering to the endothelial surface, the mature forms of infected red blood cells (IRBCs) sequester in the deep vascular beds of various tissues. Sequestration is believed to impart the severe consequences of infection, such as cerebral malaria [1].

By adulthood, individuals residing in malaria endemic areas gain immunity sufficient to reduce the frequency and severity of infection. During pregnancy, however, women suffer increased susceptibility to malaria infection compared to their nonpregnant counterparts; this susceptibility diminishes with successive pregnancies. A characteristic feature of maternal malaria is the accumulation of parasites within the placenta, sometimes in the absence of peripheral parasitemia. Recent work has elucidated the mechanism for sequestration within the placenta, and suggested a model to explain the susceptibility of pregnant women to infection [2]. Here we describe patterns of malaria parasite adhesion, and the tissue pathology which ensues from placental infection, to explain the phenomenon of maternal malaria.

## *Plasmodium* life cycle

The life cycle of *Plasmodium* parasites involves invertebrate and vertebrate hosts (Fig. 1). The parasite, in the form of a sporozoite, is transmitted at the time of a mosquito bite to the mammalian host. Following inoculation the sporozoite circulates briefly in the blood, rapidly entering the parenchymal cells of the liver, where it undergoes multiplication during a stage referred to as exoerythrocytic schizogony. Roughly 2 weeks after invasion, the hepatocyte ruptures, liberating thousands of uninucleate merozoites which attach to and enter red blood cells. The young stage within the red blood cell is known as the ring form. Over 48 h the ring develops into a trophozoite, multiplies to produce the multinucleate schizont, then causes the red blood cell to burst, releasing merozoites into the blood circulation to invade new erythrocytes. This repetitive cycle of invasion, asexual multiplication, and release is known as the erythrocytic cycle of schizogony.

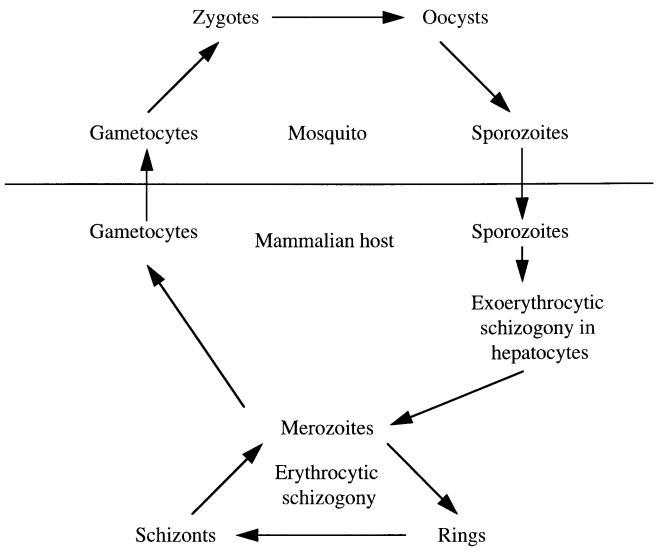


**Fig. 1** Life cycle of the *Plasmodium* parasite

In a poorly understood process, some invading parasites develop into the sexual forms, called macrogametocytes and microgametocytes. When taken up in the bloodmeal of a female anopheline mosquito, these forms emerge as a macro- and microgamete, fuse to form a zygote, then successively develop into an ookinete and an oocyst. The oocyst, lodged under the basal lamina of the external wall of the mosquito midgut, enlarges over 10–14 days until it contains hundreds of sporozoites. Upon rupture of the oocyst wall the liberated sporozoites migrate to the salivary gland of a female mosquito, poised to infect another host at the next bloodmeal [3].

## Clinical consequences of maternal malaria

In 1934 Wickramasuriya observed the devastating outcome of a malaria epidemic on pregnant women in Sri Lanka [4], and subsequent reports throughout the tropics have documented the high susceptibility of pregnant women to malaria infection [4–7]. A variety of micro-organisms, including viruses, fungi, bacteria, and protozoa other than *P. falciparum*, have also been reported to be more prevalent or more virulent during gestation [8]. However, the risk of malaria infection and disease is greatest during first gestation [4, 5], a characteristic which distinguishes maternal malaria from other infections affecting pregnant women. Our studies, conducted in a holoendemic (high transmission) area of western Kenya over the past 2 years, confirm these observations. We found that primigravid women are infected more frequently and with substantially higher parasite densities than secundigravid or multigravid women (Fig. 2). While the intensity of malaria transmission influences the clinical presentation of maternal malaria (see below), primigravid women remain uniformly more susceptible than multigravid women at any level of endemicity [5].

Pregnant women in the tropics commonly suffer from anemia, raising the risk of mortality due to postpartum hemorrhage, and predisposing toward poor fetal outcomes. Malaria, nutritional deficiencies, and cultural practices have all been implicated in the development of anemia during pregnancy. Practices such as food avoidance and clay-eating and large household size predispose to malnutrition and anemia in pregnant women [9]. In Zambia, malaria, folate deficiency (which ensues from malaria), and iron deficiency are most frequently diagnosed among pregnant women with severe anemia (below 7 g/100 ml) [10]. In studies which segregated by parity, primigravid women were more likely to develop severe anemia than multigravid women, suggesting that anemia occurs in part as a consequence of malaria [11, 12]. In western Kenya we observed significantly lower hemoglobin levels in infected primigravid women than in uninfected primigravid women; among other parities, uninfected and infected women did not differ in their mean hemoglobin level (unpublished data). Thus, while the etiology of anemia during pregnancy is multifactori-

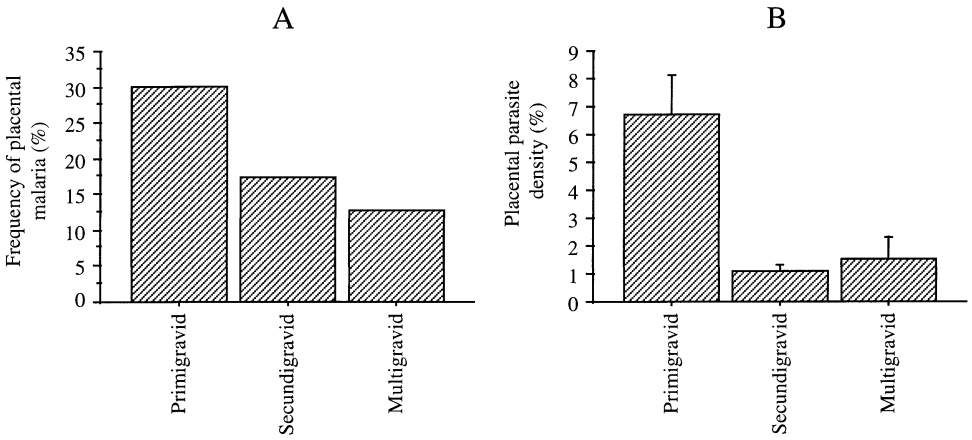


**Fig. 2A, B** Frequency and density of placental parasitemia. Frequency of placental infection (**A**) and mean density of placental parasitemia (**B**) in 1000 samples donated by women delivering at the New Nyanza Provincial General Hospital in Kisumu, Kenya, between March 1995 and September 1996. Density of parasitemia calculated as IRBCs/total red blood cells×100

al, malaria appears to be a significant contributor, particularly among primigravid women.

The clinical presentation of maternal malaria differs between residents of malaria holoendemic areas and those of low transmission areas, likely due to different levels of systemic antiparasite and antidisease immunity. Particularly in high transmission areas, the placenta may have a high parasite density while the peripheral circulation is free of parasites [5]. As a consequence of low peripheral parasitemia as well as systemic immunity, women can be asymptomatic despite a high density of placental parasites [4]. In high transmission areas febrile illness occurs more commonly among primigravid women than other women, while all parities are equally susceptible to febrile illness in low transmission areas [13]. Other potentially fatal complications, such as cerebral malaria [14, 15], hypoglycemia, and pulmonary edema, are more likely to occur in women with low immunity (residents of low transmission areas) or with no immunity (naive visitors to malarious areas), and in these cases the risk does not differ among parities [4, 16].

The level of malaria transmission also influences the pattern of fetal and neonatal mortality. Holoendemic areas experience a relatively higher proportion of preterm deliveries, with a high consequent risk for infant mortality, while stillbirths and abortions occur more frequently in areas of low endemicity/low immunity [5, 13, 17]. In nonimmune women malaria infection during the first or second trimester is associated with a high rate of abortion, and during the third trimester frequently results in premature delivery [13].

Low birthweight (LBW) increases the risk of mortality during the first year of life [18]. Studies have consistently found that birthweights are lower among infants in malaria endemic areas than nonendemic areas. The most profound effect on birthweight occurs in primigravid women, and birthweight increases with parity [4, 5, 19, 20]. In nonmalarious areas primigravid women also deliver smaller babies than other mothers. However, several lines of evidence suggest that a reduction in mean birthweight and an increase in the frequency of LBW occur in association with malaria: the frequency of LBW is higher during the rainy season than in the dry season [21, 22]; birthweight is lower in infected placentas than in uninfected placentas of primigravid women [17, 19–21]; birthweight is correlated with placental parasite density [4, 17]; and malaria prophylaxis during pregnancy reduces the risk of LBW [23].

Malaria acts in concert with maternal anemia to influence fetal outcomes. In primigravid women severe maternal anemia increases the risk of having LBW infants; this risk is greatest when the mother suffers the combination of severe anemia and placental malaria at term [24]. Maternal anemia is not uncommon in either malaria-exposed or nonexposed populations in Africa, and in both groups is associated with anemia in the newborn. However, in nonmalarious areas fetal anemia develops when the mother has severe anemia, while in malarious areas lower cord hemoglobin levels are reported in neonates born to mothers with only mild anemia [25]. Studies which clarify the relationship between low hemoglobin levels at birth and the development of anemia and severe diseases in early childhood [25] may expand our understanding of the impact of maternal malaria.

## Histological studies of *P. falciparum*-infected placentas

Malaria elicits an inflammatory response, recruiting new immune cell populations into the placenta, which may lead to tissue damage. Placental infection has been staged according to the presence and distribution of parasites and malaria pigment. Four histological categories have been described, corresponding to the absence, acquisition, and resolution of infection [26]:

– Category N: no infection – no parasites or pigment seen.
– Category 1: active infection – parasites and pigment-containing monocytes seen in the intervillous space.
– Category 2: active chronic infection – parasites, pigment-containing erythrocytes, and pigment-containing monocytes seen in the intervillous space, and pigment seen in fibrin, cells within fibrin, and/or villous syncytiotrophoblast or stroma.

– Category 3: past chronic infection – no parasites seen, pigment seen in fibrin or cells within fibrin.
In our examination of placentas collected in western Kenya we have observed that active infection is not always accompanied by pigment-containing monocytes in the intervillous spaces (unpublished data). Nevertheless, the studies emphasize the common presence of inflammatory infiltrates around trophoblastic villi of the placenta.

In healthy placentas from malaria-free areas a population of large granular lymphocytes has been characterized which resides in the decidual tissue [27]. This cell population has a natural killer cell lineage but is distinguished by a high level of CD56 on the cell surface (about 20-fold the level of circulating natural killer cells) and the absence of CD3 and CD16 [28]. The absence of CD3 and CD16 corresponds to an absence of killing activity among these cells [28]. Macrophages are also normally present in the decidua and in fibrous tissue near the placenta [29]. In malaria-infected placentas, on the other hand, monocytes/macrophages, lymphocytes, and less frequently neutrophils accumulate in the intervillous spaces which abut the maternal-fetal interface [30–32]. These inflammatory leukocytes colocalize with IRBCs and often contain malaria pigment or phagocytized IRBCs [30]. Macrophages also concentrate around necrotic syncytiotrophoblast [30]. Within the intervillous space monocytes/macrophages often appear in areas of fibrinoid deposition, where they usually contain pigment [32]. High densities of monocytes and IRBCs may form aggregates bound by intervillous fibrin, creating an inflammatory mass [33].

IRBCs, occasionally in the presence of monocytes, cluster around the chorionic villi and in the intervillous spaces and can appear contiguous to the surface of the villous epithelium [31, 32]. In ultrastructural studies scientists observed placental parasites adherent to the surface of microvilli. “Knob structures“ on the IRBC membrane, similar to those thought to mediate parasite adhesion to vascular endothelium in other tissue beds, make contact with the villous epithelium [30]. By electron microscopy erythrocytes with irregular forms and hemispheric protrusions, most likely representing IRBCs, were applied across the villous surface in infected but not uninfected placentas [30]. Although transmission EM revealed IRBC “closely applied to the syncytiotrophoblast” or “inserted into gap(s) in syncytiotrophoblast microvilli,” these images were uncommon, and parasites did not generally appear attached to the villi in this study [34]. Potentially, adherent parasites may be mechanically dislodged during delivery due to uterine contractions and passage through the vaginal canal, reducing the number of IRBCs attached to the villous surface. Future studies which examine placentas delivered through caesarean section may reflect the distribution of parasites within the intervillous space more accurately.

Other histopathological features of malaria-infected placentas include a prominent increase in fibrinoid deposits associated with syncytial necrosis and the loss of microvilli. The trophoblastic basement membrane can be thickened, usually at sites of damaged tissue and areas rich in pigment-containing monocytes and trophoblasts [33]. In the chorionic villus collagen fibers are increased in number, particularly under the trophoblastic basement membrane. In sum, pathological changes predominantly occur in the intervillous space and at the villous surface, areas where the parasite accumulates [30, 32, 33]. The consequences of these pathological changes on fetal development are unknown. Inflammatory leukocytes rarely infiltrate a normal placenta, and their presence in malaria-infected placentas reflects an altered immunological environment which, coupled with placental necrosis, may affect fetal outcome.

## Parasite sequestration

A discussion of maternal malaria requires an understanding of IRBC adhesion and sequestration. *P. falciparum* is distinguished from other human malaria parasites by its ability to sequester in the deep vascular beds of many organs. Sequestration is believed to impart the severe consequences of malaria, such as cerebral malaria which arises when parasites adhere to the vascular endothelium of the brain [1]. Sequestration confers a survival advantage on the parasite; mature parasites remain immobilized on the vascular endothelium, avoiding passage through the spleen where they may suffer immunological clearance. Further, the adherent parasites localize to postcapillary venules, offering an environment of low oxygen tension which favors the growth of *P. falciparum*.

Three adhesion processes of *P. falciparum* are known: cytoadherence is the binding of IRBCs to receptors expressed on the vascular endothelium; rosetting is the binding of IRBCs to uninfected erythrocytes [35, 36]; and agglutination is the binding of IRBCs to other IRBCs [37]. Among these processes cytoadherence has been best characterized at the molecular level. In vitro studies have demonstrated that IRBCs can bind to several endothelial surface molecules, including thrombo-

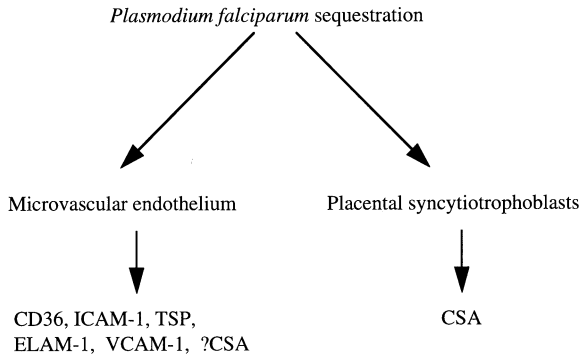


**Fig. 3** Schematic presentation of putative receptors for *P. falciparum* parasite adhesion on endothelial and syncytiotrophoblastic cells

spondin (TSP), CD36, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin (ELAM-1) and chondroitin sulfate (CS) A (Fig. 3). TSP is a 420-kDa glycoprotein first identified on activated platelets and is involved in cell-cell interactions. IRBC adhere selectively to purified TSP but not to other cytoadherence molecules such as fibronectin, and binding can be inhibited by TSP-specific monoclonal antibodies [38]. However, binding to TSP may not be sufficient to mediate cytoadherence, implying that other receptor molecules are required in this process [39].

The molecule CD36, an 88-kDa glycoprotein on the surface of multiple cells, mediates cytoadherence of IRBCs to endothelial cells in vitro [40]. Several lines of evidence suggest specific binding of IRBCs to CD36, including inhibition of IRBC binding by CD36-specific monoclonal antibodies [40–42], and inhibition of IRBC adherence to CD36-expressing cell lines by purified CD36 [41, 43]. Monocytes and platelets, which express CD36 on their surface, also bind IRBCs [41]. ICAM-1, a 90-kDa glycoprotein expressed on endothelial cells, is a ligand for lymphocyte function antigen 1, and also serves as a receptor for IRBCs [44]. ICAM-1 expression is upregulated by cytokines [45], including tumor necrosis factor-$\alpha$, an inflammatory molecule elevated in severe cases of malaria [46]. Similarly, VCAM-1 and ELAM-1 are expressed on the surface of endothelial cells, are induced by cytokines, and bind IRBCs [47]. Inflammation occurs at sites where parasites accumulate, such as the brains of cerebral malaria patients. The appearance of ICAM-1, VCAM-1, and ELAM-1 on inflamed endothelium has argued for their role as parasite adhesion receptors during cerebral malaria [47].

CSA, as other glycosaminoglycans, is a long, unbranched polysaccharide chain composed of repeating disaccharide units [48]. Glycosaminoglycans are covalently linked to a core protein to form a proteoglycan molecule. CSA molecules are part of the extracelluar matrix and are abundant in cartilage, skin and arteries [48]. CSA, whether expressed on the surface of cultured cells [49] or in purified form [50], has been shown to support parasite adhesion in vitro. While parasites in nearly all isolates from nonpregnant donors bind in substantial numbers to CD36, a minority of field isolates bind in low numbers to CSA [50, 51]. The clinical relevance of the CSA-binding phenotype was not previously understood but appears to be critical to the development of maternal malaria (see below).

Glycosaminoglycans other than CSA are also used by micro-organisms as receptors for binding or invasion. After inoculation into the bloodstream, the *Plasmodium yoelii* sporozoite rapidly invades host hepatocytes; invasion is inhibited in vitro by fucoidan, heparin, and dextran sulfate, suggesting that glycosaminoglycans are involved in this process [52]. Preincubation of various mammalian cells with heparinase or heparitinase reduces the adhesion and development of *Trypanosoma cruzi*, another protozoan parasite, implying a role for heparin/heparan sulfate in binding and invasion [53]. Similarly, attachment of the varicella zoster virus to the surface of mammalian cells is mediated by adhesion of the viral envelop protein to heparan sulfate [54].

Several molecules expressed on the surface of the IRBC have been proposed as counterreceptors for endothelial molecules such as CD36 and ICAM-1. The *var* gene family encodes a group of proteins which vary in their size (200–350 kDa) and antigenicity. These proteins have been implicated in adhesion to ICAM-1 and CD36 [55–58]. PfEMP1 is a trypsin-sensitive protein with molecular mass of 240–260 kDa, is a product of the *var* gene family, and has been associated with parasite binding to CD36, ICAM-1, and TSP [58, 59]. Another high molecular weight protein, called Sequestrin, can be immunoprecipitated from the surface of IRBC by anti-idiotype antibodies raised against the CD36-specific monoclonal antibody OKM8 [60]. Recombinant forms of Sequestrin bind CD36 and can inhibit cytoadherence (P.E. Duffy, unpublished). Band 3, a major surface protein of normal erythrocytes, is truncated in *P. falciparum* infected cells, potentially exposing cryptic domains. Peptides representing Band 3 domains, as well as antibodies against these domains, inhibit IRBC adhesion to C32 amelanotic melanoma cells [61]. Infusion of Band 3 peptides into infected primates causes mature parasites to appear in the peripheral circulation 24 h after administration, presumably due to the reversal of cytoadherence [61].

The role of adhesion in the development of disease remains an area of active inquiry – an understanding of the binding receptors involved in severe malaria syndromes will greatly influence the development of antiadhesion therapies. The vast majority of field isolates bind to CD36 [62]. Binding to melanoma cells, which express CD36 and ICAM-1 [41, 62], and to purified CD36 was higher among Thai isolates obtained from individuals with severe malaria [62, 63], but not cerebral malaria [63], than isolates taken from mild malaria patients. However, these groups of parasites had considerable overlap in cytoadherence levels. Levels of binding to purified ICAM-1 did not differ between parasites obtained from severe and mild cases of malaria [62]. In Madagascar isolates from patients with cerebral malaria, severe noncerebral malaria, or uncomplicated malaria did not differ in the level of binding to human umbilical vein endothelial cells, which express ICAM-1, or to melanoma cells [64]. While the burden of sequestered parasites in the brain is correlated with the severity of cerebral malaria [1], the endothelial and parasite molecules involved in this syndrome have not yet been elucidated.

Contradictory results have also been found in studies which examined the role of rosetting in disease. In the Gambia IRBCs obtained from children with cerebral malaria rosette to a greater degree than IRBC from children with uncomplicated malaria [65]. Similarly, parasite isolates from Kenyan children with severe malaria rosette more frequently than isolates from children with mild malaria. However, in this same study parasites from children with cerebral malaria and those from children with

severe noncerebral malaria did not differ in the frequency of rosette formation [66]. In Thailand researchers found no correlation between rosetting and biochemical indices of severe malaria [67]. Further, other Plasmodium spp. (*P. ovale* and *P. vivax*) that do not sequester and do not cause severe disease have the ability to form rosettes in vitro [68, 69]. Conceivably, rosetting may exacerbate the severity of disease by acting in combination with cytoadherence to increase the degree of vascular obstruction and anoxia.

IRBCs can agglutinate by binding to other IRBCs [37] and can also be agglutinated with human hyperimmune sera in vitro [70]. If the mechanical effect of rosetting contributes to pathology, agglutination of IRBCs, either in the form of autoagglutination or antibody-mediated agglutination, may play a similar role. Antibody-mediated agglutination has strain-dependent specificities; the degree to which an isolate agglutinates varies with different serum samples, and the same serum sample agglutinates different parasite isolates to varying degrees [71]. While the role of IRBC agglutination in vivo remains unclear, some authors have speculated that it may facilitate adhesion [37, 72]. No studies have demonstrated an association between IRBC agglutination and severity of disease.

In summary, *P. falciparum* adhesion is a complex phenomenon, encompassing a number of processes (cytoadherence, rosetting, agglutination) described in vitro, as well as several IRBC and endothelial molecules with putative roles in cytoadherence. A consensus among scientists holds that adhesion and sequestration impart the severe consequences of falciparum malaria. However, the role of a particular process or an individual receptor in a syndrome such as cerebral malaria has eluded definition.

## Adhesion of placental parasites

Adults residing in malaria endemic areas enjoy immunity which limits their frequency of infection and protects them from severe disease. Pregnant women, and primigravid women in particular, are distinguished by their susceptibility to malaria infection. This susceptibility is believed to result from pregnancy-associated immunosuppression [73–75]. In this model protective immunity is impaired during pregnancy, rendering the gravid host more susceptible to infection. Contradictory results have been reported for antibody levels against blood stage antigens in pregnant women. Previous studies describe similar antibody levels between primigravid and nonpregnant individuals [76] and between primigravid and multigravid women [74, 77, 78]. In one report primigravid women had lower antibody levels than multigravid women to the malarial antigen RESA [79]. With regard to cell-mediated immune responses, mononuclear cells from primigravid women may have diminished proliferative responses to selected, but not all malarial antigens compared to multigravid women, and placental mononuclear cells are suppressed to a greater degree than peripheral mononuclear cells from the same donor [74]. Thus pregnancy alters the immunological environment of women, ostensibly to prevent rejection of the fetal allograft, and these changes may impair antimalarial immunity, making gravid women, and primigravidas in particular, susceptible to malaria. However, further data are needed to substantiate or refute the role of immunosuppression in placental malaria.

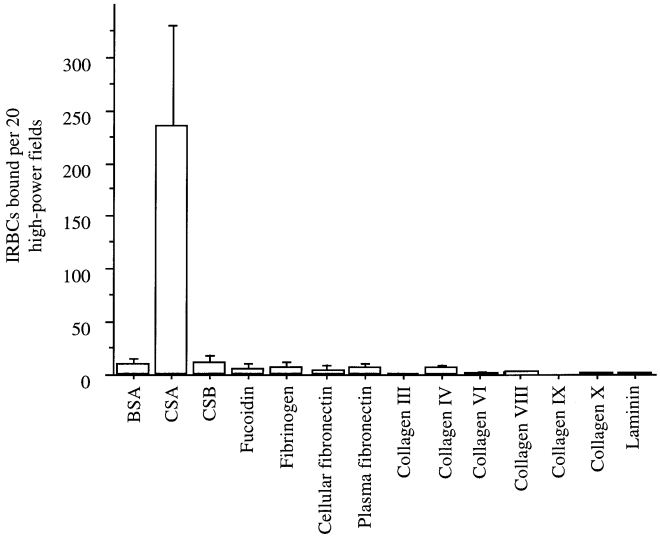


**Fig. 4** Binding of placental parasites to extracellular matrix proteins. Extracellular matrix proteins in solution (10 μg/ml) were adsorbed on a petri dish and then overlaid with an IRBC suspension of 5–20% parasitemia at 5% hematocrit. After incubation at 37°C for 1 h unbound cells were washed off with PBS and bound IRBCs were fixed with 2% glutaraldehyde. Values are presented as mean numbers of parasites counted per 20 high-power fields (×1000) from assays of 11 different placental isolates. (Reprinted with permission from [2])

Because primigravid women are particularly at risk, and the risk progressively diminishes with subsequent pregnancies, the immunosuppression of pregnancy may not offer an adequate explanation for the syndrome. During pregnancy the placenta is a preferential site for *P. falciparum* sequestration, and we speculated that the placenta may offer a distinct receptor for parasite adhesion. Further, the distinct pattern of parasite adhesion in the placenta may explain the unique susceptibility of pregnant women, especially primigravidas, to malaria infection

In assays using various immobilized extracellular matrix molecules as substrates for adhesion, all parasite isolates obtained from infected placentas uniformly bound CSA (Fig. 4). Related glycosaminoglycan molecules such as CSB, CSC, and heparan sulfate glycoprotein did not support placental parasite binding [2]. Soluble CSA competitively inhibited parasite binding to immobilized CSA in a dose-dependent manner, demonstrating the specificity of the interaction.

These results demonstrate that the placenta selects CSA-binding parasites for propagation, suggesting that parasites commonly bind to CSA in the placenta but not

in other tissue beds. Studies of glycosaminoglycan distribution indicate that CSA may be accessible for adhesion in the vasculature of the placenta but not other organs. The expression of specific proteoglycans on the surface of endothelial cells, where these molecules would be accessible for parasite adhesion, depends on tissue localization. For example, NG-2 is a proteoglycan expressed on the surface of microvessels within the central nervous system; in the vasculature of other tissues this proteoglycan is found on smooth muscle cells but not the endothelial surface [80].

CS appears in the extracellular matrix of several adherent cell types, including chondrocytes, fibroblasts, neurons, and epithelial cells [81]. Heparan sulfate and CS are found in the basal lamina but not on the surface of endothelial cells of rat splenic blood vessels, while splenic sinuses have no detectable amount of these glycoconjugates [82]. In normal blood vessels the majority of the glycosaminoglycans are synthesized by muscle cells while the endothelial cells synthesize a small fraction, mainly in the form of heparan sulfate [83]. In contrast, the surface of placental syncytiotrophoblasts contains CS, suggesting accessibility for parasite adhesion [84]. In vitro cultured Saimiri brain endothelial cells support *P. falciparum* adhesion mediated by CSA [49]. Whether this occurs in vivo, particularly in the brain vasculature of humans, is unknown.

Remarkably, none of the parasite isolates from the placenta bound to CD36 [2]. Earlier studies of parasites obtained from nonpregnant donors identified adhesion to CD36 as a nearly ubiquitous characteristic of *P. falciparum* [62]. TSP and ICAM-1 also fail to support adhesion of placental parasites [2]. In this work none of the parasites obtained from the peripheral circulation of nonpregnant individuals bound to CSA [2]; studies elsewhere have identified CSA-binding as a minor characteristic of isolates from nonpregnant donors [50, 51].

While binding to CSA may enable the parasite to sequester in the placenta, the loss of the CD36-binding phenotype may confer a separate survival advantage. By shedding the surface molecule(s) required to bind CD36 the parasite may also be shedding an antigen widely recognized by malaria-exposed individuals. Binding CSA but not CD36, the placental parasite represents a distinct subpopulation of *P. falciparum*. Because nonpregnant individuals do not support the sequestration and survival of this subpopulation, a woman may not have substantial immunological experience with this parasite until her first pregnancy. In this model primigravid women are highly susceptible to infection with the CSA-binding parasite subpopulation, and protective immunity develops only over successive pregnancies.

We developed an ex vivo model to determine whether parasites employ exclusively CSA to bind in the placenta. Thin sections of uninfected placenta were used as a platform for parasite adhesion; placental sections, immobilized on a glass slide, were overlaid with parasites, then washed to remove nonadherent IRBCs. Using this method we observed that parasites bind along the surface of the trophoblastic villi, extravillous trophoblasts, and syncytial bridges (Fig. 5). These placental structures encompass the intervillous spaces of the placenta, where parasites are known to accumulate. Soluble CSA but not other extracellular matrix molecules completely abrogated parasite binding to placental sections. Further, pretreatment of the tissue sections with chondroitinase AC, which endolytically cleaves both CSA and CSC, abrogated parasite adhesion. Thus CSA is required to mediate parasite adhesion in the placenta [2].

Notably, sections of umbilical cord do not support the adhesion of placental parasites (unpublished data). Congenital malaria in endemic areas is rare, although para-

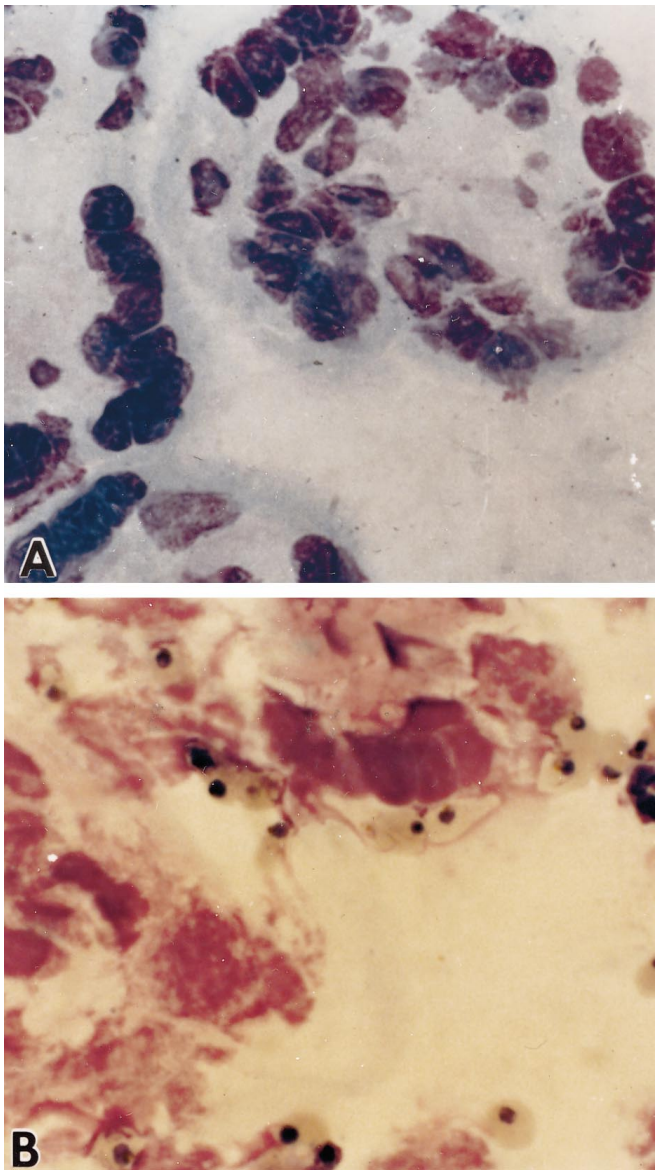


**Fig. 5A, B** Adhesion of placental IRBCs to uninfected placenta tissue. **A** Uninfected placenta tissue (fresh-frozen, Giemsa-stained). **B** Binding of placental IRBCs to uninfected placenta tissue. A section of uninfected placenta tissue was overlaid with IRBCs suspended at 5% hematocrit. After incubation for 1 h at 37°C the nonadherent cells were washed off, and the tissue was then fixed with methanol and stained with Giemsa (×400). (Reprinted with permission from [2])

sites are detected in approximately 5–8% of cord blood samples [85]. The low rate of malaria among infants has been ascribed to maternal antibodies transferred in utero, although these same antibodies clearly do not prevent the mother from becoming infected. The inability of parasites to adhere to vascular endothelium in cord sections suggests that appropriate receptors for adhesion are absent or inaccessible in the umbilical vessels. Whether parasites can adhere in other vascular beds of the newborn remains to be explored. Sequestration appears to be critical to *P. falciparum* survival and the absence of adhesion receptors in the infant could explain the inability of parasites to establish a sustained infection.

We believe the ex vivo model offers a better reflection of parasite adhesion in vivo than assays using purified proteins and are now testing different compounds for their ability to inhibit parasite adhesion to placental sections. We envision these ex vivo assays, using sections of placenta and other tissues where the parasite sequesters, as a tool to screen potential new antiadhesion therapies for malaria. Our armamentarium against malaria has dwindled as drug-resistant parasites have spread throughout the tropics. Some malarious areas will soon have no effective therapies with which to treat infection, and parasite adhesion represents a rich new area for pharmaceutical research. The description of CSA-binding parasites in the placenta offers a target to develop drugs and vaccines against maternal malaria and provides a model to study adhesion in other malaria syndromes.

**Acknowledgement** The views expressed in this report do not necessarily reflect those of the U.S. Department of Defence. M.F. is a National Research Council fellow.

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