Malaria in pregnancy: pathogenesis and immunity

Stephen J Rogerson, Lars Hviid, Patrick E Duffy, Rose F G Leke, Diane W Taylor

Understanding of the biological basis for susceptibility to malaria in pregnancy was recently advanced by the discovery that erythrocytes infected with *Plasmodium falciparum* accumulate in the placenta through adhesion to molecules such as chondroitin sulphate A. Antibody recognition of placental infected erythrocytes is dependent on sex and gravidity, and could protect from malaria complications. Moreover, a conserved parasite gene—*var2csa*—has been associated with placental malaria, suggesting that its product might be an appropriate vaccine candidate. By contrast, our understanding of placental immunopathology and how this contributes to anaemia and low birthweight remains restricted, although inflammatory cytokines produced by T cells, macrophages, and other cells are clearly important. Studies that unravel the role of host response to malaria in pathology and protection in the placenta, and that dissect the relation between timing of infection and outcome, could allow improved targeting of preventive treatments and development of a vaccine for use in pregnant women.

Lancet Infect Dis 2007; 7: 105–17

Department of Medicine, University of Melbourne, Parkville, Victoria, Australia (S J Rogerson FRACP); **Centre for Medical Parasitology, Department of Infectious Diseases, Copenhagen University Hospital, Copenhagen, Denmark** (Prof L Hviid PhD); **University of Copenhagen, Copenhagen** (L Hviid); **Seattle Biomedical Research Institute, Seattle, WA, USA** (P E Duffy MD); **Walter Reed Army Institute of Research, Silver Spring, MD, USA** (P E Duffy); **Faculty of Medicine and Biomedical Sciences, Biotechnology Center, University of Yaounde, Yaounde, Cameroon** (Prof R F G Leke PhD); **Department of Biology, Georgetown University, Washington, DC, USA** (Prof D W Taylor PhD); **and Department of Tropical Medicine and Medical Microbiology, John A Burns School of Medicine, University of Hawaii, Honolulu, HI, USA** (D W Taylor)

Correspondence to:
Dr Stephen J Rogerson, Department of Medicine (RMH/WH), University of Melbourne, Post Office Royal Melbourne Hospital, Parkville, Victoria 3052, Australia.
Tel +61 3 8344 3259; fax + 61 3 9347 1863;
sroger@unimelb.edu.au

Introduction

Over 50 million women are exposed to the risk of malaria in pregnancy every year. Pregnancy-associated malaria results in substantial maternal and especially fetal and infant morbidity, causing 75 000–200 000 infant deaths every year.[1,2] Both *Plasmodium falciparum* and *Plasmodium vivax* infections can cause adverse pregnancy outcomes, including maternal anaemia and low birthweight due to preterm delivery and fetal growth restriction, but mechanisms could differ.[3]

Pregnant women are more susceptible than non-pregnant women to malaria, and this susceptibility is greatest in first and second pregnancy. Although some other infectious diseases are also worse in pregnancy, malaria seems to be a special case. Susceptibility to pregnancy-associated malaria probably represents a combination of immunological[4] and hormonal changes associated with pregnancy (although the nature of the latter is the subject of debate[5]), combined with the unique ability of a subset of infected erythrocytes to sequester in the placenta. Extensive evidence confirms that antibodies directed against the surface of infected erythrocytes in the placenta are important in protection, and are usually absent in first pregnancy.

This review summarises current knowledge of the basis for susceptibility to pregnancy-associated malaria and of the mechanisms by which disease could lead to morbidity and mortality, and discusses how these insights can help us to develop more effective strategies to prevent and treat disease.

Current status

Malaria-associated placental changes

Central to the pathogenesis of *P falciparum* infection in pregnancy is the observation that infected erythrocytes accumulate in the maternal vascular area of the placenta—the intervillous space—to much higher densities than in the peripheral circulation.[6] Trophozoite and schizont stages, which are absent from peripheral blood, sequester in the placenta.[7] Other findings associated with pregnancy-associated malaria include increased numbers of maternal phagocytic cells—especially monocytes—in the intervillous space,[8] and deposition of haemozoin—or malaria pigment, a byproduct of parasite haemoglobin digestion—in phagocytic leucocytes and within fibrin deposits in the intervillous space. Accurate detection of placental parasitisation, and of these other findings, requires examination of histological sections of fixed placental tissue. A useful classification of placental histological changes is given in table 1. Figure 1 illustrates histological appearances of a normal placenta and of a malaria-infected placenta showing parasites and monocyte-macrophage infiltrates.

The relation between placental histological findings and birthweight was recently reviewed by Brabin and colleagues.[11] In brief, chronic infection has been most closely associated with decreased birthweight due to fetal growth restriction, whereas acute infection (especially with high parasitaemia[12,13]) has been more closely associated with preterm delivery (table 1). Chronic infection was also most closely associated with lower maternal haemoglobin or severe anaemia.[14,15] However, these observations are presently based on few studies.

Pregnancy-associated malaria is common, but is not always associated with pathology

Traditionally, microscopic examination of blood smears is used to detect pregnancy-associated malaria. In non-pregnant populations, many individuals have sub-microscopic peripheral blood parasitaemias, detectable by PCR,[16] and these are often composed of many different parasite genotypes. In one study in pregnant women, submicroscopic infections were not associated with decreased birthweight,[17] nor were they associated with lower haemoglobin in three of four studies,[18–21] and neither

	Description
Not infected	No evidence of parasites or malaria pigment (haemozoin)
Active-acute	Parasites present, with absent or minimal pigment deposition within fibrin
Active-chronic	Parasites, with substantial amounts of pigment in fibrin or in cells
Past	Presence of pigment with no parasites

Table 1: **Classification of placental pathology[9]**

pregnancy nor gravidity seem to change the complexity of infection.[20] Together, these results suggest that pregnancy-associated malaria is not caused by the presence of more parasite types in pregnancy, but instead is caused by an increase in density of parasitaemia. Women who remain able to control parasite density might not experience adverse consequences of pregnancy-associated malaria.

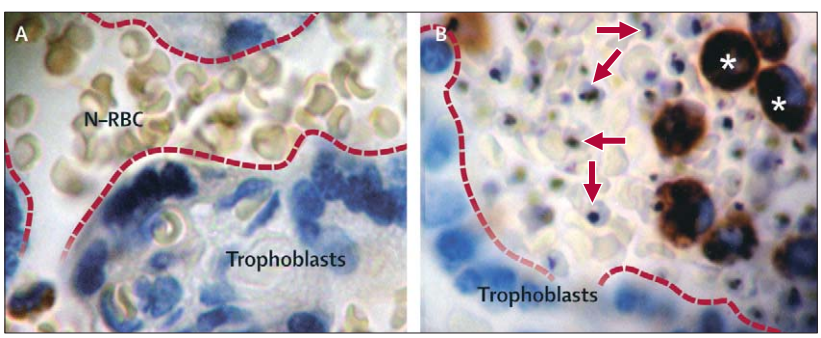

Figure 1: **Placental tissue from (A) normal and (B) malaria-infected women**
Sections were stained with monoclonal antibody to CD68, specific to monocytes and macrophages, and developed with diaminobenzidine (brown colour). Asterisks indicate CD68 staining of monocytes/macrophages in the intervillous space. Arrows indicate parasitised erythrocytes. The lines indicate the outline of the trophoblast layer. Original magnification (oil immersion) ×1000. N-RBC=non-parasitised red blood cells. Adapted with permission from Suguitan and colleagues.[10] Copyright, Infectious Diseases Society of America. Published by University of Chicago Press.

	Infected erythrocytes from the placenta of pregnant women, and infected erythrocytes selected for adhesion to chondroitin sulphate A in vitro	Infected erythrocytes from non-pregnant individuals	Reference
Adhesion to chondroitin sulphate A	Yes	No	Fried and Duffy[27] Ricke et al[28] Rogerson et al[29]
Adhesion to CD36, ICAM-1	No	Common	Fried and Duffy[27] Ricke et al[28] Rogerson et al[29]
Rosetting*	No	Common	Maubert et al[30] Carlson et al[31] Udomsangpetch et al[32] Rogerson et al[33]
Agglutination†	Variable	Common	Fried et al[25] Beeson et al[34,35] Maubert et al[36] Bull et al[37]
Non-specific binding of IgM to infected erythrocytes	Yes	No	Creasey et al[38]
Trypsin sensitivity of variant surface antigens	Variable	Generally high	Beeson et al[39] Fried et al[40] Sharling et al[41]
Sex-specific IgG recognition of variant surface antigens	Yes	No	Fried et al[25] Ricke et al[28]
Parity-dependent IgG recognition of variant surface antigens	Yes	No	Fried et al[25] Ricke et al[28]

*Adhesion of uninfected erythrocytes to infected erythrocytes. †Surface antigen-specific, antibody-mediated clumping of infected erythrocytes.

Table 2: **Characteristics of erythrocytes infected with *P falciparum* in pregnant women compared with those in non-pregnant individuals**

Pregnancy-associated malaria and malaria endemicity

Almost all our knowledge about pathogenesis of, and immunity to, pregnancy-associated malaria comes from areas of high transmission. In low transmission areas, women of all gravidities are susceptible to symptomatic and severe maternal disease; miscarriage, stillbirth, and congenital malaria are common complications; and malaria is an important cause of low birthweight.[22–24] Parasites that infect the placenta in women in non-African areas of low endemicity, and the immune responses to them, are phenotypically much the same as those from Africa, but acquisition of immunity is delayed.[25] If infection is promptly treated, placental histology is often normal.[26] Priority research areas are outlined in a recent review[24] and elsewhere in this issue.[2]

Parasites that cause malaria in pregnancy

Erythrocytes infected with *P falciparum* that are obtained from the placenta differ in important ways from infected erythrocytes isolated from non-pregnant individuals (table 2). Placental infected erythrocytes adhere to glycosaminoglycan receptors not exploited by other infected erythrocytes, and do not bind to receptors commonly used for sequestration by non-placental infected erythrocytes.[27,34] Placental sequestration seems to occur throughout the intervillous space, by contrast with sequestration in other tissues, where infected erythrocytes are usually found in close apposition to the vascular wall.[42] Chondroitin sulphate A has been consistently identified as the dominant placental adhesion receptor, although some studies point to the existence of additional receptors.[39,43,44] However, current evidence suggests that they are less important than chondroitin sulphate A,[45] which is present in the placenta as a glycosaminoglycan sidechain to the tissue anticoagulant thrombomodulin,[46] and as part of a secreted low-sulphated aggrecan in the intervillous space, postulated to function as a reversible immobiliser of hormones, cytokines, and other molecules.[42] Infected erythrocytes can bind in vitro to both forms of chondroitin sulphate A.[42,47]

Placental infected erythrocytes, and those that adhere to chondroitin sulphate A, differ from other infected erythrocytes in additional ways that are probably related to their unique adhesion receptor specificity. They tend not to be surrounded by adherent uninfected erythrocytes, forming so-called rosettes,[30,32] which are a common feature of isolates from non-pregnant individuals[33] and have been related to severe malaria in some studies.[31] Placental infected erythrocytes do not commonly clump together in the presence of serum from individuals exposed to *P falciparum* (although chondroitin sulphate A adherent lines do).[25,34–36] This agglutination, which is mediated by antibodies cross-linking antigens on the surface of infected erythrocytes,[48] is often seen with non-placental infected erythrocytes and has also been linked to disease severity.[35] Finally, chondroitin sulphate A-adhering and placental infected

erythrocytes express adhesion ligands that vary in their sensitivity to trypsin digestion[38–41,49,50] and can adsorb IgM, unlike corresponding molecules on other erythrocytes infected with *P falciparum*.[38]

Humoral immunity to variant surface antigens and other malaria antigens in pregnancy

Taken together, the above evidence indicates that the parasite antigens that serve as ligands for the adhesion of placental infected erythrocytes to chondroitin sulphate A are fundamentally different from the corresponding antigens expressed on the infected erythrocyte surface in non-placental *P falciparum* infections. This finding is important, since the surface antigens of these infected erythrocytes—collectively known as variant surface antigens—seem to be the main targets of the IgG that mediate the protective immunity that is gradually developed in response to repeated episodes of *P falciparum* malaria in non-pregnant individuals.[51–53] Thus, if placental parasites express antigens on the surface of infected erythrocytes that are immunologically distinct from (other) variant surface antigens, and if immune responses specific for these pregnancy-specific parasite antigens are important for immunity to pregnancy-associated malaria, then otherwise enigmatic features of pregnancy-associated malaria would be explainable. In particular, such a model would elegantly resolve the puzzling reappearance of susceptibility to infection with *P falciparum* in previously clinically immune women when they become pregnant, and explain the concentration of pregnancy-associated malaria in women of low gravidity in areas with intense parasite transmission.

The first direct evidence that the surface molecules on infected erythrocytes that are expressed by placenta-dwelling parasites are immunologically distinct and likely to be targets of protective immunity came when it was shown that serum IgG from multigravidae exposed to *P falciparum* could substantially inhibit the adhesion of infected erythrocytes from pregnant women to chondroitin sulphate A.[25] By contrast, serum from primigravidae and men did not inhibit the adhesion of infected erythrocytes to chondroitin sulphate A. None of the sera inhibited the binding of infected erythrocytes to CD36, a common adhesion receptor for non-placental infected erythrocytes. The observation that inhibition of adhesion to chondroitin sulphate A was independent of the geographical origin of both parasites and plasma suggested that the parasite ligand that mediated adhesion was a conserved antigen.[25] A subsequent study showed that in-vitro selection of *P falciparum*-infected erythrocytes for adhesion to chondroitin sulphate A resulted in a dramatic change in the ability of plasma IgG to recognise antigens on the surface of infected erythrocytes, pointing to the existence of pregnancy-specific variant surface antigens (VSA_{PAM}).[28] Like the infected erythrocytes that adhere to chondroitin sulphate A from pregnant women,[25] the infected erythrocytes selected for adhesion to chondroitin sulphate A[28] expressed antigens that were not recognised by plasma IgG from men, whereas antibody levels in women correlated with parity. Furthermore, the hypothesis that inhibition of adhesion to chondroitin sulphate A was mediated by antibodies to parasite antigens on the surface of infected erythrocytes surface[25] was strongly corroborated by the finding that levels of VSA_{PAM}-specific IgG, measured by flow cytometry, correlated with inhibition of adhesion of infected erythrocytes to chondroitin sulphate A.[28,35,54] Later studies have further strengthened the relation between pregnancy and VSA_{PAM}-specific IgG by showing that levels of such antibodies are not detectable until around week 20 in primigravidae, appear earlier and rise faster in multigravidae, and decline postpartum.[54,55] IgG1 is the dominant subclass of VSA_{PAM}-specific IgG.[56,57] Levels of immunity to other blood stage antigens have also been associated with reductions in placental malaria,[58] and might explain the importance of young age as an independent risk factor for pregnancy malaria,[19,59] and the relative protection from severe malaria observed in areas of high prepregnancy exposure to infection. However, antibody to pre-erythrocytic antigens or to blood stage antigens other than VSA_{PAM} is clearly not usually adequate to prevent pregnancy-associated infection.

Panel 1: **Features of VAR2CSA and the gene encoding it (*var2csa*) that lend support to the role of VAR2CSA in pregnancy-associated malaria and protective immunity to this syndrome**

- The *var2csa* gene is selectively transcribed by placental and chondroitin sulphate A-selected parasites[65–67]
- The *var2csa* gene is relatively conserved between clones[65,68]
- The *var2csa* gene is necessary for the ability to select for the adhesion of infected erythrocytes to chondroitin sulphate A[62]
- VAR2CSA is selectively expressed on the surface of infected erythrocytes expressing PAM-type variant surface antigens[69]
- VAR2CSA contains binding sites for chondroitin sulphate A[70]
- Naturally acquired VAR2CSA-specific IgG can be found in women only[69]
- Levels of VAR2CSA-specific IgG correlate with parity[69]
- High levels of VAR2CSA-specific IgG are associated with decreased risk of delivering a low-birthweight baby[69]
- VAR2CSA is an important target of naturally acquired IgG reactive with the surface of infected erythrocytes expressing VSA_{PAM}[71]
- VAR2CSA-specific IgG reactive with the surface of infected erythrocytes expressing VSA_{PAM} can be induced by subunit vaccination[71]

Molecular identification of pregnancy-specific *P falciparum* adhesion ligands

Efforts to identify VSA_{PAM} in molecular terms have largely focused on PfEMP1, a variant antigen implicated in several adhesive interactions, and initially on two variants—one often referred to as VAR1CSA—which showed both affinity for chondroitin sulphate A and substantial interclonal conservation.[60,61] However, several independent lines of evidence have since made VAR1CSA an unlikely candidate.[62–64] In its place, another PfEMP1 variant, VAR2CSA,[65] has been identified. This molecule has many of the characteristics expected of variant surface antigens involved in pregnancy-associated malaria (panel 1). Like VAR1CSA, VAR2CSA is encoded by a gene with substantial interclonal conservation, which could explain the geographical independence of antibody responses to the variant surface antigens expressed on the surface of infected erythrocytes from pregnant women.[25] Other parasite-encoded molecules might also be preferentially expressed on the surface of placental infected erythrocytes (Fried M, Seattle Biomedical Research Institute, USA, and PED, unpublished data),[72,73] but their functional roles remain unclear.

Protective antibodies to malaria in pregnancy

The strong negative association between gravidity and susceptibility to malaria in pregnancy suggests that acquired protection from this syndrome is mediated by an immune response directed against a target that is pregnancy-specific and highly immunogenic. Variant surface antigens found in pregnancy-associated malaria meet these requirements, and initial data implicated this type of antigen in protection from malaria in pregnant women.[25,27,28,34,54] This evidence has since been strengthened, since levels of antibodies inhibiting adhesion to chondroitin sulphate A correlated inversely with susceptibility to preterm delivery and low birthweight.[74] Furthermore, levels of VSA_{PAM}-specific IgG correlated with maternal haemoglobin levels and infant birthweight, whereas levels of IgG specific for variant surface antigens found in non-pregnancy-associated malaria expressed by isogenic parasites did not.[75] Although VAR2CSA seems to be a target of pregnancy-associated malaria-specific immunity and IgG-mediated protective immunity,[69] additional targets and protective mechanisms could well exist.

Role of other receptors in placental sequestration

Although many parasite lines acquire VSA_{PAM} expression in response to selection for chondroitin sulphate A adhesion in vitro, this is not always the case.[76] Furthermore, not all infected erythrocytes in the placenta bind efficiently to chondroitin sulphate A.[27,39,45] Recent data indicate that selection for adhesion to the placental cell line BeWo might be an effective alternative in selection for VSA_{PAM} expression,[76,77] although adhesion of infected erythrocytes to BeWo cells is only partly susceptible to inhibition by soluble chondroitin sulphate A or chondroitinase treatment.[77,78] Therefore, the search for additional placental adhesion receptors for infected erythrocytes must continue.

Development of monocyte infiltrates in the placental intervillous space

The sequestration of infected erythrocytes in the placenta stimulates maternal mononuclear cells to secrete β-chemokines that are chemotactic for monocytes and macrophages, including macrophage-inflammatory protein-1α and β (MIP1α and β[10,79,80]), interferon-inducible protein 10 (IP10),[10,81] monocyte chemoattractant protein 1 (MCP1),[10,79] and I309.[79] Macrophage migration inhibitory factor (MIF), a cytokine that aids in retention and activation of macrophages, is also found in raised concentrations in women with placental malaria.[81,82] Thus, induction of these chemokines provides a physiological explanation as to why monocytes and macrophages, and not other types of leucocytes, predominate in the intervillous space in response to parasite sequestration. Macrophages in the intervillous space can be activated[10] and have the ability to process and present antigens to T cells.[83]

Placental malaria changes the placental cytokine balance

During normal successful pregnancies, the cytokine balance is shifted towards a Th2-type response.[84] In mice, strong Th1 responses during pregnancy are incompatible with a successful pregnancy.[4] Although the Th1/Th2 paradigm is an over-simplification in human beings, strong Th1 responses during pregnancy are also associated with maternal anaemia, spontaneous abortions, and premature deliveries.[85] For example, substantial increases in tumour necrosis factor (TNF) α,[10,86–88] interferon γ,[87,89] interleukin 1β,[86] and interleukin 2[87] have been found in placental blood or tissue in response to malaria infection. These cytokines are known to aid in the elimination of parasites from the placenta by enhancing phagocytic activity of macrophages, generating reactive oxygen intermediates and L-arginine-derived nitric oxide, and stimulating the proliferation of T cells.[90] Thus, Th1-type responses are of parasitological importance. However, overproduction can jeopardise the pregnancy. Placental chemokines and cytokines are produced by both maternal and fetal cells.[79,91] Placental blood mononuclear cells from multigravidae without malaria produced higher levels of interferon γ in vitro than did cells from paucigravidae with and without placental malaria and from multigravidae with malaria.[89] Thus, the ability to produce interferon γ could be associated with protection from placental malaria, although we do not yet know the cell type that produces it. Upregulation of interleukin 10 has been reported in the intervillous space,[10,89,91,92] and could help prevent the pathological effects of the pro-inflammatory cytokines. To learn how the Th1/Th2 balance is maintained within the placenta is important, since it influences a number of other important immune responses—eg, isotype switching to cytophilic IgG—as well as pregnancy outcome.

Immunological changes in the placenta are associated with poor pregnancy outcomes

In studies that have related placental cytokine changes to adverse pregnancy outcomes, the clearest finding has been an association between raised levels of TNFα and babies of low birthweight,[87,88] including low birthweight caused by fetal growth restriction[86] and preterm delivery.[93] In developed countries, increased serum levels of TNFα have been associated with spontaneous abortions,[94] but the downstream sequence of events has not been elucidated. That high TNFα production has been linked to both fetal growth restriction and preterm delivery is intriguing, since fetal growth restriction seems to result from chronic infection, whereas preterm delivery is associated with high placental parasitaemias at term. Increased concentrations of interferon γ were associated with low birthweight in one study[87] but not in another,[88] and were not detected in women with fetal growth restriction.[86] In one study, preterm delivery because of malaria was associated with increased placental concentrations of TNFα and particularly of interleukin 10, resulting in a low TNFα to interleukin 10 ratio.[93] In further studies, polymorphisms in the interleukin-10 promoter associated with increased production were significantly more common in women with placental malaria and preterm deliveries than in those with placental malaria and term deliveries (p=0·02; Suguitan AL, Georgetown University, Washington, DC, USA, personal communication). Interleukin 10 can have immunosuppressive roles, and high levels are implicated in the pathogenesis of chronic disease (through effects on erythroid progenitors[95] and by reducing available iron concentrations in plasma[96,97]). Together with the strong association between anaemia and preterm delivery, this observation suggests that high interleukin 10 could contribute to anaemia that results in preterm delivery, and that there could be an important genetic component to this predisposition. Further studies of the genetic determinants of immune response to pregnancy-associated malaria, and how these influence maternal and fetal outcomes, could allow us to use human genetic data to design new treatment strategies.

To date, no-one has developed an in-vitro assay of explants of placental tissue to investigate how infected erythrocytes, haemozoin pigment, or specific malarial antigens (eg, glycosylphosphatidylinositol) might affect normal cytokine and hormone production by maternal and fetal cells, but the consequences of adhesion of infected erythrocytes to syncytiotrophoblast have begun to be explored with in-vitro models.[77]

Role of innate cells in immunity to malaria during pregnancy

Dendritic cells, macrophages, natural killer (NK) cells, NK T cells, and γδ T cells help shape the nature of the adaptive immune response to malaria. Early production of immunoregulatory cytokines by these cells and—in the case of dendritic cells—antigen presentation are probably important determinants of response to infection. In the placenta, macrophages aid in parasite elimination by phagocytosis and release of reactive oxygen intermediates as well as by enhancing innate responses through cytokines. Live infected erythrocytes adhere to NK cells and initiate production of interferon γ, and infected erythrocytes adhere to dendritic cells through CD36, modulating their functions.[98,99] Moreover, *P falciparum* impairs cross-presentation by dendritic cells and antiviral responses via interaction with toll-like receptors (TLRs).[100] Studies in human beings are scarce, but in murine studies NK1.1 T cells protect athymic mice against blood-stage malaria infections,[101] and CD1d-restricted NK T-cell responses enhance B-cell clonal expansion and antibody production in mice exposed to malaria.[102]

Asexual-stage *P falciparum* parasites are recognised by innate cells via TLRs. Interestingly, polymorphisms in TLR4 and TLR9 have been associated with low birthweight, and, in women with chronic placental infection, the TLR4 polymorphism was associated with fetal growth restriction and maternal anaemia.[103]

The important immunoregulatory roles these cell types have in malaria immunity in animal models, and in the few human studies presently available, mean that studies of their role in placental malaria are warranted. An initial study found no NK cells in the intervillous space of malaria-infected placentas,[104] whereas a more recent study, using a different antibody, showed NK cells to be increased in placental malaria.[105] Peripheral blood NK cells from primigravidae were reported to have reduced cytotoxicity against infected erythrocytes compared with NK cells from multigravidae and non-pregnant women.[106] Presently, we do not know whether infected erythrocytes that express variant surface antigens from pregnancy-associated malaria show similar interactions with dendritic cells or NK cells to those described above, and no studies have reported the presence or characteristics of dendritic cells, NK T cells, or γδ T cells in placental malaria.

Responses of T cells and B cells during pregnancy

Many researchers have suggested that suppression of cell-mediated immune responses in pregnant women accounts for their increased susceptibility to severe disease; however, data supporting this conclusion are scarce. Early studies found that in-vitro T-cell responses to malaria antigens were lower in pregnant women, especially primigravidae, than in non-pregnant women.[107] Today, that a large number of pregnant women have very low, submicroscopic parasitaemias—ie, are slide negative but PCR positive—is clear. Thus, memory T cells that are measured in in-vitro proliferation assays could be sequestered rather than circulating in the peripheral blood.[108] Therefore, decreased T-cell proliferative responses could be caused by the absence of trafficking memory T cells in the peripheral blood, not immunosuppression.

Data on how antimalarial T-cell responses change during the course of individual pregnancies and how they relate to pregnancy outcomes are not available, but Fievet and colleagues[109] studied women during their first pregnancies, postpartum, and in second pregnancies. Overall, interleukin-2 responses were suppressed to malaria and non-malaria antigens, but surprisingly proliferation in vitro was not. Interleukin 4 and interferon-γ responses were either not affected or enhanced. Similarly, in a longitudinal study of pregnant women, neither interferon-γ nor interleukin-5 responses were suppressed in primigravidae or multigravidae women during pregnancy (Megnekou R et al, University of Yaoundé 1, Cameroon, personal communication).

Parasite type could help determine cellular responses. Fievet and colleagues[110] showed that T-cell proliferative responses and cytokine production (interleukin 2, interleukin 4, interleukin 10, and interferon γ) were substantially higher in multigravidae than in primigravidae when cells were stimulated with the chondroitin sulphate A-adhering RP5 strain of *P falciparum*, but not after stimulation with the W2 strain that does not adhere to chondroitin sulphate A. Further studies should extend these observations, to determine which types of T cells interact with infected erythrocytes expressing VSA_{PAM}, and the parasite epitope(s) involved. If the parity-dependent stimulation of cytokine production by infected erythrocytes expressing VSA_{PAM} is important for immunity to pregnancy-associated malaria, to determine whether vaccines for pregnancy-associated malaria elicit similar cellular responses will be important.

High levels of VSA_{PAM}-specific IgG are produced in response to pregnancy-associated malaria, and recent data suggest that the frequency of VSA_{PAM}-specific memory B cells in recently pregnant multigravidae can be as high as 1 in 4000 B cells (with this specificity), and that conformation-dependent, surface-exposed epitopes in VAR2CSA are the main target of the antibodies produced.[111,112]

Malaria and HIV infection

HIV infection increases susceptibility to malaria in pregnancy,[113,114] in part by decreasing variant-specific immunity and possibly other forms of humoral immunity to malaria in pregnancy.[115,116] It also impairs cytokine responses to malaria. Women who are HIV positive have reduced levels of interleukin 12,[81] a cytokine that helps bias the immune response toward Th1, and consequently they have reduced interferon-γ responses, thus providing another potential explanation for increased susceptibility to placental malaria in women infected with HIV.[117] Malaria increases HIV viral load in pregnant women,[118] and has been associated with increased expression of CCR5 mRNA in placental tissue.[119] CCR5, an important co-receptor for HIV cell entry, is expressed by macrophages in the intervillous space (which are a potential virus reservoir) and by Hofbauer cells (fetal villus macrophages, a possible route of fetal infection). Despite this, the effect of malaria on mother-to-child transmission of HIV remains controversial.[120,121] HIV-induced impairment of the antibody response to malaria does not seem to explain a substantial component of maternal anaemia or low birthweight due to HIV, but it could have implications for transplacental transfer of antibody. Further studies on the interaction of malaria and HIV are needed.

***Panel 2:* Key questions**

- How does timing of malaria infection relate to pregnancy outcomes?
- How does placental malaria cause preterm delivery?
- How long do chronic infections persist, and how long after infection resolves can we see pigment, indicating past infection?
- What are the characteristics of infections that cause pathology, and of infections that lead to the development of immunity, and can they be separated?
- What is the role of human genetic polymorphisms in susceptibility to placental malaria and its complications?

Critical gaps in knowledge

A greater understanding of the pathogenesis of malaria in pregnancy could lead to improvements in our ability to prevent malarial complications, mainly by changing the timing or nature of preventive treatments (panel 2).

Timing of gestation and the effects of malaria throughout pregnancy

To ensure accurate dating of pregnancy is important to understand the pathogenesis of low birthweight. Although the Dubowitz or Ballard's scores are helpful, they must be done soon after birth, which is difficult when women deliver at home. Fetal ultrasound accurately dates gestation, if done before 20 weeks' gestation. Prospective studies with accurately dated pregnancies and sequential ultrasound monitoring of fetal growth would improve our understanding of the relation between timing of malaria episodes and outcomes of pregnancy-associated malaria such as fetal growth restriction and preterm delivery.

Doppler ultrasound studies can assess the effects of malaria on uteroplacental and fetal circulation. In one such study, malaria in late pregnancy was associated with increased resistivity in the uteroplacental arteries,[122] suggesting inadequate placental blood flow, as seen in pre-eclampsia.[123] Whether this was because of effects of concurrent placental malaria on blood flow, or because infection in early pregnancy affected placentation, and such infections might persist through pregnancy,[124] is unknown. Fetal Doppler studies during symptomatic maternal malaria have shown alterations in umbilical and cerebral vascular resistance, indicating placental dysfunction and possible fetal hypoxia.[125] Such studies can assess placental function and fetal

circulation before delivery, and could be extended to asymptomatic infection.

Pathogenesis of fetal growth restriction and preterm delivery due to malaria

Preterm delivery (birth before 37 weeks' gestation) is closely associated with malaria parasitaemia,[12,13,126] anaemia, and high levels of TNFα and, in particular, interleukin 10.[93] Beyond this, we still understand little of how malaria parasitaemia leads to initiation of parturition, and thus how we might prevent preterm births, which carry a high risk of death in early life.

Fetal growth restriction is associated with chronic malaria, probably through placental insufficiency. Whether fetal growth restriction arises mainly as a consequence of events close to delivery (eg, cytokine release, impairment of uteroplacental blood flow, or biochemical disturbance in placental nutrient transport), or whether it indicates chronic infection that insidiously compromises fetal growth, is presently unknown. Pregnancy-associated malaria might compromise placental circulation if malaria infection during trophoblast invasion impairs remodelling of uterine spiral arteries, as happens in pre-eclampsia.[123] Remodelling, which continues until 18–20 weeks' gestation[127] and into a period of high susceptibility to malaria,[128] is critical to the development of adequate placental circulation near term. Alternatively, infected erythrocytes, monocytes, and fibrin deposition[129] in the intervillous space might decrease placental blood flow by mechanical means.

Some studies,[130,131] but not others,[14,122] have found a relation between malaria and the risk of pre-eclampsia and hypertension in pregnancy. In a recent study, the risk of hypertension was increased in first-time mothers with chronic, inflammatory forms of placental malaria; this effect could have resulted from the fetal response to placental inflammation.[131]

Placental inflammation and decreased placental blood flow are known to impair the nutrient transport function of the placenta. A number of mechanisms have been implicated in fetal growth restriction due to placental insufficiency.[132–135] To understand the importance of these pathways in the pathogenesis of malarial fetal growth restriction is critical, and could lead to novel interventions. For example, increasing the maternal aminoacid supply through dietary supplementation could overcome impaired transport.[136] Some of these pathways are illustrated in figure 2.

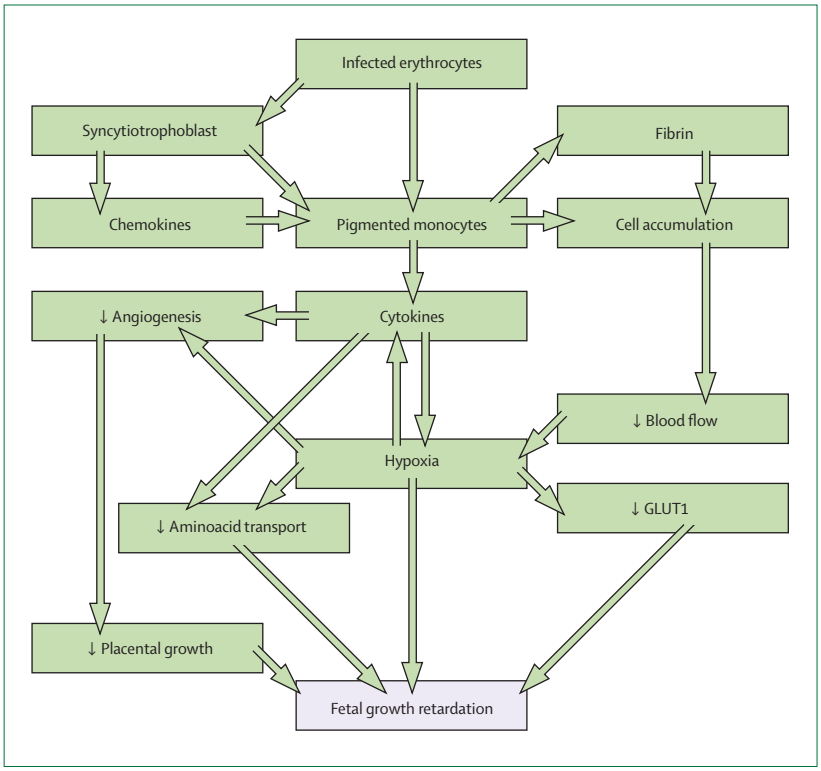

Figure 2: **Possible pathways by which placental sequestration of infected erythrocytes could activate monocytes and other cells to cause changes in placental function that result in growth retardation**
Infected erythrocytes or their products can activate both syncytiotrophoblasts and monocytes to release chemokines and cytokines. The former contribute to monocyte accumulation, whereas the latter could have direct effects of placental growth (through angiogenesis). Cytokines and cell accumulations can lead to placental hypoxia. Cytokines could directly or indirectly affect nutrient transport mechanisms. Decreased placental growth, or decreased nutrient transport, are probable final common pathways by which malaria leads to fetal growth restriction. GLUT1=glucose transporter 1.

Pathogenesis of malarial anaemia

Malarial anaemia is caused by a combination of bone marrow dysfunction and destruction of infected and uninfected erythrocytes. In pregnancy, this is often superimposed on micronutrient deficiency (eg, iron and folic acid), HIV infection, hookworm infection, or chronic inflammation.[137,138] Placental accumulation of pigmented monocytes has been associated with maternal anaemia,[15,139] perhaps because these cells release inflammatory mediators such as TNF that suppress erythropoiesis in the absence of adequate interleukin 10 or because they cause oxidative stress, altering erythrocyte membranes and leading to increased erythrocyte destruction.[140,141] Presently, to what extent anaemia results from reduced erythropoiesis or from removal of erythrocytes damaged in the placenta or elsewhere is unclear.

Duration of maternal susceptibility after delivery

Peripheral parasites obtained from infected children have not been found to express VSA_{PAM}, whereas those obtained during pregnancy from women living in areas of intense transmission of *P falciparum* mostly do.[142] This observation suggests that the latter parasites originate from a placental sequestration focus, a hypothesis that is lent support by the finding that peripheral parasitaemias in pregnant women usually resolve spontaneously within 1–2 days of delivery.[143] Nevertheless, increasing evidence shows at least some women remain at increased risk of malaria into the puerperium.[144,145] The relation between the parasites that cause malaria in pregnancy and those seen during puerperium is unclear and deserves study.

Hormones and malaria susceptibility in pregnancy

Malaria has been associated with reduced oestradiol production in late pregnancy,[146] and with raised serum cortisol levels in primigravidae with placental malaria;[147] in the latter study prolactin levels were also studied, and were not associated with malaria infection. Whether the increased cortisol levels reflect the stress response to malaria or whether higher cortisol levels throughout pregnancy suppress immune responses, thus increasing susceptibility to malaria, is unknown. Further studies of the relation between malaria and the major endocrinological changes of pregnancy are warranted.

Placental malaria and infant outcomes

Cord blood infection is common,[148,149] but clinical disease in the newborn baby is rare, probably because transplacental transfer of variant-specific and other antibodies (eg, to MSP1) protects the infant, although this remains under debate.[150–152] Fetal infection could be acquired by transplacental microtransfusion antenatally,[153] and this might be responsible for priming of B-cell and T-cell responses to malaria.[154,155] Maternal malaria also induces cord blood CD4+CD25+ T regulatory cells, increasing interleukin-10 production and decreasing interferon-γ levels,[156] which could influence the newborn baby's susceptibility to disease. The relation could be more complicated than first anticipated, and clearly deserves further study.[152,157] Under circumstances of low maternal immunity, congenital malaria can present as a severe illness 2–6 weeks after birth, and infection in utero has been associated with stillbirth.[158]

Placental malaria decreases transplacental transfer of maternal IgG antibodies to non-malarial antigens—eg, measles, *Streptococcus pneumoniae*, and others.[159–161] The effect of placental malaria on transfer of antimalarial antibodies is less clear. Infection leads to higher antibody titres, increasing antibody transfer to the infant; however, the relative proportion of antibody transferred might be decreased, and transfer of IgG1 and IgG2 subclasses could be particularly affected.[162] Detailed dissection of how placental malaria affects transfer of antibodies to key malaria antigens has not been undertaken. Placental malaria and its treatment alter T-cell response profiles. Infection is associated with increased CD4+CD25+ T cells and interleukin-10+ T cells, whereas treatment increases interferon-γ responses.[156,162]

Finally, placental malaria substantially increases perinatal mortality and is thought to cause substantial infant mortality; however, the underlying mechanisms causing death are incompletely understood. Low birthweight induced by placental malaria could be an important mediator, but few data are available to understand the effect of placental malaria on specific immunoparasitological outcomes during early life. Studies in animal models of malaria suggest that these effects could be profound and long lasting. A recent birth cohort study in Tanzania found that placental malaria substantially modifies the risk of malaria parasitaemia throughout infancy, but that this effect varied based on birth order, with decreased risk in first offspring and increased risk in subsequent offspring.[157] Additional studies in birth cohorts are needed to better delineate these relations and to examine severe malaria and mortality outcomes.

***Panel 3:* Important future studies**

- Continuing assessment of VAR2CSA as a potential vaccine candidate
- Longitudinal studies that link pregnancy descriptions, ultrasound, development of antibodies to VSA_{PAM} and to VAR2CSA, placental histology, and birth outcomes
- Studies that examine how the endocrine and immune systems interact to predispose to malaria in pregnancy, and if malaria induces labour-associated hormones in preterm delivery
- Immunological studies to identify the key maternal and fetal cells and processes that initiate the pathological processes of placental malaria
- Studies of women over their reproductive life, following over time how immunity to pregnancy malaria develops, and factors that affect this process
- *P vivax*: influence on pregnancy outcome and role of co-infection in altering the effect of *P falciparum*
- Exploration of differences in immunity and pathogenesis between areas of high and low endemicity[24]
- Infant cohorts to examine how in-utero exposure to malaria affects growth and development and evolution of malaria immunity in childhood

Research priorities

Towards vaccines against malaria in pregnancy

Malaria control through vaccination has not been realised despite recent encouraging results.[163,164] If a successful vaccine for use in children is developed, to test it for its efficacy against malaria in pregnancy will be important, since any vaccine that decreases infection rates, or slows parasite replication, might decrease the burden of malaria in pregnancy.

Malaria in pregnancy could constitute a well-defined syndrome amenable to vaccination specifically against the parasites sequestering in the placenta. Such a vaccine could be given to young women before their first pregnancy and would be aimed at inducing the high levels of VSA_{PAM}-specific IgG that are generally seen in multigravidae that are resistant to pregnancy-associated malaria. VAR2CSA seems to be a promising candidate for such a vaccine (table 2), although many unresolved issues remain. Prospective clinical studies and detailed mapping of immune responses to VAR2CSA will resolve the importance of these obstacles.

Ideally, a vaccine specific to pregnancy-associated malaria would confer protection against maternal anaemia, preterm delivery, and fetal growth restriction, but would probably have no effect on parasites that express non-pregnancy-associated malaria variant surface antigens, and thus would probably be of little direct benefit to the child.

Effect of intermittent preventive treatment in pregnancy on immunity to malaria

The presence of malarial parasites boosts antibody responses, and high titres of antibodies to many malarial antigens are often found in pregnant women.[140] A successful regime of intermittent preventive treatment in pregnancy could decrease exposure to malaria in pregnancy, and antibody titres to key malarial antigens could decline, leaving women more susceptible to postpartum malaria. Perhaps more importantly, with reduced exposure to placental malaria, primigravidae might not produce antibodies or develop memory B cells to VSA_{PAM}.[165] If intermittent preventive treatment in pregnancy curtails development of parity-specific immunity, susceptibility to placental malaria could extend to women in their second and even subsequent pregnancies.

Implications for prevention

Current WHO recommendations include at least two doses of intermittent preventive treatment at least 4 weeks apart, commencing after quickening—the detection by the mother of fetal movements—usually at about 18 to 20 weeks in primigravidae.[166] This approach reduces the risk of teratogenicity to a minimum, but means malaria during trophoblast invasion or placentation is not treated. Similarly, two doses of sulphadoxine-pyrimethamine intermittent preventive treatment, completed by 28 weeks of pregnancy, could leave women susceptible to malaria during the peak growth period of the fetus. Studies of placental blood flow and placental pathology during standard intermittent preventive treatment in pregnancy will help to determine whether the duration of drug exposure should be extended.

Future studies

We have outlined much of the current knowledge regarding malaria pathogenesis in pregnancy, but several unresolved and important questions remain. Panel 3 identifies priority areas for future study, which will obtain crucial evidence regarding the case for a pregnancy-specific vaccine, tell us whether the current timing of interventions is optimal, identify important endocrinological and immunological changes in malaria, greatly improve our understanding of pathogenesis in low transmission areas and areas where *P vivax* is common, and lead to a clear understanding of the broad effects of maternal malaria on infant growth and development.

Search strategy and selection criteria

Data for this Review were identified by searches of PubMed with the terms "pregnant", "pregnancy", "malaria", "Plasmodium", "placenta", and combinations of these, and by searches of references from relevant articles; numerous articles were identified through searches of the authors' own files. Articles in English were sourced. Where possible, references were restricted to papers published in the past 5 years.

Conclusions

Increasing numbers of longitudinal studies of malaria during pregnancy, its prevention, and its effect on the infant's health and development of malaria immunity offer new opportunities to extend our knowledge of how, when, and where malaria exerts its pathogenic effects on mothers and babies. Such studies are crucial to rational planning of antimalarial interventions.

By linking applied laboratory research to intervention studies we will create synergies between the bench and the field, and understand how interventions can affect both malaria disease and development of immunity. Exciting progress with possible vaccine candidates will lead to new challenges in understanding their potential effect on natural immunity and discovering how much they can reduce the pathology of placental malaria.

Conflicts of interest

We declare that we have no conflicts of interest.

Acknowledgments

SJR is supported by a Wellcome Trust Senior Fellowship, 063215, and by the National Health and Medical Research Council of Australia. LH is supported by funding from the Bill and Melinda Gates Foundation, the Commission of the European Communities, and the Danish Medical Research Council. PD is supported by funding from the Bill and Melinda Gates Foundation, the Foundation for NIH, the US National Institutes of Health (R01 AI52059 and U19 AI065664), and the US Department of Defense. The views expressed in this article do not necessarily reflect those of the US Department of Defense. DWT and RL are supported by the National Institute of Allergy and Infectious Diseases, NIH U01AI 43888 and 1R21 AI066184.

References

1 Steketee RW, Nahlen BL, Parise ME, Menendez C. The burden of malaria in pregnancy in malaria-endemic areas. *Am J Trop Med Hyg* 2001; **64** (1–2 suppl): 28–35.

2 Desai M, ter Kuile FO, Nosten F, et al. Epidemiology and burden of malaria in pregnancy. *Lancet Infect Dis* 2007; **7:** 93–104.

3 Nosten F, McGready R, Simpson JA, et al. Effects of *Plasmodium vivax* malaria in pregnancy. *Lancet* 1999; **354:** 546–49.

4 Raghupathy R. Th1-type immunity is incompatible with successful pregnancy. *Immunol Today* 1997; **18:** 478–82.

5 Pearson RD. Parasites, pregnancy, prolactin and pandemics? *Trends Parasitol* 2005; **21:** 555–56.

6 Duffy PE. Immunity to malaria during pregnancy: different host, different parasite. In: Duffy PE, Fried M, eds. Malaria in pregnancy: deadly parasite, susceptible host. London: Taylor & Francis, 2001: 70–126.

7 Beeson JG, Amin N, Kanjala M, Rogerson SJ. Selective accumulation of mature asexual stages of *Plasmodium falciparum*-infected erythrocytes in the placenta. *Infect Immun* 2002; **70:** 5412–15.

8 Walter PR, Garin Y, Blot P. Placental pathologic changes in malaria. A histologic and ultrastructural study. *Am J Pathol* 1982; **109:** 330–42.

9 Ismail MR, Ordi J, Menendez C, et al. Placental pathology in malaria: a histological, immunohistochemical, and quantitative study. *Hum Pathol* 2000; **31:** 85–93.

10 Suguitan AL Jr, Leke RG, Fouda G, et al. Changes in the levels of chemokines and cytokines in the placentas of women with *Plasmodium falciparum* malaria. *J Infect Dis* 2003; **188:** 1074–82.

11 Brabin BJ, Romagosa C, Abdelgalil S, et al. The sick placenta–the role of malaria. *Placenta* 2004; **25:** 359–78.

12 Menendez C, Ordi J, Ismail MR, et al. The impact of placental malaria on gestational age and birth weight. *J Infect Dis* 2000; **181:** 1740–45.

13 Tako EA, Zhou A, Lohoue J, Leke R, Taylor DW, Leke RF. Risk factors for placental malaria and its effect on pregnancy outcome in Yaounde, Cameroon. *Am J Trop Med Hyg* 2005; **72:** 236–42.

14 Shulman CE, Marshall T, Dorman EK, et al. Malaria in pregnancy: adverse effects on haemoglobin levels and birthweight in primigravidae and multigravidae. *Trop Med Int Health* 2001; **6:** 770–78.

15 Rogerson SJ, Pollina E, Getachew A, Tadesse E, Lema VM, Molyneux ME. Placental monocyte infiltrates in response to *Plasmodium falciparum* infection and their association with adverse pregnancy outcomes. *Am J Trop Med Hyg* 2003; **68:** 115–19.

16 Bottius E, Guanzirolli A, Trape JF, Rogier C, Konate L, Druilhe P. Malaria: even more chronic in nature than previously thought; evidence for subpatent parasitaemia detectable by the polymerase chain reaction. *Trans R Soc Trop Med Hyg* 1996; **90:** 15–19.

17 Mankhambo L, Kanjala M, Rudman S, Lema VM, Rogerson SJ. Evaluation of the OptiMAL rapid antigen test and species-specific PCR to detect placental *Plasmodium falciparum* infection at delivery. *J Clin Microbiol* 2002; **40:** 155–58.

18 Mockenhaupt FP, Rong B, Till H, et al. Submicroscopic *Plasmodium falciparum* infections in pregnancy in Ghana. *Trop Med Int Health* 2000; **5:** 167–73.

19 Saute F, Menendez C, Mayor A, et al. Malaria in pregnancy in rural Mozambique: the role of parity, submicroscopic and multiple *Plasmodium falciparum* infections. *Trop Med Int Health* 2002; **7:** 19–28.

20 Walker-Abbey A, Djokam RR, Eno A, et al. Malaria in pregnant Cameroonian women: the effect of age and gravidity on submicroscopic and mixed-species infections and multiple parasite genotypes. *Am J Trop Med Hyg* 2005; **72:** 229–35.

21 Mockenhaupt FP, Ulmen U, von Gaertner C, Bedu-Addo G, Bienzle U. Diagnosis of placental malaria. *J Clin Micro* 2002; **40:** 306–08.

22 Nosten F, ter Kuile F, Maelankirri L, Decludt B, White NJ. Malaria during pregnancy in an area of unstable endemicity. *Trans R Soc Trop Med Hyg* 1991; **85:** 424–29.

23 Ndyomugyenyi R, Magnussen P. Malaria morbidity, mortality and pregnancy outcome in areas with different levels of malaria transmission in Uganda: a hospital record-based study. *Trans R Soc Trop Med Hyg* 2001; **95:** 463–68.

24 Nosten F, Rogerson SJ, Beeson JG, McGready R, Mutabingwa TK, Brabin B. Malaria in pregnancy and the endemicity spectrum: what can we learn? *Trends Parasitol* 2004; **20:** 425–32.

25 Fried M, Nosten F, Brockman A, Brabin BJ, Duffy PE. Maternal antibodies block malaria. *Nature* 1998; **395:** 851–52.

26 McGready R, Davison BB, Stepniewska K, et al. The effects of *Plasmodium falciparum* and *P vivax* infections on placental histopathology in an area of low malaria transmission. *Am J Trop Med Hyg* 2004; **70:** 398–407.

27 Fried M, Duffy PE. Adherence of *Plasmodium falciparum* to chondroitin sulfate A in the human placenta. *Science* 1996; **272:** 1502–04.

28 Ricke CH, Staalsoe T, Koram K, et al. Plasma antibodies from malaria-exposed pregnant women recognize variant surface antigens on *Plasmodium falciparum*-infected erythrocytes in a parity-dependent manner and block parasite adhesion to chondroitin sulfate A. *J Immunol* 2000; **165:** 3309–16.

29 Rogerson SJ, Chaiyaroj SC, Ng K, Reeder JC, Brown GV. Chondroitin sulfate A is a cell surface receptor for *Plasmodium falciparum*-infected erythrocytes. *J Exp Med* 1995; **182:** 15–20.

30 Maubert B, Fievet N, Tami G, Boudin C, Deloron P. *Plasmodium falciparum*-isolates from Cameroonian pregnant women do not rosette. *Parasite* 1998; **5:** 281–83.

31 Carlson J, Helmby H, Hill AV, Brewster D, Greenwood BM, Wahlgren M. Human cerebral malaria: association with erythrocyte rosetting and lack of anti-rosetting antibodies. *Lancet* 1990; **336:** 1457–60.

32 Udomsangpetch R, Wahlin B, Carlson J, et al. *Plasmodium falciparum*-infected erythrocytes form spontaneous erythrocyte rosettes. *J Exp Med* 1989; **169:** 1835–40.

33 Rogerson SJ, Beeson JG, Mhango C, Dzinjalamala F, Molyneux ME. *Plasmodium falciparum* rosette formation is uncommon in isolates from pregnant women. *Infect Immun* 2000; **68:** 391–93.

34 Beeson JG, Brown GV, Molyneux ME, Mhango C, Dzinjalamala F, Rogerson SJ. *Plasmodium falciparum* isolates from infected pregnant women and children are associated with distinct adhesive and antigenic properties. *J Infect Dis* 1999; **180:** 464–72.

35 Beeson JG, Mann EJ, Elliott SR, et al. Antibodies to variant surface antigens of *Plasmodium falciparum*-infected erythrocytes and adhesion inhibitory antibodies are associated with placental malaria and have overlapping and distinct targets. *J Infect Dis* 2004; **189:** 540–51.

36 Maubert B, Fievet N, Tami G, Cot M, Boudin C, Deloron P. Development of antibodies against chondroitin sulfate A-adherent *Plasmodium falciparum* in pregnant women. *Infect Immun* 1999; **67:** 5367–71.

37 Bull PC, Kortok M, Kai O, et al. *Plasmodium falciparum*-infected erythrocytes: agglutination by diverse Kenyan plasma is associated with severe disease and young host age. *J Infect Dis* 2000; **182:** 252–59.

38 Creasey AM, Staalsoe T, Raza A, Arnot DE, Rowe JA. Nonspecific immunoglobulin M binding and chondroitin sulfate A binding are linked phenotypes of *Plasmodium falciparum* isolates implicated in malaria during pregnancy. *Infect Immun* 2003; **71:** 4767–71.

39 Beeson JG, Rogerson SJ, Cooke BM, et al. Adhesion of *Plasmodium falciparum*-infected erythrocytes to hyaluronic acid in placental malaria. *Nat Med* 2000; **6:** 86–90.

40 Fried M, Lauder RM, Duffy PE. *Plasmodium falciparum*: adhesion of placental isolates modulated by the sulfation characteristics of the glycosaminoglycan receptor. *Exp Parasitol* 2000; **95:** 75–78.

41 Sharling L, Enevold A, Sowa KM, Staalsoe T, Arnot DE. Antibodies from malaria-exposed pregnant women recognize trypsin resistant epitopes on the surface of *Plasmodium falciparum*-infected erythrocytes selected for adhesion to chondroitin sulphate A. *Malar J* 2004; **3:** 31.

42 Muthusamy A, Achur RN, Bhavanandan VP, Fouda GG, Taylor DW, Gowda DC. *Plasmodium falciparum*-infected erythrocytes adhere both in the intervillous space and on the villous surface of human placenta by binding to the low-sulfated chondroitin sulfate proteoglycan receptor. *Am J Pathol* 2004; **164:** 2013–25.

43 Flick K, Scholander C, Chen Q, et al. Role of nonimmune IgG bound to PfEMP1 in placental malaria. *Science* 2001; **293:** 2098–100.

44 Rasti N, Namusoke F, Chene A, et al. Nonimmune immunoglobulin binding and multiple adhesion characterize *Plasmodium falciparum*-infected erythrocytes of placental origin. *Proc Natl Acad Sci USA* 2006; **103:** 13795–800.

45 Fried M, Domingo GJ, Gowda CD, Mutabingwa TK, Duffy PE. *Plasmodium falciparum*: chondroitin sulfate A is the major receptor for adhesion of parasitized erythrocytes in the placenta. *Exp Parasitol* 2006; **113:** 36–42.

46 Salem HH, Maruyama I, Ishii H, Majerus PW. Isolation and characterization of thrombomodulin from human placenta. *J Biol Chem* 1984; **259:** 12246–51.

47 Rogerson SJ, Novakovic S, Cooke BM, Brown GV. *Plasmodium falciparum*-infected erythrocytes adhere to the proteoglycan thrombomodulin in static and flow-based systems. *Exp Parasitol* 1997; **86:** 8–18.

48 Eaton MD. The agglutination of *Plasmodium knowlesi* by immune serum. *J Exp Med* 1938; **67:** 857–69.

49 Rogerson SJ, Chaiyaroj SC, Ng K, Reeder JC, Brown GV. Chondroitin sulfate A is a cell surface receptor for *Plasmodium falciparum*-infected erythrocytes. *J Exp Med* 1995; **182:** 15–20.

50 Beeson JG, Mann EJ, Byrne TJ, et al. Antigenic differences and conservation among placental type *Plasmodium falciparum*-infected erythrocytes and acquisition of variant-specific and cross-reactive antibodies. *J Infect Dis* 2006; **193:** 721–30.

51 Nielsen MA, Staalsoe T, Kurtzhals JA, et al. *Plasmodium falciparum* variant surface antigen expression varies between isolates causing severe and nonsevere malaria and is modified by acquired immunity. *J Immunol* 2002; **168:** 3444–50.

52 Marsh K, Otoo L, Hayes RJ, Carson DC, Greenwood BM. Antibodies to blood stage antigens of *Plasmodium falciparum* in rural Gambians and their relation to protection against infection. *Trans R Soc Trop Med Hyg* 1989; **83:** 293–303.

53 Bull PC, Lowe BS, Kortok M, Molyneux CS, Newbold CI, Marsh K. Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria. *Nat Med* 1998; **4:** 358–60.

54 Staalsoe T, Megnekou R, Fievet N, et al. Acquisition and decay of antibodies to pregnancy-associated variant antigens on the surface of *Plasmodium falciparum*-infected erythrocytes that protect against placental parasitemia. *J Infect Dis* 2001; **184:** 618–26.

55 O'Neil-Dunne I, Achur RN, Agbor-Enoh ST, et al. Gravidity-dependent production of antibodies that inhibit binding of *Plasmodium falciparum*-infected erythrocytes to placental chondroitin sulfate proteoglycan during pregnancy. *Infect Immun* 2001; **69:** 7487–92.

56 Megnekou R, Staalsoe T, Taylor DW, Leke R, Hviid L. Effects of pregnancy and intensity of *Plasmodium falciparum* transmission on immunoglobulin G subclass responses to variant surface antigens. *Infect Immun* 2005; **73:** 4112–18.

57 Elliott SR, Brennan AK, Beeson JG, et al. Placental malaria induces variant-specific antibodies of the cytophilic subtypes immunoglobulin G1 (IgG1) and IgG3 that correlate with adhesion inhibitory activity. *Infect Immun* 2005; **73:** 5903–07.

58 Taylor DW, Zhou A, Marsillio LE, et al. Antibodies that inhibit binding of *Plasmodium falciparum*-infected erythrocytes to chondroitin sulfate A and to the C terminus of merozoite surface protein 1 correlate with reduced placental malaria in Cameroonian women. *Infect Immun* 2004; **72:** 1603–07.

59 Rogerson SJ, van den Broek NR, Chaluluka E, Qonqwane C, Mhango CG, Molyneux ME. Malaria and anemia in antenatal women in Blantyre, Malawi: a twelve month survey. *Am J Trop Med Hyg* 2000; **62:** 335–40.

60 Reeder JC, Cowman AF, Davern KM, et al. The adhesion of *Plasmodium falciparum*-infected erythrocytes to chondroitin sulfate A is mediated by PfEMP1. *Proc Natl Acad Sci USA* 1999; **96:** 5198–202.

61 Buffet PA, Gamain B, Scheidig C, et al. *Plasmodium falciparum* domain mediating adhesion to chondroitin sulfate A: a receptor for human placental infection. *Proc Natl Acad Sci USA* 1999; **96:** 12743–48.

62 Viebig NK, Gamain B, Scheidig C, et al. A single member of the *Plasmodium falciparum* var multigene family determines cytoadhesion to the placental receptor chondroitin sulphate A. *EMBO Rep* 2005; **6:** 775–81.

63 Jensen AT, Zornig HD, Buhmann C, et al. Lack of gender-specific antibody recognition of products from domains of a var gene implicated in pregnancy-associated *Plasmodium falciparum* malaria. *Infect Immun* 2003; **71:** 4193–96.

64 Rowe JA, Kyes SA, Rogerson SJ, Babiker HA, Raza A. Identification of a conserved *Plasmodium falciparum var* gene implicated in malaria in pregnancy. *J Infect Dis* 2002; **185:** 1207–11.

65 Salanti A, Staalsoe T, Lavstsen T, et al. Selective upregulation of a single distinctly structured *var* gene in chondroitin sulphate A-adhering *Plasmodium falciparum* involved in pregnancy-associated malaria. *Mol Microbiol* 2003; **49:** 179–91.

66 Tuikue Ndam NG, Salanti A, Bertin G, et al. High level of *var2csa* transcription by *Plasmodium falciparum* isolated from the placenta. *J Infect Dis* 2005; **192:** 331–35.

67 Duffy MF, Byrne TJ, Elliott SR, et al. Broad analysis reveals a consistent pattern of *var* gene transcription in *Plasmodium falciparum* repeatedly selected for a defined adhesion phenotype. *Mol Microbiol* 2005; **56:** 774–88.

68 Kraemer SM, Smith JD. Evidence for the importance of genetic structuring to the structural and functional specialization of the *Plasmodium falciparum var* gene family. *Mol Microbiol* 2003; **50:** 1527–38.

69 Salanti A, Dahlback M, Turner L, et al. Evidence for the involvement of VAR2CSA in pregnancy-associated malaria. *J Exp Med* 2004; **200:** 1197–203.

70 Gamain B, Trimnell AR, Scheidig C, Scherf A, Miller LH, Smith JD. Identification of multiple chondroitin sulfate A (CSA)-binding domains in the *var2CSA* gene transcribed in CSA-binding parasites. *J Infect Dis* 2005; **191:** 1010–13.

71 Barfod L, Nielsen MA, Turner L, et al. Baculovirus-expressed constructs induce IgG recognizing VAR2CSA on *Plasmodium falciparum*-infected erythrocytes. *Infect Immun* 2006; **74:** 4357–60.

72 Fried M, Wendler JP, Mutabingwa TK, Duffy PE. Mass spectrometric analysis of *Plasmodium falciparum* erythrocyte membrane protein-1 variants expressed by placental malaria parasites. *Proteomics* 2004; **4:** 1086–93.

73 Pouvelle B, Buffet PA, Lepolard C, Scherf A, Gysin J. Cytoadhesion of *Plasmodium falciparum* ring-stage-infected erythrocytes. *Nat Med* 2000; **6:** 1264–68.

74 Duffy PE, Fried M. Antibodies that inhibit *Plasmodium falciparum* adhesion to chondroitin sulfate A are associated with increased birth weight and the gestational age of newborns. *Infect Immun* 2003; **71:** 6620–23.

75 Staalsoe T, Shulman CE, Bulmer JN, Kawuondo K, Marsh K, Hviid L. Variant surface antigen-specific IgG and protection against clinical consequences of pregnancy-associated *Plasmodium falciparum* malaria. *Lancet* 2004; **363:** 283–89.

76 Haase RN, Megnekou R, Lundquist M, Ofori MF, Hviid L, Staalsoe T. *Plasmodium falciparum* parasites expressing pregnancy-specific variant surface antigens adhere strongly to the choriocarcinoma cell line BeWo. *Infect Immun* 2006; **74:** 3035–38.

77 Lucchi NW, Koopman R, Peterson DS, Moore JM. *Plasmodium falciparum*-infected red blood cells selected for binding to cultured syncytiotrophoblast bind to chondroitin sulfate A and induce tyrosine phosphorylation in the syncytiotrophoblast. *Placenta* 2006; **27:** 384–94.

78 Viebig NK, Nunes MC, Scherf A, Gamain B. The human placental derived BeWo cell line: a useful model for selecting *Plasmodium falciparum* CSA-binding parasites. *Exp Parasitol* 2006; **112:** 121–25.

79 Abrams ET, Brown H, Chensue SW, et al. Host response to malaria during pregnancy: placental monocyte recruitment is associated with elevated beta chemokine expression. *J Immunol* 2003; **170:** 2759–64.

80 Chaisavaneeyakorn S, Moore JM, Mirel L, et al. Levels of macrophage inflammatory protein 1 alpha (MIP-1 alpha) and MIP-1 beta in intervillous blood plasma samples from women with placental malaria and human immunodeficiency virus infection. *Clin Diagn Lab Immunol* 2003; **10:** 631–36.

81 Chaisavaneeyakorn S, Moore JM, Otieno J, et al. Immunity to placental malaria. III. Impairment of interleukin (IL)-12, not IL-18, and interferon-inducible protein-10 responses in the placental intervillous blood of human immunodeficiency virus/malaria-coinfected women. *J Infect Dis* 2002; **185:** 127–31.

82 Chaisavaneeyakorn S, Lucchi N, Abramowsky C, et al. Immunohistological characterization of macrophage migration inhibitory factor expression in *Plasmodium falciparum*-infected placentas. *Infect Immun* 2005; **73:** 3287–93.

83 Diouf I, Fievet N, Doucoure S, et al. Monocyte activation and T cell inhibition in *Plasmodium falciparum*-infected placenta. *J Infect Dis* 2004; **189:** 2235–42.

84 Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunol Today* 1993; **14:** 353–56.

85 Kwak-Kim JY, Gilman-Sachs A, Kim CE. T helper 1 and 2 immune responses in relationship to pregnancy, nonpregnancy, recurrent spontaneous abortions and infertility of repeated implantation failures. *Chem Immunol Allergy* 2005; **88:** 64–79.

86 Moormann AM, Sullivan AD, Rochford RA, et al. Malaria and pregnancy: placental cytokine expression and its relationship to intrauterine growth retardation. *J Infect Dis* 1999; **180:** 1987–93.

87 Fried M, Muga RO, Misore AO, Duffy PE. Malaria elicits type 1 cytokines in the human placenta: IFN-γ and TNF-α associated with pregnancy outcomes. *J Immunol* 1998; **160:** 2523–30.

88 Rogerson SJ, Brown HC, Pollina E, et al. Placental tumor necrosis factor alpha but not gamma interferon is associated with placental malaria and low birth weight in Malawian women. *Infect Immun* 2003; **71:** 267–70.

89 Moore J, Nahlen B, Misore A, Lal A, Udhayakumar V. Immunity to placental malaria. I. Elevated production of interferon-gamma by placental blood mononuclear cells is associated with protection in an area with high transmission of malaria. *J Infect Dis* 1999; **179:** 1218–25.

90 Taylor-Robinson AW, Smith EC. A dichotomous role for nitric oxide in protection against blood stage malaria infection. *Immunol Lett* 1999; **67:** 1–9.

91 Fievet N, Moussa M, Tami G, et al. *Plasmodium falciparum* induces a Th1/Th2 disequilibrium, favoring the Th1-type pathway, in the human placenta. *J Infect Dis* 2001; **183:** 1530–34.

92 Jakobsen PH, Rasheed FN, Bulmer JN, Theisen M, Ridley RG, Greenwood BM. Inflammatory reactions in placental blood of *Plasmodium falciparum*-infected women and high concentrations of soluble E-selectin and a circulating *P falciparum* protein in the cord sera. *Immunology* 1998; **93:** 264–69.

93 Suguitan AL Jr, Cadigan TJ, Nguyen TA, et al. Malaria-associated cytokine changes in the placenta of women with pre-term deliveries in Yaounde, Cameroon. *Am J Trop Med Hyg* 2003; **69:** 574–81.

94 Kwak-Kim JY, Chung-Bang HS, Ng SC, et al. Increased T helper 1 cytokine responses by circulating T cells are present in women with recurrent pregnancy losses and in infertile women with multiple implantation failures after IVF. *Hum Reprod* 2003; **18:** 767–73.

95 Oehler L, Kollars M, Bohle B, et al. Interleukin-10 inhibits burst-forming unit-erythroid growth by suppression of endogenous granulocyte-macrophage colony-stimulating factor production from T cells. *Exp Hematol* 1999; **27:** 217–23.

96 Tilg H, Ulmer H, Kaser A, Weiss G. Role of IL-10 for induction of anemia during inflammation. *J Immunol* 2002; **169:** 2204–09.

97 Ludwiczek S, Aigner E, Theurl I, Weiss G. Cytokine-mediated regulation of iron transport in human monocytic cells. *Blood* 2003; **101:** 4148–54.

98 Urban B, Ferguson D, Pain A, et al. *Plasmodium falciparum*-infected erythrocytes modulate the maturation of dendritic cells. *Nature* 1999; **400:** 73–77.

99 Artavanis-Tsakonas K, Eleme K, McQueen KL, et al. Activation of a subset of human NK cells upon contact with *Plasmodium falciparum*-infected erythrocytes. *J Immunol* 2003; **171:** 5396–405.

100 Wilson NS, Behrens GM, Lundie RJ, et al. Systemic activation of dendritic cells by Toll-like receptor ligands or malaria infection impairs cross-presentation and antiviral immunity. *Nat Immunol* 2006; **7:** 165–72.

101 Mannoor MK, Halder RC, Morshed SR, et al. Essential role of extrathymic T cells in protection against malaria. *J Immunol* 2002; **169:** 301–06.

102 Hansen DS, Evans KJ, D'Ombrain MC, et al. The natural killer complex regulates severe malarial pathogenesis and influences acquired immune responses to *Plasmodium berghei* ANKA. *Infect Immun* 2005; **73:** 2288–97.

103 Mockenhaupt FP, Hamann L, von Gaertner C, et al. Common polymorphisms of Toll-like receptors 4 and 9 are associated with the clinical manifestation of malaria during pregnancy. *J Infect Dis* 2006; **194:** 184–88.

104 Ordi J, Menendez C, Ismail MR, et al. Placental malaria is associated with cell-mediated inflammatory responses with selective absence of natural killer cells. *J Infect Dis* 2001; **183:** 1100–07.

105 Sartelet H, Schleiermacher D, Le-Hesran JY, et al. Less HLA-G expression in *Plasmodium falciparum*-infected third trimester placentas is associated with more natural killer cells. *Placenta* 2005; **26:** 505–11.

106 Bouyou-Akotet MK, Issifou S, Meye JF, et al. Depressed natural killer cell cytotoxicity against *Plasmodium falciparum*-infected erythrocytes during first pregnancies. *Clin Infect Dis* 2004; **38:** 342–47.

107 Riley EM, Schneider G, Sambou I, Greenwood BM. Suppression of cell-mediated immune responses to malaria antigens in pregnant Gambian women. *Am J Trop Med Hyg* 1989; **40:** 141–44.

108 Hviid L, Theander TG, Abdulhadi NH, Abu ZY, Bayoumi RA, Jensen JB. Transient depletion of T cells with high LFA-1 expression from peripheral circulation during acute *Plasmodium falciparum* malaria. *Eur J Immunol* 1991; **21:** 1249–53.

109 Fievet N, Cot M, Ringwald P, et al. Immune response to *Plasmodium falciparum* antigens in Cameroonian primigravidae: evolution after delivery and during second pregnancy. *Clin Exp Immunol* 1997; **107:** 462–67.

110 Fievet N, Tami G, Maubert B, et al. Cellular immune response to *Plasmodium falciparum* after pregnancy is related to previous placental infection and parity. *Malar J* 2002; **1:** 16.

111 Dahlbäck M, Rask TS, Andersen PH, et al. Epitope mapping and topographic analysis of VAR2CSA DBL3X involved in *Plasmodium falciparum* placental sequestration. *PLoS Pathog* 2006; **2:** e124.

112 Barfod L, Bernasconi NL, Dahlback M, et al. Human pregnancy-associated malaria-specific B cells target polymorphic, conformational epitopes in VAR2CSA. *Mol Microbiol* (in press).

113 ter Kuile FO, Parise ME, Verhoeff FH, et al. The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant women in sub-saharan Africa. *Am J Trop Med Hyg* 2004; **71** (2 suppl)**:** 41–54.

114 Ned RM, Moore JM, Chaisavaneeyakorn S, Udhayakumar V. Modulation of immune responses during HIV-malaria co-infection in pregnancy. *Trends Parasitol* 2005; **21:** 284–91.

115 Mount AM, Mwapasa V, Elliott SR, et al. Impairment of humoral immunity to *Plasmodium falciparum* malaria in pregnancy by HIV infection. *Lancet* 2004; **363:** 1860–67.

116 Ayisi JG, Branch OH, Rafi-Janajreh A, et al. Does infection with human immunodeficiency virus affect the antibody responses to *Plasmodium falciparum* antigenic determinants in asymptomatic pregnant women? *J Infect* 2003; **46:** 164–72.

117 Moore JM, Ayisi J, Nahlen BL, Misore A, Lal AA, Udhayakumar V. Immunity to placental malaria. II. Placental antigen-specific cytokine responses are impaired in human immunodeficiency virus-infected women. *J Infect Dis* 2000; **182:** 960–64.

118 Mwapasa V, Rogerson SJ, Molyneux ME, et al. The effect of *Plasmodium falciparum* malaria on peripheral and placental HIV-1 RNA concentrations in pregnant Malawian women. *AIDS* 2004; **18:** 1051–59.

119 Tkachuk AN, Moormann AM, Poore JA, et al. Malaria enhances expression of CC chemokine receptor 5 on placental macrophages. *J Infect Dis* 2001; **183:** 967–72.

120 Brahmbhatt H, Kigozi G, Wabwire-Mangen F, et al. The effects of placental malaria on mother-to-child HIV transmission in Rakai, Uganda. *AIDS* 2003; **17:** 2539–41.

121 Ayisi JG, van Eijk AM, Newman RD, et al. Maternal malaria infection and perinatal HIV transmission in a malarious area of western Kenya. *Emerg Infect Dis* 2004; **10:** 643–52.

122 Dorman EK, Shulman CE, Kingdom J, et al. Impaired uteroplacental blood flow in pregnancies complicated by falciparum malaria. *Ultrasound Obstet Gynecol* 2002; **19:** 165–70.

123 Redman CW, Sargent IL. Latest advances in understanding preeclampsia. *Science* 2005; **308:** 1592–94.

124 Cottrell G, Mary JY, Barro D, Cot M. Is malarial placental infection related to peripheral infection at any time of pregnancy? *Am J Trop Med Hyg* 2005; **73:** 1112–18.

125 Arbeille P, Carles G, Georgescu M, et al. Consequences of reduced umbilical and increased foetal cerebral flow during malaria crisis on foetal behaviour. *Parasitology* 2003; **126:** 513–19.

126 Sullivan AD, Nyirenda T, Cullinan T, et al. Malaria infection during pregnancy: intrauterine growth retardation and preterm delivery in Malawi. *J Infect Dis* 1999; **179:** 1580–83.

127 Lyall F. Priming and remodelling of human placental bed spiral arteries during pregnancy—a review. *Placenta* 2005; **26** (suppl A)**:** S31–36.

128 Brabin BJ. The risks and severity of malaria in pregnant women. Applied field research in malaria. Geneva: World Health Organization, 1991: 1–34.

129 Imamura T, Sugiyama T, Cuevas LE, Makunde R, Nakamura S. Expression of tissue factor, the clotting initiator, on macrophages in *Plasmodium falciparum*-infected placentas. *J Infect Dis* 2002; **186:** 436–40.

130 Sartelet H, Rogier C, Milko-Sartelet I, Angel G, Michel G. Malaria associated pre-eclampsia in Senegal. *Lancet* 1996; **347:** 1121.

131 Muehlenbachs AM, Mutabingwa TK, Edmonds S, Fried M, Duffy PE. Hypertension and maternal-fetal conflict during placental malaria. *PLoS Med* 2006; **3:** e446.

132 Regnault TR, de Vrijer B, Battaglia FC. Transport and metabolism of amino acids in placenta. *Endocrine* 2002; **19:** 23–41.

133 Zamudio S, Baumann MU, Illsley NP. Effects of chronic hypoxia in vivo on the expression of human placental glucose transporters. *Placenta* 2006; **27:** 49–55.

134 Giudice LC, de Zegher F, Gargosky SE, et al. Insulin-like growth factors and their binding proteins in the term and preterm human fetus and neonate with normal and extremes of intrauterine growth. *J Clin Endocrinol Metab* 1995; **80:** 1548–55.

135 Tazuke SI, Mazure NM, Sugawara J, et al. Hypoxia stimulates insulin-like growth factor binding protein 1 (IGFBP-1) gene expression in HepG2 cells: a possible model for IGFBP-1 expression in fetal hypoxia. *Proc Natl Acad Sci USA* 1998; **95:** 10188–93.

136 Regnault TR, Friedman JE, Wilkening RB, Anthony RV, Hay WW Jr. Fetoplacental transport and utilization of amino acids in IUGR—a review. *Placenta* 2005; **26** (suppl A)**:** S52–62.

137 van den Broek NR, White SA, Neilson JP. The relationship between asymptomatic human immunodeficiency virus infection and the prevalence and severity of anemia in pregnant Malawian women. *Am J Trop Med Hyg* 1998; **59:** 1004–07.

138 van den Broek NR, Letsky EA. Etiology of anemia in pregnancy in south Malawi. *Am J Clin Nutr* 2000; **72:** 247S–56S.

139 Jilly P. Anaemia in parturient women, with special reference to malaria infection of the placenta. *Ann Trop Med Parasitol* 1969; **63:** 109–16.

140 Kurtzhals JA, Adabayeri V, Goka BQ, et al. Low plasma concentrations of interleukin 10 in severe malarial anaemia compared with cerebral and uncomplicated malaria. *Lancet* 1998; **351:** 1768–72.

141 Becker K, Tilley L, Vennerstrom JL, Roberts D, Rogerson S, Ginsburg H. Oxidative stress in malaria parasite-infected erythrocytes: host-parasite interactions. *Int J Parasitol* 2004; **34:** 163–89.

142 Ofori MF, Staalsoe T, Bam V, et al. Expression of variant surface antigens by *Plasmodium falciparum* parasites in the peripheral blood of clinically immune pregnant women indicates ongoing placental infection. *Infect Immun* 2003; **71:** 1584–86.

143 Nguyen-Dinh P, Steketee RW, Greenberg AE, Wirima JJ, Mulenda O, Williams SB. Rapid spontaneous postpartum clearance of *Plasmodium falciparum* parasitaemia in African women. *Lancet* 1988; **2:** 751–52.

144 Diagne N, Rogier C, Sokhna CS, et al. Increased susceptibility to malaria during the early postpartum period. *N Engl J Med* 2000; **343:** 598–603.

145 Ramharter M, Grobusch MP, Kiessling G, et al. Clinical and parasitological characteristics of puerperal malaria. *J Infect Dis* 2005; **191:** 1005–09.

146 Watkinson M, Rushton DI, Lunn PG. Placental malaria and foetoplacental function: low plasma oestradiols associated with malarial pigmentation of the placenta. *Trans R Soc Trop Med Hyg* 1985; **79:** 448–50.

147 Bouyou-Akotet MK, Adegnika AA, Agnandji ST, et al. Cortisol and susceptibility to malaria during pregnancy. *Microbes Infect* 2005; **7:** 1217–23.

148 Tobian AAR, Mehlotra RK, Malhotra I, et al. Frequent umbilical cord-blood and maternal-blood infections with *Plasmodium falciparum, P malariae* and *P ovale* in Kenya. *J Infect Dis* 2000; **182:** 558–63.

149 Kamwendo DD, Dzinjalamala FK, Snounou G, et al. *Plasmodium falciparum*: PCR detection and genotyping of isolates from peripheral, placental, and cord blood of pregnant Malawian women and their infants. *Trans R Soc Trop Med Hyg* 2002; **96:** 145–49.

150 Riley EM, Wagner GE, Akanmori BD, Koram KA. Do maternally acquired antibodies protect infants from malaria infection? *Parasite Immunol* 2001; **23:** 51–59.

151 Branch O, Udhayakumar V, Hightower A, et al. A longitudinal investigation of IgG and IgM antibody responses to the merozoite surface protein-119-kiloDalton domain of *Plasmodium falciparum* in pregnant women and infants: associations with febrileillness, parasitemia, and anemia. *Am J Trop Med Hyg* 1998; **58:** 211–19.

152 Hviid L, Staalsoe T. Malaria immunity in infants: a special case of a general phenomenon? *Trends Parasitol* 2004; **20:** 66–72.

153 Malhotra I, Mungai P, Muchiri E, Kwiek JJ, Meshnick SR, King CL. Umbilical cord-blood infections with *Plasmodium falciparum* malaria are acquired antenatally in Kenya. *J Infect Dis* 2006; **194:** 176–83.

154 King CL, Malhotra I, Wamachi A, et al. Acquired immune responses to *Plasmodium falciparum* merozoite surface protein-1 in the human fetus. *J Immunol* 2002; **168:** 356–64.

155 Malhotra I, Mungai P, Muchiri E, et al. Distinct Th1- and Th2-type prenatal cytokine responses to *Plasmodium falciparum* erythrocyte invasion ligands. *Infect Immun* 2005; **73:** 3462–70.

156 Brustoski K, Moller U, Kramer M, et al. Reduced cord blood immune effector-cell responsiveness mediated by CD4+ cells induced in utero as a consequence of placental *Plasmodium falciparum* infection. *J Infect Dis* 2006; **193:** 146–54.

157 Mutabingwa TK, Bolla MC, Li JL, et al. Maternal malaria and gravidity interact to modify infant susceptibility to malaria. *PLoS Med* 2005; **2:** e407.

158 Wickramasuriya GAW. Some observations on malaria occurring in association with pregnancy. *J Obstet Gynaecol Br Empire* 1935; **42:** 816–34.

159 Okoko BJ, Wesuperuma LH, Ota MO, et al. Influence of placental malaria infection and maternal hypergammaglobulinaemia on materno-foetal transfer of measles and tetanus antibodies in a rural west African population. *J Health Popul Nutr* 2001; **19:** 59–65.

160 de Moraes-Pinto MI, Verhoeff F, Chimsuku L, et al. Placental antibody transfer: influence of maternal HIV infection and placental malaria. *Arch Dis Child Fetal Neonatal Ed* 1998; **79:** F202–05.

161 Okoko BJ, Wesumperuma LH, Ota MO, et al. The influence of placental malaria infection and maternal hypergammaglobulinemia on transplacental transfer of antibodies and IgG subclasses in a rural west African population. *J Infect Dis* 2001; **184:** 627–32.

162 Brustoski K, Kramer M, Moller U, Kremsner PG, Luty AJ. Neonatal and maternal immunological responses to conserved epitopes within the DBL-gamma3 chondroitin sulfate A-binding domain of *Plasmodium falciparum* erythrocyte membrane protein 1. *Infect Immun* 2005; **73:** 7988–95.

163 Alonso PL, Sacarlal J, Aponte JJ, et al. Duration of protection with RTS,S/AS02A malaria vaccine in prevention of *Plasmodium falciparum* disease in Mozambican children: single-blind extended follow-up of a randomised controlled trial. *Lancet* 2005; **366:** 2012–18.

164 Alonso PL, Sacarlal J, Aponte JJ, et al. Efficacy of the RTS,S/AS02A vaccine against *Plasmodium falciparum* infection and disease in young African children: randomised controlled trial. *Lancet* 2004; **364:** 1411–20.

165 Staalsoe T, Shulman CE, Dorman EK, Kawuondo K, Marsh K, Hviid L. Intermittent preventive sulfadoxine-pyrimethamine treatment of primigravidae reduces levels of plasma immunoglobulin G, which protects against pregnancy-associated *Plasmodium falciparum* malaria. *Infect Immun* 2004; **72:** 5027–30.

166 WHO. A strategic framework for malaria prevention and control during pregnancy in the African region. Brazzaville: World Health Organization Regional Office for Africa, 2004.