Malaria in pregnancy: pathogenesis and immunity

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Understanding of the biological basis for susceptibility to malaria in pregnancy was recently advanced by the discovery that erythrocytes infected with \textit{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—\textit{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.

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.\(^\text{1,2}\) Both \textit{Plasmodium falciparum} and \textit{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.\(^\text{3}\)

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\(^\text{4}\) and hormonal changes associated with pregnancy (although the nature of the latter is the subject of debate\(^\text{5}\)), 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

\textbf{Malaria-associated placental changes}

Central to the pathogenesis of \textit{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.\(^\text{6}\) Trophozoite and schizont stages, which are absent from peripheral blood, sequester in the placenta.\(^\text{7}\) Other findings associated with pregnancy-associated malaria include increased numbers of maternal phagocytic cells—especially monocytes—in the intervillous space,\(^\text{8}\) and deposition of haemoglobin—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.\(^\text{10}\) In brief, chronic infection has been most closely associated with decreased birthweight due to fetal growth restriction, whereas acute infection (especially with high parasitaemia\(^\text{11,12}\)) has been more closely associated with preterm delivery (table 1). Chronic infection was also most closely associated with lower maternal haemoglobin or severe anaemia.\(^\text{13,14}\) However, these observations are presently based on few studies.

\textbf{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 submicroscopic peripheral blood parasitaemias, detectable by PCR,\(^\text{15}\) and these are often composed of many different parasite genotypes. In one study in pregnant women, submicroscopic infections were not associated with decreased birthweight,\(^\text{16}\) nor were they associated with lower haemoglobin in three of four studies.\(^\text{16–19}\) and neither

![Table 1: Classification of placental pathology\(^\text{5}\)](http://infection.thelancet.com)
pregnancy nor gravidity seem to change the complexity of infection. 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.

**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. 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. If infection is promptly treated, placental histology is often normal. Priority research areas are outlined in a recent review and elsewhere in this issue.

**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. 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. Chondroitin sulphate A has been consistently identified as the dominant placental adhesion receptor, although some studies point to the existence of additional receptors. However, current evidence suggests that they are less important than chondroitin sulphate A, which is present in the placenta as a glycosaminoglycan sidechain to the tissue anticoagulant thrombomodulin, 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. Infected erythrocytes can bind in vitro to both forms of chondroitin sulphate A.

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, which are a common feature of isolates from non-pregnant individuals and have been related to severe malaria in some studies. 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). This agglutination, which is mediated by antibodies cross-linking antigens on the surface of infected erythrocytes, is often seen with non-placental infected erythrocytes and has also been linked to disease severity. Finally, chondroitin sulphate A-adhering and placental infected erythrocytes ▼

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

| Adhesion to chondroitin sulphate A | Yes | No | Fried and Duffy 25, Ricke et al 27, Rogerson et al 29, Buhl et al 26 |
| Adhesion to CD36, ICAM-1 | No | Common | Fried and Duffy 25, Ricke et al 27, Rogerson et al 29 |
| Rosetting* | No | Common | Maubert et al 39, Carlson et al 28, Udornmsangthet et al 38, Rogerson et al 29 |
| Agglutination† | Variable | Common | Fried et al 39, Beezon et al 29, Maubert et al 39, Bull et al 26 |
| Non-specific binding of IgM to infected erythrocytes | Yes | No | Creasey et al 30 |
| Trypsin sensitivity of variant surface antigens | Variable | Generally high | Beezon et al 30, Fried et al 39, Sharling et al 30 |
| Sex-specific IgG recognition of variant surface antigens | Yes | No | Fried et al 39, Ricke et al 29 |
| Parity-dependent IgG recognition of variant surface antigens | Yes | No | Fried et al 39, Ricke et al 29 |

*Adhesion of uninfected erythrocytes to infected erythrocytes. †Surface antigen-specific, antibody-mediated clumping of infected erythrocytes.
erythrocytes express adhesion ligands that vary in their sensitivity to trypsin digestion and can adsorb IgM, unlike corresponding molecules on other erythrocytes infected with *P. falciparum*.28

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.31–33 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 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 placental 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 (VSAPAM).23 Like the infected erythrocytes that adhere to chondroitin sulphate A from pregnant women,25 the infected erythrocytes selected for adhesion to chondroitin sulphate A 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 surface23 was strongly corroborated by the finding that levels of VSAPAM-specific IgG, measured by flow cytometry, correlated with inhibition of adhesion of infected erythrocytes to chondroitin sulphate A.28,33,54 Later studies have further strengthened the relation between pregnancy and VSAPAM-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 VSAPAM-specific IgG.36,57 Levels of immunity to other blood stage antigens have also been associated with reductions in placental malaria,26 and might explain the importance of young age as an independent risk factor for pregnancy malaria.26,59 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 VSAPAM 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.
- The var2csa gene is relatively conserved between clones.
- The var2csa gene is necessary for the ability to select for the adhesion of infected erythrocytes to chondroitin sulphate A.
- VAR2CSA is selectively expressed on the surface of infected erythrocytes expressing PAM-type variant surface antigens.
- VAR2CSA contains binding sites for chondroitin sulphate A.
- Naturally acquired VAR2CSA-specific IgG can be found in women only.
- Levels of VAR2CSA-specific IgG correlate with parity.
- High levels of VAR2CSA-specific IgG are associated with decreased risk of delivering a low-birthweight baby.
- VAR2CSA is an important target of naturally acquired IgG reactive with the surface of infected erythrocytes expressing VSAPAM.
- VAR2CSA-specific IgG reactive with the surface of infected erythrocytes expressing VSAPAM can be induced by subunit vaccination.
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 VARICSA—which showed both affinity for chondroitin sulphate A and substantial interclonal conservation. However, several independent lines of evidence have since made VARICSA an unlikely candidate. In its place, another PfEMP1 variant, VAR2CSA, has been identified. This molecule has many of the characteristics expected of variant surface antigens involved in pregnancy-associated malaria (panel 1). Like VARICSA, 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. 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), 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. 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. 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. Although VAR2CSA seems to be a target of pregnancy-associated malaria-specific immunity and IgG-mediated protective immunity, 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. Furthermore, not all infected erythrocytes in the placenta bind efficiently to chondroitin sulphate A. Recent data indicate that selection for adhesion to the placental cell line BeWo might be an effective alternative in selection for VSA_{PAM} expression, although adhesion of infected erythrocytes to BeWo cells is only partly susceptible to inhibition by soluble chondroitin sulphate A or chondroitinase treatment. 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 (MIP-1α and β), interferon-inducible protein 10 (IP10), monocyte chemotactant protein 1 (MCP1), and 1309. 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. Thus, induction of these chemokines provides a physiological explanation as to why monocytes and macrophages, and not other types of leukocytes, predominate in the intervillous space in response to parasite sequestration. Macrophages in the intervillous space can be activated and have the ability to process and present antigens to T cells.

Placental malaria changes the placental cytokine balance

During normal successful pregnancies, the cytokine balance is shifted towards a Th2-type response. In mice, strong Th1 responses during pregnancy are incompatible with a successful pregnancy. 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. For example, substantial increases in tumour necrosis factor (TNF) α, interferon γ, interferon 1β, interleukin 1β, and interleukin 2 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. 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. Placental blood mononuclear cells from multigravidae without malaria produced higher levels of interferon γ in vitro than did cells from uninfected women with malaria. 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, and interleukin 1β and interleukin 20 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 cytotoxic 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 TNFαs and babies of low birthweight, including low birthweight caused by fetal growth restriction and preterm delivery. In developed countries, increased serum levels of TNFα have been associated with spontaneous abortions, 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 but not in another, and were not detected in women with fetal growth restriction. 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. 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 and by reducing available iron concentrations in plasma). 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.

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. Moreover, \textit{P falciparum} impairs cross-presentation by dendritic cells and antiviral responses via interaction with toll-like receptors (TLRs). Studies in human beings are scarce, but in murine studies NK1.1 T cells protect athymic mice against blood-stage malaria infections, and CD1d-restricted NK T-cell responses enhance B-cell clonal expansion and antibody production in mice exposed to malaria.

Asexual-stage \textit{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. 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, whereas a more recent study, using a different antibody, showed NK cells to be increased in placental malaria. 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. 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. 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. 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\(^{106}\) 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\(_{\text{VAR2CSA}}\) and the parasite epitope(s) involved. If the parity-dependent stimulation of cytokine production by infected erythrocytes expressing VSA\(_{\text{VAR2CSA}}\) 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\(_{\text{VAR2CSA}}\)-specific IgG are produced in response to pregnancy-associated malaria, and recent data suggest that the frequency of VSA\(_{\text{VAR2CSA}}\)-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 VARI2CSA 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,\(^{117}\) 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.\(^{118}\)

Malaria increases HIV viral load in pregnant women,\(^{119}\) and has been associated with increased expression of CCR5 mRNA in placental tissue.\(^{120}\) 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.\(^{121,122}\) 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.

### 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,\(^{123}\) suggesting inadequate placent al blood flow, as seen in pre-eclampsia.\(^{124}\) 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,\(^{125}\) 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.\(^{126}\) Such studies can assess placental function and fetal

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**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?
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, anaemia, and high levels of TNFα and, in particular, interleukin 10. 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. Remodelling, which continues until 18–20 weeks’ gestation and into a period of high susceptibility to malaria, is critical to the development of adequate placental circulation near term. Alternatively, infected erythrocytes, monocytes, and fibrin deposition in the intervillous space might decrease placental blood flow by mechanical means.

Some studies, but not others, 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.

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. 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. Some of these pathways are illustrated in figure 2.

**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. Placental accumulation of pigmented monocytes has been associated with maternal anaemia, and 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.

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<sub>FRAM</sub>, whereas those obtained during pregnancy from women living in areas of intense transmission of *P falciparum* mostly do. 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. Nevertheless, increasing evidence shows that at least some women remain at increased risk of malaria into the puerperium. The relation between the parasites that cause malaria in pregnancy and those seen during puerperium is unclear and deserves study.

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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.
Hormones and malaria susceptibility in pregnancy
Malaria has been associated with reduced oestriol 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.163,164

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.165 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_{Nv}, 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 endemicity166
- 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.123,164 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_{Nv}-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. 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 placentarial malaria, primigravidae might not produce antibodies or develop memory B cells to VSAVAM. If intermittent preventive treatment in pregnancy curtails development of parity-specific immunity, susceptibility to placentarial 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. 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 placentarial 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 placentarial malaria.

Conflicts of interest

We declare that we have no conflicts of interest.

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