

Modulation of immune responses during HIV–malaria co-infection in pregnancy

Renée M. Ned¹, Julie M. Moore², Sujitra Chaisavaneeyakorn^{3,4} and Venkatachalam Udhayakumar¹

¹Malaria Branch, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Public Health Service, Department of Health and Human Services, Chamblee, GA 30341, USA

²Center for Tropical and Emerging Global Diseases and Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA

³Atlanta Research and Education Foundation, Decatur, GA 30033, USA

⁴Department of Microbiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

Infection with either HIV or malaria during pregnancy often results in adverse outcomes for mother and child. Co-infection further increases the risks of these events, which include maternal anemia and babies with low birth weight. The immunological bases for the increased susceptibility of HIV-infected mothers to malaria and for the effect of co-infection on mother-to-child transmission of HIV are areas of major importance in public health. In this article, we review current data about humoral and cellular responses to HIV–placental-malaria co-infection and present an immunological hypothesis to explain the epidemiological findings.

Introduction

The high prevalence of *Plasmodium falciparum* malaria in many parts of the world, combined with the expanding HIV–AIDS epidemic, has resulted in increasing percentages of the world's population being co-infected with these diseases. This has generated immense concern about potential interactions between malaria and HIV, both of which have serious consequences in pregnancy for mother and child [1–3]. This is particularly relevant to sub-Saharan Africa, where malaria is endemic and where 80% of the world's HIV-infected women reside [4]. Here, the prevalence of maternal malaria is as high as 65% [1], and HIV affects up to 40% of pregnant women [5].

Much has been learned during the past 15 years about the interaction between malaria and HIV during pregnancy [6,7]. However, many issues remain unclear, especially the modulation of immune responses during co-infection and the immunological bases for the severe pregnancy-related outcomes associated with these diseases. Much of the pathogenesis of malaria during pregnancy is mediated by massive accumulation of *P. falciparum* in the maternal blood spaces of the placenta, termed 'placental malaria'. The placenta is also the key

interface in mother-to-child transmission (MTCT) of HIV-1, especially that involving *in utero* transfer [8]. Thus, focusing on the events occurring at the placental level is crucial for understanding the interactions between HIV and malaria, and the associated disease outcomes.

Epidemiological evidence of an interaction between malaria and HIV-1 in pregnancy

Studies in largely non-pregnant adult populations have shown that the incidences of *P. falciparum* parasitemia [9] and symptomatic malaria [9,10] are increased in HIV-infected individuals. This effect is modulated by immune status because higher parasite densities and higher rates of clinical episodes were associated with falling CD4⁺ T-cell counts [9,11]. Many studies have also established that there is a strong interaction between *P. falciparum* malaria and HIV-1 during pregnancy (for review, see Refs [6,7]). A meta-analysis of 11 studies showed that HIV-infected pregnant women are consistently at increased risk for both peripheral and placental *P. falciparum* infection compared with women not infected with HIV [7]. These HIV-associated differences were more marked in multigravidae, suggesting that HIV infection disrupts development of the gravity-dependent acquired immunity to malaria that is seen in women not infected with HIV [7,12–14]. Studies that are more in depth are required to establish whether the higher susceptibility of multigravid women to malaria is related to longer exposure to HIV-1 and, thus, more-advanced HIV-induced immunodeficiency. The densities of peripheral, placental and umbilical-cord parasitemias were also increased in HIV-infected pregnant women compared with their uninfected counterparts [12,14]. Considering the HIV-related increases in the incidence and density of malaria infection, it is not surprising that HIV–malaria co-infected pregnant women are also at increased risk for maternal anemia and low-birth-weight babies [7,15,16]. Again, these increased risks are greatest in multigravidae. Considered together, these epidemiological observations suggest a severe compromise in acquired immunity to malaria in HIV-infected

Corresponding author: Udhayakumar, V. (vxu0@cdc.gov).

Available online 28 April 2005

Box 1. Immunity to *Plasmodium falciparum* malaria in pregnancy

It has long been known that women are at higher risk of malaria infection during pregnancy, which can lead to both maternal and fetal complications [1,2]. The biological basis for this increased susceptibility in a population that enjoys well-developed clinical immunity before pregnancy has been a long-standing question. It is particularly intriguing why primigravidae in endemic areas are most susceptible, whereas multigravid women develop increased resistance to maternal malaria. Investigations to answer these questions are ongoing in many laboratories.

Humoral immunity

Malaria antibody levels are reduced in pregnant women versus non-pregnant women, and in primigravidae versus multigravidae [49]. Also, there is an association between the level of antibodies to certain *P. falciparum* antigens [e.g. merozoite surface protein (MSP)-1 19kDa and Pf155/ring-infected erythrocyte surface antigen (RESA)] and the risk of placental malaria infection [50,51]. Recently, it was shown that a subpopulation of *P. falciparum* parasites sequesters in the placenta by binding preferentially to CSA [22]. It has been hypothesized that, during a first pregnancy, women are exposed to this new parasite variant for the first time and do not mount effective resistance. Through exposure to the CSA-binding parasite variants over successive pregnancies, however, women eventually acquire immunity specific to this population of parasites. In support of this hypothesis, several laboratories have shown that primigravid women generally lack, or have delayed production of, antibodies that can block the binding of *P. falciparum* parasites to CSA, whereas multigravid women possess these antibodies or acquire them earlier in pregnancy [52–54]. Also, women with high levels of anti-CSA-binding antibodies have reduced placental parasitemia [51], reduced maternal anemia and increased infant birth weight [55]. Hyaluronic acid [56] and immunoglobulins [57] might also be involved in the binding of parasites in the placenta, and it remains to be determined whether humoral immune factors have a role in controlling parasites with different adhesion phenotypes. Finally, lack

of adhesion to CD36 (a scavenger receptor that promotes phagocytosis) by CSA-binding parasites might help parasites to evade phagocytosis by monocytes [58].

Cellular immunity

Decreased cellular immunity, as measured by peripheral blood lymphoproliferative responses to malaria antigens, has been observed in pregnancy [59–62]. Elaborate studies of cellular immune response have been conducted using placental IVBMCs. An IFN- γ response elicited by IVBMCs in response to malaria antigen stimulation correlates with protection against placental malaria. Multigravid, but not primigravid, women produce high levels of this cytokine [24]. Increased IFN- γ production by placental blood cells in placental malaria is found consistently by different laboratories and study populations [63–65]. Other cytokine changes have been documented, with an increase in levels of cytokines such as TNF- α being associated with adverse birth outcomes [64–67].

Other changes in lymphocyte subsets have been described during pregnancy [68]. A decrease in NK cell cytotoxicity in primigravid women might contribute to an inability to clear the parasite [69]. Macrophages are thought to be key cellular mediators in combating *P. falciparum* infection. These cells accumulate in large numbers in the infected placenta [70–72] and have been associated with adverse birth outcomes [72,73].

Pregnancy hormones

Elevated levels of the immunosuppressive hormone cortisol have been described in pregnant women with clinical malaria, with the highest levels in primigravid women [69,74,75]. Furthermore, elevated levels of cortisol in pre-malaria antenatal clinic visits predicted subsequent susceptibility to malaria [75]. Levels of prolactin, an immunostimulatory hormone, were found to be lower in primigravidae than in multigravidae at delivery [69]. These changes might partially mediate the increased susceptibility of pregnant women to malaria.

pregnant women, resulting in a decreased ability to limit *P. falciparum* infection. This conclusion is bolstered by the finding that the prophylactic efficacy of sulfadoxine–pyrimethamine is reduced in HIV-infected pregnant women [12,17], probably because antimalarial drugs work in concert with the host immune response to combat parasitemia effectively.

Local events in the placenta during malarial infection have a major negative impact on fetal development and birth outcome [1,2]. Despite the demonstrated effects of HIV-1 on parasitemia and the adverse pregnancy outcomes, however, the extent to which HIV–malaria co-infection contributes to increased infant mortality remains unclear. One retrospective study found a significant increase in infant mortality of children born to placental malaria and HIV co-infected, compared with mono-infected, mothers [18], but this has not been confirmed in subsequent prospective studies [19]*. Clearly, further study of the interaction between HIV-1 and malaria during pregnancy, and the influence that co-infection has on pregnancy and birth outcomes is urgently needed. The identification of the underlying immunological bases for the increased susceptibility of HIV-infected women to malaria and for the associated poor clinical outcomes is central to this effort.

Although the understanding of immunity to malaria in pregnancy is incomplete (Box 1), the studies that have

been published provide a basis for understanding how immune responses to malaria are altered in HIV–malaria co-infection. Conversely, malaria infection might alter the immune response to HIV-1 in pregnancy but there are no published studies regarding the impact of co-infection on anti-viral immune responses in pregnant women.

Impact of co-infection on disease presentation and immunity

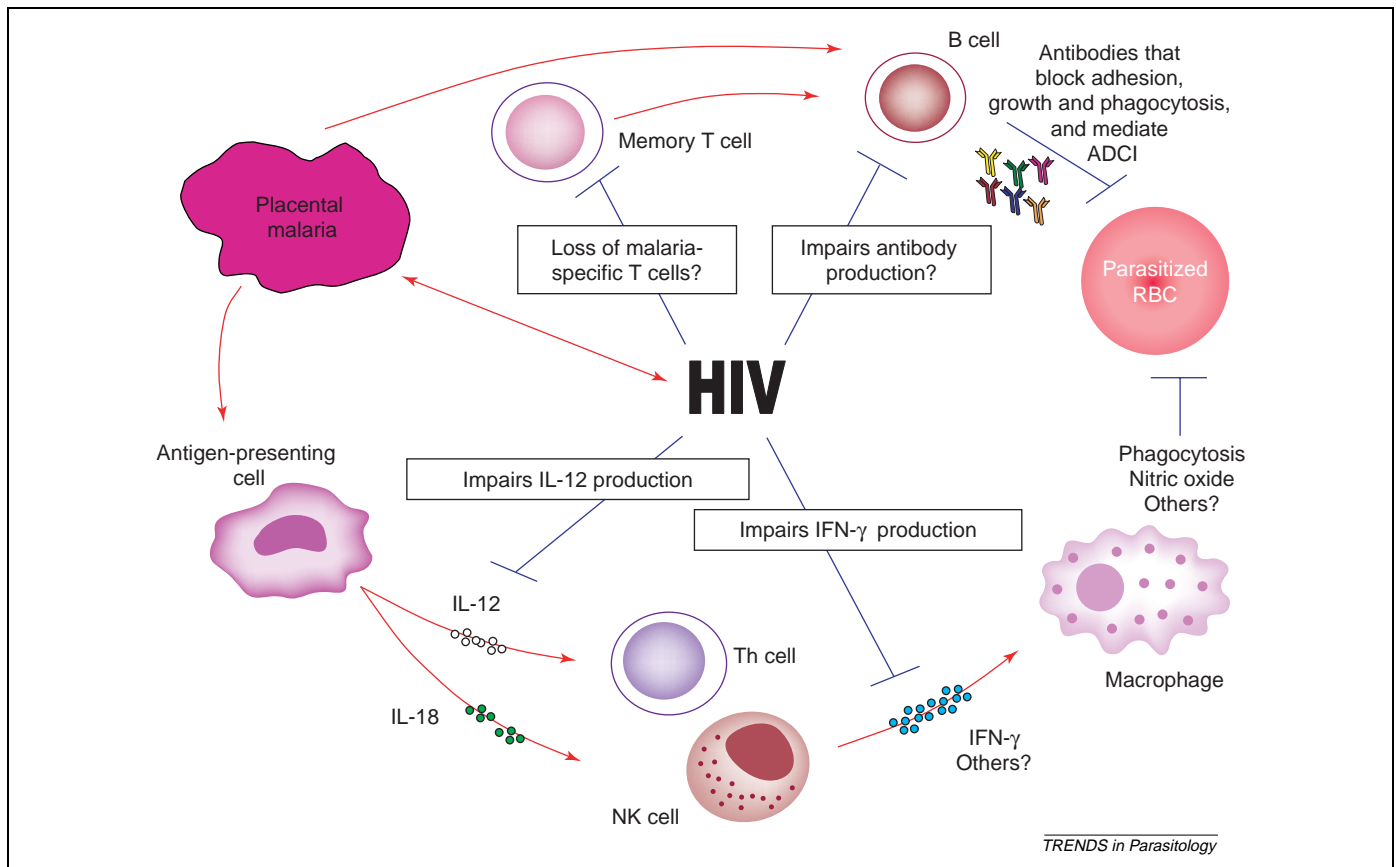
HIV-1 impact on malaria immune responses

Both humoral and cellular immune responses are known to have protective roles against malaria infection. Unfortunately, these compartments of the adaptive immune response are also profoundly altered during HIV-1 infection. Thus, as one might expect, the available evidence, which is summarized here and in Figure 1, provides a compelling argument that co-infected pregnant women suffer considerable perturbations in both humoral and cell-mediated immune responses to malaria.

Humoral responses

Antibodies against malaria antigens can mediate protection by various mechanisms, including blocking the invasion of parasites into host cells, and mediating phagocytosis and antibody-dependent inhibition of cellular growth [20]. Two studies have documented partial impairment of the humoral immune response to malaria in HIV-infected pregnant women. Ayisi *et al.* [21] reported decreased prevalence and concentration of antibodies

* van Eijk, A.M. *et al.* abstract ThPeC7605, 14th International AIDS Conference, Barcelona, July 2002.



TRENDS in Parasitology

Figure 1. Model of increased susceptibility of HIV-infected pregnant women to malaria. Image is a hypothetical representation of how HIV-1 infection impairs different immune pathways associated with protection against placental malaria. Placental malaria can lead to activation of antigen-presenting cells, resulting in the secretion of IL-12, IL-18 and other cytokines, in addition to the presentation of antigens to T cells. This can lead to T-cell activation, especially of Th1 cells that produce IFN- γ . In addition, activation of innate immune pathways involving natural killer (NK) cells can occur during placental malaria infection, resulting in the production of IFN- γ and other cytokines. IFN- γ can have a protective role by activating macrophages to kill malaria parasites through phagocytosis and/or releasing nitric oxide and other mediators. HIV-1 infection in pregnant women has been shown to impair IL-12 and IFN- γ production [25,26], thus affecting protective cellular immune pathways that can control placental malaria infection. Stimulation of malaria-antigen-specific T cells, particularly memory cells, during placental malaria infection might make these T cells prime targets for HIV infection and destruction because HIV induces loss of memory T cells, even before the onset of AIDS [29]. This loss in malaria-specific T-cell memory will have important implications for both cell-mediated (e.g. loss of IFN- γ responsiveness) and humoral (reduction in numbers of antibodies) immune responses. Placental malaria infection can also activate B cells to secrete antibodies specific to various malaria antigens, including VSAs. Anti-VSA antibodies can block parasite binding to CSA receptors in the placenta. Antibodies to different parasite surface antigens, including VSA, can inhibit parasite invasion and/or growth, and mediate phagocytosis and antibody-dependent cellular inhibition (ADCI) of growth. HIV-1 infection impairs the development of antibodies to some malaria antigens such as VSA [23] and, thus, compromises anti-malarial immunity. Abbreviation: RBC, red blood cell.

against the pre-erythrocytic-stage circumsporozoite protein (CSP)-repeat sequence NANP, which can prevent sporozoites from invading liver cells. A similar trend was observed for antibodies against EBA-175, a blood-stage antigen involved in parasite invasion of red blood cells. These effects were independent of placental malaria infection. No consistent HIV-associated differences were observed for the other antigenic determinants tested – MSP1_{19kD}, MSP-2, MSP-3 and RAP1 – which suggests that HIV infection affects antibody responses to some, but not all, malaria antigens [21].

A second study addressed the impact of HIV-1 infection on the production of antibodies against variant surface antigens (VSAs) that can block parasite adhesion to chondroitin sulfate A (CSA), which is considered to be a dominant host-cell receptor for parasite adhesion in the placenta [22]. In this recent report, pregnant HIV-infected women showed decreased prevalence and concentration of antibodies against VSA of the CSA-binding parasite line CS2, and those with a low CD4⁺ T-cell count showed the greatest impairment [23]. This decrease was found in all

gravidities, irrespective of the malaria infection status. This study is crucial because the HIV-associated decline in antibody responses to VSA could partially explain the increased susceptibility of HIV-infected pregnant women to malaria. Lack of anti-VSA antibodies could affect protective immune mechanisms in at least two ways. First, these antibodies could block parasite sequestration by CSA in the placenta. Second, anti-VSA antibodies are also likely to be crucial for mediating phagocytic uptake of CSA-binding parasites by monocytes and macrophages. Therefore, impairment of this antibody response can have negative consequences for parasite clearance. In the same study [23], antibody responses to blood-stage antigen AMA-1, but not MSP-1_{19kD}, were also found to be reduced in HIV-infected women. Collectively, these findings suggest that, although HIV-1 infection is associated with reduction in antibody responses to some *P. falciparum* malaria antigens, it does not cause a generalized suppression or reduction of humoral immune responses to malaria antigens in pregnant women. However, the reason for the selective compromise of antibody responses to only a

subset of malaria antigens is unclear. It could be because of the progressive loss of both T and B memory cells, which are required for the development and maintenance of antibody and other responses to malaria. A long-term follow up of T- and B-cell dynamics in HIV-infected pregnant women is necessary for addressing this issue.

Cellular immune responses

Many immune-cell types and the soluble immunoactive factors that they secrete are crucial for protective immunity to malaria. Several studies have focused on the cytokines and chemokines that might mediate protection against placental malaria and how they are changed in HIV-1 infection. Placental intervillous blood mononuclear cells (IVBMCs), which are cells of maternal origin in the intervillous spaces of the placenta, have been used in various studies. IVBMC production of the T helper (Th)1 cytokine interferon (IFN)- γ , which is crucial for activating monocytes and macrophages for parasite clearance, was shown to be associated with protection against placental malaria [24]. However, in HIV-infected women, irrespective of their malaria infection status, IVBMCs produced significantly lower levels of IFN- γ than in women not infected with HIV, especially in response to stimulation with crude blood-stage malaria antigens [25]. A subsequent study demonstrated that production of interleukin (IL)-12, which contributes to the regulation of IFN- γ synthesis, was impaired in HIV-infected women, with almost undetectable levels in the IVBMC culture supernatants of HIV-placental-malaria co-infected women [26]. Because the production of IL-18, another cytokine involved in the regulation of IFN- γ , was not altered, it seems that impairment of IFN- γ production in HIV-infected pregnant women is an IL-12-dependent phenomenon. Because *Plasmodium* is an intracellular parasite and its ultimate clearance requires active cellular immune mechanisms involving macrophages, it is understandable that HIV-associated impairment in this pathway is a potential cause of the increased malaria susceptibility in HIV-infected pregnant women.

Importantly, there seemed to be no generalized suppression of cytokines due to HIV-1 or HIV-placental-malaria co-infection in this and in subsequent studies evaluating levels of the cytokines tumor necrosis factor (TNF)- α , IL-4, IL-10 and macrophage migration inhibitory factor (MIF), and the chemokines macrophage inflammatory protein (MIP)-1 α and MIP-1 β [25,27,28]. Interestingly, levels of MIP-1 β were elevated in co-infected women compared with in HIV-infected women without placental malaria [27]. These observations suggest that there is a complex alteration in the cellular immune response in HIV-placental-malaria co-infection, including loss of a protective immune pathway that involves IFN- γ and potential upregulation of other cytokine and chemokine pathways.

These perturbations in cell-mediated immune function in HIV-malaria co-infected women are heavily dependent on CD4⁺ T-cell counts [25,26]. For example, IVBMC production of some cytokines, including IFN- γ , was substantially reduced in HIV-infected pregnant women with low (<500 μL^{-1}), compared with high (>500 μL^{-1}), CD4⁺ T-cell counts. This highlights the shift in cytokine

responsiveness that occurs as HIV-1 infection progresses. Therefore, comparison of results from different studies will require CD4⁺ T-cell profiles to be reported.

From the available data, we can begin to formulate a model to explain how immunity to malaria is altered in HIV-infected pregnant women (Figure 1). As outlined in the model, HIV-1 considerably downregulates production of IL-12 and IFN- γ , and impairs the production of some anti-malarial antibodies, resulting in a decreased ability to clear parasitized red blood cells from the placenta. HIV-1 infection also results in the destruction of memory T cells throughout development of the disease [29]. Thus, it is possible that the loss of protection against malaria during pregnancy in HIV-infected women is mediated by loss of malaria-antigen-specific memory T cells, even before the onset of AIDS. However, further research is needed to understand fully the biological basis for the increased susceptibility of HIV-infected pregnant women to malaria infection.

Malaria impact on HIV-1

Malaria infection can modulate HIV-1 progression by activating T cells or by inducing the release of immune factors such as TNF- α that can activate HIV-1 replication. *In vitro* studies conducted using peripheral blood mononuclear cells (PBMCs) from malaria-naïve donors showed that stimulation with malaria antigens and malaria pigment increases HIV-1 replication in a TNF- α -dependent manner [30]. Other investigators demonstrated that stimulation with *P. falciparum* schizont extract increased the permissiveness of PBMCs to HIV infection *in vitro* and could reactivate naturally acquired HIV-1 in malaria-naïve and malaria-exposed HIV-infected adults [31]. In an additional study of non-pregnant adults, the blood plasma concentration of HIV-1 RNA was increased sevenfold in patients with acute malaria compared with those without malaria co-infection [32]. Another recent study also demonstrated a significant increase in viral load during acute *P. falciparum* infection, with the largest increases occurring in individuals with high-density parasitemia and/or fever [33]. HIV-1 viral load declined within eight to nine weeks after malaria treatment [33].

In studies involving pregnant women, peripheral parasitemia is associated with statistically significant increases in peripheral HIV-1 viral load [34,35], although some reports failed to find such an association between placental malaria and peripheral viral load [35,36]. In a cross-sectional study in Malawi, however, placental malaria was associated with a twofold increase in placental HIV-1 RNA concentration in a multivariate analysis [37]. There was also a 1.7-fold increase in peripheral HIV-1 RNA concentration, and positive correlations were found between both peripheral and placental HIV-1 RNA concentrations and placental parasite density [37]. It will be important for future studies to determine both peripheral and placental viral measurements because the presence of *P. falciparum* within the placenta might increase viral load predominantly within this local environment.

These reports establish that the presence of *P. falciparum* can increase HIV-1 viral load. However, there are

no published data about the impact of malaria on the immune response to HIV-1 in pregnant women. Further studies are needed to understand to what extent malaria can increase the progression of HIV-1 infection because it will have important implications for treating malaria in HIV-infected individuals. However, in the context of pregnancy, an interaction between HIV and malaria might also impact the rate of MTCT of HIV-1.

Does placental malaria alter MTCT of HIV-1?

There is evidence that placental malaria increases HIV-1 viral load [37], which is an independent risk factor for MTCT of HIV-1 [8]. However, contrary to expectation, a definitive link between placental malaria and MTCT of HIV-1 has not been demonstrated. In a study of Ugandan HIV-infected women, Brahmhatt *et al.* [38] observed MTCT rates of 40% in mothers with placental malaria and 15.4% in those without. This resulted in a risk ratio of 2.89 for MTCT associated with placental malaria after adjustment for HIV viral load. However, the subjects were not stratified by CD4⁺ T-cell count or by the presence of severe disease. Other studies in Cameroon [39] and The Gambia [40] found increased risk of MTCT of HIV-1 when birth occurred during the rainy season, although malaria infection was not definitively assessed [39,40]. In contrast to these findings, other studies have not observed such associations. In Eastern Kenya, placental malaria did not correlate with *in utero* or periparturient transmission of HIV-1, despite the fact that placental malaria was significantly associated with HIV-1 infection [36]. Mwapasa *et al.*** also found no change in MTCT in women co-infected with HIV and placental malaria, although the rate of MTCT was still high (25.4%) despite the use of the antiviral drug nevirapine. In a study performed in Western Kenya, MTCT of HIV-1 was lower in mothers with placental malaria [35]. However, more-detailed analysis of data from that study revealed that women with low-density placental malaria (<10 000 parasites μL^{-1}) showed a significantly lower level of HIV-1 transmission (11.5%) than women with high-density (>10 000 parasites μL^{-1}) placental malaria (25% transmission) or no malaria (21.9% transmission).

The differences in outcome between these studies could be due to several factors. The studies that found no effect [36] or a protective effect [35] of placental malaria on MTCT of HIV-1 excluded women with AIDS, whereas the other investigations did not exclude study participants on this basis [38–40]. Equally importantly, the Kenyan studies employed larger sample sizes than the Ugandan study and, thus, had more statistical power to assess the impact of placental malaria on MTCT of HIV-1. Although these studies employed different criteria for defining placental malaria infection status – histological method [36,38] versus blood-film examination [35] – it is unlikely that this could explain the differences in the HIV-1 transmission rates between these studies. It is crucial for future studies to use large sample sizes and to examine the effects of CD4⁺ T-cell count, peripheral and placental

viral load, and the level of placental parasitemia to determine the effect of placental malaria on MTCT of HIV-1.

Cytokines and MTCT of HIV-1

The chemokines MIP-1 α , MIP-1 β and regulated on activation, normal T-cell expressed and secreted (RANTES) share a common receptor, C-C chemokine receptor 5 (CCR5), that also serves as a co-receptor for HIV-1 entry into host cells [41]. These three chemokines were shown to inhibit HIV-1 replication by interfering with viral entry through CCR5 [41–43]. In one study, placentas of malaria-infected women were shown to contain three times the amount of CCR5 RNA as placentas of women without malaria [44]. This increased expression of *CCR5*, if resulting in increased protein expression, might create an additional reservoir for HIV-1 in the placenta, thus locally increasing viral load and creating harmful effects for mother and fetus. Interestingly, Behbahani *et al.* [45] found that expression of *CCR5* mRNA and CCR5 protein is increased in HIV-transmitting women compared with women not transmitting HIV, and >99% of the cells in transmitting women that expressed HIV-1 *gag-pol* mRNA also expressed CCR5, versus <10% in non-transmitting women. IFN- γ , which is associated with protection against placental malaria [24,25], was shown to upregulate cell-surface expression of CCR5 on cord and adult blood mononuclear phagocytes, even though HIV-1 entry into macrophages was suppressed [46]. This suppression might have been due to the down-regulated cell-surface expression of CD4 – the major receptor for HIV-1 – and/or IFN- γ -induced secretion of MIP-1 α and MIP-1 β [46], which would, presumably, occupy CCR5 molecules and block HIV-1 entry into cells.

Malaria-infected placental intervillous blood (IVB) plasma was found to have significantly higher levels of MIP-1 β [27] and RANTES (V. Udhayakumar, unpublished) compared with placental IVB plasma from women without placental malaria, irrespective of HIV status. In addition, IVB MIP-1 α and MIP-1 β levels correlated positively with parasite density. A separate study found elevated levels of MIP-1 α , but not MIP-1 β or RANTES, in the placental plasma of malaria-infected women [47]. Collectively, these results suggest that elevated levels of β chemokines are closely associated with placental malaria infection. Theoretically, these chemokines could compete with HIV-1 for CCR5 receptors and reduce the HIV-1 transmission rate, consistent with the findings from the Kenyan MTCT study [35]. Indeed, more data are needed about the relationship among parasitemia, chemokine levels, and HIV-1 infection and replication in the placenta.

Although changes in β chemokine levels in HIV-infected women might influence HIV replication and/or MTCT of HIV-1, placental malaria might also impact HIV in other ways. Leukemia inhibitory factor (LIF) is a potent endogenous factor in the placenta that has been shown *in vitro* to inhibit HIV-1 replication in a co-receptor-independent manner [48]. In addition, the level of *LIF* mRNA and the prevalence of LIF-expressing cells were significantly greater in mothers who did not transmit

** Mwapasa, V. *et al.* abstract 21, 53rd Annual Meeting of the American Society of Tropical Medicine and Hygiene, Florida, November 2004.

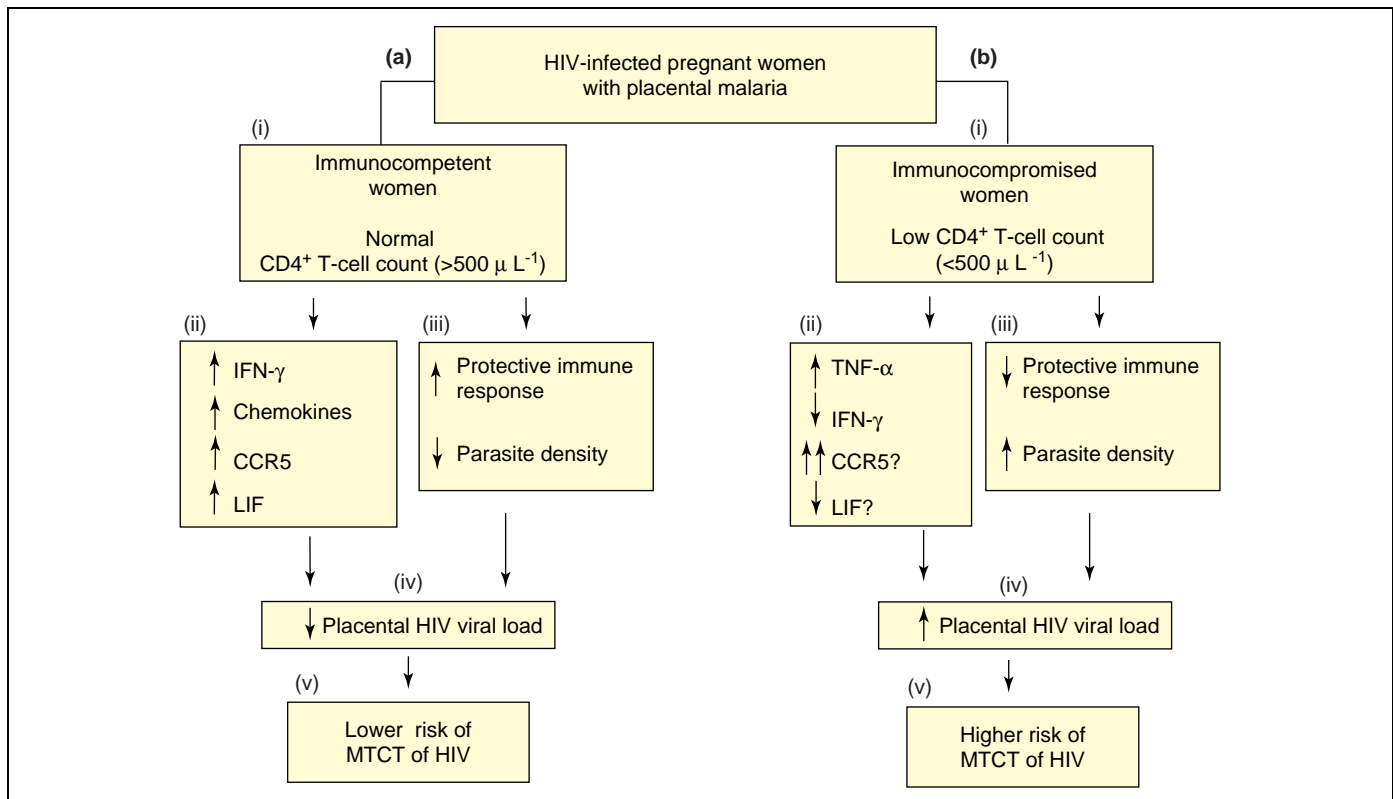


Figure 2. Hypothetical model of immune-factor modulation of MTCT of HIV-1 in HIV-placental-malaria co-infected mothers. Figure summarizes how MTCT of HIV-1 can vary in women with placental malaria infection and predicts events that probably take place in relatively immunocompetent (a) (i–v) and immunocompromised (b) (i–v) hosts that would result in different transmission outcomes. HIV-infected women who are relatively immunocompetent have normal levels of CD4⁺ T cells (>500 cells μL^{-1} of blood). These women can mount protective immune responses when exposed to placental malaria infection, and this can limit both placental malaria and HIV-1 infection. Low-density placental malaria infection can induce and sustain the production of the protective cytokine IFN- γ and chemokines such as MIP-1 α , MIP-1 β and RANTES that can block binding of HIV-1 to CCR5, a receptor for entry of HIV-1. At the same time, protective immune factors such as LIF that can reduce HIV-1 transmission might also be produced. Thus, relative levels of protective immune factors that can suppress viral replication and prevent HIV-1 transmission are at high concentrations. Consequently, MTCT of HIV-1 is much lower in these women. However, women who are relatively immunocompromised, including those with low levels of CD4⁺ T cells, have a reduced ability to mount a protective immune response to limit either placental malaria or HIV-1 infection. Consequently, these women might experience high-density placental malaria infection, which can favor overproduction of cytokines such as TNF- α . This will, in turn, further enhance HIV-1 replication. Additionally, the low level of protective immune factors (IFN- γ , chemokines and, possibly, LIF) is not adequate to control HIV-1 viral replication, leading to higher viral load in the placenta. As a result, these women have higher rates of HIV-1 transmission to their newborns.

HIV-1 to their children than in those who did. It is unclear, however, whether HIV infection itself affects LIF production or whether some placentas constitutively express lower levels of LIF and, therefore, are at greater risk of transmitting HIV-1 to the fetus. It is possible that placental malaria infection could modulate LIF production in the placenta and, therefore, could alter MTCT of HIV-1.

Model of malaria–HIV interaction with regard to MTCT of HIV-1

Although HIV-1 and placental malaria both have independent immune effects in the placental environment, co-infection has been shown to change specific aspects of the immune response and the replicative capacity of the pathogens. Considering the complex interplay between the various components of the immune system and the potential for parasite-density-dependent MTCT of HIV-1, we propose a model (Figure 2) that hypothesizes how placental malaria infection could alter HIV-1 transmission under two different immunological states. This model suggests that the immune alterations that occur during placental malaria infection ultimately influence placental HIV-1 viral load, thus modifying MTCT.

Concluding remarks

Although there is clear evidence to show that HIV-infected pregnant women are at greater risk of acquiring *P. falciparum* infection and experiencing more-severe disease complications than women not infected with HIV, the underlying immune mechanisms for these adverse outcomes are not well understood. The limited studies that have identified specific alterations in both the humoral and the cellular arms of the immune system provide some explanation of how HIV-1 infection might impair protective immune responses to malaria. In addition, the presence of malaria in the placenta could affect MTCT of HIV-1, although the few studies measuring this phenomenon have drawn contradicting conclusions. Many studies investigating the immunological and epidemiological interactions between placental malaria and HIV-1 have been limited by a variety of factors, including the use of different malaria diagnostic techniques, small sample sizes and inadequately adjusting for the stage of HIV disease, the density of parasitemia and the duration of malaria exposure in the data analysis. Further studies are necessary to understand the modulation of immune responses in co-infected women, how co-infection influences the pathogenesis of malaria and HIV-1, and what effect co-infection has on MTCT of HIV-1 (Box 2).

Box 2. Future research goals

- Establish additional studies to examine the effect of placental malaria on MTCT of HIV-1 that are adequately powered to detect significant differences in transmission rates, with measurement of the effects of CD4⁺ T-cell count, peripheral and placental HIV-1 viral load, level of placental parasitemia and histopathological changes in the placenta.
- Extend studies that examine peripheral changes in humoral and cell-mediated immunity to include local changes within the placenta. This should involve cytokine and chemokine expression patterns, in addition to changes in lymphocyte, NK cell and monocyte-macrophage populations, including memory cells. The role of the fetal trophoblast and other cell types should also be explored.
- Determine whether CD4⁺CD25⁺ regulatory T cells – which have been shown to regulate the outcome of several infectious diseases, including malaria [76,77] – are involved in HIV-mediated immunosuppression in pregnant women.
- Characterize the roles of other mediators such as hormones, prostaglandin E₂ and hemozoin (malarial pigment) in influencing the immune response to malaria.
- Consider cytokine ratios, in addition to absolute levels of cytokines, for determining effects on parasitemia and adverse birth outcomes.
- Assess the inherent variability in LIF production in normal placentas and further investigate whether HIV-1 infection and/or placental malaria infection modulates LIF levels.
- Establish analogous studies of placental malaria and placental-malaria-HIV co-infection to investigate populations in which *Plasmodium vivax* is also prevalent.

Acknowledgements

We thank Richard Steketee for his critical comments about the manuscript. R.M.N. was supported by an appointment to the Emerging Infectious Diseases Postdoctoral Fellowship Program administered by the Association of Public Health Laboratories and was funded by the Centers for Disease Control and Prevention. S.C. received financial support from the Medical Scholar Program of Mahidol University. J.M.M. was supported by the NIH (grant AI-50240).

References

- 1 Steketee, R.W. *et al.* (2001) The burden of malaria in pregnancy in malaria-endemic areas. *Am. J. Trop. Med. Hyg.* 64, 28–35
- 2 Okoko, B.J. *et al.* (2003) The epidemiology and consequences of maternal malaria: a review of immunological basis. *Acta Trop.* 87, 193–205
- 3 Brocklehurst, P. and French, R. (1998) The association between maternal HIV infection and perinatal outcome: a systematic review of the literature and meta-analysis. *Br. J. Obstet. Gynaecol.* 105, 836–848
- 4 Cock, K.M. and Weiss, H.A. (2000) The global epidemiology of HIV/AIDS. *Trop. Med. Int. Health* 5, A3–A9
- 5 UNAIDS/WHO (2003) AIDS epidemic update: December 2003. (<http://www.who.int/hiv/pub/epidemiology/epi2003/en/>)
- 6 Chandramohan, D. and Greenwood, B.M. (1998) Is there an interaction between human immunodeficiency virus and *Plasmodium falciparum*? *Int. J. Epidemiol.* 27, 296–301
- 7 ter Kuile, F.O. *et al.* (2004) 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.* 71, 41–54
- 8 Bongertz, V. (2001) Vertical human immunodeficiency virus type 1 – HIV-1 – transmission – a review. *Mem. Inst. Oswaldo Cruz* 96, 1–14
- 9 Whitworth, J. *et al.* (2000) Effect of HIV-1 and increasing immunosuppression on malaria parasitaemia and clinical episodes in adults in rural Uganda: a cohort study. *Lancet* 356, 1051–1056
- 10 Francesconi, P. *et al.* (2001) HIV, malaria parasites, and acute febrile episodes in Ugandan adults: a case-control study. *AIDS* 15, 2445–2450
- 11 French, N. *et al.* (2001) Increasing rates of malarial fever with deteriorating immune status in HIV-1-infected Ugandan adults. *AIDS* 15, 899–906
- 12 Steketee, R.W. *et al.* (1996) Impairment of a pregnant woman's acquired ability to limit *Plasmodium falciparum* by infection with human immunodeficiency virus type-1. *Am. J. Trop. Med. Hyg.* 55, 42–49
- 13 Verhoeff, F.H. *et al.* (1999) Increased prevalence of malaria in HIV-infected pregnant women and its implications for malaria control. *Trop. Med. Int. Health* 4, 5–12
- 14 van Eijk, A.M. *et al.* (2003) HIV increases the risk of malaria in women of all gravidities in Kisumu, Kenya. *AIDS* 17, 595–603
- 15 van Eijk, A.M. *et al.* (2001) Human immunodeficiency virus seropositivity and malaria as risk factors for third-trimester anemia in asymptomatic pregnant women in Western Kenya. *Am. J. Trop. Med. Hyg.* 65, 623–630
- 16 Ayisi, J.G. *et al.* (2003) The effect of dual infection with HIV and malaria on pregnancy outcome in Western Kenya. *AIDS* 17, 585–594
- 17 Parise, M.E. *et al.* (1998) Efficacy of sulfadoxine-pyrimethamine for prevention of placental malaria in an area of Kenya with a high prevalence of malaria and human immunodeficiency virus infection. *Am. J. Trop. Med. Hyg.* 59, 813–822
- 18 Bloland, P.B. *et al.* (1995) Maternal HIV infection and infant mortality in Malawi: evidence for increased mortality due to placental malaria infection. *AIDS* 9, 721–726
- 19 Verhoeff, F.H. *et al.* (2004) Post-neonatal infant mortality in Malawi: the importance of maternal health. *Ann. Trop. Paediatr.* 24, 161–169
- 20 Berzins, K. and Perlmann, P. (1996) Malaria vaccines: attacking infected erythrocytes. In *Malaria Vaccine Development: A Multi-Immune Response Approach* (Hoffman, S.L., ed.), pp. 105–143, ASM Press
- 21 Ayisi, J.G. *et al.* (2003) Does infection with human immunodeficiency virus affect the antibody responses to *Plasmodium falciparum* antigenic determinants in asymptomatic pregnant women? *J. Infect.* 46, 164–172
- 22 Fried, M. and Duffy, P.E. (1996) Adherence of *Plasmodium falciparum* to chondroitin sulfate A in the human placenta. *Science* 272, 1502–1504
- 23 Mount, A.M. *et al.* (2004) Impairment of humoral immunity to *Plasmodium falciparum* malaria in pregnancy by HIV infection. *Lancet* 363, 1860–1867
- 24 Moore, J.M. *et al.* (1999) Immunity to placental malaria. I. Elevated production of interferon- γ by placental blood mononuclear cells is associated with protection in an area with high transmission of malaria. *J. Infect. Dis.* 179, 1218–1225
- 25 Moore, J.M. *et al.* (2000) Immunity to placental malaria. II. Placental antigen-specific cytokine responses are impaired in human immunodeficiency virus-infected women. *J. Infect. Dis.* 182, 960–964
- 26 Chaisavaneeyakorn, S. *et al.* (2002) 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.* 185, 127–131
- 27 Chaisavaneeyakorn, S. *et al.* (2003) Levels of macrophage inflammatory protein 1 α (MIP-1 α) and MIP-1 β in intervillous blood plasma samples from women with placental malaria and human immunodeficiency virus infection. *Clin. Diagn. Lab. Immunol.* 10, 631–636
- 28 Chaisavaneeyakorn, S. *et al.* (2002) Immunity to placental malaria. IV. Placental malaria is associated with up-regulation of macrophage migration inhibitory factor in intervillous blood. *J. Infect. Dis.* 186, 1371–1375
- 29 Douek, D.C. *et al.* (2003) T cell dynamics in HIV-1 infection. *Annu. Rev. Immunol.* 21, 265–304
- 30 Xiao, L. *et al.* (1998) *Plasmodium falciparum* antigen-induced human immunodeficiency virus type 1 replication is mediated through induction of tumor necrosis factor- α . *J. Infect. Dis.* 177, 437–445
- 31 Froebel, K. *et al.* (2004) Activation by malaria antigens renders mononuclear cells susceptible to HIV infection and re-activates replication of endogenous HIV in cells from HIV-infected adults. *Parasite Immunol.* 26, 213–217
- 32 Hoffman, I.F. *et al.* (1999) The effect of *Plasmodium falciparum* malaria on HIV-1 RNA blood plasma concentration. *AIDS* 13, 487–494
- 33 Kublin, J.G. *et al.* (2005) Effect of *Plasmodium falciparum* malaria on concentration of HIV-1-RNA in the blood of adults in rural Malawi: a prospective cohort study. *Lancet* 365, 233–240

- 34 Kapiga, S.H. *et al.* (2002) Correlates of plasma HIV-1 RNA viral load among HIV-1-seropositive women in Dar es Salaam, Tanzania. *J. Acquir. Immune Defic. Syndr.* 30, 316–323
- 35 Ayisi, J.G. *et al.* (2004) Maternal malaria and perinatal HIV transmission, Western Kenya. *Emerg. Infect. Dis.* 10, 643–652
- 36 Inion, I. *et al.* (2003) Placental malaria and perinatal transmission of human immunodeficiency virus type 1. *J. Infect. Dis.* 188, 1675–1678
- 37 Mwapasa, V. *et al.* (2004) The effect of *Plasmodium falciparum* malaria on peripheral and placental HIV-1 RNA concentrations in pregnant Malawian women. *AIDS* 18, 1051–1059
- 38 Brahmabhatt, H. *et al.* (2003) The effects of placental malaria on mother-to-child HIV transmission in Rakai, Uganda. *AIDS* 17, 2539–2541
- 39 Ayoub, A. *et al.* (2003) Mother-to-child transmission of human immunodeficiency virus type 1 in relation to the season in Yaounde, Cameroon. *Am. J. Trop. Med. Hyg.* 69, 447–449
- 40 O'Donovan, D. *et al.* (2000) Maternal plasma viral RNA levels determine marked differences in mother-to-child transmission rates of HIV-1 and HIV-2 in The Gambia. MRC/Gambia Government/University College London Medical School working group on mother-child transmission of HIV. *AIDS* 14, 441–448
- 41 Dragic, T. *et al.* (1996) HIV-1 entry into CD4⁺ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* 381, 667–673
- 42 Cocchi, F. *et al.* (1995) Identification of RANTES, MIP-1 α , and MIP-1 β as the major HIV-suppressive factors produced by CD8⁺ T cells. *Science* 270, 1811–1815
- 43 Alkhatib, G. *et al.* (1996) CC CKR5: a RANTES, MIP-1 α , MIP-1 β receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* 272, 1955–1958
- 44 Tkachuk, A.N. *et al.* (2001) Malaria enhances expression of CC chemokine receptor 5 on placental macrophages. *J. Infect. Dis.* 183, 967–972
- 45 Behbahani, H. *et al.* (2000) Up-regulation of CCR5 expression in the placenta is associated with human immunodeficiency virus-1 vertical transmission. *Am. J. Pathol.* 157, 1811–1818
- 46 Hariharan, D. *et al.* (1999) Interferon- γ upregulates CCR5 expression in cord and adult blood mononuclear phagocytes. *Blood* 93, 1137–1144
- 47 Abrams, E.T. *et al.* (2003) Host response to malaria during pregnancy: placental monocyte recruitment is associated with elevated β chemokine expression. *J. Immunol.* 170, 2759–2764
- 48 Patterson, B.K. *et al.* (2001) Leukemia inhibitory factor inhibits HIV-1 replication and is upregulated in placenta from nontransmitting women. *J. Clin. Invest.* 107, 287–294
- 49 Mvondo, J.L. *et al.* (1992) Malaria and pregnancy in Cameroonian women. Naturally acquired antibody responses to asexual blood-stage antigens and the circumsporozoite protein of *Plasmodium falciparum*. *Trans. R. Soc. Trop. Med. Hyg.* 86, 486–490
- 50 Branch, O.H. *et al.* (1998) A longitudinal investigation of IgG and IgM antibody responses to the merozoite surface protein-1 19-kiloDalton domain of *Plasmodium falciparum* in pregnant women and infants: associations with febrile illness, parasitemia, and anemia. *Am. J. Trop. Med. Hyg.* 58, 211–219
- 51 Taylor, D.W. *et al.* (2004) 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.* 72, 1603–1607
- 52 Fried, M. *et al.* (1998) Maternal antibodies block malaria. *Nature* 395, 851–852
- 53 Ricke, C.H. *et al.* (2000) 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.* 165, 3309–3316
- 54 O'Neil-Dunne, I. *et al.* (2001) Gravity-dependent production of antibodies that inhibit binding of *Plasmodium falciparum*-infected erythrocytes to placental chondroitin sulfate proteoglycan during pregnancy. *Infect. Immun.* 69, 7487–7492
- 55 Staalsoe, T. *et al.* (2004) Variant surface antigen-specific IgG and protection against clinical consequences of pregnancy-associated *Plasmodium falciparum* malaria. *Lancet* 363, 283–289
- 56 Beeson, J.G. *et al.* (2000) Adhesion of *Plasmodium falciparum*-infected erythrocytes to hyaluronic acid in placental malaria. *Nat. Med.* 6, 86–90
- 57 Flick, K. *et al.* (2001) Role of nonimmune IgG bound to PfEMP1 in placental malaria. *Science* 293, 2098–2100
- 58 McGilvray, I.D. *et al.* (2000) Nonopsonic monocyte/macrophage phagocytosis of *Plasmodium falciparum*-parasitized erythrocytes: a role for CD36 in malarial clearance. *Blood* 96, 3231–3240
- 59 Rasheed, F.N. *et al.* (1993) Suppressed peripheral and placental blood lymphoproliferative responses in first pregnancies: relevance to malaria. *Am. J. Trop. Med. Hyg.* 48, 154–160
- 60 Fievet, N. *et al.* (1995) Malaria and pregnancy in Cameroonian primigravidae: humoral and cellular immune responses to *Plasmodium falciparum* blood-stage antigens. *Am. J. Trop. Med. Hyg.* 53, 612–617
- 61 Riley, E.M. *et al.* (1989) Suppression of cell-mediated immune responses to malaria antigens in pregnant Gambian women. *Am. J. Trop. Med. Hyg.* 40, 141–144
- 62 Fievet, N. *et al.* (2002) Cellular immune response to *Plasmodium falciparum* after pregnancy is related to previous placental infection and parity. *Malar. J.* 1, 16
- 63 Suguitan, A.L., Jr. *et al.* (2003) Changes in the levels of chemokines and cytokines in the placentas of women with *Plasmodium falciparum* malaria. *J. Infect. Dis.* 188, 1074–1082
- 64 Fievet, N. *et al.* (2001) *Plasmodium falciparum* induces a Th1/Th2 disequilibrium, favoring the Th1-type pathway, in the human placenta. *J. Infect. Dis.* 183, 1530–1534
- 65 Fried, M. *et al.* (1998) Malaria elicits type 1 cytokines in the human placenta: IFN- γ and TNF- α associated with pregnancy outcomes. *J. Immunol.* 160, 2523–2530
- 66 Moormann, A.M. *et al.* (1999) Malaria and pregnancy: placental cytokine expression and its relationship to intrauterine growth retardation. *J. Infect. Dis.* 180, 1987–1993
- 67 Rogerson, S.J. *et al.* (2003) Placental tumor necrosis factor α but not γ interferon is associated with placental malaria and low birth weight in Malawian women. *Infect. Immun.* 71, 267–270
- 68 Sholapurkar, S.L. *et al.* (1990) Cellular immunity in pregnant and non-pregnant women with malarial infection. *Asia Oceania J. Obstet. Gynaecol.* 16, 27–32
- 69 Bouyou-Akotet, M.K. *et al.* (2004) Depressed natural killer cell cytotoxicity against *Plasmodium falciparum*-infected erythrocytes during first pregnancies. *Clin. Infect. Dis.* 38, 342–347
- 70 Walter, P.R. *et al.* (1982) Placental pathologic changes in malaria. A histologic and ultrastructural study. *Am. J. Pathol.* 109, 330–342
- 71 Ismail, M.R. *et al.* (2000) Placental pathology in malaria: a histological, immunohistochemical, and quantitative study. *Hum. Pathol.* 31, 85–93
- 72 Rogerson, S.J. *et al.* (2003) Placental monocyte infiltrates in response to *Plasmodium falciparum* malaria infection and their association with adverse pregnancy outcomes. *Am. J. Trop. Med. Hyg.* 68, 115–119
- 73 Ordi, J. *et al.* (1998) Massive chronic intervillitis of the placenta associated with malaria infection. *Am. J. Surg. Pathol.* 22, 1006–1011
- 74 Vleugels, M.P. *et al.* (1987) Cortisol and loss of malaria immunity in human pregnancy. *Br. J. Obstet. Gynaecol.* 94, 758–764
- 75 Vleugels, M.P. *et al.* (1989) Cortisol and *Plasmodium falciparum* infection in pregnant women in Kenya. *Trans. R. Soc. Trop. Med. Hyg.* 83, 173–177
- 76 Mittrucker, H.W. and Kaufmann, S.H. (2004) Mini-review: regulatory T cells and infection: suppression revisited. *Eur. J. Immunol.* 34, 306–312
- 77 Hisaeda, H. *et al.* (2004) Escape of malaria parasites from host immunity requires CD4⁺ CD25⁺ regulatory T cells. *Nat. Med.* 10, 29–30