Review Article

Immune responses during gestational malaria: a review of the current knowledge and future trend of research

Amanda Maestre, Jaime Carmona-Fonseca

Grupo Salud y Comunidad, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia

Abstract

Women pregnant with their first child are susceptible to severe *P. falciparum* disease from placental malaria because they lack immunity to placenta-specific cytoadherence proteins. In subsequent pregnancies, as immunity against placental parasites is acquired, there is a reduced risk of adverse effects of malaria on the mother and fetus and asymptomatic parasitaemia is common. In the case of *vivax* malaria, with increasing reports of severe cases in Asia and South America, the effects of infection by this species during pregnancy remain to be elucidated. This review summarized the main aspects involved in the acquisition of specific antimalarial immune responses during pregnancy with emphasis in research carried out in America and Asia, in order to offer a framework of interpretation for studies on pregnant women with malaria which are recently being produced in these regions. The authors conclude that (1) Effective humoral responses during gestational malaria are mainly directed against variant surface antigens codified by genes of the *var2Csa* family of *P. falciparum*; (2) Acquisition of immunity against these variant antigens depends on the degree and intensity of transmission, and the chance increases with age and successive pregnancies; (3) Antibody development is guided by specific cellular immune responses in cases of placental and maternal infection, and (4) The study of the significance of acquisition of specific immunity against both *P. falciparum* and *P. vivax* in America, should be performed.

Key words: *P. vivax*; pregnancy; Colombia

J Infect Dev Ctries 2014; 8(4):391-402. doi:10.3855/jidc.3777

(Received 09 May 2013 – Accepted 12 August 2013)

Introduction

In countries where malaria is highly endemic, exposed subjects acquire partially protective immunity to infection as they reach adulthood. By contrast, low degree exposition to infection results in delayed immunity to *Plasmodium spp.* and, frequently, in symptomatic disease [1]. In all endemic settings, naturally acquired immunity is the result of the interplay of several host's and parasite related factors [2], and among them the issue of the species and strain dependant immunity, appears very central [3, 4].

There is consensus on the important role of naturally acquired immunity to blood stage malaria in the prevention of severe disease and death due to *P. falciparum*. However, the precise mechanisms and key determinants of such protection remain elusive [5]. The slow acquisition of a protective status is thought to represent the accumulation of protective immune responses to a repertoire of diverse antigens, probably including both PfEMP1s (*Plasmodium falciparum* erythrocyte membrane protein 1) and surface proteins. Since protective immunity against malaria is related to transmission intensity, in moderate-high malaria transmission regions, the highest risk of death from malaria is observed in infants and young children, whilst semi-immune adults remain susceptible to asymptomatic parasitaemia, but protected against clinical disease. This protective immunity is lost after a few years in the absence of exposure. Acquired immunity against malaria is also diminished in women during the first pregnancy; in fact they are susceptible to severe *P. falciparum* disease due to placental malaria as they lack immunity to placenta-specific cytoadherence proteins. In subsequent pregnancies, as immunity against placental-adherent strains develops, there is a reduced risk of adverse effects of malaria on the mother and fetus [6] and asymptomatic parasitaemia is common [7]. In the case of *vivax* malaria, with increasing reports of severe cases in Asia and South America [8,9], the consequences of infection during pregnancy remain to be elucidated, although adherence to endothelial cells [10] and sequestration of infected red blood cells in deep vasculature have been recently demonstrated [11,12].

During malaria infection in non-pregnant subjects, both humoral and cellular factors contribute to the

acquisition of immune protection. There is a general agreement that cellular immune responses are thought to be more important in controlling the pre-erythrocytic stages of malaria infection, and antibodies are thought to suppress blood-stage infection. However, the mechanism of protective immunity against malaria is poorly understood, and no particular specific immune response has been established as an essential or exclusive correlate of clinical protection. Early studies confirmed the importance of cell-mediated immune pathways in the adaptive response against malaria [13, 14], and evidenced the central role of CD4+ T cells in parasitemia clearance and host survival [15, 16]. Following activation, CD4+ T cells proliferate rapidly and acquire critical effector functions; before undergoing a dramatic contraction phase after the peak of infection [17] to become established memory T cells. Responding terminal effector T cells do not survive to the contraction period and thus do not confer protection to re-infection [18]. In murine models of malaria and chronically exposed *P. falciparum* populations, impaired memory responses are evident due to abnormal CD4 T cell mediated co-stimulation to B cells [19, 20]. *Plasmodium*-specific CD4+ T cells not only are important as a source of IFN-γ but are also essential for activation of both CD8+ T cells and B cells. It is widely accepted that B cells and CD4+ T cells are mainly activated during the blood stage infection and CD8+ T cells are activated during the pre-erythrocytic or liver stage of the *Plasmodium* life cycle [21, 22].

In addition to cell mediated immune responses, specific antibodies resulting from natural exposition, are important to confer protection against blood forms of the parasite. Anti-*Plasmodium* antibodies can prevent merozoites of infecting new red blood cells (RBCs), block cytoadherence to endotheliar capillary of infected RBCs (iRBCs), and promote phagocytosis by mononuclear cells [16,23,24]. However, persistence of significant levels of antimalarial antibodies relies on the continuous challenge resulting of chronic exposition to infection [3], probably as consequence of the impaired establishment of B cell memory [19]. This might explain the finding of short-lived antibody responses [25,26], mainly in young children [27,28]. In any case, *Plasmodium* antigens seem to elicit stable antibody responses in humans [27,29], regardless of the endemic species and even at low parasite densities [30].

While the studies on the immune response against *P. falciparum* have been controversial, the case of *P. vivax* infection has proved more challenging, mainly as a consequence of the absence of an *in vitro* long-term model of infection, and the common co-existence with *P. falciparum* in highly endemic areas [31]. Therefore, *in vivo* exploration of naturally acquired immune responses has resulted in biased and difficult to interpret results. The effect, altogether, is a significant gap in the knowledge of the immunity against *P. vivax* in non-pregnant and pregnant subjects in comparison with *P. falciparum*, with practically absolute absence of recent studies in America. Taking into account that globally at least 50 million women are exposed to malaria infection during pregnancy [32], it is easy to predict that the problem is serious. Most cases of gestational malaria occur in African countries, where in fourty-three countries malaria transmission is reported. However, significant numbers are reported in Asia and America where approximately 93 million pregnancies occur, of which 40 million take place in temperate regions with only *P. vivax* transmission and no *P. falciparum* transmission [33].

The aim of this review is to present and discuss the main aspects involved in the acquisition of specific antimalarial immune responses during pregnancy with emphasis on research carried out in America and Asia, in order to offer a framework of interpretation for studies on pregnant malaria that are recently being studied carried out in these regions.

Dynamics of gestational and placental malaria

The definition of gestational and placental malaria varies according to authors and techniques applied to establish diagnosis. According to the World health Organization (WHO), gestational malaria is defined as the presence of parasitaemia in placenta or peripheral blood [34], and thick smear examination is the recommended test for diagnosis in endemic sites [35]. In the practice the term pregnancy-associated malaria (PAM) is used to define either infection or disease by *Plasmodium spp.* during pregnancy, with three components which may concur: gestational malaria, placental malaria and congenital malaria. Within this context, gestational malaria is defined as presence of a positive result on a *Plasmodium spp.* specific test and malarial disease, placental malaria is the finding of a positive *Plasmodium spp.* specific test (including histopathology) in placenta, and congenital malaria is a positive *Plasmodium spp.* specific test in cord blood or neonate's peripheral blood.

In cases where diagnosis is made by thick smear test, low peripheral parasitaemias often go undetected. Moreover, placentas are rarely examined for presence

of parasites. Hence, all conditions (gestational, placental and neonatal malaria) are often undiagnosed due to these different causes.

Besides the limitation of microscopy to establish the diagnosis of gestational malaria, added complexity emerges when considering the different sites of infection and the weeks of pregnancy when the mother is exposed to infection. In African mothers (primiparous and multiparous) resident in areas of high endemicity, parasitaemia by *P. falciparum* appears to reach a peak between weeks 13-16 of gestation [36, 37]. Data on parasitaemia at earlier stages are biased by the method applied to determine the gestational age. In addition, many pregnancies are lost during the first trimester and, therefore, significant numbers of research subjects are lost at this stage. During week 16 of pregnancy, placental development is completed and a simultaneous decrease on peripheral parasitaemia has been reported [37]. This phenomenon has been documented in all pregnant women from endemic regions, regardless of the number of previous pregnancies [37]. For *P. falciparum*, such coincidence among the reduction of peripheral parasitaemia and the completion of placental development led to hypothesize that placental cytoadherence of parasites is the explanation for this observation [38, 39]. After delivery, a rapid clearance of parasitaemia is observed [40], although in some cases it might persist [36]. As already mentioned, placental parasitaemia is rarely explored, particularly at early stages, and in regions where *P. falciparum* is predominant, term pregnancies exhibit higher parasite densities than peripheral blood [37,41]. A similar finding has recently been reported in areas where *P. vivax* is predominant [42].

Sequestration of *P. falciparum* in the immune-privileged placenta favors parasites survival as a result of evasion to any previously acquired immune response. In addition, since the mechanism seems to involve cyto-adhesion to the highly expressed molecule chondroitin sulfate A (CSA), up-regulation of particular ligands on the parasite is required, and only after several expositions along successive pregnancies, specific immunity against these variant antigens can be observed.

Regarding *P. vivax* infections during pregnancy, mortality is rarely seen, but there is association with multiple relapses, anemia, abortion, and a reduction in birth weight in pregnant women with malaria [11, 43-45]. Pregnant women are also more likely to experience relapses than non-pregnant ones [44]. *P. vivax* sequestration has been proposed [10], and recent reports indicate disproportional organ-specific and peripheral blood parasitaemia [42]. As for the dynamics of infection throughout pregnancy, no studies have addressed the issue. Recent reports supported the concept that detection and treatment of pregnant malaria during early gestation prevents miscarriage both in *P. vivax* and *P. falciparum* infections [31,43]. At delivery, placental infection by *P. vivax* has been confirmed both in cases from America and Asia [35, 45-47].

Epidemiology of gestational malaria in non-African regions

In most African countries, *P. falciparum* is very predominant, almost exclusive; as a consequence pregnant women frequently suffer malaria due to this species. This is not the case outside Africa, particularly in most Asian and American endemic regions, where in general *P. vivax* is predominant (60%-70%) and women are exposed to both *P. vivax* and *P. falciparum*.

Asian-Pacific Region

A recent review of malaria in the Asian-Pacific region reported a median proportion of pregnant women infected with malaria in antenatal clinics of 15.3% (range 1.2–40.8), and in delivery rooms of 8.1% (range 1.6 – 18.5), according to thick smear evaluation test [45]. In addition, the median proportion of reported placental parasitaemia was 11.0% (range 1.4 –29.1). The same study reported that *P. vivax* caused a median of 21,1% (range 5 – 29) of malaria infections detected at antenatal clinics.

In the South east Asian region, a protective effect of *P. vivax* infection against subsequent episodes and severity of *P. falciparum* malaria was noted in Thailand [44,48]. However, it has been reported that an acute episode of *P. falciparum* could trigger a relapse of a previously acquired *P. vivax* infection [49]. Therefore, as the incidence of *P. falciparum* increases, the number of *P. vivax* episodes caused by relapses will eventually also increase. However, to establish the true burden of malaria in pregnancy, longitudinal follow-up of pregnant women is required. A recent study followed a small cohort of Thai pregnant women after week 12 of pregnancy in a region of low and seasonal malaria [50]. They confirmed occurrence of between one and five episodes of *vivax* malaria up until delivery with a median inter-recurrence interval of 45 days [50] . Altogether, these and other studies confirm that *P. vivax* malaria during pregnancy is a problem in many

endemic countries outside Africa and the consequences and/ or sequelae in the mother and the fetus remain unexplored [51-54].

America

In 2011, in the Americas, malaria transmission occurred in twenty-one countries with about 30% of the total population at some degree of risk. Of these countries, thirteen had achieved a reduction in malaria incidence rates of ≥ 75% between 2000 and 2011; and six (Mexico, El Salvador, Paraguay, Argentina, Costa Rica and Ecuador) are in a pre-elimination phase [55].

The burden of gestational malaria in the Americas region is less clear than the overview of the Asian-Pacific region. In Peru, the incidence of malaria in pregnant women due to any species was 43.1% [56]. Meanwhile, in Venezuela, the reported incidence was 27.4% [57]. In Brazil, the country reporting most malaria cases in the region, no recent studies addressed the problem. Altogether, this stresses the need for adequate follow up studies in order to identify both incidence and prevalence patterns of gestational malaria in the Americas.

In Colombia, molecular studies showed rates of maternal/gestational malaria of 32%, versus 13% by using a microscopy test [35]. In the same region, simultaneous application of blood microscopy and Now ICT malaria Pf/Pv test was conducted in 143 samples of maternal peripheral blood, 133 samples of placental blood and 110 cord blood samples, for a total of 386 samples examined. The authors reported prevalence of infection of 14 % in mothers, 10% in placenta and 1% in cord blood. Sensitivities of the rapid test for *P. vivax* were 75,0 (CI 95%; 50.6 - 90.4) in the mother, 57,14 (CI 95%; 29.6 – 81.2) in placenta and 50,00 (CI 95%; 2.7 – 97.3) in cord blood [58]. Finally, recent reports using a quantitative real time PCR technique to establish the diagnosis of infection, confirmed rates of infection of up to 45% by either *P. falciparum* or *P. vivax* [42].

Regulation of the immune system during pregnancy

During pregnancy the maternal immune system is modified in order to achieve tolerance toward paternal antigens expressed on fetal cells [59]. In this process, cytokines act in a coordinated fashion either at the maternal-foetal interface or systemically [60,61]. Evidence of such process is observed in the mother as cytokine and soluble cytokine receptor serum levels change throughout the different trimesters of pregnancy. In the systemic circulation of pregnant healthy women, IL-10, IL-4, IL-6, and IL-13 production progressively increases, while serum levels of most Th1-type cytokines (IL-1α, IL-1β, IL-2, IL-12, IFN-γ) significantly decrease in the third trimester compared with those observed in the first trimester of pregnancy [62-64]. TNF-α serum levels do not seem to vary during pregnancy, while those of sTNFR (Soluble tumor necrosis factor receptor) increase, probably in order to protect the foetus from the consequences of increased TNFα: risk of preeclampsia, intrauterine growth retardation, and pathologic labour [65, 66].

Several studies have observed that the increase in cortisol, progesterone, oestradiol and testosterone concentrations during pregnancy is associated with an increased production of Th2 cytokines and a reduced expression of Th1 cytokines, resulting in a Th2 polarisation of immune response [67-70]. Th1 cells produce IL-2, INF-γ, TNF-α, and IL-12 and are thought to drive tissue damage in some inflammatory diseases including malaria [71]. Th2 cells secrete IL-4, IL-5, IL-13 which mediate B cell activation and antibody production and it is well known that these two pathways reciprocally inhibit each other [72-74]. Therefore, the consequences of Th2 polarisation during normal pregnancy include inhibition of cell mediated Th1 cytokines immunity and enhancement of Th2-mediated humoral responses [75]. However, a deregulation of cytokine networks can lead to adverse pregnancy outcomes including spontaneous abortion, preterm labour, pre-eclampsia, and intrauterine growth restriction [76,77]. Such is the case of gestational malaria, in which a pro-inflammatory status has been confirmed and contributes to explain the deleterious effect of *P. falciparum* infection in mother and fetus [78].

Specific antimalarial immunity during pregnancy

Plasmodium falciparum is the main species responsible of infection due to the high transmission rates observed in Africa, but also due to the particular properties of cytoadherence exhibited by the parasite, which results in a sequestration phenomena. Among the several parasite proteins and host's ligands implicated in sequestration, variant surface antigens of *P. falciparum* parasitized red blood cells allow adherence to the vascular endothelium via the ubiquitous endothelial surface proteins intercellular adhesion molecule-1 (ICAM-1) and CD36, as well as chondroitin sulphate A (CSA) and hialuronic acid largely expressed in the placental endothelium [79].

It has become clear that the process of acquisition of immunity against malaria during pregnancy only starts when the women resident in an endemic area becomes pregnant, since previously acquired immunity fails to protect against maternal and placental infection [38, 80]. Therefore, the repeated exposition to particular parasites phenotypes throughout several pregnancies, might result in some degree of protection. In this process, the human placenta provides the parasite a particular immune environment that induces antigen switching in *var2CSA* genes in the case of *P. falciparum* and contributes to the clonal selection observed in placental parasites [39,81]. The variant nature of the antigens codified by these gene families renders pregnant women highly susceptible to develop disease and complications, regardless of the extent of previous exposure to the parasite [81-83]. Levels of antibodies to surface antigens of placental-binding infected erythrocytes are low before pregnancy and increase with successive pregnancies in women exposed to *P. falciparum* [84]. Despite increased susceptibility during pregnancy to *P. vivax* infection remains to be clarified, experts coincide in the observation of complications due to severe anemia and induction of pre-term delivery [45,50,85].

Immediately after fecundation, pro-inflammatory cytokines contribute with implantation and remodeling of spiral arteries to guarantee adequate blood supply to the embryo [86]. Afterwards, once implantation has taken place, a healthy pregnancy is characterized by a cytokine balance biased towards a predominant T helper (Th) 2 subset of CD4+ T lymphocytes [87]. The objective of this involves maintenance of a suitable environment in which the fetus is tolerated. Such Th2 predominant response is the result of high levels of progesterone and oestrogen produced throughout all pregnancy [88]. The cytokine balance is directed toward a Th2 predominant response mainly after the second trimester of gestation [67] with strong Th1 immune responses during pregnancy resulting in maternal anaemia, spontaneous abortions and pre-term delivery [89].

Infection of placenta by malaria parasites stimulate production of TNF-α [90-93] and IFN-γ [92, 94], IL-1β [91] and IL-2 [92]. These cytokines induce T cell proliferation and enhance phagocytic activity of macrophages by production of reactive oxygen intermediates and nitric oxide [95]. Therefore, in the infected placenta strong pro-inflammatory responses are observed and aimed at controlling parasite's proliferation. Albeit the immense importance of cell derived factors in the immune response against *Plasmodium spp.*, their overproduction might pose a risk for the success of the pregnancy. Since both placental and fetal cells exposed to antigens can be the source of pro-inflammatory cytokines, they may jeopardize full pregnancy completion [96, 97].

The main source of IFN-γ in cases of gestational malaria are CD8+ T cells, CD4+ T cells, NK cells and the foetal trophoblast [90]. In general, increased levels of IFN-γ are associated with protection against gestational malaria [94]. Preliminary studies in northwest Colombia, both in cases of *P. vivax* and *P. falciparum* infection, confirmed that infected placentas had high levels of IFN- γ and TNF-α [98]; whereas high levels of IFN-γ, and also TNF-α and IL1-β, correlated with placental damage in cases of placental malaria [99].

TNF-α has been reported to exert important dual actions during malaria infection. On one hand is responsible for most clinical symptoms and complications of malaria in non- pregnant [100,101] and pregnant patients [102]. On the other hand, it has immune effects since promotes monocyte recruitment [103]. However, the latter phenomena might be particularly deleterious for placenta, by contributing to a switch towards a pro-inflammatory status. Changes in levels of these cytokines in association with parity had proved variable [93, 94].

In regions where different species of malaria parasites are endemic, it has been observed that immune response to *P. falciparum* interferes with the immune factors observed in *P. vivax* malaria [104] as confirmed by the strain-specific and serological cross-reactive immunity between blood stage antigens of both species [5,105,106]. Nevertheless, cell-mediated response seems unaffected as evidenced in studies during the convalescent period in *P. vivax*-infected hosts, thus confirming a T cell-specific response, which was also reactive to *P. falciparum* antigens [104,107].

Studies on the Th1/Th2 balance in the placenta in populations with different degrees of endemicity and according to the infecting species are of the utmost importance since these influence a number of other immune responses, including isotype switching to cytophilic IgG antibodies.

Specific antibody responses

Natural immunity against *P. falciparum* malaria appears to depend on the gradual acquisition of a broad repertoire of IgGs against the surface of erythrocytes infected by mature forms of the parasite

[108]. This immunity is acquired as a result of antigenic stimulation through repeated parasite infections from early childhood onwards [109] but exposition to infection during fetal stages lead to impairment of memory responses and increased rates of infection later in life [110]. Susceptibility to malaria during pregnancy has been attributed to lack of antibodies able to block the binding of *P. falciparum* to CSA in the placenta [111] and to facilitate opsonic uptake by phagocytes [112-114]. The CSA adhesion phenotype is specific to placental parasites and it has been linked to the expression of a unique *var* gene (*var2csa*) [80, 82]. The observation that men and children have equally poor IgG levels against parasite isolates compared to pregnant women and CSA-binding laboratory lines is consistent with the concept that immunity against VAR2CSA is acquired specifically during pregnancy [115]. Therefore, in Africa, immunity to CSA-binding parasites appears to be gender specific (thus men exposed to malaria lack these antibodies) [116,117] and parity related (thus antibodies increase during successive pregnancies) [111,116,117].

Exposition to infection throughout successive pregnancies has been associated with a lower risk of placental parasitemia [111], maternal anemia [118], low birth weight [115,118] and congenital malaria [119]. However, antibodies against *P. falciparum* antigens are not specifically associated with pregnancy, which have also been shown to increase with parity [115,120,121]. Moreover, a significant number of women at delivery have antibodies against placental parasites, but their placentas remain infected [111,116]. Several studies have failed to show an association between levels of anti-CSA binding IgG and a reduced frequency of adverse consequences of malaria during pregnancy [122-124].

Comparatively lower anti-CSA IgG antibodies have been reported at delivery in parturient from Mozambique, which partly explains the high incidence of malaria episodes observed a few weeks after delivery in this population [125].

Regarding the association between presence of placental infection and antibody responses, contradictory results have been reported [111,118,121,126-129]. However, it seems well-known the observation that women with low specific antimalarial IgG exhibit high frequency of cord blood infection [130].

Very few studies addressed the subject of antibody responses to non–*P. falciparum* malaria during pregnancy, particularly to *P. vivax*, which is widespread in Asia and South America. Recent studies confirmed in an endemic region of Thailand, where both *P. falciparum* and *P. vivax* exhibit low endemicity, highly variable presence of antibodies to both species over time, with maintenance of high levels of antimalarial antibodies and highly dynamic responses resulting from intermittent exposure to infection [84]. In addition, evidence of boosting with each successive infection for *P. falciparum* responses was described, suggesting the presence of immunological memory. However, the half-lives of anti-*Plasmodium* antibody responses were relatively short [84].

Significance of submicroscopic parasitaemia in acquisition of antimalarial immunity during pregnancy

The widespread use of molecular techniques applied to the diagnosis of gestational or placental malaria has resulted in the discovery of a large gap in positivity between microscopic and molecular diagnosis [35,42]. Health authorities remain to address this problem, particularly for endemic settings where most diagnosis is performed in the field. The situation has been reported in moderate and highly endemic regions [42,131-133]. In the reports available, submicroscopic infection by *P. falciparum* is common. Undergoing studies in India (Madhya Pradesh and Chhattisgarh states) and eastern Indonesia showed that PCR detected up to twice as many *P. vivax* and *P. falciparum* episodes as malaria smears or rapid diagnostic tests did [44]. In Colombia, diagnosis of submicroscopic infection ranges from around 30% to around 60%, depending on the sensitivity of the molecular test applied [35]. However, the effect of underestimating pregnancy-associated malaria on the acquisition of specific antimalarial immunity remains to be studied.

In non-pregnant subjects low-level parasitaemia results in impairment of dendritic cells, important antigen presenting cells during malaria infection, by induction of apoptosis [134]. Such cell population has a central role in the initiation of cellular and antibody specific immune responses. Therefore the characterization of the cellular populations infiltrating infected placentas, in the case of microscopic and submicroscopic parasitaemia tests, should be addressed to identify inflammation driven changes such as enhanced apoptosis of antigen presenting cells. This would guide future treatment actions in regions where high rates of submicroscopic infection are confirmed.

The following can be concluded regarding the immune responses in gestational malaria:

1. Effective immune responses during gestational malaria are mainly directed against variant surface antigens codified by genes of the *var2Csa* family of *P. falciparum*.
2. Acquisition of antibodies against variant antigens depends on the degree and intensity of transmission, and increases with age and successive pregnancies.
3. Antibody development is guided by specific cellular immune responses in cases of placental and maternal infection.
4. The significance of specific immunity against both *P. falciparum* and *P. vivax* in America remains to be elucidated.

Research needs and directions in gestational malaria

Diagnosis of gestational and placental infection

The accuracy of associations between the status of *Plasmodium spp.* infection in the mother or in the placenta, relies on the ability to detect the parasite in the host. Therefore, all studies of this kind are required to apply the test that exhibits the highest sensitivity and specificity. In this sense, in all endemic regions, molecular approaches have proved effective [42,131] to establish infection even in cases of very low parasitaemias. However, in most field settings, laboratory conditions and personnel are limited and made unpractical this approach. Similarly, examination of the placenta by histology, which has proved suitable to diagnose past and current infection [47,135], is unpractical due to the need for specialized personnel and technical requirements.

Some of the host's biomarkers of placental infection have been described. In general, they detect infection resulting after the inflammation process secondary to malaria. Molecules such as zinc erythrocyte protoporphyrin [136], IL-10, TNF-α, soluble TNF-α receptors, IL-1, ferritin, leptin and C5a, among others [137], have been associated to gestational and/or placental infection. Others have studied the correlation between these markers with clinical outcomes, for instance the inverse relation between increased soluble CD163 (sCD163), a specific marker of monocyte/macrophage activation, and hemoglobin levels [138] has been reported. Nevertheless, the association between host biomarkers and presence of submicroscopic infection in placenta or the mother, remains to be explored and the complicated interactions between the host and the parasite in pregnant subjects are a hot topic of research.

Biological significance of placental infection.

The presence of placental infection does not always result in placental pathology and vice versa [47]. This means that resolved infections can leave sequelae in the placenta and significant parasitaemia or a chronic-persistent placental invasion are required to evidence the effect in this tissue. Few authors have addressed this question, and its exploration is a priority due the emerging reports on high frequency of infections according to molecular techniques [42] and the seldom consequences observed by histopathology examination of the placenta.

Co-infection and co-morbidity.

Very often malaria infection is present in communities where other pathogens are prevalent or in ethnic communities in which different pregnancy associated pathologies are common. For instance, helminth and HIV infections are being reported in highly affected malaria groups in Africa [139], and a high risk of mother's undernourishment and anemia has been confirmed. Undoubtedly, co-infection with other pathogens results in an altered immune response against malaria and it might render women prone to complications. In America it is of particular interest the effect that the current Dengue epidemic might have on pregnant populations affected by malaria. Being both, Dengue virus and *Plasmodium spp.,* important pro-inflammatory agents, their effects in a pregnant host probably superimpose and result in greater morbidity and mortality. Both in Africa and in America, co-infection with other pathogens, different from HIV, is rarely addressed, and recent reports of high frequency of histology alterations in non-malaria affected placentas, indicate that this is a field worthwhile to study [47].

Finally, pre-eclampsia and eclampsia are a common co-morbidity in malaria affected populations. A study in Senegal confirmed a high risk of gestational hypertension in cases of placental infection [140]. However, previous reports based on monitoring of antimalarial antibodies failed to find a co-relation between the two conditions [141]. Given that anti-malarial antibodies are poor indicators of current placental infection and that the two conditions have a common physiopathology, exploration of their effect on placental development and clinical outcome, is required.

In the immediate future the research of immune responses during gestational malaria should explore the issue of cross-antigenic stimulation in areas where both *P. vivax* and *P. falciparum* co-exist since the understanding of the non-African immune scenario is of major relevance for vaccine development with emphasis in pregnant populations.

Funding

Universidad de Antioquia, Estrategia de Sostenibilidad 2013-2014.

References

1. Grimwade K, French N, Mbatha DD, Zungu DD, Dedicoat M, Gilks CF (2004) HIV infection as a cofactor for severe *falciparum* malaria in adults living in a region of unstable malaria transmission in South Africa. AIDS 18:547-554.
2. Riley EM, Stewart VA (2013) Immune mechanisms in malaria: new insights in vaccine development. Nat Med 19:168-178.
3. Langhorne J, Ndungu FM, Sponaas AM, Marsh K (2008) Immunity to malaria: more questions than answers. Nat Immunol 9:725-732.
4. Eckhoff PA (2012) Malaria parasite diversity and transmission intensity affect development of parasitological immunity in a mathematical model. Malar J 11:419.
5. Doolan DL, Dobaño C, Baird JK (2009) Acquired immunity to malaria. Clin Microbiol Rev 22:13-36, Table of Contents.
6. Duffy P (2007) *Plasmodium* in the placenta: parasites, parity, protection, prevention and possibly preeclampsia. Parasitology 134(Pt 13).1877-1881.
7. Arango E, Maestre A, Carmona-Fonseca J (2010) [Effect of submicroscopic or polyclonal *Plasmodium falciparum* infection on mother and gestation product: systematic review]. Rev Bras Epidemiol 13:373-386.
8. Lacerda MV, Mourão MP, Alexandre MA, Siqueira AM, Magalhães BM, Martinez-Espinosa FE, Filho FS, Brasil P, Ventura AM, Tada MS, Couto VS, Silva AR, Silva RS, Alecrim MG (2012) Understanding the clinical spectrum of complicated *Plasmodium vivax* malaria: a systematic review on the contributions of the Brazilian literature. Malar J 11:12.
9. Nurleila S, Syafruddin D, Elyazar IR, Baird JK (2012) Serious and fatal illness associated with *falciparum* and *vivax* malaria among patients admitted to hospital at West Sumba in eastern Indonesia. Am J Trop Med Hyg 87:41-49.
10. Carvalho B, Lopes S, Nogueira P, Orlandi P, Bargieri D, Blanco Y, Mamoni R, Leite J, Rodrigues M, Soares I, Oliveira T, Wunderlich G, Lacerda M, Del Portillo H, Araújo M, Russell B, Suwanarusk R, Snounou G, Rénia L, Costa F (2010) On the Cytoadhesion of *Plasmodium vivax*-Infected Erythrocytes. J Infect Dis. doi: 10.1086/654815
11. Anstey NM, Russell B, Yeo TW, Price RN (2009) The pathophysiology of *vivax* malaria. Trends Parasitol 25:220-227.
12. Anstey NM, Handojo T, Pain MC, Kenangalem E, Tjitra E, Price RN, Maguire GP (2007) Lung injury in *vivax* malaria: pathophysiological evidence for pulmonary vascular sequestration and posttreatment alveolar-capillary inflammation. J Infect Dis 195:589-596.
13. Grun JL, Long CA, Weidanz WP (1985) Effects of splenectomy on antibody-independent immunity to *Plasmodium* chabaudi *adami* malaria. Infect Immun 48:853-858.
14. Grun JL, Weidanz WP (1983) Antibody-independent immunity to reinfection malaria in B-cell-deficient mice. Infect Immun 41:1197-1204.
15. Balmer P, Phillips HM, Maestre AE, McMonagle FA, Phillips RS (2000) The effect of nitric oxide on the growth of *Plasmodium falciparum*, *P.* chabaudi and *P.* berghei *in vitro*. Parasite Immunol 22:97-106.
16. Taylor-Robinson AW (2010) Regulation of immunity to *Plasmodium*: implications from mouse models for blood stage malaria vaccine design. Exp Parasitol 126:406-414. 8
17. Taylor JJ, Jenkins MK (2011) CD4+ memory T cell survival. Curr Opin Immunol 23:319-323.
18. Xu H, Wipasa J, Yan H, Zeng M, Makobongo MO, Finkelman FD, Kelso A, Good MF (2002) The mechanism and significance of deletion of parasite-specific CD4(+) T cells in malaria infection. J Exp Med 195:881-892.
19. Illingworth J, Butler NS, Roetynck S, Mwacharo J, Pierce SK, Bejon P, Crompton PD, Marsh K, Ndungu FM (2013) Chronic exposure to *Plasmodium falciparum* is associated with phenotypic evidence of B and T cell exhaustion. J Immunol 190:1038-1047.
20. Sun T, Holowka T, Song Y, Zierow S, Leng L, Chen Y, Xiong H, Griffith J, Nouraie M, Thuma PE, Lolis E, Janse CJ, Gordeuk VR, Augustijn K, Bucala R (2012) A *Plasmodium*-encoded cytokine suppresses T-cell immunity during malaria. Proc Natl Acad Sci U S A 109:E2117-2126.
21. Good MF, Doolan DL (1999) Immune effector mechanisms in malaria. Curr Opin Immunol 11:412-419.
22. Chandele A, Mukerjee P, Das G, Ahmed R, Chauhan VS (2011) Phenotypic and functional profiling of malaria-induced CD8 and CD4 T cells during blood-stage infection with *Plasmodium yoelii*. Immunology 132:273-286.
23. Wipasa J, Elliott S, Xu H, Good MF (2002) Immunity to asexual blood stage malaria and vaccine approaches. Immunol Cell Biol 80:401-414.
24. Beeson JG, Osier FH, Engwerda CR (2008) Recent insights into humoral and cellular immune responses against malaria. Trends Parasitol 24:578-584.
25. Dorfman JR, Bejon P, Ndungu FM, Langhorne J, Kortok MM, Lowe BS, Mwangi TW, Williams TN, Marsh K (2005) B cell memory to 3 *Plasmodium falciparum* blood-stage antigens in a malaria-endemic area. J Infect Dis 191:1623-1630.
26. Cavanagh DR, Elhassan IM, Roper C, Robinson VJ, Giha H, Holder AA, Hviid L, Theander TG, Arnot DE, McBride JS (1998) A longitudinal study of type-specific antibody responses to *Plasmodium falciparum* merozoite surface protein-1 in an area of unstable malaria in Sudan. J Immunol 161:347-359.
27. Taylor RR, Egan A, McGuinness D, Jepson A, Adair R, Drakely C, Riley E (1996) Selective recognition of malaria antigens by human serum antibodies is not genetically determined but demonstrates some features of clonal imprinting. Int Immunol 8:905-915.
28. Akpogheneta OJ, Duah NO, Tetteh KK, Dunyo S, Lanar DE, Pinder M, Conway DJ (2008) Duration of naturally acquired antibody responses to blood-stage *Plasmodium falciparum* is age dependent and antigen specific. Infect Immun 76:1748-1755.

29. Drakeley CJ, Corran PH, Coleman PG, Tongren JE, McDonald SL, Carneiro I, Malima R, Lusingu J, Manjurano A, Nkya WM, Lemnge MM, Cox J, Reyburn H, Riley EM (2005) Estimating medium- and long-term trends in malaria transmission by using serological markers of malaria exposure. Proc Natl Acad Sci U S A 102:5108-5113.
30. Wipasa J, Suphavilai C, Okell LC, Cook J, Corran PH, Thaikla K, Liewsaree W, Riley EM, Hafalla JC (2010) Long-lived antibody and B Cell memory responses to the human malaria parasites, *Plasmodium falciparum* and *Plasmodium vivax*. PLoS Pathog 6:e1000770.
31. Anstey NM, Douglas NM, Poespoprodjo JR, Price RN (2012) *Plasmodium vivax*: clinical spectrum, risk factors and pathogenesis. Adv Parasitol 80:151-201.
32. Steketee RW, Nahlen BL, Parise ME, Menendez C (2001) The burden of malaria in pregnancy in malaria-endemic areas. Am J Trop Med Hyg 64:28-35.
33. Dellicour S, Tatem AJ, Guerra CA, Snow RW, ter Kuile FO (2010) Quantifying the number of pregnancies at risk of malaria in 2007: a demographic study. PLoS Med 7:e1000221.
34. WHO (2007) Technical Expert Group meeting on intermittent preventive treatment in pregnancy (IPTp). In WHO HEADQUARTERS, GENEVA, 11–13 JULY 2007 Available at: http://www.jhpiego.org/files/malarialrp/english/referenceMaterials/files/Technical_expert_group_meeting_on_IPTp_report.pdf Technical Expert Group. Accesed on 09/09/2013.
35. Campos IM, Uribe ML, Cuesta C, Franco-Gallego A, Carmona-Fonseca J, Maestre A (2011) Diagnosis of gestational, congenital, and placental malaria in Colombia: comparison of the efficacy of microscopy, nested polymerase chain reaction, and histopathology. Am J Trop Med Hyg 84:929-935.
36. Desai M, ter Kuile FO, Nosten F, McGready R, Asamoa K, Brabin B, Newman RD (2007) Epidemiology and burden of malaria in pregnancy. Lancet Infect Dis 7:93-104.
37. Brabin BJ (1983) An analysis of malaria in pregnancy in Africa. Bull World Health Organ 61:1005-1016.
38. Beeson JG, Duffy PE (2005) The immunology and pathogenesis of malaria during pregnancy. Curr Top Microbiol Immunol 297:187-227.
39. Beeson JG, Brown GV (2002) Pathogenesis of *Plasmodium falciparum* malaria: the roles of parasite adhesion and antigenic variation. Cell Mol Life Sci 59:258-271.
40. Beeson JG, Rogerson SJ, Brown GV (2002) Evaluating specific adhesion of *Plasmodium falciparum*-infected erythrocytes to immobilised hyaluronic acid with comparison to binding of mammalian cells. Int J Parasitol 32:1245-1252.
41. Brabin B, Romagosa C, Abdelgalil S, Menéndez C, Verhoeff F, McGready R, Fletcher K, Owens S, D'Alessandro U, Nosten F, Fischer P, Ordi J (2004) The sick placenta-the role of malaria. Placenta 25:359-378.
42. Arango EM, Samuel R, Agudelo OM, Carmona-Fonseca J, Maestre A, Yanow SK (2013) Molecular detection of malaria at delivery reveals a high frequency of submicroscopic infections and associated placental damage in pregnant women from northwest Colombia. Am J Trop Med Hyg 89:178-183.
43. McGready R, Lee SJ, Wiladphaingern J, Ashley EA, Rijken MJ, Boel M, Simpson JA, Paw MK, Pimanpanarak M, Mu O, Singhasivanon P, White NJ, Nosten FH (2012) Adverse effects of *falciparum* and *vivax* malaria and the safety of antimalarial treatment in early pregnancy: a population-based study. Lancet Infect Dis 12:388-396.
44. Nosten F, McGready R, Simpson J, Thwai K, Balkan S, Cho T, Hkirijaroen L, Looareesuwan S, White N (1999) Eff ects of *Plasmodium vivax* malaria in pregnancy. Lancet 354:546-549.
45. Rijken MJ, McGready R, Boel ME, Poespoprodjo R, Singh N, Syafruddin D, Rogerson S, Nosten F (2012) Malaria in pregnancy in the Asia-Pacific region. Lancet Infect Dis 12:75-88.
46. Souza RM, Ataíde R, Dombrowski JG, Ippólito V, Aitken EH, Valle SN, Álvarez JM, Epiphanio S, Epiphânio S, Marinho CR (2013) Placental histopathological changes associated with *Plasmodium vivax* infection during pregnancy. PLoS Negl Trop Dis 7:e2071.
47. Carmona-Fonseca J, Arango E, Maestre A (2013) Placental Malaria in Colombia: Histopathologic Findings in *Plasmodium vivax* and *P. falciparum* Infections. Am J Trop Med Hyg. 88:1093-1101.
48. Luxemburger C, Ricci F, Nosten F, Raimond D, Bathet S, White NJ (1997) The epidemiology of severe malaria in an area of low transmission in Thailand. Trans R Soc Trop Med Hyg 91:256-262.
49. Looareesuwan S, White NJ, Chittamas S, Bunnag D, Harinasuta T (1987) High rate of *Plasmodium vivax* relapse following treatment of *falciparum* malaria in Thailand. Lancet 2:1052-1055.
50. Imwong M, Boel ME, Pagornrat W, Pimanpanarak M, McGready R, Day NP, Nosten F, White NJ (2012) The first *Plasmodium vivax* relapses of life are usually genetically homologous. J Infect Dis 205:680-683.
51. Poespoprodjo JR, Fobia W, Kenangalem E, Lampah DA, Hasanuddin A, Warikar N, Sugiarto P, Tjitra E, Anstey NM, Price RN (2009) Vivax malaria: a major cause of morbidity in early infancy. Clin Infect Dis 48:1704-1712.
52. McGready R, Davison BB, Stepniewska K, Cho T, Shee H, Brockman A, Udomsangpetch R, Looareesuwan S, White NJ, Meshnick SR, Nosten F (2004) The effects of *Plasmodium falciparum* and *P. vivax* infections on placental histopathology in an area of low malaria transmission. Am J Trop Med Hyg 70:398-407.
53. Bachschmid I, Soro Bç, Coulibaly A, Philippe E, Kingston L, Kien T, Rey J (1991) Malaria infection during childbirth and in newborns in Becedi (Ivory Coast). Bull Soc Pathol Exot 84:257–265.
54. Jelliffe E (1968) Low birth-weight and malarial infection of the placenta. Bull World Health Organ 38:69–78.
55. WHO (2012) World Malaria Report 2011. In. WHO, Geneve.
56. Parekh F, Hernandez J, Krogstad D, Casapia W, Branch O (2007) Prevalence and risk of *Plasmodium falciparum* and *P. vivax* malaria among pregnant women living in the hypoendemic communities of the Peruvian Amazon. Am J Trop Med Hyg 77:451-457.
57. Gómez E, López E, Ache A (2009) [Malaria and pregnancy. San Isidro parish, municipality Sifontes, state of Bolívar, Venezuela, 2005-2006]. Invest Clin 50:455-464.
58. Carmona-Fonseca J, Franco A, Arango E, Agudelo O, Maestre A (2010) Now ICT malaria Pf/Pv® frente a microscopía (gota gruesa-extendido) para diagnóstico de malaria en Urabá (Colombia). Iatreia 23:137-145.
59. Zen M, Ghirardello A, Iaccarino L, Tonon M, Campana C, Arienti S, Rampudda M, Canova M, Doria A (2010) Hormones, immune response, and pregnancy in healthy women and SLE patients. Swiss Med Wkly 140:187-201.

60. Orsi NM (2008) Cytokine networks in the establishment and maintenance of pregnancy. Hum Fertil (Camb) 11:222-230.
61. Orsi NM, Tribe RM (2008) Cytokine networks and the regulation of uterine function in pregnancy and parturition. J Neuroendocrinol 20:462-469.
62. Buyon JP, Petri MA, Kim MY, Kalunian KC, Grossman J, Hahn BH, Merrill JT, Sammaritano L, Lockshin M, Alarcón GS, Manzi S, Belmont HM, Askanase AD, Sigler L, Dooley MA, Von Feldt J, McCune WJ, Friedman A, Wachs J, Cronin M, Hearth-Holmes M, Tan M, Licciardi F (2005) The effect of combined estrogen and progesterone hormone replacement therapy on disease activity in systemic lupus erythematosus: a randomized trial. Ann Intern Med 142:953-962.
63. Szereday L, Varga P, Szekeres-Bartho J (1997) Cytokine production by lymphocytes in pregnancy. Am J Reprod Immunol 38:418-422.
64. Halonen M, Lohman IC, Stern DA, Spangenberg A, Anderson D, Mobley S, Ciano K, Peck M, Wright AL (2009) Th1/Th2 patterns and balance in cytokine production in the parents and infants of a large birth cohort. J Immunol 182:3285-3293.
65. Ellis J, Wennerholm UB, Bengtsson A, Lilja H, Pettersson A, Sultan B, Wennergren M, Hagberg H (2001) Levels of dimethylarginines and cytokines in mild and severe preeclampsia. Acta Obstet Gynecol Scand 80:602-608.
66. Bahar AM, Ghalib HW, Moosa RA, Zaki ZM, Thomas C, Nabri OA (2003) Maternal serum interleukin-6, interleukin-8, tumor necrosis factor-alpha and interferon-gamma in preterm labor. Acta Obstet Gynecol Scand 82:543-549.
67. Lin H, Mosmann TR, Guilbert L, Tuntipopipat S, Wegmann TG (1993) Synthesis of T helper 2-type cytokines at the maternal-fetal interface. J Immunol 151:4562-4573
68. Kanik KS, Wilder RL (2000) Hormonal alterations in rheumatoid arthritis, including the effects of pregnancy. Rheum Dis Clin North Am 26:805-823.
69. Kovats S (2012) Estrogen receptors regulate an inflammatory pathway of dendritic cell differentiation: mechanisms and implications for immunity. Horm Behav 62:254-262.
70. Denney JM, Nelson EL, Wadhwa PD, Waters TP, Mathew L, Chung EK, Goldenberg RL, Culhane JF (2011) Longitudinal modulation of immune system cytokine profile during pregnancy. Cytokine 53:170-177.
71. Grau GE, Craig AG (2012) Cerebral malaria pathogenesis: revisiting parasite and host contributions. Future Microbiol 7:291-302.
72. O'Garra A (1998) Cytokines induce the development of functionally heterogeneous T helper cell subsets. Immunity 8:275-283.
73. Murphy KM, Ouyang W, Farrar JD, Yang J, Ranganath S, Asnagli H, Afkarian M, Murphy TL (2000) Signaling and transcription in T helper development. Annu Rev Immunol 18:451-494.
74. Akdis M, Palomares O, van de Veen W, van Splunter M, Akdis CA (2012) TH17 and TH22 cells: a confusion of antimicrobial response with tissue inflammation versus protection. J Allergy Clin Immunol 129:1438-1449.
75. Sykes L, MacIntyre DA, Yap XJ, Teoh TG, Bennett PR (2012) The Th1:th2 dichotomy of pregnancy and preterm labour. Mediators Inflamm 2012:967629.
76. Prins JR, Gomez-Lopez N, Robertson SA (2012) Interleukin-6 in pregnancy and gestational disorders. J Reprod Immunol 95:1-14.
77. Lee SK, Kim JY, Lee M, Gilman-Sachs A, Kwak-Kim J (2012) Th17 and regulatory T cells in women with recurrent pregnancy loss. Am J Reprod Immunol 67:311-318.
78. Flanagan KL, Halliday A, Burl S, Landgraf K, Jagne YJ, Noho-Konteh F, Townend J, Miles DJ, van der Sande M, Whittle H, Rowland-Jones S (2010) The effect of placental malaria infection on cord blood and maternal immunoregulatory responses at birth. Eur J Immunol 40:1062-1072.
79. Fried M, Duffy PE (1996) Adherence of *Plasmodium falciparum* to chondroitin sulfate A in the human placenta. Science 272:1502-1504.
80. Duffy P, Fried M (2005) Malaria in the pregnant woman. Curr Top Microbiol Immunol 295:169-200.
81. Gamain B, Smith JD, Viebig NK, Gysin J, Scherf A (2007) Pregnancy-associated malaria: parasite binding, natural immunity and vaccine development. Int J Parasitol 37:273-283.
82. Salanti A, Staalsoe T, Lavstsen T, Jensen AT, Sowa MP, Arnot DE, Hviid L, Theander TG (2003) Selective upregulation of a single distinctly structured var gene in chondroitin sulphate A-adhering *Plasmodium falciparum* involved in pregnancy-associated malaria. Mol Microbiol 49:179-191.
83. Salanti A, Dahlbäck M, Turner L, Nielsen MA, Barfod L, Magistrado P, Jensen AT, Lavstsen T, Ofori MF, Marsh K, Hviid L, Theander TG (2004) Evidence for the involvement of VAR2CSA in pregnancy-associated malaria. J Exp Med 200:1197-1203.
84. Fowkes FJ, McGready R, Cross NJ, Hommel M, Simpson JA, Elliott SR, Richards JS, Lackovic K, Viladpai-Nguen J, Narum D, Tsuboi T, Anders RF, Nosten F, Beeson JG (2012) New insights into acquisition, boosting, and longevity of immunity to malaria in pregnant women. J Infect Dis 206:1612-1621.
85. Costa FT, Lopes SC, Albrecht L, Ataíde R, Siqueira AM, Souza RM, Russell B, Renia L, Marinho CR, Lacerda MV (2012) On the pathogenesis of *Plasmodium vivax* malaria: Perspectives from the Brazilian field. Int J Parasitol 42:1099-1105.
86. Piccinni MP (2010) T cell tolerance towards the fetal allograft. J Reprod Immunol 85:71-75.
87. Wegmann TG, Lin H, Guilbert L, Mosmann TR (1993) Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today 14:353-356.
88. Thellin O, Coumans B, Zorzi W, Igout A, Heinen E (2000) Tolerance to the foeto-placental 'graft': ten ways to support a child for nine months. Curr Opin Immunol 12:731-737.
89. Kwak-Kim JY, Gilman-Sachs A, Kim CE (2005) T helper 1 and 2 immune responses in relationship to pregnancy, nonpregnancy, recurrent spontaneous abortions and infertility of repeated implantation failures. Chem Immunol Allergy 88:64-79.
90. Suguitan AL, Leke RG, Fouda G, Zhou A, Thuita L, Metenou S, Fogako J, Megnekou R, Taylor DW (2003) Changes in the levels of chemokines and cytokines in the placentas of women with *Plasmodium falciparum* malaria. J Infect Dis 188:1074-1082.
91. Moormann AM, Sullivan AD, Rochford RA, Chensue SW, Bock PJ, Nyirenda T, Meshnick SR (1999) Malaria and pregnancy: placental cytokine expression and its relationship

to intrauterine growth retardation. J Infect Dis 180:1987-1993.
92. Fried M, Muga RO, Misore AO, Duffy PE (1998) Malaria elicits type 1 cytokines in the human placenta: IFN-gamma and TNF-alpha associated with pregnancy outcomes. J Immunol 160:2523-2530.
93. Rogerson SJ, Brown HC, Pollina E, Abrams ET, Tadesse E, Lema VM, Molyneux ME (2003) Placental tumor necrosis factor alpha but not gamma interferon is associated with placental malaria and low birth weight in Malawian women. Infect Immun 71:267-270.
94. Moore JM, Nahlen BL, Misore A, Lal AA, Udhayakumar V (1999) Immunity to placental malaria. I. Elevated production of interferon-gamma by placental blood mononuclear cells is associated with protection in an area with high transmission of malaria. J Infect Dis 179:1218-1225.
95. Taylor-Robinson AW, Smith EC (1999) A dichotomous role for nitric oxide in protection against blood stage malaria infection. Immunol Lett 67:1-9.
96. Abrams ET, Brown H, Chensue SW, Turner GD, Tadesse E, Lema VM, Molyneux ME, Rochford R, Meshnick SR, Rogerson SJ (2003) Host response to malaria during pregnancy: placental monocyte recruitment is associated with elevated beta chemokine expression. J Immunol 170:2759-2764.
97. Fievet N, Moussa M, Tami G, Maubert B, Cot M, Deloron P, Chaouat G (2001) *Plasmodium falciparum* induces a Th1/Th2 disequilibrium, favoring the Th1-type pathway, in the human placenta. J Infect Dis 183:1530-1534.
98. Agudelo OM, Aristizabal BH, Yanow SK, Arango E, Carmona-Fonseca J, Maestre A (2014) Submicroscopic infection of placenta by *Plasmodium* produces Th1/Th2 cytokine imbalance, inflammation and hypoxia in women from north-west Colombia. Malar J 13:122.
99. Yasnot MF, Perkins DJ, Corredor M,Yanow S, Carmona-Fonseca J, Maestre A (2013) The effects of *Plasmodium vivax* gestational malaria on the clinical and immune status of pregnant women in Northwestern Colombia. Colombia Medica 44:46.
100. Kabyemela ER, Muehlenbachs A, Fried M, Kurtis JD, Mutabingwa TK, Duffy PE (2008) Maternal peripheral blood level of IL-10 as a marker for inflammatory placental malaria. Malar J 7:26.
101. Davison BB, Kaack MB, Rogers LB, Rasmussen KK, Rasmussen TA, Henson EW, Henson MC, Parekh FK, Krogstad DJ (2006) The role of soluble tumor necrosis factor receptor types I and II and tumor necrosis factor-alpha in malaria during pregnancy. J Infect Dis 194:123-132.
102. Ismaili J, van der Sande M, Holland MJ, Sambou I, Keita S, Allsopp C, Ota MO, McAdam KP, Pinder M (2003) *Plasmodium falciparum* infection of the placenta affects newborn immune responses. Clin Exp Immunol 133:414-421.
103. Renaud SJ, Sullivan R, Graham CH (2009) Tumour necrosis factor alpha stimulates the production of monocyte chemoattractants by extravillous trophoblast cells via differential activation of MAPK pathways. Placenta 30:313-319.
104. Jangpatarapongsa K, Xia H, Fang Q, Hu K, Yuan Y, Peng M, Gao Q, Sattabongkot J, Cui L, Li B, Udomsangpetch R (2012) Immunity to malaria in *Plasmodium vivax* infection: a study in central China. PLoS One 7:e45971.
105. Diggs CL, Sadun EH (1965) Serological cross reactivity between *Plasmodium vivax* and *Plasmodium falciparum* as determined by a modified fluorescent antibody test. Exp Parasitol 16:217-223.
106. Woodberry T, Minigo G, Piera KA, Hanley JC, de Silva HD, Salwati E, Kenangalem E, Tjitra E, Coppel RL, Price RN, Anstey NM, Plebanski M (2008) Antibodies to *Plasmodium falciparum* and *Plasmodium vivax* merozoite surface protein 5 in Indonesia: species-specific and cross-reactive responses. J Infect Dis 198:134-142.
107. Kassa D, Petros B, Mesele T, Hailu E, Wolday D (2006) Characterization of peripheral blood lymphocyte subsets in patients with acute *Plasmodium falciparum* and *P. vivax* malaria infections at Wonji Sugar Estate, Ethiopia. Clin Vaccine Immunol 13:376-379.
108. Marsh K, Howard RJ (1986) Antigens induced on erythrocytes by *P. falciparum*: expression of diverse and conserved determinants. Science 231:150-153.
109. McGregor IA (1987) Malarial immunity: current trends and prospects. Ann Trop Med Parasitol 81:647-656.
110. Hartman TK, Rogerson SJ, Fischer PR (2010) The impact of maternal malaria on newborns. Ann Trop Paediatr 30:271-282.
111. Fried M, Nosten F, Brockman A, Brabin BJ, Duffy PE (1998) Maternal antibodies block malaria. Nature 395:851-852.
112. Tebo AE, Kremsner PG, Luty AJ (2002) Fcgamma receptor-mediated phagocytosis of *Plasmodium falciparum*-infected erythrocytes *in vitro*. Clin Exp Immunol 130:300-306.
113. Keen J, Serghides L, Ayi K, Patel SN, Ayisi J, van Eijk A, Steketee R, Udhayakumar V, Kain KC (2007) HIV impairs opsonic phagocytic clearance of pregnancy-associated malaria parasites. PLoS Med 4:e181.
114. Feng G, Aitken E, Yosaatmadja F, Kalilani L, Meshnick SR, Jaworowski A, Simpson JA, Rogerson SJ (2009) Antibodies to variant surface antigens of *Plasmodium falciparum*-infected erythrocytes are associated with protection from treatment failure and the development of anemia in pregnancy. J Infect Dis 200:299-306.
115. Mayor A, Rovira-Vallbona E, Machevo S, Bassat Q, Aguilar R, Quintó L, Jiménez A, Sigauque B, Dobaño C, Kumar S, Singh B, Gupta P, Chauhan VS, Chitnis CE, Alonso PL, Menéndez C (2011) Parity and placental infection affect antibody responses against *Plasmodium falciparum* during pregnancy. Infect Immun 79:1654-1659.
116. Ricke CH, Staalsoe T, Koram K, Akanmori BD, Riley EM, Theander TG, Hviid L (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.
117. Staalsoe T, Megnekou R, Fievét N, Ricke CH, Zornig HD, Leke R, Taylor DW, Deloron P, Hviid L (2001) Acquisition and decay of antibodies to pregnancy-associated variant antigens on the surface of *Plasmodium falciparum*-infected erythrocytes that protect against placental parasitemia. J Infect Dis 184:618-626.
118. Staalsoe T, Shulman CE, Bulmer JN, Kawuondo K, Marsh K, Hviid L (2004) Variant surface antigen-specific IgG and protection against clinical consequences of pregnancy-associated *Plasmodium falciparum* malaria. Lancet 363:283-289.
119. Carlier Y, Truyens C, Deloron P, Peyron F (2012) Congenital parasitic infections: a review. Acta Trop 121:55-70.
120. Brabin L, Brabin BJ (1992) Parasitic infections in women and their consequences. Adv Parasitol 31:1-81.

121. Mvondo JL, James MA, Sulzer AJ, Campbell CC (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.
122. Fievet N, Le Hesran JY, Cottrell G, Doucoure S, Diouf I, Ndiaye JL, Bertin G, Gaye O, Sow S, Deloron P (2006) Acquisition of antibodies to variant antigens on the surface of *Plasmodium falciparum*-infected erythrocytes during pregnancy. Infect Genet Evol 6:459-463.
123. Cox SE, Staalsoe T, Arthur P, Bulmer JN, Hviid L, Yeboah-Antwi K, Kirkwood BR, Riley EM (2005) Rapid acquisition of isolate-specific antibodies to chondroitin sulfate A-adherent plasmodium *falciparum* isolates in Ghanaian primigravidae. Infect Immun 73:2841-2847.
124. Serra-Casas E, Menéndez C, Bardají A, Quintó L, Dobaño C, Sigauque B, Jiménez A, Mandomando I, Chauhan VS, Chitnis CE, Alonso PL, Mayor A (2010) The effect of intermittent preventive treatment during pregnancy on malarial antibodies depends on HIV status and is not associated with poor delivery outcomes. J Infect Dis 201:123-131.
125. Diagne N, Rogier C, Sokhna CS, Tall A, Fontenille D, Roussilhon C, Spiegel A, Trape JF (2000) Increased susceptibility to malaria during the early postpartum period. N Engl J Med 343:598-603.
126. Beeson JG, Mann EJ, Elliott SR, Lema VM, Tadesse E, Molyneux ME, Brown GV, Rogerson SJ (2004) Antibodies to variant surface antigens of *Plasmodium falciparum*-infected erythrocytes and adhesion inhibitory antibodies are associated with placental malaria and have overlapping and distinct targets. J Infect Dis 189:540-551.
127. Maubert B, Fievet N, Tami G, Cot M, Boudin C, Deloron P (1999) Development of antibodies against chondroitin sulfate A-adherent *Plasmodium falciparum* in pregnant women. Infect Immun 67:5367-5371.
128. Oleinikov AV, Rossnagle E, Francis S, Mutabingwa TK, Fried M, Duffy PE (2007) Effects of sex, parity, and sequence variation on seroreactivity to candidate pregnancy malaria vaccine antigens. J Infect Dis 196:155-164.
129. O'Neil-Dunne I, Achur RN, Agbor-Enoh ST, Valiyaveettil M, Naik RS, Ockenhouse CF, Zhou A, Megnekou R, Leke R, Taylor DW, Gowda DC (2001) Gravidity-dependent production of antibodies that inhibit binding of *Plasmodium falciparum*-infected erythrocytes to placental chondroitin sulfate proteoglycan during pregnancy. Infect Immun 69:7487-7492.
130. Naniche D, Serra-Casas E, Bardají A, Quintó L, Dobaño C, Sigauque B, Cisteró P, Chauhan VS, Chitnis CE, Alonso PL, Menéndez C, Mayor A (2012) Reduction of antimalarial antibodies by HIV infection is associated with increased risk of *Plasmodium falciparum* cord blood infection. J Infect Dis 205:568-577.
131. Omer S, Khalil E, Ali H, Sharief A (2011) Submicroscopic and multiple plasmodium *falciparum* infections in pregnant Sudanese women. N Am J Med Sci 3:137-141.
132. Adam I, A-Elbasit IE, Salih I, Elbashir MI (2005) Submicroscopic *Plasmodium falciparum* infections during pregnancy, in an area of Sudan with a low intensity of malaria transmission. Ann Trop Med Parasitol 99:339-344.
133. Walker-Abbey A, Djokam RR, Eno A, Leke RF, Titanji VP, Fogako J, Sama G, Thuita LH, Beardslee E, Snounou G, Zhou A, Taylor DW (2005) Malaria in pregnant Cameroonian women: the effect of age and gravidity on submicroscopic and mixed-species infections and multiple parasite genotypes. Am J Trop Med Hyg 72:229-235.
134. Woodberry T, Minigo G, Piera KA, Amante FH, Pinzon-Charry A, Good MF, Lopez JA, Engwerda CR, McCarthy JS, Anstey NM (2012) Low-level *Plasmodium falciparum* blood-stage infection causes dendritic cell apoptosis and dysfunction in healthy volunteers. J Infect Dis 206:333-340.
135. Rogerson SJ, Mkundika P, Kanjala MK (2003) Diagnosis of *Plasmodium falciparum* malaria at delivery: comparison of blood film preparation methods and of blood films with histology. J Clin Microbiol 41:1370-1374.
136. Senga EL, Koshy G, Brabin BJ (2012) Zinc erythrocyte protoporphyrin as marker of malaria risk in pregnancy - a retrospective cross-sectional and longitudinal study. Malar J 11:249.
137. Conroy AL, McDonald CR, Kain KC (2012) Malaria in pregnancy: diagnosing infection and identifying fetal risk. Expert Rev Anti Infect Ther 10:1331-1342.
138. Chua CL, Brown GV, Hamilton JA, Molyneux ME, Rogerson SJ, Boeuf P (2013) Soluble CD163, a product of monocyte/macrophage activation, is inversely associated with haemoglobin levels in placental malaria. PLoS One 8:e64127.
139. Ivan E, Crowther NJ, Rucogoza AT, Osuwat LO, Munyazesa E, Mutimura E, Njunwa KJ, Zambezi KJ, Grobusch MP (2012) Malaria and helminthic co-infection among HIV-positive pregnant women: prevalence and effects of antiretroviral therapy. Acta Trop 124:179-184.
140. Ndao CT, Dumont A, Fievet N, Doucoure S, Gaye A, Lehesran JY (2009) Placental malarial infection as a risk factor for hypertensive disorders during pregnancy in Africa: a case-control study in an urban area of Senegal, West Africa. Am J Epidemiol 170:847-853.
141. Conde-Agudelo A, Villar J, Lindheimer M (2008) Maternal infection and risk of preeclampsia: systematic review and metaanalysis. Am J Obstet Gynecol 198:7-22.

Corresponding author

Amanda Maestre
Carrera 51D Numero 62-29, oficina 303, Grupo Salud y Comunidad,
Facultad de Medicina, Universidad de Antioquia
Medellín, Colombia,
Tel +574 2196024
Fax +574 2196000
E-mail: aemaestre@gmail.com

Conflict of interests: No conflict of interests is declared.