Review Article

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

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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


(Received 09 May 2013 – Accepted 12 August 2013)

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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
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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 Plasmodium 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 endothelial 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 Plasmodium 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 forty-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, preeclampsia, 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...
This immunity is acquired as a result of antigenic stimulation through repeated parasite infections from early childhood onwards [109] but exposure 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 *Plasmodium 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 antimalarial 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.

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Conflict of interests: No conflict of interests is declared.