Novel biomarkers in the pathogenesis of placental malaria in sub-Saharan Africa

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8. GENERAL DISCUSSION
8.1 Overview

This thesis presents original data on various biomarkers of placental malaria not commonly featured in previous medical literature but which may provide fresh insights into the pathogenesis of this condition. These include some common red cell antigenic variants linked with disease susceptibility, previously unreported observations of parasite-placental interactions, new components of the parity-specific malaria immune response to infection, and functional consequences of placental malaria for the neonatal immune system. These specific findings are considered more broadly here in relation to current understanding of the effects of placental malaria on maternal, placental and neonatal immune responses.

8.2 Placental malaria and the maternal immune response

Pregnancy is characterised by a transient depression of cell-mediated immunity in women, which allows retention of the fetal allograft, but also interferes with maternal resistance to infectious diseases from a variety of pathogens. The findings in chapters 2, 5 and 6 of this thesis refer to parity-specific differences in maternal immune responses and susceptibility to placental malaria. The conventional model of parity-related susceptibility in placental malaria is based on the gradual acquisition of specific antibodies to VAR2CSA expressed by infected erythrocytes, developed over several pregnancies, which prevent parasite binding and sequestration in the intervillous spaces of the placenta (1). This model suggests that VAR2CSA is an attractive target for vaccination against malaria in pregnancy, and is well-supported by the available evidence (2). However, in addition to the production of anti-VAR2CSA antibodies, placental malaria also stimulates the production of antibodies which react with a diverse range of antigens, including those of malaria isolates not commonly associated with pregnancy and even other pathogens (3-5). Some women generate anti-VAR2CSA antibodies and yet still develop placental malaria (6) (7) and several studies have failed to show a reduced frequency of adverse consequences of malaria in pregnancy in the presence of such antibodies (8, 9) (10). In some cases, poor pregnancy outcomes have been associated with peripheral parasitaemia in the absence of placental malaria (11) and primigravidae have been reported to be at increased risk of such episodes for several weeks after into the postnatal period (12). Taken together, these data suggest that other mechanisms additional to the paucity of anti-adhesion antibodies to CSA-binding parasites may also be involved in the increased susceptibility of pregnant woman to malaria, particularly among primiparae. These mechanisms may reflect nonspecific modulation of previously acquired immune responses to malaria during first pregnancies, including effects on cell-mediated (13) and innate systems (14).
In Mozambique primigravidae without placental malaria had reduced total and specific IgG concentrations against a range of placental and non-placental *P. falciparum* isolates compared to uninfected multigravidae, even after adjustment for age and other common confounding factors (15). Cellular immune responses to *P. falciparum* antigens are reported to be depressed in pregnant women in comparison with non-pregnant controls [reviewed in (16) and in Cameroon, where malaria transmission is perennial, cellular immune responses to a placental-adherent line of *P. falciparum* were closely and positively related to parity (17). In a malaria-endemic area of Gabon, multiparous women had higher NK cell cytotoxic activity against *P. falciparum*–infected erythrocytes than did primiparous women (18) and crude schizont lysate-stimulated NK cells from primiparae produced significantly more IFNγ than those from multiparous women (19), indicating a parity-specific effect of malaria exposure on innate immune function. Such effects may also be manifested in phagocytic function. In Kenya, CSA-binding IE were cleared by opsonic phagocytosis in a sex-specific and parity-dependent manner, in association with the presence of IgG1 and IgG3 VAR2CSA-specific antibodies and this process was impaired by maternal HIV infection (14). It is well known that HIV-coinfection obliterates parity differences in pregnancy malaria susceptibility, increasing the risk for multiparae without significantly affecting the risk in primiparae (20). Although the mechanisms for these effects are not well understood it seems likely that they are a function of a generalised dismantling of immunological memory in multiparae which extends beyond the reduction in anti-VAR2CSA antibodies.

This thesis points to additional effects of parity on non-specific and innate immune pathways mediated by androgen hormones, blood groups and antiphospholipid antibodies which may improve parasite phagocytosis and reduce inflammation in the placenta. These pathways are components of the general immune response to malaria which is developed pre-pregnancy in most women living in highly-endemic areas, and their failure or modulation in the context of placental malaria is suggestive of a more pervasive suppression of immunity in first pregnancies. Natural immunity against *P. falciparum* malaria depends on the gradual acquisition of a broad repertoire of IgGs against the surfaces of erythrocytes infected by mature forms of the parasite (21). This immunity is acquired as a result of antigenic stimulation through repeated malaria infections from early childhood onwards, developing more rapidly after puberty (22-24). During first pregnancies, aspects of this immunity may be lost or modified, at least in relation to specific parasite variants.

In non-pregnant individuals co-factor independent antiphospholipid antibodies (aPL) including anticardiolipin (aCL) are common in malaria infection and the
concentrations are inversely proportional to the clinical severity of disease (25-27). In mouse models, injection of aPL faciliates recovery from malaria (28). In humans it is thought that aPL are directed against parasite phospholipid antigens on red cell membranes, such as phosphatidylinositol, inhibiting the mechanism by which TNFα is produced, especially in severe malaria (29). The study outlined in chapter 2 is the first, and at the time of writing only, report of the presence of aPL in placental malaria. The data suggest a protective role for aCL in the clearance of parasites from the placenta but the effect was found only in multiparae.

Malaria has been associated with reduced oestradiol production in late pregnancy (30) and with raised serum cortisol levels in primigravidae with placental malaria (31). It is unclear whether increased cortisol is a marker of the maternal stress response to malaria or whether it actively suppresses immune responses, increasing susceptibility to malaria. Other hormones have not been investigated and further studies of the relationship between malaria and the major endocrinological changes of pregnancy are warranted. In chapter 6, an association between placental malaria and another adrenal hormone, dihydroepiandrosterone sulphate (DHEAS) is explored. Many studies have now shown that DHEAS has significant immune modulatory functions, exhibiting both immune stimulatory and antiguocorticoid effects (32). Previous studies in pubertal young men and women suggested that DHEAS production is associated with a more rapid development of malarial immunity and resistance to re-infection in non-pregnant adults (22, 23). The current study is the first to report such a relationship in placental malaria. DHEAS concentrations were negatively associated with increasing maternal age and with parity, and there was a trend toward a lower prevalence of placental malaria in women with higher concentrations of DHEAS at delivery, but only in primiparae. The findings are difficult to interpret since the lowest concentrations of DHEAS were found in multiparae who also had the lowest prevalence of malaria. One explanation might be that some primiparae, being generally younger, were still developing the robust prenatal immunity characteristic of adults living in holo-endemic areas, in a process which is intensified around adolescence and which appears to be dependent on adrenal hormones (22, 23). In Malawi, maternal age as a marker of this process, was a stronger negative predictor of malaria in pregnancy than was parity ). However another explanation is that malaria-infected placentas metabolised DHEAS more rapidly than uninfected placenta. Given that the major placental metabolites of DHEAS are oestrogens and placental malaria is associated with diminished oestrogen production (30), this seems unlikely.

Another aspect of the inherent, non-immune-mediated susceptibility to malaria which may be 'unmasked' during first pregnancies, is that predicted by the commonest red cell polymorphism, the ABO blood group. As discussed in detail in chapter 4's systematic review, there is good evidence that the prevalence of ABO blood group
antigens varies around the world according to strong selection pressures applied by *P. falciparum* throughout human evolutionary history. Blood group A has consistently been associated with increased susceptibility to severe clinical disease in non-pregnant individuals through the mechanisms of increased cytoadherence leading to rosetting, and more efficient erythrocyte invasion by malaria parasites. Conversely, blood group O has been associated with protection against infection. In contrast to some studies of pregnancy malaria, chapter 5 reports the re-emergence of blood group A as a risk factor for placental malaria but again, only in primiparae. It might be postulated that this is another example of pre-pregnancy susceptibility to malaria being highlighted in the context of immunomodulation of pregnancy. However none of the ABO studies have considered the placenta as a fetal organ, antigenically distinct from the maternal tissue-type. Since placental malaria is characterised by cytoadherence and sequestration of parasitised red cells in apposition to the fetal interface of this organ, it would be interesting to examine the role of fetal ABO blood group phenotypes in placental malaria.

Another area of concern to malaria susceptibility in pregnancy not covered in this thesis is the nutritional modulation of placental malaria, especially by iron. It has recently been argued that “a better understanding of the mechanism(s) by which supplementary iron might increase the incidence of malaria and other infections is needed to ensure the safe and effective delivery of iron interventions in malaria-prone areas.” (33). Studies to address these issues are needed urgently in order to better inform public health efforts to address the global pandemic of maternal micronutrient malnutrition and anaemia.

### 8.3 Placental responses to malaria

#### 8.3.1 How does the syncytiotrophoblast respond to placental malaria?

The mechanisms by which malaria-infected erythrocytes (IE) sequester in the placenta are well described and are summarised in Chapter 1. In this model, the
syncytiotrophoblast (ST) may be viewed in passive terms, simply as the target organ in the pathogenesis of the infection. In fact there is evidence that ST responds actively to malaria infection but the processes involved are as yet poorly understood.

Chapter 4 reports upregulated α2,6-linked sialic acid expression on ST with chronic placental malaria in a small number of Zambian placentas. It was hypothesised that placental infection increased ST activity of sialyltransferases, which would be required in order to add sialic acid in α2,6-linkage to the galactose residues on N-linked glycoprotein chains. Such upregulation has previously been reported in endothelial cells as a result of stimulation with interleukin (IL)1, tumour necrosis factor (TNF)α and IL4 (34) and it is known that placental malaria elicits comparable systemic cytokine production (16). Possible roles for α2,6-linked sialic acid in IE repulsion at the materno-fetal interface or in the blocking of parasite invasion of uninfected erythrocytes in the intervillous space were postulated. Although upregulation of α2,6 sialic acid may not represent a malaria-specific ST response, as some patchy increases were seen in uninfected samples, and conditions resulting in local cytokine production may also elicit a similar response, the study gives an additional insight into ST as a reactive tissue in malaria infection.

ST is a unicellular fetal epithelium which modulates the maternal immune system in order to maintain the conceptus as an alien allograft in what would otherwise be a hostile immunological environment (35). In the context of malaria, binding of infected erythrocytes to ST in vitro was shown to induce tyrosine phosphorylation of a number of proteins, demonstrating for the first time that cellular activation in ST is induced by malaria IE (36).

Later the same in vitro system was used to assess the biochemical and immunological changes induced in ST by ST-adherent IE (37). The study demonstrated that ST mitogen-activated protein kinase (MAPK) C-Jun N-terminal kinase 1 (JNK1) was activated following IE/ST interaction, and modest increases in transcript expression of transforming growth factor (TGF)-β and IL-8 ensued. In addition, this interaction increased secretion of macrophage migration inhibitory factor (MIF) and macrophage inflammatory protein (MIP)-1α by ST and induced migration of maternal mononuclear cells towards IE-stimulated ST. This study showed that ST plays an active immunological role in response to placental malaria and that it is capable of influencing the local maternal immune environment during the infection.

8.3.2 Placental malaria and transfer of immunoglobulin across the syncytiotrophoblast

In Chapter 6 the impact of placental malaria on the passive immunisation of newborns against a specific infant pathogen is reported. Women with placental malaria transferred 30% less maternal measles antibody (MMA) to their babies by delivery than women without placental malaria. Among those without placental malaria at delivery, women who recalled taking anti-malaria medication transferred 30% more MMA than women who did not. This chapter was originally published as a short report and its data benefit from further discussion. MMA is an example of immunoglobulin isotype G (IgG), the only antibody isotype to cross the placenta in significant quantities during pregnancy. Newborn infants are particularly susceptible to infections because of the functional immaturity of their immune systems. Transplacentally acquired maternal IgG is therefore a vital component of that immune system, providing passive protection against the range of
In a further study by the same group, stimulation of ST with parasite-derived hemozoin induced another MAPK, extracellular signal-regulated kinase (ERK)1/2, to phosphorylate (38). Treated cells then secreted IL-8, MIP-1α, MIP-1β and TNF and released soluble intercellular adhesion molecule (ICAM)-1. Hemozoin-stimulated cells elicited specific migration of maternal mononuclear cells and induced the upregulation of ICAM-1 on primary monocytes. As outlined in Chapter 1, hemozoin characterises long-standing or recently resolved placental malaria and the observations from this latter study are consistent with those outlined in Chapter 4 in that they indicate a specific ST response to chronic placental malaria.

Taken together, all four studies suggest that a number of cellular activation pathways may be activated in placental malaria and elicit a variety of responses from ST and the maternal immune system (36-39). Future studies might now examine ST activation by parity and in relation to anti-adhesion antibodies which block CSA-binding by IE and are the hallmark feature of protective immunity to placental malaria. ST activation might lie at the heart of placental malaria pathogenesis and the adverse fetal outcomes of the infection. It is not known if IE/ST interactions affect non-immunological ST functions such as nutrient and gas exchange, although the high-prevalence of fetal growth restriction and the findings described in Chapter 6 suggest that ST transfer capacity is adversely affected by malaria.

### 8.3.2 Placental malaria and transfer of immunoglobulin across the syncytiotrophoblast

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MMA is an example of immunoglobulin isotype G (IgG), the only antibody isotype to cross the placenta in significant quantities during pregnancy. Newborn infants are particularly susceptible to infections because of the functional immaturity of their immune systems. Transplacentally acquired maternal IgG is therefore a vital component of that immune system, providing passive protection against the range of
specific pathogens previously encountered by the mother while autologous humoral immunity is still developing (40).

The transport of IgG across the placenta is an active, selective, intracellular process specifically mediated by the neonatal Fc receptor (FcRn) expressed in ST (40). It begins in the second trimester, increasing in rate as pregnancy progresses (41). By 33 weeks of gestation, maternal and fetal IgG are at equivalent concentrations. By term, the IgG concentration in fetal blood exceeds that of the mother. The efficiency of this process depends on multiple factors including placental integrity, the total and specific IgG concentrations in maternal blood, the specificity, subclass and avidity of the IgG molecule and the gestational age of the fetus at birth.

Several studies have reported comparable findings to the present study, not only with measles antibody but also with other pathogen-specific antibodies. These data suggest that the effects of malaria on immunoglobulin transfer across the ST are mediated both by the chronicity and the density of placental parasite sequestration, as well as by the presence of co-morbidities such as HIV infection, hypergammaglobulinaemia (itself a consequence of malaria and HIV infection) and preterm delivery.

In the earliest published study of this type, Brair et al. compared concentrations of tetanus antibody in paired maternal-cord sera from 224 women living in a malarious area of Papua New Guinea. With heavy placental infection (>35 parasites per 200 white cells) the concentration of tetanus antibody in cord blood was 18% (95% CI 12, 26) of that found in maternal blood, compared to 23% (95% CI 14, 34) with light infection (< 35 parasites per 200 white cells) and 82% (95% CI 57, 121) with no infection (42). It was reported that 10% of babies born with heavily infected placentas failed to acquire protective concentrations of tetanus antibody despite adequate maternal concentrations. In the current study, based on serological criteria defined by the ELISA manufacturer (Ridascreen, Biopharm GmbH, Germany), 10% of cord sera (95% CI 1, 22) from malaria-infected placentas had ‘negative’ or ‘equivocal’ concentrations of measles antibody, despite ‘positive’ paired maternal titres, compared with 5% (1,11) of those from uninfected placentas but the difference was not significant (p=0.189).

In Malawi, placental malaria was associated with a reduction in placental IgG antibody transfer to S. pneumoniae and to measles to 82% and 81%, respectively. In contrast to the previous study by Brair et al., there was no effect on the transfer of maternal antibodies to tetanus toxoid (43).
In The Gambia, the transfer of maternal antibodies to herpes simplex virus 1, respiratory syncytial virus, and varicella-zoster virus was reduced by placental malaria infection by 69%, 58%, and 55%, respectively (44). In a second publication from the same study, placental malaria was associated with a reduction in the transplacental transfer of measles antibody of 72% (95% CI 67, 84) but again there was no effect on the transfer of tetanus antibodies (5).

In Kenya, there was no overall association between placental malaria infection and the ratio of cord:maternal (CMR) MMA concentration (p=0.15). However, a reduction of 10.0% (95% CI 1.3%–17.9%) was observed in the subset of women with active-chronic malaria infection (45). In a later study by the same group, tetanus antibody levels were reduced by 48% (95% CI, 26, 62) in newborns whose mothers had active-chronic or past placental malaria (46).

The mechanisms by which malaria infection of the placenta impairs materno-fetal transfer of IgG are unknown but presumably reflect the distortion of delicate placental architecture described particularly with chronic and past malaria infection and damage to the cellular machinery of ST. These changes include thickening of the basement membrane and massive infiltrates of inflammatory cells and villitis (47, 48). No studies were identified which quantified the expression of FcRn in placentas with and without malaria infection. Alternatively, hypergammaglobulinaemia resulting from non-specific clonal stimulation of maternal B-cells in the presence of malaria could ‘saturate’ the ST FcRn receptors and thereby impair binding to pathogen-specific modalities. Hypergammaglobulinaemia has been associated with diminished placental antibody transfer in other African studies (5, 43, 44). In the present study maternal total IgG concentrations were typically elevated compared to European values, with a geometric mean titre (GMT) of 27.0 g/l (95% 25.7, 28.4). All of the women were hypergammaglobulinaemic by standard definition (total IgG > 15 g/l). On univariate analysis maternal total IgG and CMR were negatively correlated (p=0.016). However, as in a previous study (43), the effects of placental malaria on placental transfer were independent of the total concentration of maternal IgG.

In the study from Malawi maternal HIV infection was associated with a reduction in IgG antibody transfer to S. pneumoniae to 79% (43). Kenyan infants born to HIV-infected mothers had 35.1% (95% CI, 9.8, 53.2) lower levels of measles antibodies (45) and 52% (95% CI, 30, 67) lower levels of tetanus antibodies (46) than did those born to HIV-uninfected mothers. In contrast to these studies, the present study was
carried out in an urban centre with low HIV-prevalence. A small number of HIV-positive women were excluded in order to avoid possible confounding interactions.

Transplacental IgG transport begins as early as 13-weeks gestation but the majority of IgG is acquired by the fetus during the last month of pregnancy, such that by full term fetal IgG concentration may be 20-30% higher than maternal concentration (40)). In the present study there was a trend for higher cord:maternal transfer ratios (CMR) of MMA with increasing gestational age but as the sample size of babies with known gestational age was small and almost all of these were born at term, this did not reach significance (p=0.068).

Certain subclasses of IgG are transmitted more efficiently than others, with the order usually described as IgG1>IgG4>IgG3>IgG2 (49). The present study did not examine subclasses in the context of MMA which is generally classified as IgG1 (50), however this observation may explain why antibody specificities elicited by some pathogens and vaccines are transmitted less efficiently than others, in particular those to encapsulated bacteria. Differential stimulation of antibody specificities and avidities by wild-type and vaccine-type measles viruses, together with boosting effects of repeated virus exposure, in different populations of women could explain why placental malaria affected MMA transfer in some studies and not others.

In common with previously published data, the present study found no relationship between maternal age, weight and parity and placental antibody transfer.

8.4 Placental malaria and the infant immune system

8.4.1 Placental malaria and susceptibility to measles

Measles remains the leading cause of vaccine-preventable death among infants and young children around the world, estimated to account for 164000 deaths in 2008 (51). MMA provides infants with passive immunity to infection with measles virus in the early months of postnatal life, when the case fatality rate is highest (52). As with maternal antibodies to other antigens, the concentration of MMA declines rapidly after birth and infants become susceptible to measles infection once it is lost. Typically MMA is absent before the end of the first year but the precise timing of this event varies according to the baseline concentration of MMA in neonatal sera at delivery and the rate of its decay during infancy. While decay rates do no seem to
vary much across populations, the baseline concentration of neonatal MMA is primarily dependent on the concentration of maternal MMA during late pregnancy and the efficiency of MMA transfer across the placenta (50).

At a population level, there is circumstantial evidence for earlier loss of MMA in infants in developing countries but the prevalence of MMA varies significantly across infant populations in different countries and socio-economic strata. Infants born to women with vaccine-induced immunity become susceptible to measles at a younger age than those born to women with naturally acquired immunity (53). Maternal serum concentrations of MMA during pregnancy vary considerably across populations, reflecting the boosting effects of natural exposure to measles virus especially in developing countries, and vaccine coverage. The impact of either variable is difficult to discern in isolation and it is unclear whether women in developing countries have higher titres of MMA than women in developed countries. The broader geographic significance of other factors identified in single-country studies, for example age, race, parity, and socioeconomic status, is also unknown. There is no evidence that pregnancy malaria is associated with higher titres of MMA.

Almost all studies carried out in different parts of the world have shown that the geometric mean titres of MMA in cord blood are higher than those in maternal blood at delivery but there is a trend for more efficient transfer in developed countries compared with developing countries (50). This could reflect a greater incidence of preterm delivery in developing countries, as MMA concentration varies directly with gestational age. However, as outlined above, there is substantial evidence that maternal infections such as HIV and malaria interfere with placental transfer of maternal antibodies and this may have important policy implications for when countries choose to vaccinate their children.

As well as providing short-term protection against infant infections, transplacentally-acquired IgG impacts on infant vaccine responses and so helps determines the optimal age for immunisation. If vaccines are given too early, higher circulating levels of maternal IgG in infant serum interfere with seroconversion, diminishing vaccine effectiveness. If vaccines are given too late, when circulating maternal antibodies have declined to sub-protective levels, there may be a period when infants are left vulnerable to early-onset infection. In the case of measles, the optimal age for immunisation is therefore a balance of risks between the probability of seroconversion in response to vaccination at any given age and the probability of acquiring measles infection before that age.
Most seroprevalence profiles revealed a nadir in the prevalence of antibody to measles virus at age <9 months. All other things being equal, this finding tends to support the current WHO strategy of measles vaccination at age 9 months in countries with a high burden of mortality from measles (52). Measles vaccination according to this schedule is administered in many developing countries and between 2000 and 2007, deaths from measles fell by 74% worldwide (51). However, epidemic measles is re-emerging in countries with low incidence and two dose vaccination programmes in place, as shown by recent outbreaks (54, 55). The coverage of universal immunisation programmes influences the concentration of maternal MMA during pregnancy: a higher coverage reduces the probability of natural boosting from measles infection. The mean age at first pregnancy is increasing in many countries, as a function of improved education of girls, and the time since maternal measles vaccination is increasing with it. These factors may serve to reduce the baseline concentration of MMA in neonatal sera and as countries improve control of measles transmission at a population level, the period of highest risk may shift from childhood to infancy. A recent study in Belgium described a very early susceptibility to measles in a cohort of 207 infants such that by 6-months of age; more than 99% of infants of vaccinated women and 95% of infants of naturally immune women had lost maternal antibodies (56). In the Tropics, this process will be exacerbated by maternal infections in pregnancy such as malaria, which impair transmission of already diminished concentrations MMA across the placenta to the fetus.

Public health authorities should be aware of and monitor the possibility of early loss of maternal protection against measles in their own populations. Clinical recognition of cases of measles in young infants is important for surveillance of the disease. This is also important for decision-making on individual infants presenting with febrile illness in endemic areas and supports ongoing research on early vaccination. Most importantly, it confirms that the timely administration of the first dose of measles vaccine is a critical fixture in infant health in developing countries and that malaria control during pregnancy may have far-reaching benefits for children in addition to improved birth weight.

8.4.2 Placental malaria and cellular immune responses to vaccination in infancy

Despite improvements in vaccine coverage, vaccine-preventable diseases still kill up to 2 million African children each year (57). These deaths highlight inadequate vaccine-efficacy in resource poor settings. Failure to respond to vaccination in developing countries is frequently associated with markers of severe poverty, including malnutrition, anaemia and chronic parasitic infection. The inter-
relationships between these factors are complex and multifactorial but there is clinical evidence emerging that chronic antenatal parasitic infections may significantly alter infant immune responses to standard childhood vaccinations (58).

In sub-Saharan Africa, a quarter of women have evidence of malaria infection in the placenta. If untreated, these chronic infections can persist throughout the third trimester of pregnancy and as a consequence, soluble malaria antigens cross the placenta, priming or tolerising the fetal immune system. Evidence to support this theory includes several reports of T- and B-cell responses to crude schizont extracts and blood-stage antigens in cord blood lymphocytes, a cell population that represents circulating fetal lymphocytes present at the time of birth (59-63) [6–9]. It is postulated that such exposure in utero exacerbates the Th2-biased, IL10-mediated, immuno-tolerant phenotype of infancy, and affects the development of Th1-biased protective responses to antigens included in the childhood vaccination schedule, and increases susceptibility to infectious diseases (64).

Infant immune tolerance in placental malaria may arise through three basic pathways. It may be due to the loss of clones of specific antigen-reactive memory cells in utero and subsequent failure to recall such antigens later in infancy. Secondly, clonal anergy may result from inadequate costimulation of CD4+ T-cells by antigen-presenting cells (APC) because of down-regulation of major histocompatibility complex (MHC) receptors. Finally, prenatal exposure to malaria or other parasites has been reported to lead to the creation of a distinct subset of immunoregulatory CD4+CD25+ T-cells (T_{reg}) (65) arising from CD34+ stem cells in response to stimulation with IL10, TGFβ and IFNα (66, 67). T_{reg} cells are antigen-specific and produce quantities of IL10, IFNα, TGFβ and IL5 but little IL2 and no IL4.

Imprinting of the fetal immune system may also play a significant role in infant infectious disease susceptibility and vaccine responses. It has recently been shown that maternal micronutrient supplementation during early pregnancy is associated with extensive gender-dependent methylation changes across the human genome, including several genes associated with immune responses (68). It is thought that these effects are mediated by the availability of methyl donors such as folate in the uterine environment in early pregnancy. Similarly, disturbances in folate metabolism are likely to be impacted by placental malaria from early in pregnancy (69). There are no published studies of epigenetic modification of the fetal genome in the context of placental malaria.
Whatever the mechanism, immunomodulation may limit the inflammation of chronic parasite infections in the placenta but cause a concomitant reduction in the ability to respond to acute bacterial infections and to vaccination in later childhood. This position is consistent with studies of malaria in children, showing reduced antibody responses to vaccination with bacterial polysaccharide, glycoconjugate and protein antigens (70). However there is little comparable direct clinical evidence for such effects in placental malaria.

It is well-recognised that the effectiveness of Bacille Calmette-Guérin (BCG) vaccine for tuberculosis is reduced in tropical countries (71). Although the aetiology of this observation is likely to be multifactorial, defects in IFNγ signalling are strongly predictive for tuberculosis and it is therefore likely that T-cell responses to BCG play an important part in generating immunity to tuberculosis (72-74). In another Gambian study, placental malaria predicted a weak IFNγ response to tuberculin purified protein derivative (PPD) at 12-months in infants who had received BCG at birth; few children in the cohort had presented with malaria in the intervening period (75).

The numbers in this latter study were too small to analyse for the specific effects of acute, chronic and past placental malaria, however a previous study in The Gambia, showed that active and past malaria infections were associated with different immune profiles in neonates (65). Culture of CBMC exposed to malarial antigens in utero resulted in the expansion of malaria-specific FOXP3+ Treg and more generalized FOXP3+ CD4+ Treg in chronic and resolved placental malaria, but increased Th1 pro-inflammatory responses (IFNγ, TNFα, IFNγ:IL10) in resolved placental malaria infection. These observations clearly demonstrate that exposure to malarial antigens in fetal life impacts on the immune environment at birth, with a regulatory response dominating in neonates born in the context of chronic malaria, while those with resolved infection produce both regulatory and inflammatory responses.

8.4.3 Placental malaria and risk of malaria in offspring

Although cord parasitaemia is relatively common, clinical malaria is rare in neonates born in highly endemic areas (76). The reasons for this are incompletely understood but may involve the protective effects of variant-specific and other antibodies, for example to merozoite surface protein-1, which are transmitted across the placenta (77). Whatever the mechanism, such protection is relatively short-lived because epidemiological studies demonstrate that placental malaria and high titres of anti-pregnancy-associated malaria antibodies in cord blood predict enhanced susceptibility to malaria from 6-months of age (78, 79). In a recent epidemiological study, infants
born with placental malaria had a 2.13 fold increased risk of malaria infection during the first year than those born from a non-infected placenta, when sleeping in a house with an insecticide-treated bednet (80).

The basis for the modified susceptibility to postnatal malaria of infants born with placental malaria is not yet clear. Uterine exposure to malaria antigens may induce fetal immune tolerance to malaria through the mechanisms described above. In Kenya, 90% of babies born with placental malaria failed to generate Th1-type cytokine responses, compared with approximately 50% of other children, and the risk of future infection tended to be higher in this immune tolerant group (81). T-cells from such children tended to produce more malaria-driven IL10, an effect which persisted throughout childhood, suggesting the presence of T regulatory cells specific for malaria blood-stage antigens. In other studies, placental malaria was also shown to impact fetal dendritic cell maturation (82), toll-like receptor ligand–induced cytokine responses (83) and Vγ2 repertoire of γδT-cells (84).

The failure to develop a robust Th1-based cytokine profile in response to malaria in utero may have long-lasting implications, including inadequate T-cell support for antibody production and/or antibody-dependent cell mediated immunity. However, there is likely to be a range of fetal immune responses elicited by placental malaria and not all infants will develop the fully tolerant immune phenotype described above. The precise position on this spectrum may depend on the type and amount of malaria antigen exposure, and when exposure occurs during gestation.

**8.4.4 Conclusions**

The causes of reduced vaccine efficacy and increased susceptibility to infectious diseases in infancy are multifactorial but there is increasing evidence that parasitic infections during pregnancy and childhood are implicated. The effects of intermittent preventative treatment of placental malaria on fetal immune modulation are difficult to predict and further research specifically on the role of optimal malaria control in pregnancy on infant immune responses is indicated. Such research may include randomised controlled trials of improved chemoprophylaxis during pregnancy but ultimately a vaccine for malaria during pregnancy is likely to be introduced (2). It will be important to establish the optimal timing of such a vaccine in order to minimise the deleterious effects of pregnancy malaria on fetal immune development.
The role of late pregnancy antimalarial treatment to clear placental malaria at the time of delivery should be assessed, as improved control of late pregnancy malaria might enhance infant immune response characteristics. This is particularly relevant as placental malaria has been clearly associated with increased infant malaria risk, anaemia and morbidity.

The deleterious effects of multiple parasite infections during childhood on host immunity also warrant further study. It has been shown that chronic parasitic infections have a substantial impact on child cognitive and intellectual development and educational performance, effects which can be reversed with appropriate antiparasitic treatment (85) (86-88). It is unclear what effects such treatment may have on infant vaccine responses but experimental studies have shown that parasitic infections in childhood lead to decreased vaccine efficacy and an inability to resist new infection (89-92). Multiple and concurrent infections are likely to have additive or synergistic effects on immune responses. Prospective studies are needed to assess the duration of these effects.
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