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INVITED REVIEW

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Abstract

Malaria causes tremendous early childhood morbidity and mortality, providing an urgent impetus for the development of a vaccine that is effective in neonates. However, the infant immune response to malaria may be influenced by events that occur well before birth. Placental malaria infection complicates one quarter of all pregnancies in Africa and frequently results in exposure of the fetus to malaria antigens *in utero*, while the immune system is still developing. Some data suggest that *in utero* exposure to malaria may induce immunologic tolerance that interferes with the development of protective immunity during childhood. More recently, however, a growing body of evidence suggests that fetal malaria exposure can prime highly functional malaria-specific T- and B-cells, which may contribute to postnatal protection from malaria. *In utero* exposure to malaria also impacts the activation and maturation of fetal antigen presenting cells and innate lymphocytes, which could have implications for global immunity in the infant. Here, we review recent advances in our understanding of how various components of the fetal immune system are altered by *in utero* exposure to malaria, discuss factors that may tilt the critical balance between tolerance and adaptive immunity, and consider the implications of these findings for malaria prevention strategies.

KEYWORDS
fetal, immune development, placental malaria, pregnancy, T-cells, tolerance

1 | INTRODUCTION

Malaria caused by *Plasmodium falciparum* is a leading killer of infants and children. Efforts to develop a protective vaccine have been hindered by the parasite's numerous evasion strategies, which include the induction of immunoregulatory mechanisms in the human host. Indeed, several promising vaccine candidates that induce robust protection in malaria-naive populations have been shown to have much lower efficacy when administered to individuals residing in endemic settings,[1,2] suggesting that immunomodulatory mechanisms induced by prior malaria exposure may pose a critical barrier to vaccine-mediated protection. In endemic regions, many individuals are first exposed to malaria *in utero*, when *P. falciparum*-infected red blood cells sequester in the placenta and parasite antigens cross the syncytiotrophoblast barrier, gaining entry into the fetal circulation. Therefore, even during the neonatal period, vaccination may be influenced by prior malaria exposure. Here, we examine the consequences of *in utero* antigen exposure for fetal immune development and postnatal immunity.

The worldwide burden posed by malaria-related complications of pregnancy is profound. More than 125 million pregnancies occur annually in regions at risk for malaria transmission,[3] and one in four pregnant women in sub-Saharan Africa have evidence of infection with malaria at parturition.[4] Although most placental malaria (PM) infections are attributable to *P. falciparum*, recent evidence indicates that *P. vivax* is also associated with poor pregnancy outcomes, and some data suggest that it too may sequester in the placenta.[5,6] Pregnancy-associated malaria results in tremendous obstetrical and pediatric morbidity,

including maternal anemia, intrauterine growth retardation, low birth weight, prematurity, miscarriage, and stillbirth, and it has been estimated to contribute to 100 000 infant deaths per year.[4] While the obstetric complications of PM have been studied extensively, it remains unclear whether prenatal exposure to malaria has a lasting detrimental impact on the infant. Notably, several observational studies have reported that infants born to women with PM are themselves at higher risk of malaria during early life,[7-14] and may even have increased vulnerability to non-malarial febrile illnesses.[15]

In this review, we summarize recent advances in our understanding of how the fetal immune system responds to malaria antigens encountered as a result of *P. falciparum* infection during pregnancy. A number of recent studies have shed light on how the infant's immune response is shaped by malaria antigen exposure during fetal life. Some evidence suggests that *in utero* malaria exposure results in the development of tolerance, which could contribute to poor immunity to malaria in early life.[11,16,17] However, other recent studies have found evidence that the fetus, even before birth, mounts a functional adaptive immune response to malaria antigens that may protect against postnatal infection.[18-21] We will highlight knowledge gaps in our understanding of human fetal immune ontogeny, the functional competence of fetal T-cells and antigen-presenting cells (APCs), and factors governing effector vs regulatory T-cell differentiation *in utero*. Finally, we will consider the implications of these findings for the development of immunity to malaria and other pathogens during childhood, and how malaria control interventions may—favorably or adversely—impact this process.

2 | MALARIA IN PREGNANCY

To understand how maternal infection with *P. falciparum* may impact the infant *in utero* and beyond, it is important to consider the immunopathologic mechanisms by which malaria parasites invade, sequester, and induce local inflammation in the placenta, and how the timing of these events intersects with critical junctures in fetal immune development (Figure 1). Here, we discuss the biology of PM, as well as host factors associated with increased vulnerability to PM and potential avenues for intervention and prevention.

2.1 | The biology and histopathology of placental malaria

Placental malaria occurs when *P. falciparum*-infected erythrocytes adhere to placental receptors and sequester within the intervillous
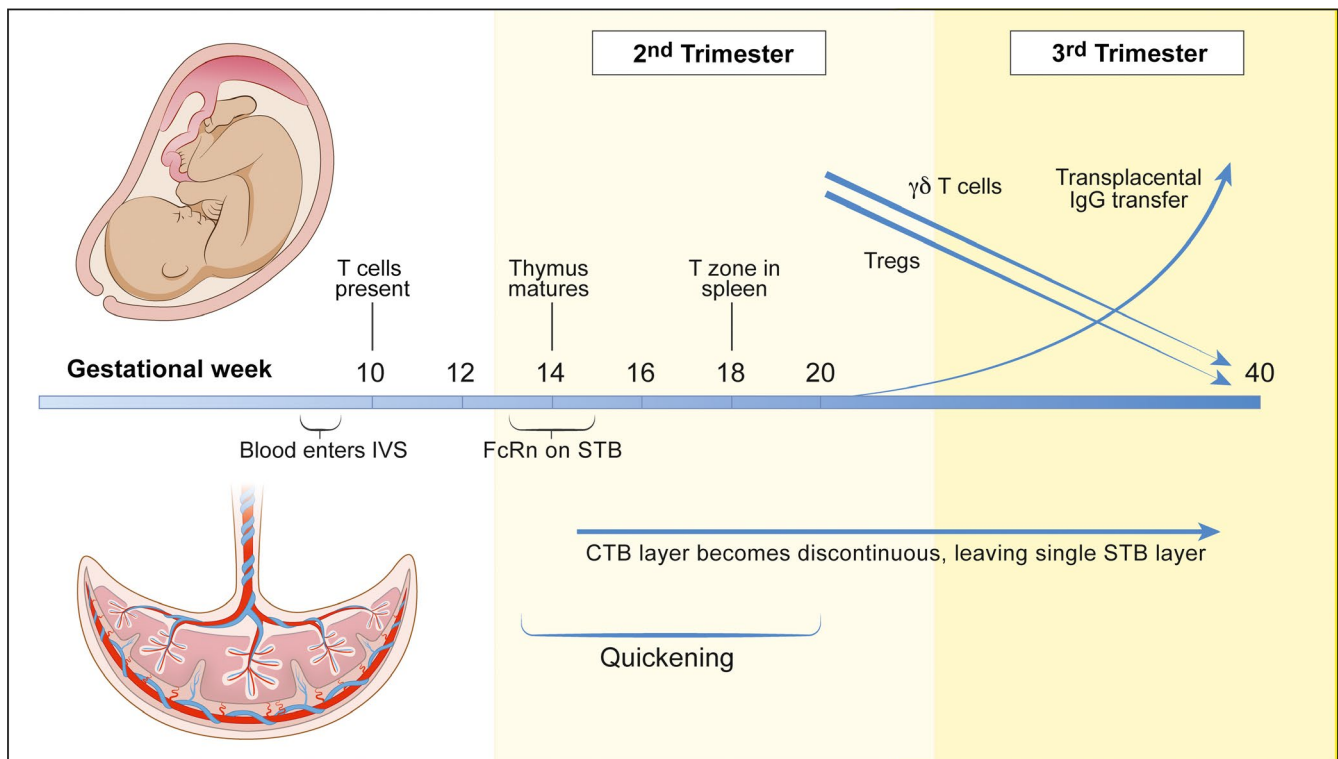

FIGURE 1 Developmental changes in the placenta and fetal immune system during gestation. Maternal blood first enters the intervillous space at 8-9 wks gestation, enabling *Plasmodium falciparum*-infected red blood cells to contact the placenta. This is followed by progressive loss of the cytotrophoblast layer beginning at week 14, leaving the fused syncytiotrophoblast (STB) layer as the sole barrier between maternal and fetal blood. At 13-16 wks gestation, STB cells begin expressing FcRn, which transports IgG and immune complexed antigens across the placenta. Transplacental transfer of IgG increases exponentially until term. Meanwhile, T-cells are evident in the fetus as early as week 10 of gestation, thymic architecture is mature at week 14, and the splenic T zone is evident at week 18. Fetal Foxp3+ CD4 Tregs and γδ T-cells are most abundant in the fetus at mid-gestation, but decline progressively between weeks 20-40

spaces of the placenta. The presence of infected erythrocytes stimulates maternal mononuclear cells to secrete β-chemokines that are chemotactic for maternal monocytes and macrophages (MIP1α, MIP1β, IP10, and MCP1), resulting in variable degrees of inflammation. Histologically, PM is characterized by breakdown of syncytiotrophoblast layer, with focal denudement; infiltration of maternal monocytes; sequestration of parasitized erythrocytes; and deposition of hemozoin and other erythrocyte breakdown products that are themselves toxic and inflammatory.[22] Rogerson et al proposed three histologic categories of PM: "active-acute" in which parasites are detected but there is minimal or no deposition of pigment or fibrin; "active-chronic" in which both parasites and substantial pigment and perivillous fibrin are seen; and "past" or resolved infection in which pigment is observed, but no parasites are detected.[23] Of note, this categorization does not consider the degree of placental inflammation, which, along with the chronicity of infection has been found to correlate with the risk of fetal growth restriction.[23,24]

2.2 | Risk factors and timing of placental infection

The risk of malaria is higher in pregnant women than in non-pregnant adults, and even women who have acquired substantial immunity to malaria following repeated life-long exposure become susceptible again once pregnant. Among pregnant women, primagravidity is the strongest host risk factor for PM. The decline in the risk of PM in subsequent pregnancies is believed to be due to the acquisition of antibodies that block the adhesion of parasitized RBCs to chondroitin sulfate A and other adhesion molecules expressed in the placenta,[22] as recently reviewed by Fried et al[25] The conserved parasite gene *var2csa* encodes a pregnancy-specific adhesion ligand that is thought to be an important mediator of placental adherence. In some studies, antibodies against *var2csa* have been shown to correlate with protection from PM, and this antigen is now the target of a major PM vaccine development effort.[25] There are, however, additional gravidity-associated differences in the immunology of the fetal-maternal interface, including activation of macrophages and "trained memory" of uterine NK cells, which could also theoretically contribute to the reduced risk of PM infection seen in multigravid women.[26,27]

The earliest window of vulnerability to PM has not been definitively established. It is known that the placenta can become infected by 9-12 weeks gestation and perhaps even earlier, when maternal blood first enters the intervillous space and begins to perfuse the placenta at 8-9 weeks gestation.[28-30] A recent modeling study used placental histopathology results, parity, and transmission intensity data to estimate that 63% of infections become established by the end of the first trimester, with the highest risk occurring at the end of the third month.[31] The risk of PM correlates with the frequency and parasite density of maternal *P. falciparum* infections during pregnancy, but even low-density infections (ie those that are detectable by sensitive PCR or LAMP tests but not blood smear) can result in placental infection.[32] Importantly, many maternal infections that result in PM are clinically silent.[33,34] This poses a barrier to accurate

determination of the timing of infection, as well as a practical impediment to treatment and prevention strategies.

2.3 | Strategies for prevention of placental malaria

In low resource settings, antenatal care is often not established until after "quickening," the onset of perceptible fetal movements, which typically occurs at 13-20 weeks gestation. Primagravidas, who are at highest risk for PM, tend to experience quickening later in gestation than multigravidas. Thus, PM infection is often already established by the time a woman presents for prenatal care, making intervention challenging.[5,34] Currently, WHO recommends intermittent presumptive treatment (IPTp) of pregnant women in malaria-endemic regions with sulfadoxine-pyrimethamine (SP), which is to be given at least three times during pregnancy. However, the efficacy of SP is limited by the fact that in some regions, particularly East Africa, antifolate resistance is nearly universal among circulating *P. falciparum* strains. To address this, recent trials have tested the efficacy of other antimalarial drugs, such as dihydroartemisinin-piperaquine (DP), for use as IPTp.[35,36] DP is an artemisinin combination therapy (ACT) that combines the rapid killing of dihydroartemisinin with a prolonged postadministration prophylactic effect due to the long half-life of piperaquine, such that monthly administration provides nearly continuous prophylaxis against reinfection. In a study conducted in Uganda, Kakuru et al found that DP had superior efficacy to SP in preventing maternal parasitemia, symptomatic malaria, and PM, and when given monthly DP reduced the rate of adverse birth outcomes.[35] DP therefore represents an attractive alternative for IPTp in areas of high antifolate resistance. However, the long-term utility of the artemisinin drug class for IPTp is threatened by the emergence of artemisinin-resistant *P. falciparum* strains, which are now increasingly prevalent in Southeast Asia.[37] Moreover, regardless of the antimalarial drug used, the efficacy of IPTp as a strategy is limited by the fact that vulnerability to PM begins in the first trimester, when few women are enrolled in prenatal care. Concerns for teratogenicity further limit antimalarial drug options in the first trimester. For all of these reasons, alternate strategies, including maternal vaccination and vector control approaches, will likely be needed to reduce the risk of placental infection in early pregnancy.

3 | MALARIA ANTIGEN EXPOSURE *IN UTERO*

True congenital malaria—in which intact parasites cross into the fetal circulation and establish a self-propagating blood stage infection—has been clearly documented, but it is rare.[38] Nonetheless, several lines of evidence indicate that malaria antigens gain access to the fetal circulation after crossing the placenta.[39-42] Exactly how they do this is not entirely clear. The placenta is an effective physical barrier that protects the fetus against many pathogens (Figure 2).[43] The syncytiotrophoblast layer, comprised of fused multinucleated cells that line the chorionic villi and directly contact maternal blood,
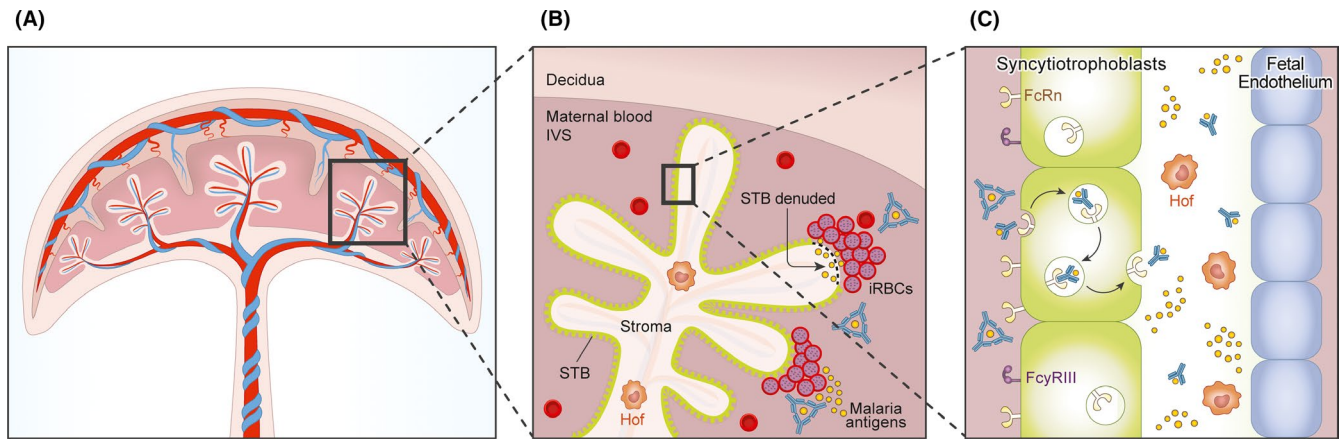
FIGURE 2 The maternal-fetal interface and potential routes of antigen transfer. A, Anatomically, the human placenta is comprised of tree-like chorionic villi that directly contact maternal blood in the intervillous space. B, Chorionic villi are lined by a single layer of multinucleated syncytiotrophoblast (STB) cells. *Plasmodium falciparum*-infected red blood cells (iRBCs) adhere to syncytiotrophoblasts, and attract inflammatory mediators which may lead to focal STB denudement (dotted line). This disruption of the STB barrier may cause free malaria antigens to contact the villous stroma, which is patrolled by fetal Hofbauer cells (Hof). C, FcRn mediates transfer of IgG, and possibly immune complexes, across the STB layer to the fetus. FcγRIII may also contribute to antigen internalization

lacks intercellular junctions and has a dense actin cytoskeleton network that confers elasticity and contributes to its physical barrier function.[44,45] Direct contact between maternal blood and syncytiotrophoblasts does not occur until the end of the first trimester, when the extra-villous trophoblast plug erodes, allowing maternal blood to flood the intervillous space. During the first trimester, a second cell layer comprised of cytotrophoblasts underlies the syncytiotrophoblast layer, but this degenerates as the placenta grows, leaving only the single syncytiotrophoblast layer separating the maternal and fetal blood.[28] While low molecular weight compounds (<500 Da) can simply diffuse across this placental barrier, larger compounds are generally prevented from traversing an intact placenta in the absence of an active transport mechanism.[46] At least some malaria antigens appear to be transported across the placenta as immune complexes,[19,39] which are ferried across the syncytiotrophoblast cell layer by FcRn, the neonatal Fc receptor. FcRn is expressed by syncytiotrophoblast cells and acts as a pH-dependent shuttle. Syncytiotrophoblast cells also express FcγRIIIa, which may play a role in antigen uptake from the intervillous space.[28,47] In addition to receptor-mediated transport, malaria antigens may access the fetal circulation more directly, as inflammatory PM can cause disruption of placental barrier function, including focal denudement and necrosis of the syncytiotrophoblast layer,[22] bringing the stromal core of villi in direct contact with maternal blood. This may permit larger molecules or even cells to enter into the fetal circulation.[42,48] Once across the syncytiotrophoblast barrier, it is unclear how exogenous antigens traverse the fetal endothelium, which represents the last barrier to entry into the fetal bloodstream, although FcgRI on endothelial cells could play a role. Presumably, malaria antigens and/or immune complexes are subsequently taken up by fetal APCs, although this process has received little or no research attention. Fetal macrophages termed Hofbauer cells reside within the villous stroma and are increased in PM, but their functional role has not been well

characterized.[49] Two key distinctions between transplacental exposure to malaria antigens and that which occurs with natural malaria are that the fetus is exposed only to blood stage antigens (as pre-erythrocytic stage parasites are not present in the placenta), and fetal exposure is likely of "low dose," contrasting with the extremely high antigen burden characteristic of malaria.

In addition to malaria antigens, PM may result in exposure of the fetus to cytokines, microbial products and metabolites, and other inflammatory mediators that could impact the fetal immune system. Placental perfusion studies indicate that cytokines themselves generally cannot cross the term placenta,[50] although their levels are often similar between maternal and cord blood at delivery, suggesting that there may be some coordinate regulation.[51] Syncytiotrophoblast cells themselves produce type III interferons and chemokines.[28] Local inflammation in the placenta recruits maternal macrophages, monocytes, and lymphocytes and leads to their activation, and such inflammation is associated with placental insufficiency even in the absence of direct pathogen invasion. Perhaps the most critical consequence of inflammation at the fetal-maternal interface is an increase in the risk of preterm birth, a key complication of PM.[5,52] In women with PM, levels of cytokines and chemokines including CXCL9, IFNγ, IL-10, and IL-1β have been associated with poor pregnancy outcomes including fetal loss, low birthweight, and preterm delivery.[53] It is likely that transfer of immune-modulating factors into the fetal circulation may have additional, currently unknown, influences on fetal immune development.

The gestational timing of *in utero* antigen exposure may play a large role in determining its impact on fetal immunity. In individual cases, it is not usually possible to discern the timing of fetal malaria exposure using non-invasive testing methods. In clinical trials, a variety of diagnostic methods, varying in sensitivity, have been used to define PM. These include histopathologic examination of the placenta and testing of placental intervillous blood obtained at delivery by blood smear,

antigen-based rapid diagnostic tests (RDTs), or more sensitive nucleic acid amplification tests (PCR and LAMP). The presence of visible parasites, parasite antigens, and/or plasmodial DNA in placental blood generally indicates an infection that is "active" at the time of delivery. Placental histopathology is frequently positive in cases in which placental blood testing is negative; however, this is not merely an issue of sensitivity, but also reflects the timing and chronicity of infection. Visualization of malaria pigment (hemozoin) and fibrin on placental histopathology indicates an infection that is chronic or one that occurred earlier in gestation but has since resolved, either spontaneously or due to drug treatment, including IPTp. Hence examination of the placenta offers a cumulative record of infections that occurred over the entire course of pregnancy, and when used in combination with sensitive PCR or LAMP testing of placental blood, provides the most complete picture of the infant's *in utero* exposure. It is not surprising, given the dynamic changes in the fetal immune system that occur during gestation, that the timing and chronicity of antigen exposure might influence the fetal response. As discussed in detail below, a great deal of evidence indicates that chronic, active, and resolved placental infections may differ in their impact on fetal immune cell populations.

4 | PLACENTAL MALARIA AND MATERNAL MICROCHIMERISM

In addition to foreign antigens, it is increasingly appreciated that substantial numbers of maternal cells can enter the fetal circulation, even during normal pregnancy. These maternal cells establish a variable degree of microchimerism in the fetus that persists throughout life. As "non-self," these semi-allogeneic maternal cells play an important role in the development of fetal tolerance to non-inherited maternal alleles (NIMA) by inducing NIMA-specific T regulatory cells (T$_{regs}$) that persist and remain functionally suppressive into adulthood.[54] PM has been associated with higher levels of maternal microchimerism in cord blood, particularly when placental histopathology shows evidence of significant inflammation.[48] The short- and long-term consequences of this maternal microchimerism for infant immunity have not yet been fully explored, but increased induction of T$_{regs}$ during gestation could lead to dampened pathogen-specific immunity. It has also been noted that cord blood immune cell populations, including T-cells, B-cells, NK cells, and monocytes frequently include cells of maternal origin, which are particularly enriched among memory T-cells.[55] Although unproven, this raises the possibility that maternal-origin immune effector cells may directly contribute to the fetal immune defense against malaria and other pathogens.

5 | THE FETAL IMMUNE SYSTEM: POISED TOWARD TOLERANCE BUT READY FOR ACTION

In order to understand the impact of malaria antigen exposure *in utero*, it is important to consider the ontology of the fetal immune system and the functional capabilities of its constituent cells, which evolve rapidly over the course of gestation. It has been known since Medawar's classic transplantation experiments of the 1950s that the fetus is uniquely disposed toward the induction of tolerance upon encounter with foreign antigens.[56] This predisposition arises from the need for reciprocal immune tolerance between a mother and her semi-allogeneic fetus, in order to avoid inflammatory responses to non-self-antigens that could trigger labor and threaten the pregnancy. Much of our mechanistic understanding of fetal tolerance derives from murine models. However, fetal immune development differs markedly between humans and mice. Mice are profoundly immunodeficient at birth, with most T-cell development occurring postnatally; for instance, murine T-cells immigrate from the thymus only after birth. During human gestation, lymphocytes bearing phenotypic markers of mature T-cells can be detected in the fetal liver during week 10 of gestation,[57] fetal thymic architecture is mature in appearance by week 14-16, and T-cell zones can be seen in the spleen during week 18. As in mice, human fetal T-cells exhibit a propensity toward tolerance, but it has become increasingly evident that under appropriate conditions, they are also capable of mounting a highly functional adaptive immune response.[58-61] Thus, rather than viewing the neonatal immune system as deficient or immature, it is appropriate to view it as a distinct entity with unique functional attributes that have evolved to meet the challenges of intrauterine life. As the fetus progresses toward term gestation, it must balance the competing demands of maternal tolerance with the need to mount effector T-cell responses against pathogens encountered after birth.

At the time of birth, the vast majority of T-cells in the human infant is naive in phenotype. While it had long been believed that the intrauterine environment is sterile, it is increasingly clear that this is not true in all cases, as demonstrated by a high prevalence of bacterial DNA and other microbial products in amniotic fluid.[62] Upon encounter with non-self-antigens, naive fetal CD4$^+$ T-cells preferentially differentiate into FoxP3$^+$ regulatory T-cells (T$_{regs}$), which suppress T-cell activation and production of inflammatory cytokines.[54] T$_{regs}$ are particularly abundant in the human fetus during mid-gestation.[63] Fetal CD4 T-cells have an intrinsic bias away from CD4 Th1 differentiation and inflammatory cytokine production *in utero* that is due in part to their epigenetic programming, including hypermethylation of *IFNG* locus.[64] Priming of inflammatory T-cell responses is further inhibited by extrinsic factors, such as immaturity of fetal antigen-presenting cell (APC) function and the presence of regulatory cell inhibition. Studies in both mice and humans suggest that during fetal life, APCs are relatively inefficient in their ability to prime adaptive immune responses.[65-67] This is likely related to their reduced expression of MHC II and costimulatory molecules and lower production of Th1-polarizing cytokines, relative to their adult counterparts. In particular, neonatal dendritic cells (DCs) produce much lower quantities of IL-12, the key cytokine required for Th1 polarization, which has been linked to epigenetic regulation of its p35 subunit.[65,67-69] Elevated expression of arginase-2 by fetal DCs has been shown to inhibit T-cell production of TNFα.[70] Monocytes in the fetus do not efficiently upregulate costimulatory and antigen

presentation machinery in response to IFNγ, as adult monocytes do. Lastly, in addition to suppressive FoxP3⁺ T_regs, other regulatory cell populations that are unique to the neonate, including CD71⁺ erythroid cells,[71] myeloid-derived suppressor cells,[72] and B regulatory cells,[73] have been reported to actively suppress T-cell activation and cytokine production. Together, these mechanisms suppress inflammation in response to maternal antigens, but also present a barrier to mounting an effective adaptive T and B-cell response to pathogens in the intrauterine environment.

As pregnancy progresses, the fetal immune system gradually evolves from one that is skewed toward tolerance to one that is poised to fight foreign pathogens. Over the past decade, it has become increasingly clear that the fetus is capable of mounting robust T-cell responses under certain conditions. Fetal CD4 and CD8 T-cells that are reactive to maternal alloantigens can become activated and secrete Th1 cytokines (TNFα and IFNγ) that stimulate uterine contractions, contributing to preterm labor.[28] Inflammation at the maternal-fetal interface may be the initial signal which triggers the maturation of fetal DCs and subsequent activation of fetal T-cells. Zhang et al demonstrated that human fetal CD4⁺ effector-memory T-cells that are capable of producing both Th1 and Th2 cytokines develop during healthy pregnancy, even in the absence of known intrauterine pathogen exposure.[27] Among infants congenitally infected with viruses such as CMV, virus-specific CD4 and CD8 cells that produce inflammatory cytokines can often be detected in the cord blood.[58,61] Indeed, tetramer studies have demonstrated that in some fetuses, virus-specific T-cells are primed *in utero* by maternal influenza vaccination during pregnancy.[74] These data make it clear that the essential machinery for generation of robust T-cell response to pathogen-derived antigens is present during fetal life. A better understanding of the conditions that foster the priming and development of functionally competent pathogen-specific T-cells will be of fundamental importance for the development of vaccines that are immunogenic in infancy.

6 | WHAT HAPPENS WHEN THE FETAL IMMUNE SYSTEM "SEES" MALARIA ANTIGENS?

6.1 | Cord blood cytokine production

A number of studies have identified differences in cytokine production by cord blood cells following stimulation with malaria antigens, mitogens, and TLR agonists. In aggregate, findings from these studies strongly indicate that exposure to malaria *in utero* alters the fetal immune system. However, many of these studies relied upon measurement of cytokines in culture supernatants following bulk stimulation, which precludes identification of the precise immune cell populations (eg αβ T-cells, γδ T-cells, NK cells, or APCs) and pathways that are impacted by malaria exposure. Furthermore, substantial heterogeneity in cytokine production has been observed, both within and among cohorts. Malaria antigen stimulation has been shown to induce in vitro production of both Th1 and Th2 cytokines,

with statistical differences between exposed and unexposed infants reported in a few but not all studies.[11,17,21,75,76] Several studies have concluded that cytokine production by cord blood cells is modified both by maternal gravidity and/or by the timing, duration, and chronicity of placental infection,[16,17,75,77-79] which may account for some of the observed variability among published findings. There is remarkably little published evidence for differences in cord blood plasma cytokine levels between exposed and unexposed infants, and our own data indicate that these differences are minimal (unpublished observations). Natama et al reported that whole cord blood samples from placental malaria-exposed infants exhibit lower spontaneous production of numerous pro-inflammatory and anti-inflammatory cytokines, but higher production of many of the same cytokines in response to TLR7/8 stimulation, suggesting that the innate immune response is programmed in the fetus before birth.[16] However, as frequencies of individual cellular populations were not assessed, it is not clear whether the observed differences were due to an impact of malaria exposure on immune cell function, or merely reflect differing frequencies of constituent immune cell populations in the cord blood of exposed infants.[16]

6.2 | Fetal antigen presenting cells

Antigen-presenting cells, including DCs and monocytes, are key orchestrators of the immune response, influencing the development of both immunological memory and tolerance. DCs play a paramount role in the initiation and regulation of adaptive immune responses through priming of antigen-specific CD4⁺ T-cells. *Plasmodium falciparum* has been demonstrated to modulate dendritic cell function and maturation both in vivo and in vitro, leading to decreased expression of maturation, cell adhesion and costimulatory markers.[77,80] The resulting immature, or "tolerized" DCs have a diminished capacity to activate effector T-cells.[80] However, this effect appears to require intact parasite-infected erythrocytes, which are not frequently observed in fetal blood.[80] It is therefore unclear whether the tolerizing impact of *Plasmodium* on DCs is relevant in the fetus, in whom DCs already have an intrinsically higher threshold for activation. Another question that merits further investigation is whether distinct antigen presenting cell populations may be important during fetal life. For instance, γδ T-cells are particularly abundant in the fetus and possess robust antigen presentation capabilities upon activation,[81,82] suggesting that they could play a role in the induction of adaptive immune responses *in utero*.

The impact of malaria antigen exposure on professional APCs in the fetus has been directly assessed in only a few studies. We and others have observed that frequencies of myeloid DCs (mDCs), but not plasmacytoid DCs, are markedly elevated in the cord blood of malaria-exposed infants.[83,84] The functional competence of mDCs and other APCs in the uptake, processing, and presentation of malaria antigens to T-cells *in utero* is not fully understood. Data regarding the degree to which fetal mDC mature and upregulate costimulatory molecules in response to PM are somewhat mixed, and suggest that the duration or persistence of parasite exposure may

play a modulatory role. For instance, it has been reported that infants born to mothers with active PM do not exhibit significant mDC upregulation of MHC II,[84] while those with chronic infection upregulate MHC II but not CD86 on cord blood mDCs.[85] Likewise, fetal monocytes appear to upregulate MHC II in past/resolved PM but not in active infection.[77] In the latter study, co-culture of cord blood mononuclear cells with *P. falciparum*-infected RBCs dramatically reduced both MHC I and II expression on monocytes from infants with active PM, while having no impact in infants with past or no infection. Together, these data suggest that the sustained presence of parasites inhibits infant APC maturation through mechanisms that remain unclear. Furthermore, they support the notion that the threshold for activation and maturation of fetal APCs is high, and exposure to transplacentally transferred antigen may be insufficient to drive costimulatory signals needed for efficient antigen presentation function *in utero*. Further investigation of phenotypic and functional features of infant DCs is warranted, given their critical role in priming of neonatal vaccine responses.

6.3 | Regulatory fetal T-cells

Several studies have specifically addressed the hypothesis that fetal exposure to malaria antigens induces the development of regulatory CD4 T-cells, a mechanism by which the fetus could actively acquire peripheral tolerance to malarial antigens.[79] Two major populations of regulatory CD4 T-cells are induced by malaria and play potentially important roles in its immunopathogenesis: FoxP3^{+} Treg cells, and IL10-producing CD4 cells of the Tr1 phenotype. One of the earliest studies to hypothesize a role for regulatory T-cell induction *in utero* reported that frequencies of T_{regs} are higher among infants born to women with PM.[79] However, this study was performed before the role of FoxP3 was appreciated, and defined T_{regs} based on elevated expression of the high affinity IL2 receptor alpha chain (CD25^{hi} CD4 T-cells), which might include effector T-cells that express CD25 transiently upon activation. Subsequent studies that used a more comprehensive set of T_{reg} markers have failed to replicate this association of T_{reg} frequencies with PM.[76,83,86] Nonetheless, depletion of cord blood T_{regs} has been shown to augment the malaria-specific IFNγ production in vitro and to abrogate production of the immunoregulatory cytokine IL-10,[76,79,87] indicating that fetal T_{regs} are functionally suppressive. Flanagan et al found that cord blood frequencies of FoxP3^{+} T_{regs} do not differ ex vivo, but differentiation of T-cells into T_{regs} following in vitro antigen stimulation is higher among infants born to women with PM.[86] Another factor that may influence induction of FoxP3^{+} T_{regs} is the timing of malaria exposure relative to fetal immune development. In a longitudinal study of mother-infant pairs followed from 12-20 weeks gestation, we found that cord blood FoxP3^{+} T_{reg} frequencies did not differ between those with and without histopathologic evidence of PM; however, T_{regs} were modestly elevated in infants whose mothers were already parasitemic at enrollment into the study.[83] This could suggest that first or early second trimester malaria exposure is more prone to induce T_{reg} differentiation, whereas infections occurring closer to term may not

have the same effect. Such a model would be consistent with the demonstrated propensity for naive T-cells in the mid-gestation fetus to differentiate into FoxP3^{+} T_{regs} upon foreign antigen exposure.[54]

IL10-producing Tr1 cells, which can also suppress malaria-specific T-cell proliferation, dominate the malaria-specific CD4 T-cell response in heavily exposed children.[88] Tr1 cells frequently co-produce IFNγ along with IL10 and express the canonical Th1 transcription factor T-bet. In studies of malaria-exposed Gabonese infants, CD4 T-cells that produce IL10 in response to stimulation with parasitized red blood cells were non-significantly elevated in the cord blood of infants with active (but not past/resolved) PM, and antibody blockade of IL10- enhanced malaria-specific CD4 T-cell IFNγ production in these infants.[77,79] However, among infants in a recent maternal chemoprevention trial, we did not observe IL10 production by CD4 T-cells, even in those with active PM.[18] These discrepant findings could potentially be explained by the fact that participants in the latter trial received at least three doses of IPTp during pregnancy, which may have cleared placental parasites. It is plausible that IL10 production by CD4 cells *in utero* modulates fetal immunity but is abrogated upon parasite clearance, making this phenomenon challenging to study in the setting of widespread IPTp implementation.

6.4 | Malaria-specific effector T-cells

We have recently shown that some infants develop highly functional malaria-specific CD4 and CD8 T-cells following *in utero* malaria exposure,[18] adding to the growing body of evidence that fetal T-cells, under appropriate circumstances, can be primed and differentiate into competent effector cells prior to birth.[58-60] In Ugandan infants born to mothers with active PM, we observed markedly elevated frequencies of effector-memory CD4 T-cells (T_{EM}) in cord blood, as well as higher frequencies of CD4 T-cells expressing Ki67, a marker for in vivo proliferation. Cord blood CD4 T_{EM} cells were confirmed by fluorescence in situ hybridization (FISH) of X and Y chromosomes to be of fetal, not maternal, origin.[18] These findings strongly indicate that fetal T-cells of malaria-exposed infants undergo antigen-driven expansion prior to birth. The fetal CD4 T_{EM} population was comprised of phenotypically diverse T-helper populations, including Th1, Th2, and T_{reg} subsets; transcription factor expression was likewise heterogeneous, with significant expression of factors associated with Th1 (*Prdm1*, *Tbx21*, and *BATF*), Th2 (*Gata3*), and Th17 (RoRγT) cells.[18] Expansion of CD4 T_{EM} cells was also seen, though to a lesser degree, in infants with past/resolved PM. Notably, CD4 and CD8 T-cells in malaria-exposed infants were highly functional, demonstrating both robust malaria-specific proliferation and production of inflammatory cytokines. Antigen-specific proliferation was observed in response to both whole parasite antigen (*P. falciparum* schizont extract) and the immunodominant MSP1 blood stage antigen.[18] The frequency of proliferating cells was highest among infants born to mothers with active PM, but those with past/resolved infection also demonstrated greater proliferation, in both the CD8 and CD4 T-cell compartment, than unexposed infants. This proliferation was abrogated by MHC I and II blockade, respectively, confirming that this response is TCR-dependent. Higher frequencies of CD4 and CD8

T-cells producing TNFα and IFNγ following in vitro mitogen stimulation were also seen in exposed infants (again, with stronger responses in those with active than past/resolved infection), contrasting with the findings of Brustoski et al, who reported T-cell IFNγ production to be higher in infants with resolved infection.[77,79] IL10 production by fetal T-cells was notably absent among infants in our study.

The most striking finding from this study is that malaria-specific CD4^{+} T-cell proliferation correlated with prospective protection from both *P. falciparum* infection and symptomatic malaria during the first 2 years of life.[18] This is somewhat surprising, as a protective role for CD4 T-cells has been difficult to establish, even in older children with greater immunological maturity.[89] While the association between CD4 responses and protection certainly requires confirmation in future prospective studies, it raises the intriguing prospect that functional malaria-specific T-cell responses can be generated *in utero* and afford protection against pathogen exposure in early childhood.

Why might the fetus mount a protective T-cell response, when it is not clear that young children can? One potential reason is that postnatal malaria infection results in an extremely high antigen burden, which triggers numerous immunoregulatory mechanisms including skewing of the CD4 response toward a Tr1 phenotype characterized by production of IL10.[88,89] Such regulatory responses interfere with the generation of functional, durable, proliferation-competent T-cell responses.[88] In contrast, PM results in a low dose exposure to blood stage antigens, which have been shown in other settings to prime robust, highly proliferative CD4 T-cell responses and induce sterilizing immunity.[90,91]

Further research is needed to determine how malaria exposure enables fetal T-cells to overcome the general bias away from inflammation *in utero*, and this will likely require a better understanding of the antigen-presenting capabilities of the fetus. In this regard, it is notable that not only CD4 but also CD8 T-cells from exposed infants exhibit malaria-specific responses. Priming of CD8 cells *in utero* would presumably require MHC I cross-presentation of exogenous malaria antigens taken up by fetal antigen presenting cells, which may be facilitated by transplacental transfer of malaria antigen in the form of immune complexes.[19,39] As TNFα and IFNγ secretion by fetal T-cells can stimulate uterine contractions, contributing to preterm labor,[60] this inflammatory bias in the malaria-specific fetal T-cell response could help to explain the elevated risk of prematurity seen with PM.

6.5 | Gamma Delta (γδ) T-cells

Gamma delta (γδ) T-cells are the first T-cells to develop in the human fetus, and they comprise 2%-5% of circulating T lymphocytes in adults. They exhibit rapid, innate-like effector function that is not dependent on prior antigen exposure nor on priming by DCs, which are functionally immature in the fetus. Therefore, γδ T-cells are in many ways uniquely suited to protection of the fetus and infant. Indeed, in murine models of parasitic infection, γδ T-cells are required for protection in the young, but not in mature animals.[92] Moreover, γδ T-cells are remarkably conserved across vertebrate species and they are the first T-cells to develop in all species studied

to date. It has been hypothesized that the primary selective advantage driving this conservation is their role in neonatal protection.[82]

Like conventional αβ T-cells, γδ T-cells recognize antigen through a T-cell receptor (TCR), which is generated by recombination of Vγ and Vδ gene segments. A single pairing consisting of δ2 and γ9 chains accounts for 50%-75% of all γδ T-cells in adult peripheral blood. Vγ9Vδ2 T-cells recognize non-peptide phosphoantigens that are abundantly produced by the *Plasmodium* apicoplast. Phosphoantigen recognition by Vγ9Vδ2 cells is TCR-dependent but does not require processing or presentation by professional APCs. Instead, phosphoantigens bind the ubiquitously expressed molecule butyrophilin 3A1, inducing a conformational shift that enables recognition by the γδ TCR.[93,94] As a result, malaria antigens stimulate a marked proliferative expansion of Vγ9Vδ2 cells both in vitro and in vivo (comprising up to 30% of peripheral blood T-cells).[95] Vγ9Vδ2 cells exhibit intrinsic reactivity to malaria antigens and can act as innate-like effectors, rapidly degranulating and producing inflammatory cytokines such as IFNγ and TNFα upon phosphoantigen stimulation, even in malaria-naive individuals. Activated Vγ9Vδ2 cells inhibit parasite growth and kill extracellular merozoites via release of granulysin,[96] and in human clinical trials they have been associated with protection from malaria.[97,98] However, in settings of repeated malaria exposure, chronic antigenemia results in the eventual loss and dysfunction of circulating Vγ9Vδ2 cells.[99]

At mid-gestation, fetal blood is highly enriched for Vγ9Vδ2 cells that can be rapidly activated to produce IFNγ and granzyme A upon stimulation.[100] This "fetal wave" of Vγ9Vδ2 T-cell development supplies the infant with innate-like T-cells capable of rapid activation and effector function. Intriguingly, mid-gestation Vγ9Vδ2 T-cells exhibit a markedly restricted TCR repertoire, with almost half of CDR3γ9 sequences encoded by the germline Vγ9-Jγ1.2 sequence[100] that is known to react to malaria-derived phosphoantigens.[101] The Vγ9Vδ2 population steadily declines as a proportion of circulating lymphocytes between 20 and 40 weeks gestation.[100,102] However, we have found that infants born to mothers with PM have elevated frequencies of Vγ9Vδ2 cells in cord blood (unpublished data), and others have observed that cord blood Vγ9Vδ2 T-cells are preferentially activated, produce more IFNγ,[103] and exhibit greater memory differentiation[104] following *in utero* malaria exposure. While many questions remain, it is attractive to hypothesize that γδ T-cells equip the infant with an army of ready-made innate effector cells that protect against fatal malaria during early childhood, while adaptive immunity develops.

6.6 | NK Cells and Other Innate Lymphocytes

The relationship between prenatal malaria exposure and other innate lymphocyte populations, including NK cells and other more recently described ILCs, has not been well studied. At least one study observed expansion of CD56^{dim}CD16^{+} NK cells in malaria-exposed neonates at birth,[105] but the frequencies of other NK populations and their potential antimalarial functions have not been assessed. Despite their immaturity, neonatal NK cells are highly responsive to

immune complexes[47] and exhibit robust antibody-dependent functions including IFNγ production and degranulation, which suggests that they could be an important antimalarial effector population *in utero* and in the newborn. Further studies of their role in the fetal and infant immune response are warranted.

6.7 | Malaria-specific Antibodies

Transplacental transfer of maternal-origin antibodies is essential for protecting the infant from pathogens encountered during the initial months of life. During pregnancy, maternal IgG antibodies are transferred to the fetus via an active transport mechanism mediated by FcRn, the neonatal Fc receptor, which selectively transfers IgG but not other antibody classes. The amount of antibody transported to the infant is influenced by multiple factors including IgG subclass, glycosylation, gestational age, and total maternal IgG concentration, as well as pathologic states including maternal HIV and placental infection (recently reviewed by Wilcox et al[46]). Several Fc region characteristics, including secondary glycan structures,[47] have been shown to modulate the efficiency of transplacental IgG transfer, which can vary across pathogens and even across antigen specificities within an individual.[106,107] Overall, transfer efficiency is highest for the IgG1 subclass, followed by IgG4, IgG3, and IgG2. FcRn is expressed on syncytiotrophoblasT-cells beginning at around 13 weeks gestation.[108] Transfer of IgG increases exponentially as pregnancy progresses, with a sharp increase in IgG transfer during the final month of a term pregnancy.[46,108] In the full-term neonate, IgG levels often exceed those of the mother. While term infants have higher absolute levels of maternal IgG than preterm infants, the repertoire of epitope specificities is largely similar between premature and full-term infants.[107]

Chronic maternal infections including HIV and malaria have been associated with reduced transfer of IgG across the placenta, which appears to vary by antigen specificity and subclass, with relative preservation of IgG3 transfer efficiency.[109] However, most studies examining the impact of PM on antibody transfer have focused on the IgG transfer ratio rather than absolute IgG levels in cord blood. This is a key point because, similar to HIV, malaria can cause maternal hypergammaglobulinemia, and FcRn-mediated transport saturates in the setting of high total IgG levels. Therefore, a lower proportional transfer of maternal malaria-specific antibodies does not necessarily translate into low absolute antibody levels in the fetus. As reviewed in detail elsewhere,[110] there are conflicting data regarding the impact of PM on the transfer of antibodies against both malaria and other specificities, with some studies showing a reduction in absolute or relative IgG transfer, and others showing no impact. In a recent randomized clinical trial of intensive malaria chemoprevention during pregnancy, there was no impact on cord blood levels of antibodies to 19 malaria antigens, despite a dramatic reduction in maternal and PM.[111]

In addition to antibodies transferred from the mother, the fetus is capable of producing its own malaria-specific antibodies *in utero*. In several studies, malaria-specific antibodies of the IgM class (which cannot cross the placenta) have been detected in the cord blood of malaria-exposed infants, suggesting *in utero* priming of fetal B-cells.[19-21] Tassi Yunga et al reported that malaria-specific IgM can be detected as early as 22 weeks gestation, and malaria-specific fetal B-cells can undergo class-switching to IgG prior to delivery.[19] This raises the possibility that fetal B-cells could be sensitized by maternal malaria vaccination, as has been reported to occur with tetanus and influenza vaccination.[74,112]

The importance of both maternal-origin and fetal-origin antibodies in protection from malaria following birth remains unclear. It has been widely maintained that malaria-specific antibodies of maternal origin play an important role in protecting the infant during the early months of life. However, a thorough review of the published literature on this topic concluded that there is scant evidence to support such a protective role, and that instead most evidence suggests that malaria-specific IgG in cord blood represents a biomarker of maternal malaria exposure.[113] However, this analysis was based on measurement of total IgG levels and did not take into account more recent evidence indicating that cytophilic IgG subclasses, particularly IgG3, may play a more important role in protection, likely due to their ability to fix complement and/or engage of FcR-bearing lymphocytes and phagocytes.[114] Future studies should carefully address the role of antibody subclass, glycosylation, and FcR-mediated engagement of cellular immunity in infant protection from malaria.

7 | SEX DIFFERENCES IN THE INFANT IMMUNE RESPONSE

The immune response to many infections differs by sex, both in children and in adults.[115,116] In general, females exhibit more robust innate and adaptive immunity than males, including higher postvaccination antibody titers, more rapid antigen clearance, and lower levels of chronic viremia. However, females are more susceptible to autoimmune and inflammatory disease and have higher rates of postvaccination adverse reactions, both local and systemic.[115] To date, few studies have examined sex differences in malaria susceptibility, clinical outcomes, or immunity. Limited but intriguing data suggest a more inflammatory response to malaria in females. For instance, a retrospective analysis of data from phase III trials of the leading malaria candidate vaccine RTS,S/AS01 found that vaccination in infancy was associated with a highly significant increase in all-cause mortality among female but not male children.[117] Furthermore, there was a tendency toward an increased risk of fatal malaria in females, but not males.[117] These findings echo the increased vaccine-associated mortality seen in females following introduction of the high-dose measles vaccine (HTMV), when all-cause mortality among girls (but not boys) doubled, leading to its eventual withdrawal from the market.[118] Additionally, in a recent clinical trial, the impact of maternal IPTp on infant malaria outcomes appeared to be modified by sex, with female but not male infants showing increased susceptibility to malaria during the first two years of life.[111] The biologic underpinnings of these sex difference in malaria outcomes have not been identified.

Even as newborns, boys and girls differ in their cord blood immune cell composition, indicating that sexual dimorphism in the immune response emerges during fetal life. In a recent birth cohort

study of Ugandan infants, we found that male newborns have significantly higher frequencies of regulatory FoxP3$^+$ CD4 cells (T$_{regs}$) in cord blood.[119] As most infants in this cohort were exposed to malaria *in utero*, this could suggest an enhanced propensity for antigen-driven T$_{reg}$ differentiation in males. However, another recent study also found higher T$_{reg}$ frequencies in males at birth, in the absence of known pathogen exposure,[120] suggesting an intrinsic male propensity toward regulatory differentiation that is independent of pathogen exposure. CD5$^+$ naive/immature B-cell frequencies are also higher in male newborns, and correlate with levels of dihydrotestosterone at birth, suggesting a hormonal influence on immune development.[121] Hormonal differences between males and females first emerge during gestation, with testosterone produced by male testes as early as the tenth gestational week.[122] In addition to potential hormonal effects, genes encoded by sex chromosomes may contribute to a sexually dimorphic immune response. Many genes critical for immune function and immunoregulation are encoded by the X chromosome (eg those encoding FoxP3, TLR7, TLR9, and IRAK), and it is estimated that 15% of the 1000 human X chromosome genes that lack a homologue on the Y chromosome may escape X inactivation, making them subject to gene dosage effects.[123] It is critical that the potential for sex-based differences receive careful attention in future studies of the immune response to malaria, both in naturally exposed populations and in malaria vaccine trials.

8 | DOES *IN UTERO* ANTIGEN EXPOSURE IMPACT THE DEVELOPMENT OF IMMUNITY TO MALARIA IN INFANCY?

The most important question regarding *in utero* malaria exposure is whether it has an impact – good or bad – on the development of immunity during childhood. It is biologically plausible that fetal exposure to malaria during a critical developmental window could induce tolerance, mediated by regulatory T-cells or other mechanisms that might hinder the priming of malaria-specific B and T-cell responses postnatally. Indeed, there is precedent for such an effect with helminths, as maternal filarial infection is associated with a 13-fold increase in the risk of filaria in offspring.[124] Consistent with this model, several studies have reported that infants born to women with PM are themselves at higher risk of malaria during early life.[8-11,125,126] However, this association has not been observed in all cohorts,[127-129] and indeed one study found that infants of primagravidas with PM actually have a lower risk of parasitemia during infancy.[7]

A parsimonious explanation for the repeated observation of higher infant malaria risk following *in utero* exposure is that these studies are confounded by variability in exposure. In other words, women with *P. falciparum* infections during pregnancy have greater environmental exposure to infected mosquitoes, and their infants would share this higher exposure risk. Such confounding can be circumvented by examining infant malaria outcomes in the setting of a randomized intervention that prevents malaria during pregnancy, and thus "uncouples" maternal and infant exposure. A recent trial in which women were randomized at 12-20 weeks gestation to receive standard IPTp vs intensive chemoprophylaxis found that despite a dramatic reduction in PM, infants whose mothers received the intensified chemoprevention regimen (monthly DP) actually had a higher incidence of clinical malaria episodes and parasitemia during the first 2 years of life, and a shorter time to first malaria episode, than those receiving standard IPTp.[111] Therefore, in this trial, prenatal exposure to malaria appeared to *reduce* the infant's risk of malaria after birth.

How can these divergent findings be reconciled? One possible explanation relates to the gestational timing of fetal exposure. The impact of *in utero* antigen exposure may differ between early and late pregnancy, leading to tolerance with early exposure and priming of adaptive malaria-specific T and B-cells with late gestation exposure. Initiation of intensive maternal chemoprevention during the second trimester, as in the trial cited above, would reduce fetal exposure late in pregnancy but not during the first and early second trimester, when fetal T-cells are most prone toward tolerance. If true, chemopreventive interventions could have the paradoxical effect of pre-empting malaria-specific fetal T and B-cell responses that might contribute to postnatal protection from malaria.[18] The timing of fetal malaria exposure will be a critical variable to consider in future studies of interventions to PM, which should include prospective clinical follow-up of infants with detailed analysis of their cellular and humoral immune responses. At present, the long-term clinical impact of *in utero* exposure to malaria remains an open question.

Beyond its impact on malaria-specific immunity, it has been hypothesized that *in utero* malaria exposure could have a global tolerizing impact on infant immunity, resulting in heightened vulnerability to non-malarial pathogens. Specifically, malaria-induced tolerization of fetal APCs and/or innate lymphocyte populations could have broad effects on the infant's immune responsiveness to infection and vaccination. To date, limited clinical data support this notion.[15] Future randomized trials designed to reduce *in utero* malaria exposure could provide a useful framework for empirically testing this hypothesis.

9 | CONCLUDING REMARKS

Despite the incomplete and somewhat conflicting data currently available, there is strong evidence that PM impacts the fetal immune system. In a high proportion of affected pregnancies, the fetus is directly exposed *in utero* to malaria antigens that cross the placenta, yet there appears to be substantial heterogeneity in how the infant responds. In some infants, fetal exposure to malaria may result in T-cell tolerance, providing a plausible biological mechanism by which PM may increase the risk of malaria during childhood. However, identification of malaria-specific antibodies and T-cells in cord blood raises the intriguing prospect that functional and protective malaria-specific immune responses can be generated *in utero*, and may even contribute to postnatal protection from malaria.

In recent years, efforts to prevent PM have gained substantial traction. These efforts include trials of new artemisinin-based regimens for IPTp and implementation of vector-targeted interventions, such as

insecticide-treated bed nets and pesticide spraying. Nonetheless, fewer than half of pregnant women in regions at risk sleep under an insecticide-treated net or receive IPTp in accordance with current WHO recomendations.[6] Innovative approaches are needed to reduce the enormous burden of pregnancy-associated malaria on maternal and child health. Before new malaria control interventions are widely implemented, however, questions about their potential downstream consequences for the fetus must be carefully considered, as their impact may be paradoxical. For instance, studies performed in Mozambique following a dramatic decline in local malaria transmission intensity found that PM was associated with increased parasitemia levels in both peripheral and placental blood, and a greater adverse impact on infant birth weight, than in historical controls.[130] Thus, while the prevalence of PM declined, its harmful impact on the fetus (and potentially fetal antigen exposure) increased. On the other hand, if transplacental transfer of malaria antigens occurs largely in the form of immune complexes, waning population immunity may result in lower maternal levels of malaria-specific IgG, and hence less antigen transfer and fetal exposure. In light of this complexity, the possible implications of future control measures on *in utero* malaria exposure should receive careful consideration prior to implementation.

It is essential that future studies further define the factors – which may include the timing, duration, and extent of placental infection and fetal antigen exposure - that influence the fetal immune response. Very little is currently known about the mechanisms by which PM results in fetal growth restriction and preterm birth, including the potential role of maternal and infant immune responses. Another important area for future investigation is the role and functional capabilities of various antigen presenting cell populations in the fetus, and how they might be manipulated to engender adaptive immune responses that do not result in excess inflammation that threatens continuation of the pregnancy. Additionally, the role of innate and semi-innate lymphocyte populations, including NK and γδ T-cells, in malaria-exposed infants has received insufficient research attention. By harnessing the specificity of maternal-origin antibodies, these FcR-expressing effector lymphocyte populations could provide a valuable first line of defense in the fetus and neonate, prior to the development of robust adaptive immunity. Finally, future studies of infant immunity should pay careful heed to sex differences in both the innate and adaptive immune response to malaria in the infant.

The past decade has seen great progress toward reducing malaria mortality and addressing its harmful impact on pregnancy. As we look hopefully toward an era of malaria vaccination, it is time to address a refined set of questions regarding the impact of prenatal malaria exposure on individual fetal immune cell populations and molecular pathways. Ultimately, questions regarding the clinical impact of *in utero* malaria exposure, and interventions to prevent it, must be answered empirically through careful studies of the infant immune response to vaccination and natural pathogen exposure in infancy.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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