Review

Malaria, primigravidae, and antibodies: knowledge gained and future perspectives

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Pregnant women have an increased risk of malaria infection, independent of previously acquired immunity. Women in their first pregnancy and children under the age of five are the primary victims of malaria worldwide. Pregnant women develop antibodies against placenta-adhesive parasites in a parity-dependent manner. Various efforts to understand the targets, quality, and quantity of this antibody response could aid the design of an effective vaccine against placental malaria. This review focuses on the research that has led to the current understanding of the antibody response that primigravidae (PG) acquire to *Plasmodium falciparum* malaria and draws from this knowledge to suggest serology and PG as sentinels for malaria transmission.

PG and serology: why look at it?

The study of malaria in pregnancy was, until the beginning of the 1980s and much throughout the following 15 years, based on observations that could not easily be explained. Pregnant women were known to be more susceptible to malaria than their non-pregnant counterparts [1–3]; PG (see Glossary) were the most affected, suffering from severe anaemia and delivering low birth-weight (LBW) babies more frequently than multigravidae (MG) [1–3]. In the 1960s and early 1970s, studies showed that antimalarial antibodies in adults, including pregnant women, were mainly of the Ig class IgG [4–6], but the role of antibody-mediated immune responses during malaria in pregnancy was unknown. This review chronologically examines the increasing understanding over the past 30 years of the importance of antibodies against *P. falciparum* in PG (Table 1, Box 1). We highlight the value of future studies, in the context of declining malaria transmission and changing malaria epidemiology, as well as the role of measurement of antimalarial antibodies in PG as a malaria surveillance tool.

1980 to 1994: paradigms before chondroitin sulfate A (CSA)

Although the susceptibility of PG to the risks of malaria during pregnancy was well known, understanding why remained elusive into the late 1970s. In 1980 a longitudinal study on the acquisition of antimalarial antibodies during pregnancy showed that antibodies to *P. falciparum*, measured by an immunofluorescence assay (IFA), declined in the third trimester, although the study failed to identify PG as a separate group deserving special attention [7]. By contrast, levels of total IgG, IgM, IgA, and *P. falciparum*-specific antibodies (measured by IFA), declined during gestation in a group of 60 Nigerian PG who were receiving malaria chemoprophylaxis [8]. Malaria-specific IgG titres and total IgG correlated with one another and were reported to be lower than levels previously reported for women not receiving treatment [8].

Following a comprehensive review of the epidemiology of malaria in pregnancy, which highlighted the particular susceptibility of PG [9], an explanation was pursued by comparing the levels of antibodies against ring-infected erythrocyte surface antigen (RESA) between nulligravidae (NG), PG, and MG [10]. In this small study, antibodies to RESA failed to predict susceptibility at enrolment and exhibited higher levels in MG than in PG, although the authors did not correct for age. In that same year, the levels of antimalarial antibodies were analysed between pregnant and non-pregnant women, and the ability to generate lymphoproliferative responses to *P. falciparum* antigens was compared between the two groups [11]. Pregnant women displayed a diminished capacity to generate lymphoproliferative responses, but there was no difference in the titres of antimalarial antibody between pregnant and non-pregnant women or PG and MG. Together, these studies suggested that antimalarial antibody might not differ significantly between pregnant and non-pregnant women.

In 1992, attention again turned to anti-RESA antibodies confirming earlier findings demonstrating that these were significantly lower in pregnant versus non-pregnant women, in PG more so than in MG, and also inversely

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Glossary

Agglutinating antibodies: cause parasites to clump together when bound to an antigen on the parasite surface; potentially important as part of the immune response needed to control parasite numbers. Can in theory by any Ig isotype.
Antenatal clinic (ANC): healthcare facility where pregnant women receive attention during their pregnancy. Routine exams performed will depend on the local setting and conditions.
Anti-adhesion antibodies: prevent parasite adhesion to receptors when bound to the surface of infected erythrocytes; can in theory be any isotype.
Antibodies: Igs composed of four peptide chains (two light chains and two heavy chain. Antibodies are produced by B cells and can be secreted into the blood or lymph in response to an antigen. Antibodies are classified into five different isotypes according to the class of their heavy chains. These five antibody isotypes are: IgA, IgD (mostly bound to B cell surfaces), IgE, IgG, and IgM.
Antigen: substance (foreign or self) that causes the activation of the immune system.
Antimalarial antibody: any antibody that recognises *Plasmodium* antigens.
Apical membrane antigen-1 (AMA-1): well-conserved merozoite protein involved in the process of erythrocyte invasion. It is one of the major targets for vaccine development
Chondroitin sulfate A (CSA): chondroitin sulfates belong to a family of glycosaminoglycans that are present in a variety of tissues. Their functions range from structural stability to surface receptors and signaling molecules, or even essential components of embryonic development. In the placenta, CSA acts as a receptor for IE expressing VAR2CSA.
Duffy binding-like (DBL) protein domains: adhesive cysteine-rich protein domains present on PfEMP1 and initially identified as part of the Duffy binding protein of *Plasmodium vivax*; involved in parasite:receptor adhesion to both protein (e.g., ICAM-1) and carbohydrate (e.g., CSA). Six different DBL domains have been identified.
Expanded Program on Immunization (EPI): World Health Organization initiative aiming to improve access to standardised vaccination schedules.
Gravidae (nulli-, primi-, secundi- or multi-): the number of a woman's current pregnancy (none, first, second, or third and higher).
Infected erythrocytes (IEs): erythrocytes where one or more *Plasmodium* merozoites have invaded and are developing asexually. During this development stage in the blood, the parasite secretes several molecules to the surface of the IE, among them VSA.
Insecticide-treated bed-nets (ITN): usually made of polyester and treated with pyrethroids. These nets form a protective barrier between the mosquito vector and people, and act by killing or repelling the mosquitoes. ITNs have been associated with a decrease in disease burden in malaria endemic regions.
Intermittent preventive treatment, pregnancy (IPTp): public health intervention where pregnant women receive antimalarials at various time-points during pregnancy, regardless of infection status, with the aim of both clearing any current infections and preventing new ones.
Lymphoproliferative responses: cell-mediated proliferation in response to a specific antigen. It can be a measure of the cellular immunity of an individual to that antigen.
Merozoite surface protein 1, 19 kDa fragment (MSP1-19): 19 kDa fragment of the 200 kDa merozoite protein that coats the surface of the parasite and is processed and cleaved into several fragments before erythrocyte invasion. It is one of the leading candidates for vaccine development.
Opsonizing antibodies: promote uptake of parasites by phagocytic cells (e.g., monocytes, macrophages, neutrophils) expressing the appropriate Fc receptors on their surface; are predominantly IgG1 and IgG3.
Phagocytic cells: any cell that is able to perform phagocytosis, either through recognition of opsonins on the surface of an antigen (e.g., antibodies, complement) or through recognition of the antigen by scavenger receptors (e.g., CD36 on the surface of macrophages). The most common phagocytic cells are neutrophils, macrophages, and monocytes.
Placental blood: blood collected from the placenta. Usually composed of a mix of both maternal and foetal blood.
***Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1):** predominant family of proteins expressed on the surface of infected erythrocytes. These proteins are encoded by the *var* genes (of which there are 60 different variants) and are actively transported to the surface of the erythrocyte during the middle third of the intraerythrocytic maturation stage of the parasite. Only one variant of PfEMP1 is expressed at any one time. Structurally, PfEMP1 molecules are mainly composed of several DBL domains, cysteine-rich interdomain regions, a transmembrane region, and an intracellular acidic-terminal sequence. This family of proteins is involved in parasite–receptor binding (e.g., to ICAM-1 or to CSA)
Peripheral blood: blood from the peripheral circulation.
Pregnancy-specific malaria antibodies: antibodies towards erythrocytes that are able to bind to CSA-binding infected erythrocytes and/or the CSA-binding protein, VAR2CSA. These antibodies are predominately of the IgG class.
Ring-infected surface antigen (RESA): also known as Pf155 due to its molecular weight of 155 kDa, this protein is released from merozoites and associates with the membrane of the erythrocyte. Recombinant fragments of this protein have been used in Phase II trials of malaria vaccines.
Seroconversion: acquisition of detectable serum levels of antibodies to a given antigen.
Syncytiotrophoblast: continuous, multinucleated epithelial layer that lines the placental intervillous space where maternal/foetal exchanges occur.
VAR2CSA (*var2csa*): PfEMP1 variant expressed on the surface of IE commonly associated with placental infections and with parasite lines selected *in vitro* to bind CSA. Composed of six DBL domains and four interdomain regions. Several of the DBL domains can adhere to CSA.
Variant surface antigen (VSA): variant *Plasmodium* antigens present on the surface of the parasite or the IE. Several families have been identified and have been shown to be involved in biological processes of the parasite, pathological processes, and/or immune evasion. PfEMP1 is one of the families of VSA present on the surface of IE.

correlated with parasitaemia [12]. This could, however, be explained by a general immune suppression attributed to pregnancy. Also in 1992, mean specific IFA IgG titres were found to increase with parity in pregnant women from western Kenya when they were screened at their first antenatal visit [13].

The following year, antimalarial antibodies from 46 Papua New Guinean women of varying gravidities were measured using an enzyme-linked immunosorbent assay (ELISA) and western blots against *P. falciparum* extracts [14]. In this small cohort, ELISA results showed no significant difference with regard to gravidity. However, western blots revealed that antibodies from MG recognised a higher number of low molecular-weight proteins than those of PG. At around this time, others were examining the cellular immune responses to *P. falciparum* and other antigens during pregnancy [15]. The placental and peripheral blood lymphoproliferative responses of 102 Gambian women to all antigens indicated that the proliferation of mononuclear cells in PG was lower when compared to MG, whereas levels of antibodies to the malarial antigen did not vary with parity [15]. Elevated serum cortisol levels, previously reported in PG and malaria-infected women [16], were speculated to underlie the susceptibility of PG to malaria, and humoral immunity was discarded as an important factor.

1995 to 2002: antibodies rise to fame, and parity-dependency is explained

As we have seen, subsequent to 1995, the prevailing idea in the field was that antibodies to malarial antigens during pregnancy did not explain the observed parity-dependent susceptibility to *P. falciparum*; instead, lower cellular immune responses in PG were thought to be responsible for that susceptibility as shown in three studies that addressed this topic in 1995–1997 [17–19]. In the first of these studies, the proliferative responses of peripheral mononuclear cells to malarial antigen in 97 Gambian women and their neonates were evaluated [17]. As previously noted, PG were less responsive than MG. The two remaining studies evaluated the production of interleukin (IL)-4, interferon (IFN)-γ, IL-2, and the cellular proliferative responses to crude malarial antigen, purified RESA, and non-malarial antigen [18,19]. In the first, peripheral blood mononuclear cells from PG showed less proliferation than those from NG in response to both malarial and non-malarial antigens, again suggesting an overall immune depression attributable to pregnancy, which could be more