Malaria and HIV Infection in Mozambican Pregnant Women Are Associated With Reduced Transfer of Antimalarial Antibodies to Their Newborns

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Background. Malaria and human immunodeficiency virus (HIV) infection during pregnancy affect the transplacental transfer of antibodies against several pathogens from mother to fetus, although the effect of malaria and HIV infection on the transfer of antimalarial antibodies remains unclear.

Methods. Levels of total immunoglobulin G (IgG), immunoglobulin M (IgM), and IgG subtypes against the following Plasmodium falciparum antigens were measured in 187 pairs of mother-cord plasma specimens from Mozambique: 19-kDa fragment of merozoite surface protein 1 (MSP119), erythrocyte binding antigen 175 (EBA175), apical membrane antigen 1 (AMA1), and parasite lysate. Placental antibody transfer was defined as the cord-to-mother ratio (CMR) of antibody levels.

Results. Maternal malaria was associated with reduced CMR of EBA175 IgG (P = .014) and IgG1 (P = .029), AMA1 IgG (P = .002), lysate IgG1 (P = .001), and MSP1 IgG3 (P = .01). Maternal HIV was associated with reduced CMR of MSP1 IgG1 (P = .022) and IgG3 (P = .023), lysate IgG1 (P = .027) and IgG3 (P = .025), AMA1 IgG1 (P = .001), and EBA175 IgG3 (P = .001). Decreased CMR was not associated with increased adverse pregnancy outcomes or augmented risk of malaria in the infant during the first year of life.

Conclusions. Placental transfer of antimalarial antibodies is reduced in pregnant women with malaria and HIV infection. However, this decrease does not contribute to an increased risk of malaria-associated morbidity during infancy.

Keywords. malaria; HIV; pregnancy; antibody; placenta; transfer.
transfer minimizes deficiencies in antibody production in the fetus and provides short-term passive immunity [5], conditioning the degree and length of protection [4] and the success of vaccination in newborns [6, 7]. However, the effect of placental transfer of antimalarial antibodies on infant’s immune responses and susceptibility to malaria during the first months of life remains unclear [8–11].

The efficiency of placental antibody transfer is affected by maternal antibody levels, receptor density and functionality, immunoglobulin G (IgG) subclass, avidity, antigen nature, and gestational age [12–14]. Maternal hypergammaglobulinemia, prematurity, low birth weight, multigravidity, undernutrition, and maternal infections have been associated with decreased transfer [4, 14–16], although these factors seem to vary with the study population [5]. Maternal HIV infection has been consistently associated with reduced placental passage of antibodies against several common viral and bacterial antigens [4, 14, 17–19]. Placental malaria has been associated with maternal hypergammaglobulinemia [20, 21] and reduced transfer of antibodies against measles virus, Clostridium tetani, Streptococcus pneumoniae, and varicella-zoster virus in some studies [4, 18, 22–24] but not in others [14]. However, the information about factors affecting placental transfer of specific antimalarial antibodies, defined as the cord-to-mother ratio (CMR) of antibody levels, is scarce, and, to our knowledge, no previous work analyzed the effect of maternal malaria infection. Only 1 study in Kenya addressed the effect of maternal HIV infection and found that placental passage of antibodies against the circumsporozoite protein but not against other malarial antigens was reduced in HIV-infected mothers, compared with noninfected mothers [25].

Therefore, the aim of this study was to investigate maternal factors affecting the maternal-fetal transfer of antimalarial antibodies and the potential relationship between antibody transfer and adverse pregnancy outcomes among mothers and malaria risk among newborns. We hypothesized that P. falciparum and HIV infections during pregnancy might decrease the placental transfer of antimalarial antibodies, owing to alterations in placenta architecture [26, 27], thus contributing to an increased risk of malaria in the newborn. To address this, levels of P. falciparum–specific immunoglobulin G (IgG), immunoglobulin M (IgM), and IgG subclasses against several asexual-blood-stage vaccine candidates and parasite lysate were measured in maternal peripheral and cord plasma specimens. The effect of HIV infection and malaria, as well as other maternal factors (antibody levels, age, gravidity, preventive treatment, and anemia) on placental transfer of antibodies was assessed. Information regarding placental transfer of antibodies against critical P. falciparum merozoite antigens involved in invasion will help in the development of antimalarial vaccines specifically for pregnant women and increase our understanding of the effects of malaria and HIV infection on possible strategies of maternal vaccination in malaria-endemic areas.

MATERIALS AND METHODS

Study Population

This study was nested in a placebo-controlled trial of intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) for malaria prevention conducted at the Manhiça Health Research Center in Manhiça district, southern Mozambique, during 2003–2006 [28]. Malaria transmission in this semirural area is perennial, with some seasonality, and P. falciparum is the predominant species [29]. Women were enrolled at the antenatal clinics of the Manhiça District Hospital if they had a gestational age ≤28 weeks, no reported allergies to sulfa drugs, and resided permanently in the study area. After providing written informed consent, women received a long-lasting insecticide-treated net, were randomly assigned to receive SP or placebo, and were screened for HIV if they agreed [28].

Maternal HIV type 1 infection was diagnosed with the Determine HIV-1/2 (Abbott Laboratories) and confirmed with the Unigold rapid test (Trinity Biotech). At delivery, maternal peripheral and cord blood were collected by venipuncture into ethylenediaminetetraacetic acid–containing vacutainers, and thin and thick smears were prepared. Blood specimens were centrifuged, and plasma specimens was stored at −80°C. Hematocrit level was quantified in a microcapillary tube after centrifugation. Peripheral, cord, and placental blood specimens were collected onto filter papers (903’TM; Schleicher & Schuell). Tissue samples (area, 2.5 cm2) were removed from the maternal side of the placenta in an off-center position, placed in 25 mL of 10% neutral-buffered formalin, and embedded in paraffin wax by standard techniques. Paraffin sections 4-mm thick were stained with hematoxylin–eosin and Giemsa [30].

The last 200 women recruited into the main trial receiving either placebo or SP were included in this ancillary study [21]. Of these, 187 mothers had complete demographic and clinical data, as well as samples from maternal, placental, and cord blood for the immunologic analysis. The study was approved by the Mozambican National Bioethics Committee and the Hospital Clinic of Barcelona Ethics Review Committee.

P. falciparum Detection by Microscopy, Placental Histological Analysis, and Quantitative Polymerase Chain Reaction (qPCR)

Thin and thick smears were stained with Giemsa and examined for malarial parasites according to quality-control procedures [31]. Placental biopsy specimens were processed for histological examination and classified according to previous criteria [32]. DNA was extracted from 50-µL blood drops onto filter paper with an ABI Prism 6700 Automated Nucleic Acid Workstation (Applied Biosystems) and resuspended in 200 µL of water. Five microliters of DNA samples were screened for P. falciparum by qPCR targeting the 18S ribosomal RNA gene, as described elsewhere [21]. Malaria episodes were recorded for infants during
the first year of life through a passive case detection system at the outpatient clinic of Manhiça District Hospital.

**Measurement of Antibodies Against Merozoite Antigens and Parasite Lysate**

Maternal and cord plasma samples were tested by enzyme-linked immunosorbent assay (ELISA) for the presence of IgG, IgM, and IgG subclasses specific for the recombinant merozoite antigens merozoite surface protein 1 (19-kDa fragment, 3D7; MSP119), erythrocyte binding antigen 175 (region F2, Camp; EBA175), and apical membrane antigen 1 (full ectodomain, 3D7; AMA1) [21, 33]. High-binding 96-well microplates (Nunc Maxisorp) were coated overnight at 4°C with 200 ng/well of recombinant antigen diluted in carbonate-bicarbonate buffer. After blocking with 2% bovine serum albumin at 4°C for 8 hours, 100-µL samples of plasma diluted 1:500 (for IgG and IgM) or 1:200 (for IgG subtypes) were tested in duplicate. Secondary peroxidase-conjugated antibodies were used as follows: goat anti–human IgG (1:30 000), IgM (1:2000; both from Sigma); and sheep anti–human IgG1 (1:6000), IgG2 (1:3000), IgG3 (1:6000), and IgG4 (1:3000; all from The Binding Site). Reactions were developed, and optical densities (ODs) were read at 492 nm.

Whole-parasite lysate was prepared by 3 freezing/thawing cycles of asynchronous in vitro cultures of 3D7 and HB3 laboratory strains (MRA-102 and MRA-155, respectively, both from MR4, ATCC) at a 5% level of parasitemia and 1% hematocrit. Noninfected erythrocyte (NIE) lysate prepared in the same way was used as a control. Plates were coated with 50 µL/well of parasite extract. Wells were blocked with 300 µL of 5% skim milk at 4°C for 8 hours. One hundred microliters of plasma samples were tested in duplicate for IgG (dilution 1:6400) and for IgM and IgG subclasses (1:1600 each). Incubation of antibodies and reaction development were performed as described above. *P. falciparum*–specific antibody recognition was evaluated by subtracting the mean OD of NIEs from the mean OD of infected erythrocytes. A pool of plasma samples from 8 Mozambican adults was used to normalize data from different ELISAs [21, 33].

**Definitions and Statistical Methods**

Pregnant women were classified as primigravidae (first pregnancy), secundigravidae (second pregnancy), or multigravidae (at least 2 previous pregnancies). Age was categorized as ≤20, 20–24, or ≥25 years, based on maternal age terciles in this population. Maternal anemia was defined as a hematocrit level <33%. Malaria in the pregnant woman was considered if parasites were detected by histological analysis or microscopy in placenta and/or periphery specimens, respectively, or by qPCR in any of the compartments. Pregnant women were considered to have submicroscopic infection if parasites were detected in periphery or placenta specimens by qPCR but not by microscopy (for periphery specimens) or histological analysis (for placenta specimens). Clinical malaria was defined as the presence of parasitemia in a blood smear plus fever (defined as an axillary temperature of ≥37.5°C).

For each *P. falciparum*–specific immunoglobulin, mothers or newborns were considered seropositive if plasma samples had an OD above the median OD (plus three standard deviations) determined for 10 healthy controls from a non–malaria-endemic area. The efficiency of antibody placental passage was defined as the CMR of ODs in cord and mother peripheral plasma specimens among those women who were seropositive for each specific immunoglobulin. Univariate (evaluated by the Student t test) and multivariate linear regression models were used to estimate the association of HIV infection, malaria, and other clinical and demographic covariates with antibody levels in cord blood or CMRs after log transformation. Linear regression models were used to estimate associations between CMRs and pregnancy outcomes (gestational age, birth weight, and hematocrit), and binomial regression models were used to estimate associations between CMRs and malaria incidence in the first year of infant’s life. Multivariate models were adjusted for maternal antibody levels, maternal HIV infection and malaria, parity, age, maternal anemia, and IPTp group. For all regression models, crude proportions and adjusted effects with their corresponding 95% confidence interval (CI) were computed. Statistical analysis was performed using GraphPad Prism, version 6 (GraphPad Software), and Stata Statistical Software, version 11.0 (StataCorp).

**RESULTS**

**Characteristics of the Study Population**

The prevalence of maternal *P. falciparum* infection in peripheral blood was 9.6% (18/187) by light microscopy but increased to 29.9% (56/187) by qPCR. Parasites were found in 26 placental sections by histological analysis (13.9%), and 61 (32.6%) infections were detected by qPCR in the placenta specimens. In total, 116 women (62.0%) had parasites detected in one or both compartments by any of the techniques. Thirty-eight women (20.3%) had peripheral submicroscopic infections, 40 (21.4%) placental submicroscopic infections, and only 1 (0.53%) presented with clinical malaria at delivery.

Fifty-seven women (30.5%) were HIV positive. The characteristics of the 187 women at delivery, according to their HIV status, are presented in Table 1. The subset included in this study and the 1030 women participating in the randomized trial were comparable in terms of IPTp group, parity, age, HIV infection, peripheral and placental malaria, and hematocrit level. Similarly, no differences were found in parity, age, malaria status, and IPTp intervention between HIV-negative and HIV-positive women. The prevalence of anemia was higher among mothers with HIV infection, compared with mothers without HIV infection (Table 1).
Factors Associated With Levels of Antimalarial Antibodies in Cord Blood

Levels of antimalarial antibodies in maternal peripheral and cord plasma samples are shown in Figure 1. IgG and IgG subtype levels were comparable between mother-cord pairs (Figure 1 and Table 2), in contrast to IgM levels, which were much lower in cord samples. Maternal and cord blood levels of IgG and IgG subclasses were highly correlated, but IgM levels were not (Supplementary Table 1). The presence of parasites in cord blood was associated with increased seroprevalence of IgG4 against parasite lysate (P = .036), but this association was not present for other IgG subtypes or IgM. Given the very low levels of IgG2 and IgG4 against the 3 antigens and parasite lysate, both in mother and cord plasma specimens (Figure 1), these subclasses were not further included in the analysis.

Maternal antibody levels and other clinical (maternal HIV infection, malaria, anemia, and IPTp group) and demographic (maternal age and gravidity) factors that could influence levels of antimalarial antibodies in cord blood were included in a univariate and multivariate regression analyses. In both analyses, maternal antibody levels were associated with cord levels for all of the IgGs (Table 2). The univariate analysis showed that maternal HIV infection was associated with a significant reduction of cord IgG, IgG1, and IgG3 levels against all the antigens, with the exception of MSP1 IgG and AMA1 IgG3 (Figure 2A). After adjustment (Table 2), HIV infection remained associated with significantly decreased levels of cord IgG1 and IgG3 against MSP1 and lysate, of cord IgG1 against AMA1, and of cord IgG3 against EBA175.

Malaria in the mother was associated with increased cord levels of IgG3 against MSP1 in the univariate analysis (Figure 2B). In the multivariate model, malaria was associated with a significant decrease in the levels of IgG against AMA1 and EBA175 and of IgG1 and IgG3 against MSP1 (Table 2). No statistically significant associations were found between cord antibody levels and IPTp group, gravidity, age or maternal anemia.

Factors Associated With Placental Transfer of Antimalarial Antibodies

The CMR was used as a measure of placental transfer of antibodies from mother to fetus. A 2-fold increase in maternal antibody levels was associated with decreasing CMRs of IgG1 and IgG3 against all the antigens but not against parasite lysate (Table 3). Both in the univariate (Figure 3A) and multivariate (Table 3) analyses, maternal HIV infection was associated with a significant reduction in the CMRs of IgG1 against AMA1, of IgG1 and IgG3 against lysate, and of IgG3 against MSP1 and EBA175. This was also the case for the CMR of IgG1 against AMA1 in the univariate analysis, but the significance was lost after adjustment.

Malaria in the mother was associated with reduced CMRs of IgG against EBA175 and AMA1, of IgG1 against all the antigens, and of IgG3 against MSP1 in the univariate model (Figure 3B). In the multivariate analysis, reduced CMRs of IgG and IgG1 against EBA175, of IgG against AMA1, and of IgG3 against MSP1 remained significantly associated with malaria (Table 3). There were no statistically significant differences in CMRs by IPTp group, gravidity, age, or maternal anemia.

Placental Transfer of Antimalarial Antibodies, Pregnancy Outcomes, and Malaria Incidence in the Infant

Relationships between adverse pregnancy outcomes (preterm delivery, low birth weight, and anemia in cord blood) or malaria incidence during the first year of life and the CMR were assessed by multivariate or binomial regression analysis, respectively. No significant associations were found, with a few exceptions. Only a 2-fold increase in the CMR of lysate IgG was associated with augmented gestational age (difference in weeks, 0.51 [95% CI, .01–1.00]; P = .047).

With respect to malaria incidence in the infant, a 2-fold increase in the CMRs of lysate IgG and AMA1 IgG1 was significantly associated with an increased risk of malaria during the first year of life (incidence ratios, 1.48 [95% CI, 1.01; 2.18; P = .046] and 3.11 [95% CI, 1.11; 8.71; P = .031], respectively).

Table 1. Demographic and Clinical Factors of Mothers at Delivery, According to Their Human Immunodeficiency Virus (HIV) Status

<table>
<thead>
<tr>
<th>Factor</th>
<th>HIV Status, No. (%)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Noninfected (n = 130)</td>
<td>Infected (n = 57)</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>51 (39.2)</td>
<td>19 (33.3)</td>
</tr>
<tr>
<td>20–24</td>
<td>40 (30.8)</td>
<td>16 (28.1)</td>
</tr>
<tr>
<td>≥25</td>
<td>39 (30.0)</td>
<td>22 (38.6)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primigravida</td>
<td>36 (27.7)</td>
<td>15 (26.3)</td>
</tr>
<tr>
<td>Secundigravida</td>
<td>25 (19.2)</td>
<td>12 (21.1)</td>
</tr>
<tr>
<td>Multigravida</td>
<td>69 (53.1)</td>
<td>30 (52.6)</td>
</tr>
<tr>
<td>Anemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>87 (66.9)</td>
<td>23 (40.3)</td>
</tr>
<tr>
<td>Yes</td>
<td>43 (33.1)</td>
<td>34 (59.7)</td>
</tr>
<tr>
<td>Malaria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>76 (58.5)</td>
<td>35 (61.4)</td>
</tr>
<tr>
<td>Positive</td>
<td>54 (41.5)</td>
<td>22 (38.6)</td>
</tr>
<tr>
<td>IPTp group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>65 (50.0)</td>
<td>23 (40.3)</td>
</tr>
<tr>
<td>SP</td>
<td>65 (50.0)</td>
<td>34 (59.7)</td>
</tr>
</tbody>
</table>

Abbreviations: IPTp, intermittent preventive treatment in pregnancy; SP, sulfadoxine-pyrimethamine
* By the χ² test.
Figure 1. Levels of antimalarial antibodies in maternal (gray triangles) and cord (black triangles) samples. Data were determined by enzyme-linked immunosorbent assays and are represented as optical densities (ODs). Black lines correspond to the geometric mean of the population. Abbreviations: AMA1, apical membrane antigen 1; EBA175, erythrocyte binding antigen 175; IgG, immunoglobulin G; IgM, immunoglobulin M; MSP1, merozoite surface protein 1.
The four IgG subclasses have been shown to cross the placenta [35]; however, low levels of IgG2 and IgG4 both in mother and cord blood found in our samples cannot confirm a reduced transfer for these isotypes in the case of antimalarial antibodies [37]. This is in accordance with previous articles showing that IgG1 and IgG3 are the predominant subclasses produced in response to malarial parasite antigens [40] and present high affinity for Fc receptors, which suggests a preferential transfer [35, 37, 41–43]. Fc receptors, key players of the immune modulation that contribute to the release of inflammatory mediators, are upregulated in several inflammatory conditions [44]. Future studies analyzing expression of Fc receptors in the context of malaria during pregnancy and HIV infection will be of great interest in the attempt to understand the underlying molecular mechanisms.

Malaria at delivery was associated with a reduced CMR of antimalarial IgGs. This is the first study to show such a reduced transfer of antibodies against P. falciparum from mother to fetus. Placental damage caused by malaria [32] may alter Fc receptors and, together with maternal hypergammaglobulinemia [20, 21], probably explains the observed reduction in placental transfer. Similarly, HIV infection leads to a reduction in the CMR of IgGs against AMA1, MSP1, and parasite lysate. This reduction in antibody transfer is stronger than that shown in the only previous report [25] that found a decrease in IgGs against the antigenic determinant (NANP)5 of CSP but not against other antigens. Possible explanations for inefficient transfer of antibodies associated with maternal HIV infection could be the formation of immune complexes impairing transplacental IgG passage, the production of defective IgGs unable to bind to the Fc receptor, direct decrease of receptor levels associated with HIV infection [14], or direct competition by HIV-specific antibodies for a finite number of Fc receptors [17], although further study is required to establish the mechanisms.

As a result of this decreased placental transfer of antibodies, we found a reduction in cord levels of several specific antimalarial antibodies in association with maternal malaria or HIV infection. This reduction will be of great interest in the attempt to understand the underlying molecular mechanisms.

**DISCUSSION**

This study is the first to show that Plasmodium falciparum malaria in pregnant women at delivery is associated with reduced CMRs of several antimalarial antibodies, mainly IgG and IgG1 against EBA175, IgG1 against parasite lysate, and IgG3 against MSP1. In addition, these results demonstrate that HIV infection in pregnant women is associated with decreased placental transfer of specific antimalarial IgG1 and IgG3 to the newborn. However, this decrease does not contribute to an increased risk of malaria-associated morbidity during infancy. Our results raise the concern about the potential for HIV infection and malaria to limit the effectiveness of infant immunization strategies that are based on maternal vaccination during pregnancy [34].

This study confirms that IgGs against malaria parasites, but not IgMs, are transferred through the placenta [35, 36]. The lack of mother-cord correlation for IgM excludes the possibility of blood contamination and suggests that low IgM levels in some cord blood samples could be of fetal origin due to in utero exposure [37–39]. The four IgG subclasses have been shown to cross the placenta [35]; however, low levels of IgG2 and IgG4 both in mother and cord blood found in our samples cannot confirm a reduced transfer for these isotypes in the case of antimalarial antibodies [37]. This is in accordance with previous articles showing that IgG1 and IgG3 are the predominant subclasses produced in response to malarial parasite antigens [40] and present high affinity for Fc receptors, which suggests a preferential transfer [35, 37, 41–43]. Fc receptors, key players of the immune modulation that contribute to the release of inflammatory mediators, are upregulated in several inflammatory conditions [44]. Future studies analyzing expression of Fc receptors in the context of malaria during pregnancy and HIV infection will be of great interest in the attempt to understand the underlying molecular mechanisms.

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As a result of this decreased placental transfer of antibodies, we found a reduction in cord levels of several specific antimalarial antibodies in association with maternal malaria or HIV infection.
Figure 2. Cord levels of antimalarial antibodies, by maternal human immunodeficiency virus (HIV) infection (A) and malaria (B) status, in the univariate analysis. Data were determined by enzyme-linked immunosorbent assays and are represented as optical densities (ODs). Horizontal black lines correspond to the geometric mean of the population. *P < .05, **P < .01, and ***P < .001, by the Student t test. Abbreviations: AMA1, apical membrane antigen 1; EBA175, erythrocyte binding antigen 175; IgG, immunoglobulin G; MSP1, merozoite surface protein 1.
Table 3. Multivariate Linear Regression Analysis of the Association Between the Cord-to-Mother Ratio (CMR) of Antimalarial Antibodies (Abs) and Maternal Ab Levels, Human Immunodeficiency Virus (HIV) Infection, and Malaria

<table>
<thead>
<tr>
<th>Antimalarial Ab</th>
<th>Maternal Ab Level</th>
<th>HIV Infection</th>
<th>Malaria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effect&lt;sup&gt;a&lt;/sup&gt; (95% CI)</td>
<td>P Values</td>
<td>Effect&lt;sup&gt;a&lt;/sup&gt; (95% CI)</td>
</tr>
<tr>
<td>IgG MSP1 (n = 110)</td>
<td>0.91 (.75–1.10)</td>
<td>.326</td>
<td>1.01 (.79–1.30)</td>
</tr>
<tr>
<td>IgG EBA175 (n = 176)</td>
<td>0.92 (.83–1.03)</td>
<td>.165</td>
<td>0.87 (.73–1.02)</td>
</tr>
<tr>
<td>IgG AMA1 (n = 184)</td>
<td>1.05 (.96–1.14)</td>
<td>.285</td>
<td>0.91 (.81–1.02)</td>
</tr>
<tr>
<td>IgG lysate (n = 142)</td>
<td>0.87 (.64–1.17)</td>
<td>.358</td>
<td>0.97 (.67–1.40)</td>
</tr>
<tr>
<td>IgG1 MSP1 (n = 151)</td>
<td>0.83 (.76–.90)</td>
<td>&lt;.001</td>
<td>0.85 (.74–.98)</td>
</tr>
<tr>
<td>IgG1 EBA175 (n = 183)</td>
<td>0.85 (.79–.91)</td>
<td>&lt;.001</td>
<td>0.89 (.80–1.00)</td>
</tr>
<tr>
<td>IgG1 AMA1 (n = 187)</td>
<td>0.94 (.88–1.00)</td>
<td>.041</td>
<td>0.88 (.81–.94)</td>
</tr>
<tr>
<td>IgG1 lysate (n = 159)</td>
<td>0.94 (.80–1.09)</td>
<td>.408</td>
<td>0.83 (.70–.98)</td>
</tr>
<tr>
<td>IgG3 MSP1 (n = 121)</td>
<td>0.89 (.82–.98)</td>
<td>.015</td>
<td>0.82 (.69–.97)</td>
</tr>
<tr>
<td>IgG3 EBA175 (n = 139)</td>
<td>0.76 (.68–.84)</td>
<td>&lt;.001</td>
<td>0.73 (.61–.88)</td>
</tr>
<tr>
<td>IgG3 AMA1 (n = 170)</td>
<td>0.90 (.83–.97)</td>
<td>.009</td>
<td>1.02 (.89–1.17)</td>
</tr>
<tr>
<td>IgG3 lysate (n = 163)</td>
<td>0.91 (.80–1.03)</td>
<td>.140</td>
<td>0.79 (.65–.97)</td>
</tr>
</tbody>
</table>

For each malaria-specific antibody, only women seropositive for that antibody were included.

Abbreviations: AMA1, apical membrane antigen 1; CI, confidence interval; EBA175, erythrocyte binding antigen 175; IgG, immunoglobulin G; IPTp, intermittent preventive treatment in pregnancy; MSP1, merozoite surface protein 1.

<sup>a</sup> Defined as the proportional increase in cord Ab levels per doubling of Ab levels in the mother. The analysis adjusted for the following maternal factors: antibody levels, HIV infection and malaria, parity, age, anemia, and IPTp group.

<sup>b</sup> Defined as the ratio of the mean cord IgG levels for infected women with respect to uninfected women. The analysis adjusted for the following maternal factors: antibody levels, HIV infection and malaria, parity, age, anemia, and IPTp group.

Figure 3. Cord-to-mother ratio (CMR) of antimalarial antibody levels, by maternal human immunodeficiency virus (HIV) infection (A) and malaria (B) status, in the univariate analysis. Horizontal black lines correspond to the geometric mean of the population. *P < .05, **P < .01, and ***P < .001, by the Student t test. Abbreviations: AMA1, apical membrane antigen 1; EBA175, erythrocyte binding antigen 175; IgG, immunoglobulin G; MSP1, merozoite surface protein 1.
infected mothers to hematological complications when infected has been suggested as one of the possible mechanisms explained by compartment of infection or diagnostic method used (microscopy and/or histological analysis vs qPCR, the most sensitive detection method [45]) or the statistical analysis performed (ie, adjustment for levels of maternal antibodies or use of antibody levels in the cord as a measure of transplacental transfer, instead of CMR, the more appropriate metric) may account for differences with our results.

The reduction of placental antibody transfer was not consistently associated with adverse pregnancy outcomes or an increased risk of incident malaria during the first year of life. Although a decrease in the transfer of antimalarial antibodies has been suggested as one of the possible mechanisms explaining the increased predisposition of children born to HIV-infected mothers to hematological complications when infected with malaria [3, 46], our results do not support this hypothesis. Higher transfer of IgG against lysate and IgG1 against AMA1 was associated with an increased risk of incident malaria during the first year of life, probably pointing toward these antibodies as markers of the risk of infection rather than protection, as previously suggested [10, 11, 47]. It is therefore plausible that other physiological factors, such as the presence of fetal hemoglobin, lactoferrin, and secretory immunoglobulin A or a reduced level of para-aminobenzoic acid, are involved in the relative protection against malaria of infants aged <6 months [10, 48].

This study presents several limitations. First, it was not possible to assess the effect of HIV-associated immunosuppression on the transfer of antimalarial antibodies [11, 17], as data on CD4+ T-cell counts and viral loads were not available for all HIV-infected women. Second, the presence of malaria in pregnant women was determined at delivery and did not account for malaria occurring earlier during gestation, which could also affect the response and transfer of antimalarial antibodies. The number of women included in this study did not allow further stratification by compartment of infection or diagnostic method; therefore, further research will be required to clarify the effect of peripheral infection by itself, as well as the effect of chronic placental malaria, which was previously associated with reduced antibody transfer [14, 18]. Potential differences between symptomatic and asymptomatic infections, as well as between submicroscopic or microscopic malaria, on antibody passage should be addressed. Finally, our observational approach describes potential associations, but causal relationships cannot be inferred. Although IPTp with SP was not associated with changes in antimalarial antibody transfer in this population, interventions reducing the malaria burden might be expected to revert the adverse impact of \textit{P. falciparum} on antibody transfer. Future studies should analyze the effect of different IPTp drugs and regimens on placental IgG passage.

In summary, this study shows that malaria and HIV infection during pregnancy are independently associated with a decrease in placental transfer of antibodies against asexual blood-stage \textit{P. falciparum} antigens from mother to fetus. The high prevalence of HIV infection in Mozambique and other parts of Southeast Africa [2], together with declines in malaria transmission [49], may translate into a reduction of antimalarial immunity in pregnant women and affect antibody transfer and immunity development in their infants. The role that antimalarial antibodies transferred from mother to fetus have in infant protection and the consequences of the reduction of antibody passage associated with maternal malaria and HIV infection should be further investigated for the design of effective vaccines in pregnancy.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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