The Effect of Intermittent Preventive Treatment during Pregnancy on Malarial Antibodies Depends on HIV Status and Is Not Associated with Poor Delivery Outcomes

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Background. Intermittent preventive treatment during pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) is recommended for malaria prevention in sub-Saharan Africa. However, studies reporting the effect of IPTp on malaria-specific immunity are scarce and are based on findings in human immunodeficiency virus (HIV)–negative primigravidae.

Methods. Plasma samples obtained from 302 pregnant women (177 who were HIV negative, 88 who were HIV positive, and 37 who were of unknown HIV status) participating in a placebo-controlled trial of IPTp with SP (IPTp-SP) were analyzed for the presence of antibodies against merozoite antigens, whole asexual parasites, and variant surface antigens from chondroitin sulfate A–binding and nonbinding lines. Antibody levels were compared between intervention groups, and their association with morbidity outcomes was assessed.

Results. HIV-positive mothers receiving SP had lower levels of peripheral antibodies against apical membrane antigen–1 and variant surface antigens, as well as lower levels of cord antibodies against erythrocyte-binding antigen–175 and parasite lysate, than did HIV-positive placebo recipients. No difference between intervention groups was observed among HIV-negative mothers. High antibody levels were associated with maternal infection and an increased risk of a first malaria episode in infants. Antibody responses were not consistently associated with reduced maternal anemia, prematurity, or low birth weight.

Conclusions. The IPTp-associated reduction in antibodies in HIV-infected women, but not in HIV-uninfected women, may reflect a higher efficacy of the intervention in preventing malaria among HIV-positive mothers. This reduction did not translate into an enhanced risk of malaria-associated morbidity in mothers and infants.

Trial registration. Clinicaltrials.gov identifier NCT00209781.
(HIV) pandemic, because maternal HIV infection has been shown to reduce humoral immune responses against malaria [8, 9].

It has been suggested that the reduction in exposure to *Plasmodium falciparum* brought about by malaria prevention strategies, such as chemoprophylaxis, might interfere with the development of malaria-specific immunity in children and adults [10–15]. The rationale behind the use of intermittent preventive treatment regimens is to reduce the clinical consequences of malaria without preventing the development or maintenance of immune responses. This seems to be the case for intermittent preventive treatment in infants [16]. However, studies assessing the effect of interventions during pregnancy on maternal immunity are scarce [17–19], and only one study has specifically addressed the immunologic consequences of IPTp in HIV-negative primigravidae [7]. In that study, IPTp with SP (IPTp-SP) was associated with lower levels of immunoglobulin G (IgG) against *P. falciparum* variant surface antigens (VSAs) expressed by parasites that adhere to chondroitin sulfate A (CSA). The clinical relevance of CSA-binding parasites in pregnancy is suggested by the finding that this adhesion phenotype is exclusive to placental parasites [20]. The parity-dependent development of antibodies that block parasite sequestration in the placenta [21, 22] has been associated with a reduction in the deleterious consequences of malaria in pregnancy [23, 24]. Therefore, prevention strategies that reduce exposure to placental parasites might potentially impair the acquisition of pregnancy-specific malaria antibodies, especially during first pregnancies, or the maintenance of maternal immune responses during subsequent pregnancies. Moreover, IPTp might also have an effect on the immune responses of the newborn by affecting transplacental passage of maternal antibodies [25] or fetal in utero exposure to malaria antigens [26, 27]. However, controversial results about the effect of maternal chemoprophylaxis on newborn immune responses have been obtained only from observational studies [17, 28], and no randomized controlled trial has addressed the consequences of malaria prevention during pregnancy on neonatal immunity.

To evaluate the effect of IPTp on the humoral immune responses of mothers at delivery, maternal antibodies against *P. falciparum* were analyzed in 302 pregnant women in Mozambique, who were participating in a randomized, placebo-controlled trial of IPTp-SP following malaria prevention during pregnancy (ClinicalTrials.gov identifier NCT00209781) [30]. After providing written informed consent, pregnant women received a long-lasting ITN and were randomized to receive SP or placebo. Women went through the voluntary counseling program for mother-to-child prevention of HIV and were screened for HIV if they agreed to testing [30]. At the time of delivery, peripheral and cord blood samples were collected into EDTA (ethylenediaminetetraacetic acid) vacutainers and on filter papers (no. 903TM; Schleicher & Schuell). Tissue samples obtained from the maternal side of the placenta were placed into 10% neutral-buffered formalin, and placental blood spots were also collected in filter paper. Eight weeks after delivery, a maternal capillary blood sample was collected for parasitologic determinations. Malaria episodes were recorded for infants during the first year of life, through use of a passive case detection system based on the reporting of all malaria cases detected in children attending the Manhiça outpatient clinic. The last 309 women recruited into the main trial were included in the immunologic study. The study was approved by the national Mozambican ethics review committee and the Hospital Clinic of Barcelona ethics review committee.

### HIV and parasitologic examination.

Maternal HIV type 1 (HIV-1) status was determined using the Determine HIV-1/2 Rapid Test (Abbott Laboratories) and confirmed using the Uni-gold rapid test (Trinity Biotech). Thin and thick smears of maternal and infant peripheral blood were Giemsa stained and examined for malarial parasites according to quality-control procedures [31]. Placental biopsy specimens were processed for histologic examination and classified according to criteria previously reported elsewhere [32]. Peripheral and placental blood samples collected on filter papers were screened for *P. falciparum* DNA by use of a species-specific quantitative polymerase chain reaction (qPCR) targeting the 18S ribosomal RNA gene, as described elsewhere [33].

### Measurement of antibodies against merozoite recombinant antigens and whole-parasite extract.

Maternal and cord plasma samples were tested by enzyme-linked immunosorbent assay for the presence of IgG, IgM, and IgG subclasses specific against the recombinant antigens merozoite surface protein–1 (MSP-119; 19-kD fragment, 3D7), erythrocyte-binding antigen–
175 (EBA-175; region F2, Camp), and apical membrane antigen–1 (AMA-1; full ectodomain, 3D7) from ICGEB, as well as against *P. falciparum* blood-stage lysate. High-binding 96-well microplates (Nunc Maxisorp) were coated overnight at 4°C with 200 ng per well of recombinant antigen diluted in 0.05 mol/L carbonate-bicarbonate buffer. After blocking with 2% bovine serum albumin at 4°C for 8 h, 100 μL of plasma diluted at 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 (Sigma); sheep anti–human IgG1, 1:6000; IgG2, 1:3000; IgG3, 1:6000; and IgG4, 1:3000 (The Binding Site). Reactions were developed as described elsewhere [16], and optical density (OD) values 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, MR4, ATCC) at a 5% level of parasitemia and 1% hematocrit. Noninfected erythrocyte (NIE) lysate prepared in the same way as whole-parasite lysate was used as a control for each plasma sample. Plates were coated with 50 μL/well of extract. Wells were blocked with 300 μL of 5% skim milk at 4°C for 8 h. One hundred microliters of plasma sample were tested in duplicate for IgG (dilution, 1:6400) and for IgM and IgG subclasses (dilution, 1:1600). Incubation of antibodies and development of the reaction were performed as described above. Malaria-specific antibody recognition was evaluated by subtracting the mean OD value of NIEs from the mean OD value of infected erythrocytes (IEs). A pool of plasma samples obtained from 8 Mozambican adults was used to normalize the data from different enzyme-linked immunosorbent assays. Cutoff values for positivity were calculated as the arithmetic mean plus 3 standard deviations of 15 plasma samples from Spanish pregnant women who had never been exposed to malaria.

**Measurement of antibody responses against VSAs.** Peripheral plasma samples collected from the pregnant women were tested by flow cytometry for recognition of the CSA-binding strain CS2 (MRA-96, MR4, ATCC) and a non–CSA-binding isolate that was obtained from a Mozambican child and adapted to in vitro culture (MOZZ2). Mature trophozoites at parasitemia levels of 1%–3% were cryopreserved in multiple aliquots to minimize interassay variations among different experiments [34]. After thawing, 95 μL of the parasite suspension at 1% hematocrit were incubated in 96-well round-bottom plates with 5 μL of test plasma at room temperature for 30 min. Cell suspensions were sequentially incubated for 30 min with 100 μL of polyclonal rabbit anti–human IgG (DakoCytomation) diluted at 1:200 and 100 μL of AlexaFluor donkey anti–rabbit IgG diluted at 1:1000 (Invitrogen) plus 10 μg/mL ethidium bromide. Data from 1000 events in the channel for ethidium bromide–labeled erythrocytes were acquired with a Becton-Dickinson FACSCalibur flow cytometer. Reactivity against the IE surface was expressed as the difference between the mean fluorescence intensity (MFI) of IEs and the MFI of NIEs. The mean (plus 3 standard deviations) of MFI values obtained for 15 negative control plasma samples collected from malaria-naive pregnant Spanish women was subtracted from all tested MFI values. A pool of plasma samples obtained from 15 Mozambican pregnant women was used to normalize the data from different assays.

**Statistical methods and definitions.** Peripheral infection was defined as the presence of parasites detected in peripheral blood by optical microscopy or by qPCR. Placental infection was defined as the presence of parasites and/or pigment detected by histologic examination or by qPCR. Maternal anemia was considered to develop when the hematocrit was <33%. A birth occurring before 37 weeks of gestation was classified as preterm, and LBW was considered if birth weight was <2500 g. Infant malaria episodes were defined by a positive blood smear result plus an axillary temperature ≥37.5°C and/or fever reported within the previous 24 h. Women were classified as primigravidae (PG), if they were in their first pregnancy, and multigravidae (MG), if they reported having ≥1 previous pregnancy.

OD and MFI values were log transformed, and the averages within groups were presented as geometric means plus 95% confidence intervals. Cord blood IgM data were analyzed in terms of seroprevalence. Differences between intervention groups in terms of antibody levels and the seropositivity of cord IgM responses were estimated using Student’s *t* test and Fisher’s exact test, respectively. Linear regression models were used to compare IgG levels between intervention groups stratified by HIV (parity adjusted) and parity (HIV adjusted). Interaction between SP administration and HIV infection was assessed by analysis of variance. Correlation between maternal and corresponding cord blood IgG levels was assessed by Spearman’s correlation test. Linear regression models were used to evaluate the association between antibody levels and maternal infection both at delivery and postpartum, maternal anemia, prematurity, and LBW, after adjusting for HIV status and parity as potential confounding variables. Cox regression models were estimated to evaluate the association of antibody responses with time to the first malaria episode in infants. Adjustment for multiple comparisons was done by Monte Carlo permutation tests with 1000 random permutations [35]. A permuted *P* value of <0.05 was considered to denote statistical significance. Data analysis was performed using Stata software (version 10.0; Stata).

**RESULTS**

**Characteristics of the women participating in the study.** The analysis of antibody responses finally included 302 women (7 peripheral plasma samples were not available), 152 of whom had received SP and 150 of whom had received placebo. There
were 297 singleton deliveries, 4 twin deliveries, and 1 triplet delivery. A total of 258 cord plasma samples were tested for antibody responses (50 samples were not available as a result of home deliveries). Peripheral infection was detected in 32% and 15% of the mothers at the time of delivery or postpartum, respectively. Placental infection was detected in 60% of the women. Maternal anemia at delivery was diagnosed in 44% of the mothers. The prevalence of preterm births and delivery of LBW infants was 5.4% and 13.4%, respectively. Seventy-three of the women (24%) were PG, and 228 (76%) were MG (with data missing for 1 woman). Mean parity (for the placebo group vs the SP group, 23.9 vs 24.6; \( P = .348 \)) and mean maternal age (for the placebo group vs the SP group, 23.9 vs 24.6; \( P = .348 \)) were comparable between intervention groups. HIV status was determined for 265 of the women, and HIV sero-prevalence was similar between mothers receiving placebo (39 [30%] of 129 women) and those receiving SP (49 [36%] of 136 women) (\( P = .297 \)).

**Effect of IPTp on maternal antibody responses against P. falciparum at delivery.** Maternal IgG levels showed no significant difference between the 2 intervention groups in the crude analysis, except for IgGs against VSAs expressed by MOZ2 (MOZ2VSA), which were found to be lower in the SP group than in the placebo group (3.26 vs 3.23; \( P = .348 \)) and mean maternal age (for the placebo group vs the SP group, 23.9 vs 24.6; \( P = .348 \)) were comparable between intervention groups. HIV status was determined for 265 of the women, and HIV sero-prevalence was similar between mothers receiving placebo (39 [30%] of 129 women) and those receiving SP (49 [36%] of 136 women) (\( P = .297 \)).

**Effect of IPTp on fetal antibody responses against P. falciparum.** Maternal and cord levels of IgGs specific for all antigens were significantly correlated (with \( r^2 \) ranging from 0.532 to 0.809; all \( P \) values were <.001). Crude analysis showed that cord IgG levels were not significantly different between the 2 intervention groups, except for IgGs against EBA-175 and \( P. falciparum \) lysate, which were found to be lower in the SP group

### Table 1. Effect of Intermittent Preventive Treatment during Pregnancy (IPTp) with Sulfadoxine-Pyrimethamine (SP) on Maternal and Cord Immunoglobulin G (IgG) Levels

<table>
<thead>
<tr>
<th>Antibodies, antigen</th>
<th>Geometric mean (95% CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal IgGs (n = 302)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSP-1</td>
<td>0.41 (0.37–0.46)</td>
<td>.059</td>
</tr>
<tr>
<td>EBA-175</td>
<td>0.60 (0.54–0.67)</td>
<td>.078</td>
</tr>
<tr>
<td>AMA-1</td>
<td>1.11 (1.01–1.21)</td>
<td>.253</td>
</tr>
<tr>
<td>Lysate</td>
<td>0.98 (0.84–1.14)</td>
<td>.317</td>
</tr>
<tr>
<td>CS2\textsubscript{vsA}</td>
<td>88.1 (64.6–120.3)</td>
<td>.322</td>
</tr>
<tr>
<td>MOZ2\textsubscript{vsA}</td>
<td>107.2 (94.1–122.2)</td>
<td>.030</td>
</tr>
<tr>
<td>Cord IgGs (n = 258)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSP-1</td>
<td>0.29 (0.24–0.33)</td>
<td>.120</td>
</tr>
<tr>
<td>EBA-175</td>
<td>0.40 (0.35–0.46)</td>
<td>.045</td>
</tr>
<tr>
<td>AMA-1</td>
<td>1.00 (0.88–1.14)</td>
<td>.170</td>
</tr>
<tr>
<td>Lysate</td>
<td>0.72 (0.61–0.85)</td>
<td>.033</td>
</tr>
</tbody>
</table>

**NOTE.** Geometric mean values are expressed as optical density measurements, except for CS2\textsubscript{vsA} and MOZ2\textsubscript{vsA}, which are expressed as the mean fluorescence intensity. All comparisons were corrected by the Monte Carlo permutation test (1000 random permutations). AMA-1, apical membrane antigen–1; CI, confidence interval; CS2\textsubscript{vsA}, variant surface antigens expressed by the chondroitin sulfate A–binding strain CS2; EBA-175, erythrocyte-binding antigen–175; MOZ2\textsubscript{vsA}, variant surface antigens expressed by a non–CSA-bind- ing field isolate obtained from a Mozambican child; MSP-1, merozoite surface protein–1.

\( a \) Student’s \( t \) test.

\( b \) Linear regression models adjusted by human immunodeficiency virus (HIV) status and parity. Because of unknown parity and/or HIV status, 37 values were missing for maternal IgGs and 31 for cord IgGs.

\( c \) Data were missing for 6 women.
Effect of IPTp on Maternal and Cord Immunoglobulin G (IgG) Levels, by Human Immunodeficiency Virus (HIV) Status

Table 2. Effect of Intermittent Preventive Treatment during Pregnancy (IPTp) with Sulfadoxine-Pyrimethamine (SP) on Maternal and Cord Immunoglobulin G (IgG) Levels, by Human Immunodeficiency Virus (HIV) Status

| Antibodies, antigen | HIV negative | | | HIV positive | | |
|---------------------|-------------|------------------|------------------|
|                     | Adjusted effect | (95% CI) | P a | Adjusted effect | (95% CI) | P a |
| Maternal IgG 5       |             |             |     |             |             |     |
| MSP-1               | 0.89 (0.72–1.09) | .263 | 0.81 (0.63–1.04) | .089 |
| EBA-175             | 0.90 (0.73–1.11) | .330 | 0.75 (0.56–1.01) | .052 |
| AMA-1               | 1.00 (0.84–1.19) | .997 | 0.72 (0.55–0.94) | .013 |
| Lysate              | 0.97 (0.76–1.25) | .822 | 0.73 (0.52–1.03) | .063 |
| CS2 VSA             | 1.22 (0.72–2.06) | .447 | 0.31 (0.15–0.67) | .002 |
| MOZ2 VSA c          | 0.95 (0.76–1.19) | .657 | 0.57 (0.33–0.98) | .041 |
| Cord IgG 8          |             |             |     |             |             |     |
| MSP-1               | 0.90 (0.69–1.16) | .438 | 0.74 (0.52–1.05) | .097 |
| EBA-175             | 0.93 (0.72–1.19) | .537 | 0.68 (0.48–0.97) | .031 |
| AMA-1               | 0.98 (0.78–1.22) | .836 | 0.73 (0.50–1.06) | .107 |
| Lysate              | 0.89 (0.67–1.18) | .394 | 0.50 (0.30–0.83) | .004 |

**NOTE.** Effects refer to the ratio of antibody levels between women receiving SP and women receiving placebo. All comparisons were corrected by the Monte Carlo permutation test (1000 random permutations). AMA-1, apical membrane antigen–1; CI, confidence interval; CS2 VSA, variant surface antigens expressed by the chondroitin sulfate A–binding strain CS2; EBA-175, erythrocyte-binding antigen–175; MOZ2 VSA, variant surface antigens expressed by a non–CSA-binding field isolate obtained from a Mozambican child; MSP-1, merozoite surface protein–1.

a Linear regression models adjusted by parity.

b A total of 177 HIV-negative women and 88 HIV-positive women were assessed.

c Data were missing for 6 women.

d A total of 153 HIV-negative women and 74 HIV-positive women were assessed.

than in the placebo group (Table 1). However, for anti-EBA-175 antibodies, statistical significance was lost after adjusting for maternal HIV status and parity (Table 1). Similarly, cord IgG-subtype levels did not differ between intervention groups in the adjusted analysis, with the exception of IgG against MSP-1 (P = .049) and IgG 1,4 against P. falciparum lysate (P = .022 and P = .006, respectively), which were found to be lower in mothers receiving SP than in those receiving placebo. The analysis stratified by HIV status and adjusted by parity showed that the intervention did not significantly affect IgG levels in cord samples from HIV-negative mothers (Table 2). However, HIV-positive mothers receiving SP had significantly lower levels of cord IgGs against EBA-175 and P. falciparum lysate than did mothers receiving placebo (Table 2). Again, the interaction terms from analysis of variance showed that the effect of IPTp on cord levels of IgG against P. falciparum–lysate is modified by HIV infection: infants of HIV-positive women who received IPTp-SP had lower levels of antibody against this antigen than did infants of HIV-negative women (P = .031). The analysis stratified by parity and adjusted by HIV status showed no significant effect of IPTp on cord IgG levels among PG or MG, with the exception of cord IgGs against EBA-175 and P. falciparum–lysate, which were found to be lower among MG receiving SP than among MG receiving placebo (P = .031 and P = .021, respectively).

IgM specific for MSP-1 was detected in 8 (3%) of the 258 cord plasma samples analyzed, in 12 (5%) for EBA-175, in 41 (16%) for AMA-1, and in 17 (7%) for P. falciparum lysate. Prevalence of IgM seropositivity did not differ between cord blood samples obtained from the 2 intervention groups in the adjusted analysis (data not shown).

**Association between antibody levels and morbidity outcomes.** Maternal levels of IgG against all antigens were found to be significantly higher in mothers presenting with malaria infection at delivery or postpartum, except for antibodies against MOZ2 VSA, which were not increased in those mothers with postpartum infection (Table 3). No association was found between antibody levels and maternal anemia at delivery, prematurity, or LBW, except for IgG levels against AMA-1, which were found to be lower in mothers delivering a LBW infant, and IgGs against MSP-1, which were found to be higher in mothers with a preterm delivery (Table 3). Overall, 75 first or only episodes of malaria were observed during the first year of life of the infant (rate, 24.18 episodes/1000 person-months at risk). An increase in maternal levels of IgG against MSP-1, AMA-1, P. falciparum–lysate, and CS2 VSA and in cord IgGs against MSP-1 and P. falciparum–lysate was significantly associated with the risk of first or only episodes of malaria in infants (Tables 4 and 5, the latter of which appears only in the electronic version of the Journal). No significant association
Table 3. Association between Maternal Immunoglobulin G Levels at Delivery and Morbidity Outcomes

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Peripheral infection at delivery</th>
<th>Placental infection</th>
<th>Postpartum peripheral infection</th>
<th>Maternal anemia at delivery</th>
<th>Low birth weight</th>
<th>Prematurity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adj. effect (95% CI)</td>
<td>P&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Adj. effect (95% CI)</td>
<td>P&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Adj. effect (95% CI)</td>
<td>P&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MSP-1</td>
<td>1.25 (1.06–1.48) .006</td>
<td>1.35 (1.15–1.60) &lt;.001</td>
<td>1.29 (1.02–1.61) .032</td>
<td>1.12 (0.95–1.31) .182</td>
<td>0.93 (0.74–1.18) .582</td>
<td>1.47 (1.02–2.14) .041</td>
</tr>
<tr>
<td>EBA-175</td>
<td>1.24 (1.04–1.47) .023</td>
<td>1.39 (1.17–1.65) &lt;.001</td>
<td>1.35 (1.06–1.70) .014</td>
<td>1.07 (0.91–1.26) .422</td>
<td>1.00 (0.78–1.27) .983</td>
<td>1.22 (0.84–1.79) .313</td>
</tr>
<tr>
<td>AMA-1</td>
<td>1.19 (1.03–1.39) .016</td>
<td>1.52 (1.30–1.76) &lt;.001</td>
<td>1.38 (1.12–1.69) .004</td>
<td>1.04 (0.90–1.20) .577</td>
<td>0.74 (0.60–0.91) .005</td>
<td>0.80 (0.57–1.12) .193</td>
</tr>
<tr>
<td>Lysate</td>
<td>1.35 (1.08–1.68) .010</td>
<td>1.64 (1.34–2.01) &lt;.001</td>
<td>1.38 (1.04–1.85) .017</td>
<td>1.13 (0.91–1.39) .277</td>
<td>1.11 (0.81–1.50) .513</td>
<td>1.59 (0.98–2.57) .057</td>
</tr>
<tr>
<td>CS2&lt;sub&gt;CSA&lt;/sub&gt;</td>
<td>2.98 (1.93–4.61) &lt;.001</td>
<td>4.33 (2.85–6.59) &lt;.001</td>
<td>2.70 (1.48–4.91) &lt;.001</td>
<td>1.27 (0.82–1.96) .299</td>
<td>1.07 (0.57–2.02) .825</td>
<td>2.05 (0.76–5.52) .159</td>
</tr>
<tr>
<td>MOZ2&lt;sub&gt;CSA&lt;/sub&gt;</td>
<td>1.52 (1.20–1.91) &lt;.001</td>
<td>1.51 (1.19–1.90) &lt;.001</td>
<td>1.28 (0.93–1.75) .124</td>
<td>0.89 (0.71–1.12) .315</td>
<td>1.16 (0.84–1.61) .345</td>
<td>1.10 (0.66–1.83) .732</td>
</tr>
</tbody>
</table>

**NOTE.** Effects refer to the ratio of antibody levels between the group with the morbidity outcome and the group with the normal outcome. All comparisons were corrected by the Monte Carlo permutation test (1000 random permutations). Adj., adjusted; AMA-1, apical membrane antigen–1; CI, confidence interval; CS2<sub>CSA</sub>, variant surface antigens expressed by the chondroitin sulfate A–binding strain CS2; EBA-175, erythrocyte-binding antigen–175; MOZ2<sub>CSA</sub>, variant surface antigens expressed by a non–CSA-binding field isolate obtained from a Mozambican child; MSP-1, merozoite surface protein–1.

* Linear regression models were adjusted by human immunodeficiency virus status and parity.
was found between cord IgM seropositivity and the risk of malaria episodes in children during the first year of life (Tables 4 and 5, the latter of which appears only in the electronic version of the Journal).

**DISCUSSION**

The results of the present study show that 2 doses of IPTp-SP administered to HIV-negative pregnant women who were concurrently using long-lasting ITNs for malaria prevention is not associated with a reduction in *P. falciparum*–specific antibody responses at delivery in women and their newborns. In contrast, IgG levels were reduced in HIV-positive mothers receiving SP, compared with HIV-positive mothers receiving placebo. Interestingly, this decrease was found both for antibodies against pregnancy-specific antigens (VSAs from the CSA-binding line CS2) and for non–pregnancy-associated antigens (AMA-1 and VSAs from the pediatric isolate MOZ2). Furthermore, maternal infection and maternal antibody levels against all antigens were positively associated, suggesting that IgG levels at delivery may reflect maternal exposure to the parasite. The differences in the effect of the intervention on malaria-specific responses between HIV-infected and -uninfected pregnant women may be explained by the fact that IPTp-associated reduction in placental infection (ie, exposure to parasite antigens) was greater in HIV-positive mothers [30]. Moreover, a reduction in exposure may have a greater effect on the humoral responses of HIV-positive women, whose immune systems are impaired by viral infection, compared with HIV-negative women [8, 9].

Although our findings were based on a small sample size, levels of antibodies against pregnancy-specific antigens in PG were not found to be affected by the intervention. These results contrast with the only previous study reporting that HIV-negative Kenyan PG who received IPTp-SP had lower antibodies against CSA-binding parasites than did women who received placebo [7]. The discrepancy in the results may be explained by a difference in the number of SP doses, because the effect of IPTp with 3 SP doses on the immunity of Kenyan mothers was more pronounced than the effect noted after 1 or 2 doses [7]. In the present study, women received only 2 SP doses during pregnancy, which might be a critical number of antimalarial doses to allow the development of antibody responses to placental parasites in HIV-negative mothers. Alternatively, levels of antibodies may be reduced by the use of ITNs in such a way that no further reduction can be observed after administration of the IPTp regimen [36].

Of great concern is the possibility that the reduction in antibodies associated with IPTp might potentially translate into an increased risk of poor pregnancy outcomes and infant malaria. Nevertheless, no significant association was observed between pregnancy-specific antibody levels (IgGs against CS2VSA) at delivery and such morbidity outcomes as maternal anemia, prematurity, or LBW, suggesting that other immune mechanisms might be involved in protection against the adverse effects of malaria in pregnancy. Alternatively, antiadhesion antibodies may have a more relevant role during the initial phases of pregnancy. Susceptibility of mothers to malaria postpartum also was not enhanced by the IPTp-associated reduction in antibodies at delivery. Similarly, the decrease in transplacental IgGs in newborns did not increase their risk for clinical malaria during the first year of life. On the contrary, maternal and cord IgG levels were found to be associated with an increased risk

**Table 4. Antibody Responses and the Risk of a First Malaria Episode during the First Year of Life**

<table>
<thead>
<tr>
<th>Antibody finding, antigen</th>
<th>Adjusted HR (95% CI)</th>
<th>P*</th>
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<tbody>
<tr>
<td>Maternal IgG level (n = 265)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSP-1</td>
<td>1.41 (1.15–1.74)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>EBA-175</td>
<td>1.16 (0.92–1.45)</td>
<td>.210</td>
</tr>
<tr>
<td>AMA-1</td>
<td>1.35 (1.01–1.81)</td>
<td>.044</td>
</tr>
<tr>
<td>Lysate</td>
<td>1.44 (1.20–1.73)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CS2VSA</td>
<td>1.35 (1.21–1.50)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>MOZ2VSA</td>
<td>1.28 (0.98–1.67)</td>
<td>.083</td>
</tr>
<tr>
<td>Cord IgG level (n = 227)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSP-1</td>
<td>1.28 (1.05–1.56)</td>
<td>.018</td>
</tr>
<tr>
<td>EBA-175</td>
<td>1.08 (0.89–1.32)</td>
<td>.467</td>
</tr>
<tr>
<td>AMA-1</td>
<td>1.25 (0.97–1.62)</td>
<td>.088</td>
</tr>
<tr>
<td>Lysate</td>
<td>1.41 (1.16–1.72)</td>
<td>.001</td>
</tr>
<tr>
<td>Cord IgM seropositivity (n = 227)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSP-1</td>
<td>1.61 (0.47–5.55)</td>
<td>.487</td>
</tr>
<tr>
<td>EBA-175</td>
<td>1.47 (0.51–4.22)</td>
<td>.506</td>
</tr>
<tr>
<td>AMA-1</td>
<td>1.04 (0.51–2.11)</td>
<td>.924</td>
</tr>
<tr>
<td>Lysate</td>
<td>1.64 (0.70–3.83)</td>
<td>.264</td>
</tr>
</tbody>
</table>

*NOTE.* For immunoglobulin G (IgG) levels, the hazard ratio (HR) is the effect of a 2-fold increase in IgG levels on the risk of first or only malaria episode occurring in infants (ratio between the predicted hazards for infants whose mothers differ in terms of a 2-fold increase in IgG levels). For immunoglobulin M (IgM) seropositivity, the HR is the effect of a positive response on the risk of first or only episode of malaria occurring in infants, compared with a negative response. All comparisons were corrected by the Monte Carlo permutation test (1000 random permutations). AMA-1, apical membrane antigen–1; CI, confidence interval; CS2VSA, variant surface antigen (CS2) expressed by the chondroitin sulfate A–binding strain CS2; EBA-175, erythrocyte-binding antigen–175; MOZ2VSA, variant surface antigens expressed by a non–CSA-binding field isolate obtained from a Mozambican child; MSP-1, merozoite surface protein–1.

* Effect of IPTp on Malarial Antibodies

**Table 5. Incidence of Malaria Episodes in Infants during the First Year of Life, According to Maternal and Cord Antibody Responses**

This table is available in its entirety in the online edition of *The Journal of Infectious Diseases.*

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of malaria in offspring, as it had been previously suggested in similar studies [37, 38]. It could be argued that high antibody levels in pregnant women reflect maternal infection and, thus, an increased risk of fetal exposure to P. falciparum antigens, which may lead to enhanced malaria susceptibility in the offspring [39, 40]. However, in the present study, the presence of IgM in cord blood (an indicator of in utero exposure) was not associated with an increased risk of malaria during the first year of life. Alternatively, the positive association between maternal antibody levels and the risk of infection in the offspring might be explained by the similar risk of exposure to the parasites in mothers and infants living in the same household. Likewise, the strong association observed between high antibody levels at delivery and maternal infection postpartum may indicate that women who are infected during pregnancy are also at higher risk of infection after delivery.

In conclusion, a reduction in maternal and cord humoral immune responses to malaria was observed after administration of 2 IPTp-SP doses to HIV-positive pregnant women, but not to HIV-negative mothers, probably reflecting the combined effect of HIV immunosuppression and the reduction in exposure to malaria antigens resulting from the intervention. Of importance, this reduction in P. falciparum-specific antibodies did not translate into an enhanced risk of malaria-associated morbidity in mothers and infants, indicating that IPTp regimens can be recommended to all pregnant women, regardless of their HIV status. Finally, it remains to be established whether humoral responses at earlier stages of pregnancy or other immunologic mechanisms, such as cellular immunity, could be affected by IPTp regimens with SP or with alternative antimalarial drugs currently under evaluation.

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References


