Umbilical Cord–Blood Infections with *Plasmodium falciparum* Malaria Are Acquired Antenatally in Kenya

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**Background.** It is unknown whether the presence of *Plasmodium falciparum* malaria parasites in umbilical cord blood denotes infection acquired antenatally or contamination with infected maternal blood at delivery.

**Methods.** Parasites were quantified by real-time quantitative polymerase chain reaction (RTQ-PCR) and were genotyped in paired maternal- and cord-blood samples obtained from 632 pregnant Kenyan women and their newborns. Placental alkaline phosphatase (PLAP) and polyclonal immunoglobulin E levels were also quantified in paired maternal- and cord-blood samples, as markers of admixture of maternal blood with cord blood.

**Results.** Sixty-six cord-blood samples (10.4%) contained falciparum malaria, as detected by RTQ-PCR. For 25 of the infected cord-blood samples, either absence of infection was noted in paired maternal-blood samples at delivery (n = 16) or amplicon levels in cord-blood samples were 10-fold higher than those in maternal-blood samples (n = 9). Of the paired maternal- and cord-blood samples that were both infected, 57% showed discordant malaria parasite strains. There was no correlation between maternal parasitemia and levels of PLAP and immunoglobulin E in cord blood. PLAP levels, however, were significantly higher in cord-blood samples obtained from newborns of primigravid or secundigravid women with placental malaria, compared with cord-blood samples obtained from newborns of women without placental malaria or multigravid women. These findings indicate that parity and placental malaria are risk factors for maternofetal transfusion.

**Conclusions.** Malaria parasites identified in cord blood are acquired antenatally by transplacental transmission of infected erythrocytes. Primigravid and secundigravid women with placental malaria are at increased risk for congenital infection.

Pregnant women experience an increased risk of *Plasmodium falciparum* infection during pregnancy. In countries where malaria is endemic, this risk represents a major health problem that often results in severe maternal anemia, low-birth-weight newborns, and increased infant mortality.

Sequestration of *P. falciparum*-infected erythrocytes in the placenta during pregnancy is mediated by *P. falciparum* erythrocyte membrane protein 1, which binds to chondroitin sulfate A and hyaluronic acid on the placental syncytiotrophoblastic membrane [1, 2]. The host response to parasites in the placenta produces intervillous inflammation that is especially severe among women experiencing their first or second pregnancies [3] and that can damage the syncytiotrophoblastic membrane [4]. This inflammation may increase the risk of transplacental transmission of infected erythrocytes to the fetus during pregnancy.

*P. falciparum*-infected erythrocytes have been identified in umbilical cord blood with varying frequency. Some reports have suggested that up to 27% of cord-blood samples from malaria-endemic areas contain malaria parasites that can be identified by blood-smear analysis; however, most studies have indicated the prev-
alence to be ≤5% [5, 6]. The recent use of the more sensitive polymerase chain reaction (PCR) assays has routinely identified falciparum malaria parasites in 10%–32% of cord-blood samples obtained from individuals in areas where malaria is endemic [7–10], suggesting that the presence of malaria parasites in cord blood occurs with greater frequency than previously was appreciated. Often, the presence of malaria parasites in cord blood correlates with the presence and density of malaria parasites in intervillous placental blood (IVPB) or maternal peripheral blood; this finding suggests that the presence of malaria parasites may arise from admixture of maternal blood with cord blood at birth and not in utero [11, 12]. This distinction is important because prenatal exposure to malaria or the soluble products of malaria parasites may have an important effect on the fetal immune response, either by priming fetal immunity or inducing tolerance. If tolerance is induced, this may adversely affect the efficacy of blood-stage vaccines administered to infants. Congenital malaria may also contribute to intrauterine growth retardation and may have adverse long-term effects during infancy and childhood.

A comprehensive study to determine whether infected erythrocytes cross the placenta antenatally or whether they are acquired during the peripartum period has not been undertaken. Several observations have suggested that in utero exposure to malaria parasites or their soluble products occurs with considerable frequency [12, 13]. First, fetal lymphocytes are primed to blood-stage malaria antigens. Parasite-specific IgM and IgE have been detected in 11%–25% of cord-blood samples obtained from individuals in areas of endemicity [9, 14–16]. Because IgM does not cross the placenta during gestation, the presence of P. falciparum–specific IgM indicates activation of B cells by malaria parasites in utero. In addition, up to 60% of newborns have demonstrated lymphocyte reactivity to blood-stage malaria antigens in cord-blood mononuclear cells from newborns of malaria-infected women [17, 18]. Second, malaria parasite strains identified in cord blood were often different from those found in maternal peripheral or placental blood [8], indicating that malaria parasites in cord blood can be acquired antenatally.

In the current study, we quantified P. falciparum parasites in paired maternal peripheral-blood, IVPB, and cord-blood samples obtained from women and their newborns living in a malaria-endemic area of Kenya, by use of a real-time quantitative PCR (RTQ-PCR), because it is sensitive, specific, quantitative, and correlates with clinical outcomes of malaria [12]. We characterized the genotypes of infecting parasites in paired maternal and newborn blood samples, and we measured levels of placental alkaline phosphatase (PLAP) and polyclonal IgE, 2 molecules that are found in high concentrations in maternal blood but do not cross the placenta, as markers of admixture of maternal and newborn blood at delivery.

SUBJECTS, MATERIALS, AND METHODS

**Study population.** Healthy pregnant women residing in Kwale District, Coast Province, Kenya, where malaria is holoendemic, were recruited from the antenatal clinic at Msambweni District Hospital, as described elsewhere [7, 15]. Women received pyrimethamine-sulfadoxine prophylaxis at the beginning of their second and third trimesters. Newborns were weighed using a triple-beam balance with precision to ±10 g. During the peripartum period, maternal peripheral venous blood samples were collected, and IVPB samples were obtained by canulation of the intervillous space and removal of ~1 mL of free-flowing blood into a heparinized syringe. DNA was extracted from 200 μL of whole IVPB and from 200 μL of erythrocyte pellets from peripheral blood, as described elsewhere [15]. Cord blood was collected by canulation of the umbilical vein after inversion of the placenta and cleaning of the umbilical cord. Written, informed consent was obtained from all study participants. The study was approved by the institutional review boards of University Hospitals of Cleveland and the Kenya Medical Research Institute.

**Determination of HIV infection status and the presence of HIV or helminths.** For blood-smear analysis, thick and thin films were prepared from freshly collected whole blood, stained with 4% Giemsa, and examined for asexual P. falciparum in 100 oil-immersion fields in thick smears, as described elsewhere [12]. RTQ-PCR for the detection of multicopy, small-subunit, ribosomal RNA P. falciparum genes was performed exactly as described elsewhere [12, 15]. DNA was extracted from 200 μL of erythrocyte pellets from maternal peripheral and cord blood. The number of amplicons per microliter of whole blood was determined assuming an average volume of red blood cells of 0.3; thus, the number of amplicons was multiplied by 0.3. DNA was extracted from 200 μL of whole IVPB, and this conversion was not applied. For all samples, 2.5 μL of DNA was amplified. The presence of schistosomiasis, lymphatic filariasis, HIV antibodies in maternal blood, and intestinal helminths was measured as described elsewhere [19].

**Merozoite surface protein (MSP) genotyping.** Thirty PCR-positive samples of paired cord and maternal blood were genotyped for msp-1, msp-2, and glutamine-rich protein (glurp) genes, by use of nested PCR performed with internal primers specific for the 3 known allelic families of the block 2 region of the gene coding for MSP1, the 2 allelic families of the central polymorphic region of msp-2, and glurp as described elsewhere [20].

**PLAP assay and detection of total IgE.** The PLAP level was measured in maternal- and cord-blood samples, by use of the method of Kaneda et al. [21] as modified by Kwiek et al. [22]. The PLAP level in cord and maternal blood was determined by interpolation from a standard curve of purified hu-
Table 1. Factors associated with Plasmodium falciparum parasitemia in umbilical cord blood.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Newborns with cord-blood parasitemia and the risk factor specified, %</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primigravid or secundigravid</td>
<td>43</td>
<td>3.76 (1.25–7.89)</td>
</tr>
<tr>
<td>Maternal HIV infection</td>
<td>21</td>
<td>2.89 (1.03–11.34)</td>
</tr>
<tr>
<td>Intrauterine growth retardation</td>
<td>27</td>
<td>2.44 (1.35–8.56)</td>
</tr>
<tr>
<td>Coinfection with helminths(^a)</td>
<td>20</td>
<td>2.11 (0.95–7.07)</td>
</tr>
<tr>
<td>Delivery during the rainy season(^b)</td>
<td>18</td>
<td>1.62 (0.88–3.95)</td>
</tr>
<tr>
<td>Female sex of infant</td>
<td>47</td>
<td>0.91 (0.78–2.84)</td>
</tr>
</tbody>
</table>

**NOTE.** CI, confidence interval; OR, odds ratio.
\(^a\) Schistosomiasis, lymphatic filariasis, and/or presence of intestinal helminths.
\(^b\) Five months total.

man PLAP (Sigma) fit to a quadratic equation. The ELISA for total IgE was performed as described elsewhere [23].

**Statistics.** Data on the number of parasite copies were log-normally distributed. The values were expressed as geometric mean ± SE values, and statistical tests were performed using log-transformed values. To evaluate the association of maternal peripheral blood and IVPB with the presence of parasites in cord blood, samples were divided into 4 groups, on the basis of RTQ-PCR results: parasite-negative and parasite-positive samples with low, intermediate, and high parasitemia levels based on terciles. For the subset of paired maternal-infant blood samples for which we examined the association of PLAP and IgE levels with cord-blood parasitemia, samples were divided into quartiles. To determine the association with the primary outcome (i.e., the presence or absence of malaria parasites in cord blood), in addition to a number of different exposure variables (such as parity, levels of parasitemia in maternal blood, maternal HIV infection, sex of the newborn, and season of birth) and the potential interactions of these variables, we performed a forward stepwise logistic-regression analysis (binary logistic command) by use of SPSS software (version 13.0; SPSS). The factors in the univariate analysis and interaction terms were removed if they were not significantly associated with the presence of cord-blood parasitemia (P > .05) after adjustment for the variables shown in table 1 in the final model. For continuous variables, the significance of differences between groups was evaluated using Student’s t test, and associations between variables were examined using simple linear regression.

**RESULTS**

A total of 632 paired maternal- and cord-blood samples and 521 IVPB samples were examined, and the frequencies of malaria infection determined by RTQ-PCR and blood-smear analysis are shown in table 2. The frequency of malaria parasites, as assessed by RTQ-PCR, was 10.4% in cord blood, 15.9% in IVPB, and 36.4% in maternal peripheral venous blood.

**Factors associated with cord-blood parasitemia.** To determine the factors that affect the presence of malaria parasites in umbilical cord blood, we examined the association of parasitemia levels in maternal peripheral-blood samples and IVPB samples (estimated as the number of amplicons per microliter, by use of RTQ-PCR) with positive cord-blood results (table 3). Newborns of women with the highest levels of parasitemia in IVPB or peripheral blood were more likely to have malaria parasites in cord blood, compared with newborns of women who had the lowest parasite densities or who were lacking malaria parasites altogether (χ² = 88; 3 df; P < .001, for the association with peripheral blood parasitemia; χ² = 61; 3 df; P < .001, for the association with IVPB parasitemia density). However, among women who had falciparum malaria infection at delivery, there was no correlation of peak parasite densities (as determined by RTQ-PCR) in maternal peripheral-blood and/or IVPB samples with those in paired cord-blood samples (r² = 0.02; P = .5), which suggests infrequent admixture of maternal with fetal blood at delivery.

Additional factors associated with falciparum malaria in cord blood are shown in table 1. In a univariate analysis, birth to a primigravid or secundigravid woman, low birth weight, and presence of HIV were also significantly associated with the presence of P. falciparum in cord blood (table 1). Helminth coinfection was weakly associated with the presence of cord-blood parasitemia but not with the season of birth or the sex of the newborn (table 1). We used a logistic-regression model that included all factors in the univariate analysis, and log-transformed numbers of amplicons in maternal and peripheral blood identified the following variables as significant predictors of cord-blood infection: the logarithm of the number of placental amplicons, the logarithm of the number of maternal
Table 2. Frequency of malaria infection in pregnant women and their newborns, as determined by blood-smear analysis and real-time quantitative polymerase chain reaction (RTQ-PCR).

<table>
<thead>
<tr>
<th>Blood sample</th>
<th>Blood-smear analysis, n/N (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RTQ-PCR, n/N (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal PB</td>
<td>57/632 (9.0)</td>
<td>211/632 (33.4)</td>
</tr>
<tr>
<td>IVPB</td>
<td>14/521 (2.7)</td>
<td>83/521 (15.9)</td>
</tr>
<tr>
<td>Umbilical cord</td>
<td>14/626 (2.2)</td>
<td>66/626 (10.4)</td>
</tr>
</tbody>
</table>

NOTE. IVPB, intervillous placental blood; PB, peripheral blood.

<sup>a</sup> The no. of samples for which results were positive/total no. of samples tested (% of samples for which results were positive).

Peripheral-blood amplicons, the interaction term of numbers of placental and peripheral-blood amplicons, and birth to a primigravid or secundigravid woman (odds ratio, 2.95; 95% confidence interval, 1.31–7.25). Thus, parity and maternal malaria infection are important independent risk factors for cord-blood infection.

Of the 66 cord-blood samples in which malaria parasites were identified, 16 had paired maternal peripheral-blood and IVPB samples that lacked detectable parasites, as determined by RTQ-PCR (figure 1A). Nine additional cord-blood samples had parasite amplicon levels that were at least 1 log-fold greater than the number of amplicons observed in the maternal peripheral-blood or IVPB samples (figure 1B). Therefore, if malaria parasites in cord blood occurred by admixture with maternal blood at delivery, it would be expected that malaria parasites should be present and have at least similar or higher densities in maternal-blood samples, compared with paired cord-blood samples.

**Parasite genotypes in cord blood and maternal blood.** We examined whether the parasite strains in cord blood differed from those examined in maternal blood by genotyping the highly polymorphic loci of *msp-1*, *msp-2*, and *glurp* for most of the remaining paired maternal- and cord-blood samples for which the number of amplicons was lower than that observed in maternal blood at birth (n = 30) (table 4). One or more alleles of *msp-1* were detected in 92% of paired maternal- and cord-blood samples, ≥1 alleles of *msp-2* were detected in 89% of such samples, and ≥1 alleles of *glurp* were detected in 63% of such samples. Mixed infections were common in samples of maternal peripheral blood (median, 3 samples; range, 1–6 samples), IVPB (median, 4 samples; range, 1–6 samples), and cord blood (median, 2 samples; range, 1–5 samples). The genotypes of malaria parasites identified in cord blood were con-

Table 3. The proportion of newborns with *Plasmodium falciparum* parasitemia in umbilical cord blood, according to parasite density in maternal peripheral or intervillous placental blood based on real-time quantitative polymerase chain reaction.

<table>
<thead>
<tr>
<th>Sample, parasitemia level&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Parasite density, amplicons/μL&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Newborns with parasites in cord blood&lt;sup&gt;c&lt;/sup&gt;, n/N (%)&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal peripheral blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No infection</td>
<td>0</td>
<td>25/384 (6.5)</td>
</tr>
<tr>
<td>Lowest tercile</td>
<td>157</td>
<td>6/83 (7.2)</td>
</tr>
<tr>
<td>Middle tercile</td>
<td>13,368</td>
<td>11/82 (13.4)</td>
</tr>
<tr>
<td>Highest tercile</td>
<td>1,226,016</td>
<td>36/83 (43.4)</td>
</tr>
<tr>
<td>Intervillous placental blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No infection</td>
<td>0</td>
<td>20/429 (4.7)</td>
</tr>
<tr>
<td>Lowest tercile</td>
<td>1808</td>
<td>4/31 (12.9)</td>
</tr>
<tr>
<td>Middle tercile</td>
<td>48,754</td>
<td>5/30 (16.7)</td>
</tr>
<tr>
<td>Highest tercile</td>
<td>2,232,800</td>
<td>13/30 (43.3)</td>
</tr>
</tbody>
</table>

<sup>a</sup> The no. of women with positive results based on blood-smear analysis of maternal peripheral-blood samples, according to whether the parasitemia level of the sample was considered to be in the lowest, middle, or highest tercile, was 11 (13.3%) of 83 women, 20 (24.3%) of 82 women, and 31 (37.3%) of 83 women, respectively. Only 14 women had intervillous placental-blood samples that were found to be positive for malaria parasites by blood-smear analysis, with 9 women having parasitemia levels in the highest tercile.

<sup>b</sup> Geometric mean number of amplicons.

<sup>c</sup> According to real-time quantitative polymerase chain reaction.

<sup>d</sup> Data are no. of newborns with parasites in cord blood/total no. of newborns evaluated (% of newborns with parasites in cord blood).
considered to be concordant with those observed in maternal peripheral or placental blood, if the banding patterns of the alleles for 3 loci were identical or if a "subset" was identical (e.g., lacking some bands but otherwise the same as that observed in maternal blood). Parasite populations in cord blood were considered to be discordant if a banding pattern for \( \geq 1 \) loci was different from that observed in maternal blood. Overall, 57% of parasite genotypes identified in cord blood did not match those identified in maternal peripheral or placental blood at delivery.

**Association of PLAP and IgE levels in maternal blood with those in cord blood.** We measured, in paired maternal- and cord-blood samples, the levels of PLAP and polyclonal IgE, 2 molecules that are produced at high levels in women but not in the fetus [21]. Because these molecules do not cross the intact placenta during gestation [21], they have been previously used as markers of maternofetal transfusion during the peripartum period [7, 21, 24, 25]. PLAP and IgE levels were measured in a subset of 274 paired maternal- and cord-blood samples that included all parasite-positive cord-blood samples and that were obtained from all women who were found to have malaria infection at delivery, as well as from an equal number of women without malaria who were matched with malaria-infected women on the basis of age (\( \pm 3 \) years) and parity. The geometric mean \( \pm \) SE PLAP level in peripheral venous blood samples obtained from pregnant women was 1635 \( \pm 119 \) ng/mL (range, 25–5819 ng/mL) and 58 \( \pm 6 \) ng/mL (range, 25–3717 ng/mL) in cord blood. PLAP levels exceeded 1000 ng/mL in 89% of women, whereas only 4 cord-blood samples (1.4%) had PLAP levels >1000 ng/mL, 18 samples (6.6%) had PLAP levels of 200–1000 ng/mL, and 33 samples (12%) had PLAP levels of 100–199 ng/mL. There was no correlation between the PLAP levels in maternal and cord blood (\( r^2 = 0.017; \ P = .4 \)).

The geometric mean \( \pm \) SE serum IgE level was 143.3 \( \pm 13.6 \) \( \mu \)g/mL (range, 5–2506 \( \mu \)g/mL) in maternal-blood samples and 1.3 \( \pm 0.1 \) \( \mu \)g/mL (range, 0–63.5 \( \mu \)g/mL) in cord-blood samples. Only 1 infant had a serum IgE level >50 \( \mu \)g/mL, 5 infants (1.8%) had a serum IgE level >10 \( \mu \)g/mL, and 38 infants (13.9%) had a level >2.0 \( \mu \)g/mL. There was no correlation between polyclonal IgE levels in maternal and cord blood (\( r^2 = -0.001 \)). IgE and PLAP levels in cord blood were correlated (\( r^2 = 0.31; \ P < .0001 \)). Infants with PLAP levels >200 ng/mL (the highest 8% of samples) also had the highest IgE levels in cord blood (the highest 5.7% of samples).

Cord-blood samples with elevated PLAP and IgE levels are likely to have admixture with maternal blood at delivery. Therefore, cord-blood samples were divided into quartiles on the basis of their PLAP and IgE levels, because it is unknown exactly what PLAP and IgE levels denote admixture of blood considered to be significant enough to merit examination of the association between the levels of these molecules and the presence of malaria parasites in cord blood (table 5). It was determined that the proportion of newborns with malaria parasites in cord blood was similar when cord-blood samples with the highest and lowest PLAP and IgE levels were evaluated. In addition, there was no correlation between PLAP or IgE levels and parasite amplicon levels in cord blood (\( r^2 = 0.013; \ P = .6 \)).

PLAP and IgE levels in cord blood were often discordant, with high PLAP levels and low IgE levels usually noted. Because both PLAP and IgE levels should be high as a result of admixture of maternal and cord blood at delivery, this finding suggested that microtransfusions may have occurred antenatally, because PLAP has a longer half-life (5–6 days) [21] than IgE (1–2 days) [26]. To examine the hypothesis that placental malaria may increase the risk of occurrence of microtransfusions during gestation, as measured by elevated levels of PLAP,
Table 5. Frequency of malaria parasites in umbilical cord blood, according to whether placental alkaline phosphatase (PLAP) and IgE levels were high or low in cord-blood samples.

<table>
<thead>
<tr>
<th>Level in cord blood, quartile</th>
<th>Cord-blood samples with malaria parasites, no. (%)</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLAP</td>
<td></td>
<td>1.4</td>
<td>&gt;.2</td>
</tr>
<tr>
<td>Highest ($n = 66$)</td>
<td>13 (19.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest ($n = 66$)</td>
<td>8 (12.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgE</td>
<td></td>
<td>1.8</td>
<td>&gt;.2</td>
</tr>
<tr>
<td>Highest ($n = 65$)</td>
<td>22 (33.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest ($n = 65$)</td>
<td>15 (23.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

compared with levels of IgE, we examined the levels of these 2 molecules in cord blood in association with the presence of placental malaria and parity. PLAP levels in cord-blood samples obtained from newborns of women with placental malaria (geometric mean, 93.1 ng/mL) were higher than those in cord-blood samples obtained from newborns of women without placental malaria (geometric mean, 51.8 ng/mL) ($P = .04$). This difference in PLAP levels in cord blood was more striking in infants born to primigravid and secundigravid women with placental malaria than in those born to multigravid women (figure 2A). By contrast, PLAP levels in cord blood were not different in newborns of women with or without malaria in peripheral blood, irrespective of parity (figure 2B). There was no difference in IgE levels in cord blood for any of the same comparisons described above (data not shown).

DISCUSSION

The present study shows that malaria parasites, as detected by RTQ-PCR, are present in 10.4% of cord-blood samples in a malaria-holoendemic area on the coast of Kenya. The levels of parasitemia in peripheral blood and IVPB, as estimated by RTQ-PCR, positively correlated with the frequency and intensity of umbilical cord-blood parasitemia. This association, however, raises the important question of whether the presence of parasites in cord blood results from admixture of maternal and fetal blood at delivery or denotes malaria infections acquired antenatally. Undoubtedly, some malaria-infected erythrocytes mix with fetal blood at delivery, in a manner similar to the mechanisms of vertical transmission of hepatitis B virus and HIV during the peripartum period; however, we propose that this occurrence applies to the minority of samples. Our findings indicate that most malaria parasites identified in cord blood are acquired antenatally, on the basis of discordant levels of malaria parasitemia, parasite genotypes, and the observation that PLAP and IgE levels, which have been used as markers of admixture of maternal with fetal blood at delivery, fail to correlate with cord-blood parasitemia. We cannot, however, determine an exact proportion of parasites in cord blood that causes infection antenatally or perinatally, because of the imprecision of these assays (specifically, the failure to detect a malaria genotype by PCR does not exclude its presence, and the specific PLAP and IgE levels that denote significant admixture of maternal blood with fetal blood at birth are unknown).

The presence of HIV in pregnant women, low birth weights, parity, and, to a lesser extent, helminth coinfections were also associated with an increased risk for the detection of malaria parasites in cord blood in univariate analysis. However in multivariate analysis, these associations, with the exception of parity and the levels of parasitemia in maternal IVPB and peripheral blood, failed to reach statistical significance. This finding is not surprising, because low birth weights and the presence of HIV
are associated with placental malaria [3, 12, 19, 27]. Parity is also strongly associated with placental malaria, because women acquire immunity to recurrent infections with parasite strains that sequester in the placenta with each pregnancy [28]. Parity, however, remained an independent variable predicting cord-blood parasitemia, because it may detect the risk for placental malaria that occurs earlier during pregnancy but may have resolved by term.

We propose that malaria-infected erythrocytes or their soluble products transfer from the maternal IVPB to the fetus during gestation. This may occur through the intact placenta, because maternal erythrocytes can cross to the fetus in normal pregnancies [29]. Our results suggest that this transplacental transfer of malaria-infected erythrocytes increases during placental malaria, especially among primigravid and secundigravid women, in whom placental malaria occurs more frequently [4]. The correlation of elevated PLAP levels but not IgE levels in cord blood with placental malaria and parity supports this hypothesis of increased antenatal microtransfusions. The host immune response to sequestered parasites in the intervillous space in the placenta, or intervillitis, may compromise the integrity of the placenta or impede growth or repair of tears in the syncytiotrophoblastic membrane, which is a normal process of placental growth during pregnancy. A previous study found that the presence of cord-blood parasitemia correlated with the severity of placental inflammation resulting from placental malaria [4]. This transplacental passage of malaria parasites or their soluble products may occur as early as the beginning of the second trimester of pregnancy.

Malaria most commonly infects pregnant women between weeks 13 and 20 of gestation [30, 31]. At this time, chondroitin sulfate A is first expressed in the placenta, and whole blood begins to perfuse through the intervillous space [32]. This permits infected red blood cells to sequester in the placenta. As pregnancy progresses, the mother develops antibodies to infected erythrocytes, thereby reducing their ability to sequester and thus decreasing the parasite burden later in pregnancy [32]. It is possible that placental damage is most severe during the second trimester, thereby permitting infected erythrocytes to cross from the maternal circulation to the fetal circulation. Parasites might then be cleared in the maternal circulation and yet persist in the fetal circulation, because the fetus has not developed adequate cellular and humoral immunity, and because passive transfer of maternal antibodies may be insufficient or incomplete. This could explain (1) the presence of malaria parasites in cord blood at delivery and not in maternal blood or (2) the presence of discordant parasite strains. This explanation is also consistent with the observation that parity remains an independent risk factor for the presence of malaria parasites in cord blood at delivery.

There are important limitations in the current study. First, assessment of placental malaria was primarily determined by measuring parasites in the IVPB by use of RTQ-PCR and not by blood-smear analysis or placental biopsy. The low frequency of blood-smear-positive results for IVPB occurred because blood-smear analysis was not always performed immediately, resulting in lysis of erythrocytes. Second, failure to examine placental biopsy specimens permitted determination of only acute placental malaria and not determination of either the severity of placental malaria or whether chronic changes had occurred. Moreover, we cannot determine whether parasites in the IVPB arise from the peripheral circulation or from sequestration in the placenta. However, once detected, amplicon densities were higher in IVPB than in peripheral venous blood, indicating some sequestration. The use of RTQ-PCR may account for the observation of a lower frequency of malaria infection in IVPB, compared with peripheral venous blood, in the current study; this observation is the opposite of the findings of most other studies [9–11]. Third, the presence of malaria in pregnant women was determined only at delivery, and it is likely that additional women had malaria infection earlier during gestation. Finally, the lower parasite densities in cord blood, compared with maternal blood, may have resulted from a greater failure to detect rare parasite clones and, therefore, may have created a bias toward discordance of parasite genotypes.

Antenatal exposure to malaria parasites may have profound effects on the fetus. Malaria in a fetus may adversely affect fetal growth and development in utero. Exposure of a fetus to malaria may prime immune responses or induce immune tolerance that may subsequently affect susceptibility to infection and disease during infancy [33]. This information may have important implications with respect to the frequency of administration of malaria chemoprophylaxis during pregnancy and may affect vaccine strategies used to protect children from malaria.

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