Changes in the Levels of Chemokines and Cytokines in the Placentas of Women with *Plasmodium falciparum* Malaria

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*Plasmodium falciparum*–infected erythrocytes often are sequestered in the placenta and stimulate the accumulation of maternal mononuclear cells. In this study, the role that chemokines and cytokines play in mediating the inflammatory response was investigated. Placental parasites elicited a statistically significant increase in the levels of interferon (IFN)–γ, tumor necrosis factor (TNF)–α, and interleukin (IL)-10, in plasma collected from the intervillous space. Explants of fetal tissue from malaria-positive placentas also secreted significantly enhanced amounts of IFN-γ. Culture supernatant of maternal intervillos leukocytes obtained from infected placentas contained significantly higher levels of TNF-α, IL-10, monocyte chemotactic protein–1, macrophage inflammatory protein (MIP)–1α, MIP-1β, and IFN-γ inducible protein–10 than did cultures of white blood cells obtained from uninfected placentas. Taken together, these results show that both fetal and maternal cells secrete inflammatory and immunoregulatory cytokines in response to *P. falciparum* and suggest that β-chemokines produced by maternal cells contribute to the accumulation of macrophages in the intervillous space.

*Plasmodium falciparum* infection during pregnancy can create a problem for both the mother and the developing fetus [1–3]. It is estimated that ∼200,000 infants die annually in sub-Saharan Africa as a result of their mothers becoming infected with malaria during pregnancy [4]. Malarial parasites often accumulate in the placenta [5, 6], creating a condition known as “placental malaria.” Ultrastructural, histological, and immunohistochemical studies have found that the number of inflammatory cells, particularly monocytes and macrophages, increases within the intervillous space of women with placental malaria [7–13]. The resulting inflammatory response can alter trophoblast morphology and influence the cytokine balance in the placenta [7–12, 14–16]. In a recent study, Menendez et al. reported a significant association between massive monocyte infiltration within the intervillous space and an increased risk of delivering a baby with a low birth weight [12].

A successful pregnancy is characterized by the predominance of Th2-type cytokines at the fetal-maternal interface that down-regulate Th1-type responses that could be detrimental to the fetus [17, 18]. Since malarial parasites elicit a dominant Th1-type cell-mediated response [19], sequestration of *P. falciparum*–infected erythrocytes in the placenta causes a shift toward a Th1-type cytokine profile. Several investigators have reported an increase in the levels of the proinflammatory cytokines interferon (IFN)–γ and tumor necrosis factor (TNF)–α, in the placentas of women infected with malaria [14–16]. Although maternal white blood cells (WBCs) are important in controlling parasite density, they can also mediate an inflammatory response that leads to the release of inflammatory cytokines that, if left unregulated, are deleterious to the developing fetus.

Chemokines are important in the initiation of the inflammatory cascade because they attract and activate
different leukocyte populations. These chemotactic cytokines are categorized into 2 broad families: the CXC or α-chemokines (e.g., interleukin [IL]–8, IFN-γ inducible protein [IP]–10, and platelet factor 4), which are mainly chemoattractant for polymorphonuclear cells, and the CC or β-chemokines (e.g., monocyte chemotactic protein [MCP]–1, macrophage inflammatory protein [MIP]–1α, MIP-1β, and RANTES), which act primarily on monocytes, macrophages, and lymphocytes [20]. Interest in chemokines has grown rapidly since it became clear that they play a significant role also in angiogenesis, hematopoiesis, and development [21, 22]. Some chemokines also contribute to Th1/Th2 polarization [23], whereas others are involved in the inflammatory response against protozoa and other intracellular pathogens [24]. It remains unclear, however, whether P. falciparum affects the expression of chemokines in the placenta and whether a differential chemokine-secretion profile alters the pattern of leukocyte migration into the organ.

Recent studies have demonstrated that fetal villous tissues also secrete a number of inflammatory and immunoregulatory cytokines [16, 25], as well as chemokines [26–29], making it possible that both fetal and maternal tissues contribute to the cytokine and chemokine balance at the fetal-maternal interface. In this study, we sought to (1) confirm that malaria induces an increase in the concentrations of placental proinflammatory cytokines in our study population, (2) determine whether malaria parasites induce chemokine responses and how the presence of α- and β-chemokines correlates with the accumulation of leukocytes within the intervillous space, and (3) establish whether the enhanced levels of cytokines and chemokines are produced by maternal leukocytes, fetal villous tissue, or both.

SUBJECTS, MATERIALS, AND METHODS

Subjects. In the initial study, placental blood and tissue samples were consecutively collected from women who gave birth at the Central Maternity and Biyem-Assi Hospitals in Yaoundé, Cameroon, between August 1996 and August 2000. Approximately 17.2% of the women had placental malaria (malaria positive) at the time of delivery. Eighty-nine samples for which >750 μL of placental plasma was available for study, obtained from malaria-positive women, were selected and evaluated. An approximately equal number of samples (n = 83) from malaria-negative women with sufficient placental-plasma volume, which were collected during the same period, were also chosen for comparison. Informed consent was obtained from all patients in accordance with guidelines of the authors’ institutions. The study was approved by the Institutional Review Board of Georgetown University, the National Ethical Committee, Ministry of Health, Cameroon, and is covered by Single Project Assurance S-9601-01.

In a follow-up study, placental blood and tissue samples were consecutively collected from 68 women (18 malaria positive and 50 malaria negative) between June 2001 and August 2001. Levels of cytokines and chemokines were also measured in these plasma samples. In addition, cytokine and chemokine production by fetal and maternal tissues was investigated in vitro, as described below.

All 240 women from the initial and follow-up studies were delivered of singleton infants by vaginal deliveries and were healthy at the time of delivery. No other complicating factors, in addition to malaria, were identified. Although the human immunodeficiency virus (HIV) status of the women is unknown, the prevalence of HIV among pregnant women attending antenatal-care clinics in 2001 in urban areas in Cameroon is estimated to be 4.0%–13.6% [30], making it unlikely that HIV had a major effect on the results. A questionnaire was used to record clinical data, including age, chemoprophylaxis usage, last menstrual period, and previous number of pregnancies.

Collection of samples. Maternal placental blood and pieces of the placenta were collected immediately after delivery. Placental blood was collected by use of the biopsy-pool method. In brief, a block of tissue (2 × 2 × 2 cm) was excised from the basal side of the placenta, resulting in a large pool of intervillous blood at the excision site. Heparinized blood was quickly withdrawn and was stored at 4°C until being processed. For histological studies, a placental section (2 × 2 cm) was fixed in 50 mL of neutral, buffered formalin, for subsequent paraffin embedding, tissue sectioning, and staining with hematoxylin-eosin (HE). For villous tissue culture, a piece of the placenta (5 × 5 cm) was placed in 120 mL of sterile Dulbecco’s PBS (Gibco) supplemented with 0.1% heparin (Sigma), 2% streptomycin and penicillin (Gibco), and 1% fungizone (Gibco).

Determination of parasitemia and packed-cell volume (PCV). Impression smears of placental tissues, as well as thin and thick smears of placental blood, were prepared, were stained with Diff-Quick solution (Baxter Scientific Products), and were examined for the presence of parasites. Parasitemia was determined by counting the number of infected red blood cells (RBCs)/2000 RBCs and expressing this value as a percentage. On the basis of the presence or absence of parasites in placental impression, placental blood smears, and/or placental histological sections, women were considered to be either malaria positive or malaria negative, for placental malaria.

An aliquot of maternal peripheral blood was used to measure the PCV. In brief, a microhematocrit tube was filled with blood, 1 end was sealed with clay, and the tube was centrifuged at 400 g for 5 min. The PCV was determined by use of a hematocrit reader. On the basis of the World Health Organization definition, a woman was considered to be anemic if her hematocrit was <30%.
Isolation of maternal placental blood plasma. Maternal placental blood was centrifuged at 400 g for 10 min, and the plasma was stored at −80°C until being used.

Histopathology. Histological studies were conducted on placental tissue sections that had been fixed in neutral formalin, embedded in paraffin, cut to 4-μm sections, and stained with HE solution. Women were classified as either noninfected, having an active infection, having an active-chronic infection, or having had a past-chronic infection, depending on the presence and localization of parasites and/or hemozoin pigment [31].

Immunohistochemical staining. Placental tissues embedded in paraffin blocks were deparaffinized and were hydrated in xylene and a series of graded alcohol. Antigen retrieval was performed by placing the slides in a boiling solution of citrate buffer (pH 6.0; Zymed Laboratories), for 10 min and allowing the sections to cool for 20 min at room temperature. Endogenous peroxidase activity was blocked for 10 min in 0.03% H₂O₂. Protein block (BioGenex) was applied to the sections for 10 min, to minimize nonspecific binding. The slides were incubated with the primary antibody for CD68 (clone KP1; DAKO) for 45 min at room temperature and then were washed with the washing buffer. Biotinylated anti-antibody was added to the sections, followed by the addition of the streptavidin label (BioGenex). The slides were washed, were incubated with diaminobenzidine substrate–chromogen solution, were washed again, were counterstained with hematoxylin, were dehydrated, and were mounted.

Morphometric analysis was used to quantitate the number of CD68+ cells infiltrating the intervillous space, by a reader who did not know the malaria-infection status of the samples. The slides were examined by use of a 100× objective with a diameter-of-field view of 8.8 mm. The surface area of each field was 60.8 mm². Because of uneven distribution of cells, a total of 10 fields were counted on each slide: 5 of these fields were representative of areas where the cells were abundant, and the remaining 5 fields were from areas where the cell number was low. The median number of cells in each sample is reported as the number of cells per millimeter squared.

Villous tissue and maternal WBC cultures. A piece of the placenta was carefully teased and was flushed with sterile PBS to remove maternal blood. Then, a 2.0-g piece was transferred to a 25-cm² culture flask (Corning) containing 10 mL of RPMI-1640 medium (Gibco) supplemented with 1% sodium bicarbonate (Gibco), 1% penicillin and streptomycin (Gibco), 10% fetal bovine serum (HyClone), and 1% fungizone (Gibco). Tissue explants were cultured for 24 h at 37°C in a humid atmosphere of 5% CO₂/95% air, and the supernatant from each culture was harvested and was stored at −80°C until being used.

Maternal placental blood cells, flushed from the intervillous space, were filtered through a sterile gauze to remove tissue debris and clotted material. The cell suspension then was centrifuged at 400 g for 10 min. The cells were resuspended in the medium used for villous tissue culture, described above. Differential WBC counts were determined by preparing thin smears from the cell suspensions, staining the slides with Diff-Quick solution, and determining the percentage of each WBC type, on the basis of counting 300 WBCs. A total of 1 × 10⁶ viable WBCs was added to a 25-cm² culture flask containing 10 mL of the culture medium. Maternal WBCs were cultured as described above.

During the process of collecting placental tissue samples, some fetal blood vessels were cut, allowing some fetal blood to flow into the intervillous space. Because lymphocytes from human fetuses are known to strongly suppress a variety of adult lymphocyte functions [32], the level of fetal-cell contamination in the isolated intervillous WBC population was assessed on the basis of detecting the percentage of RBCs containing fetal hemoglobin. Smears were stained for fetal hemoglobin (Simmler), and contamination was measured by counting the number of positively-stained RBCs/1000 RBCs.

Measurement of levels of cytokines and chemokines, in placental plasma and culture supernatants. The levels of cytokines and chemokines in placental plasma and in culture supernatants were measured by use of commercially available kits for granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, IL-2, IL-4, IL-10, IL-12, transforming growth factor (TGF)–β, and TNF-α (OptEIA ELISA kits; BD Pharmingen) and for IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, and RANTES (DuoSet ELISA Development System; R&D Systems). The protocol was followed in accordance with the manufacturer's recommendations. The sensitivity of the assay for each cytokine is as follows: 2 pg/mL for GM-CSF, IFN-γ, and IL-10; 5 pg/mL for IL-2, IL-4, IL-8, MIP-1α, RANTES, and TNF-α; 10 pg/mL for MCP-1 and MIP-1β; 30 pg/mL for IL-12 and IP-10; and 50 pg/mL for TGF-β.

Statistical analyses. Pearson’s χ² test was used for univariate, between-group comparisons of binomial or multinomial variables (e.g., anemia and the use of chemoprophylaxis during pregnancy). The Wilcoxon signed rank test was used to compare the peripheral and placental parasitemias for each subject. The Wilcoxon rank sum test was used for univariate, between-group comparisons of continuous variables—including age, proportion of different WBC types, and number of monocytes and macrophages in the placental intervillous space—and for analysis of the levels of cytokines and chemokines. The same test was used for comparisons of the extent of monocytic infiltration between the different stages of placental-malaria infection. Spearman’s correlation coefficient was measured to determine the association between concentrations of plasma TNF-α and IL-10, in both infected and noninfected women. In examining the association between the various cytokines and chemokines and the proportion of different WBCs present in the intervillous space, the
robust information-sandwich estimate was computed by use of the general estimating equation (GEE) model, to avoid overdispersion, along with the inclusion of covariates, such as age and malaria-infection status. In this model, the covariate matrix of the scores was estimated empirically, and the type-3 P values of score statistics were reported. The test-wise, nominal P values were used for the determination of statistical significance. All descriptive and inferential statistics were obtained by use of SAS 8.2 (SAS Institute).

RESULTS

Composition of the study groups. Table 1 provides a comparison of the malaria-positive and malaria-negative women included in the initial and follow-up studies. As expected, in women with placental malaria, the level of parasitemia was significantly higher in the placenta than in the peripheral blood (P < .001, for both studies). With respect to the other parameters considered in the present study, there was little difference between women in the malaria-positive group and women in the malaria-negative group (table 1).

Levels of cytokines and chemokines, in placental plasma. By combining the data on cytokines from the 2 studies, the levels of IFN-γ, TNF-α, and IL-10 were significantly higher in plasma from malaria-positive placentas than in in plasma from noninfected placentas (P = .056, P = .067, and P < .001, respectively) (table 2). After adjusting for age and anemia, the levels of IFN-γ, TNF-α, and IL-10 remained significantly higher in malaria-infected placentas (P = .043, P = .028, and P = .023, respectively). IL-10 concentrations were significantly correlated with levels of TNF-α, within the same individual, in both malaria-positive and malaria-negative women (Spearman’s r, 0.60 and 0.45, respectively; P < .0001, for both). No significant differences, between malaria-infected and noninfected women, were found in the concentrations of either the cytokines IL-2, IL-4, IL-12, TGF-β, and GM-CSF or the chemokines IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, and RANTES (table 2).

Increased numbers of monocytes/macrophages in malaria-positive placentas. Immunohistochemical staining using antibodies against the macrophage-specific marker CD68 confirmed the accumulation of macrophages in the intervillous space of infected placentas (figure 1). Morphometric analysis showed that the number of monocytes and macrophages infiltrating the intervillous space of infected placentas was significantly higher than that infiltrating the intervillous space of noninfected placentas (medians, 0.53 cells/mm² and 0.09 cells/mm², respectively; P < .001). The number of monocytes and macrophages in the intervillous space was also associated with the length and severity of placental P. falciparum infection—that is, the intervillous space of placentas undergoing active-chronic infections had nearly 7 times more inflammatory cells than did those of noninfected placentas (medians, 0.61 cells/mm² and 0.09 cells/mm², respectively; P < .001), whereas only a marginally significant difference was found between the number of infiltrating CD68+ cells in placentas undergoing active infections and that in noninfected placentas (medians, 0.52 cells/mm² and 0.09 cells/mm², respectively; P = .072); there was no significant difference between the number of infiltrating CD68+ cells in placentas undergoing past-chronic infections and that in noninfected placentas (medians, 0.03 cells/mm² and 0.09 cells/mm², respectively; P = .094).

Cytokines and chemokines secreted by maternal WBCs in vitro. Maternal WBCs from infected placentas contained a significantly greater proportion of monocytes/macrophages than did WBCs from noninfected placentas (medians, 14% and 4%, respectively; P < .01). There was no significant difference in the proportion of neutrophils, lymphocytes, eosinophils, and basophils between the 2 groups (data not shown). The population of maternal WBCs recovered from the intervillous space was minimally contaminated with fetal cells, since it was found that a mean ± SD of 6.8% ± 3.4% of the RBCs in the samples

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Table 1. Description of the pregnant women in the present study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Initial study</th>
<th>Follow-up study</th>
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<tbody>
<tr>
<td></td>
<td>Malaria negative</td>
<td>Malaria positive</td>
</tr>
<tr>
<td>Subjects, no.</td>
<td>83</td>
<td>89</td>
</tr>
<tr>
<td>Age, mean ± SD, years</td>
<td>25.4 ± 6.2</td>
<td>24.0 ± 4.9</td>
</tr>
<tr>
<td>Primigravidae, %</td>
<td>39</td>
<td>47</td>
</tr>
<tr>
<td>Women who reported taking prophylaxis, %</td>
<td>90</td>
<td>91</td>
</tr>
<tr>
<td>Parasitemia, mean ± SD, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placental</td>
<td>0</td>
<td>0.076 ± 0.154</td>
</tr>
<tr>
<td>Peripheral</td>
<td>0</td>
<td>0.005 ± 0.011</td>
</tr>
<tr>
<td>Packed-cell volume, mean ± SD, %</td>
<td>33.3 ± 6.3</td>
<td>31.9 ± 6.9</td>
</tr>
<tr>
<td>Anemia, %a</td>
<td>37</td>
<td>46</td>
</tr>
</tbody>
</table>

a Packed-cell volume <30%.
contained fetal hemoglobin, a finding indicating that the level of fetal WBC contamination was low.

The contribution of maternal WBCs to the cytokine balance in the placenta was evaluated. Although maternal WBCs secreted detectable amounts of all the cytokines and chemokines examined, maternal WBCs from malaria-infected women secreted significantly higher concentrations of TNF-α (P = .015) and IL-10 (P = .012) than did the maternal WBCs from non-infected women (table 2). There was no indication that fetal-cell contamination had significant effects on the amounts of cytokines and chemokines secreted by maternal WBCs in vitro.

Maternal intervillous WBCs from malaria-infected women secreted significantly higher concentrations of IP-10 (P = .035), MCP-1 (P = .009), MIP-1α (P = .010), and MIP-1β (P = .005). There were no significant differences in the secretory pattern displayed by maternal WBCs for the other cytokines and chemokines studied (table 2). Thus, in the intervillous space of malaria-positive placentas, there was an increase in the number of macrophages and significantly higher levels of the cytokines TNF-α and IL-10 and of the chemokines IP-10, MCP-1, MIP-1α, and MIP-1β.

Levels of cytokines and chemokines secreted by maternal WBCs correlate with macrophage infiltration. Univariate analyses showed that higher levels of TNF-α, IL-10, IP-10, MCP-1, MIP-1α, and MIP-1β were associated with an increased proportion of monocytes and macrophages present in the cultures (table 3). On the other hand, none of the cytokines or chemokines was associated with the proportion of lymphocytes (table 3) or neutrophils (data not shown). After adjusting for age and malaria-infection status and by including all 3 types of WBCs (monocytes, lymphocytes, and neutrophils) in a GEE model, all the adjusted P values were similar to those in univariate analyses, except that the association between the proportion of monocytes/macrophages and IL-10 was no longer significant. These results suggest that the increasing proportion of monocytes and macrophages was responsible for the elevated secretion of TNF-α, IP-10, MCP-1, MIP-1α, and MIP-1β, regardless of age and malaria-infection status.

Table 2. Median levels of cytokines and chemokines (pg/mL) in placental plasma and in supernatants of cultured maternal intervillous white blood cells (WBCs) and fetal villous explants.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Placental plasma</th>
<th>WBC culture</th>
<th>Villous tissue culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Malaria negative (n = 132)</td>
<td>Malaria positive (n = 107)</td>
<td>Malaria negative (n = 37)</td>
</tr>
<tr>
<td>Cytokine</td>
<td>(n = 48)</td>
<td>(n = 18)</td>
<td>(n = 46)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>9.7 (5.8–16.1)</td>
<td>11.9 (7.3–18.6)</td>
<td>9.9 (6.5–17.6)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>10.6 (6.2–21.0)</td>
<td>13.4 (7.3–30.9)</td>
<td>24.2 (10.2–36.1)</td>
</tr>
<tr>
<td>IL-2</td>
<td>16.0 (9.1–23.8)</td>
<td>15.1 (8.2–25.1)</td>
<td>9.2 (5.6–14.9)</td>
</tr>
<tr>
<td>IL-12</td>
<td>50.6 (35.6–76.1)</td>
<td>52.3 (32.0–94.0)</td>
<td>33.9 (21.3–50.7)</td>
</tr>
<tr>
<td>IL-4</td>
<td>9.0 (5.3–14.3)</td>
<td>8.3 (5.6–14.1)</td>
<td>7.5 (4.0–11.8)</td>
</tr>
<tr>
<td>IL-10</td>
<td>16.0 (9.4–25.3)</td>
<td>32.0 (11.4–105.1)</td>
<td>6.1 (3.1–8.3)</td>
</tr>
<tr>
<td>TGF-β</td>
<td>104.3 (67.4–164.6)</td>
<td>107.9 (68.9–175.3)</td>
<td>87.4 (60.0–172.3)</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>6.9 (4.1–11.0)</td>
<td>6.7 (4.2–10.2)</td>
<td>6.1 (4.2–7.5)</td>
</tr>
<tr>
<td>Chemokine</td>
<td>(n = 28)</td>
<td>(n = 12)</td>
<td>(n = 49)</td>
</tr>
<tr>
<td>IL-8</td>
<td>263.9 (209.1–447.9)</td>
<td>585.2 (166.9–687.4)</td>
<td>69.8 (8.6–641.5)</td>
</tr>
<tr>
<td>IP-10</td>
<td>22.1 (10–137.1)</td>
<td>73.5 (0–222.8)</td>
<td>0 (0–4.8)</td>
</tr>
<tr>
<td>MCP-1</td>
<td>18.9 (5.5–55.3)</td>
<td>21.8 (0–102.1)</td>
<td>0 (0–4.8)</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>62.8 (20.6–100.0)</td>
<td>66.5 (26.3–165.1)</td>
<td>22.7 (3.9–48.0)</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>71.7 (45.5–100.9)</td>
<td>67.3 (49.4–375.1)</td>
<td>8.6 (0–15.9)</td>
</tr>
<tr>
<td>RANTES</td>
<td>633.9 (610.5–667.0)</td>
<td>666.9 (603.0–691.7)</td>
<td>89.0 (57.1–143.5)</td>
</tr>
</tbody>
</table>

**Note.** Data are median (interquartile range). The Wilcoxon rank sum test was used; P < .05 was considered to be significant. GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IP, IFN-γ-inducible protein; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; TGF, transforming growth factor; TNF, tumor necrosis factor.

- .05 < P < .07
- .01 < P < .05
- used 49 and 17 placental plasma samples from malaria-negative and malaria-positive women, respectively.
- used 49 and 18 placental plasma samples from malaria-negative and malaria-positive women, respectively.
Figure 1. Placental tissue from malaria-infected (A) and noninfected (B) women treated with monoclonal anti-CD68 antibodies. Arrows indicate CD68 staining of monocytes/macrophages in the intervillous space. Original magnification (oil immersion), ×1000; bar, 10μm.

Cytokines and chemokines secreted by fetal villous tissue in vitro. Supernatants from cultured villous tissues contained detectable levels of all the cytokines and chemokines measured (table 2). Results show that the villous tissue from malaria-infected placentas secreted higher levels of IFN-γ (P = .007) than did villous tissue from noninfected placentas (table 2). For the other cytokines and chemokines measured in association with malaria, no significant differences were detected in the secretory pattern of the villous tissue (table 2).

DISCUSSION

The accumulation of monocytes and macrophages within the intervillous space is a hallmark of placental malaria [7–13]. These cells help control malarial parasites by phagocytosing merozoites and parasite-infected erythrocytes [33]. Activated macrophages also release potent antimicrobial molecules (e.g., reactive oxygen intermediates, nitric oxide, and TNF-α) to aid in elimination of parasites. If left unchecked, however, excessive
Lymphocytes

fore, it is likely that results from women in Yaoundé will be the primary cause of macrophage accumulation. There-
tor identified by attending physicians, malaria appears to have been the only complicating risk fac-
tions of the placenta revealed no evidence of bacterial infection. There-
were macrophages (figure 1). Examination of histological sec-
tas, especially when there was evidence of chronic placental
increased risk of having a baby with a low birth weight [12].
It has recently been shown that excessive accumulation of
mononuclear cells in the placenta is associated with an in-
creased risk of having a baby with a low birth weight [12].
Furthermore, since malaria was the only complicating risk fac-
production of inflammatory mediators can damage host tissues. It has recently been shown that excessive accumulation of mononuclear cells in the placenta is associated with an increased risk of having a baby with a low birth weight [12]. Therefore, we sought to determine whether malarial parasites sequestered in the placenta induced the expression of chemokines that influence the migration and retention of leukocytes to the intervillous space.

Consistent with results from previous studies [7–13], a signifi-
cant increase of macrophages in the intervillous space was found in malaria-infected Cameroonian women. Differential WBC counts of blood collected from the intervillous space revealed an increase in macrophages in malaria-infected pla-
centas, especially when there was evidence of chronic placental infections (P < .001). Morphometric analysis of placental sections treated with CD68 confirmed that the accumulating cells were macrophages (figure 1). Examination of histological sec-
tions of the placenta revealed no evidence of bacterial infection. Furthermore, since malaria was the only complicating risk factor identified by attending physicians, malaria appears to have been the primary cause of macrophage accumulation. Therefore, it is likely that results from women in Yaoundé will be applicable to women with placentarial malaria elsewhere.

Initially, we hypothesized that levels of β-chemokines, but not of α-chemokines, would be elevated within the intervillous space and that they would be associated with increased numbers of macrophages. Results show that, when maternal intervillous WBCs from women with placentarial malaria were cultured in vitro, they secreted increased levels of the β-chemokines MCP-1, MIP-1α, and MIP-1β (table 2). Multivariate analysis shows that macrophages were the primary source of these chemokines (table 3). MCP-1, MIP-1α, and MIP-1β are chemoattractant for mononuclear cells and, thus, could play an active role in macrophage recruitment. Macrophages are known to secrete MIP-1α and MIP-1β, in response to malarial hemoglobin pig-
ment [34], which is highly abundant in placentas, especially during chronic infections [31].

In opposition to our initial hypothesis, however, the α-
chemokine IP-10 was also elevated. IP-10 is produced in re-
sponse to IFN-γ and is chemoattractant for T cells. In a recent study, Chaisavaneyakorn et al. reported increased production of IP-10 in placentarial malaria, especially among HIV-positive women [35]. In the present study, explants of fetal villous tissue from women with placentarial malaria secreted increased levels of IFN-γ, compared with those of noninfected women (table 2). The expression of IFN-γ by trophoblasts is thought to confer protection to the fetus, especially from infectious microorganisms that traverse the placental barrier [36]. Thus, a “dialogue” between fetal and maternal cells appears to be involved in the response to placentarial parasites.

Other researchers have reported an increase in mRNA ex-
pression of IL-8 in placentarial malaria [15]. Elevated concentra-
tions of IL-8 have been associated with peripheral parasitemias in adults [37]. In the present study, however, levels of IL-8 in plasma and in supernatants of maternal placental WBCs were higher in malaria-positive women than in noninfected women, but the difference was not statistically significant (table 2). Since the proportion of neutrophils declined slightly in the intervillous space of women with placentarial malaria (data not shown), the role of IL-8 in placentarial malaria remains unclear. No differences in levels of RANTES were observed, but it is possible that a small number of residual platelets might have remained in the plasma and released RANTES during storage. Therefore, a difference in levels of RANTES could have been missed, although this is unlikely. The above results demonstrate that levels of several β-chemokines and of the α-chemokine IP-10 are increased during placentarial malaria and may contribute to the accumulation of inflammatory-type leukocytes in the placenta.

At the time of delivery, a significant increase in levels of IFN-
γ and TNF-α also was observed in plasma collected from the intervillous space of women with placentarial malaria (table 2). These results are similar to those reported for women living in Kenya and Malawi [14, 15]. Since IFN-γ is important in controlling asexual-stage parasites [38] and since TNF-α has antiparasitic effects when present at appropriate levels [39], it is important that these cytokines are produced during placentarial malaria. In the present study of Cameroonian women, however, elevated levels of IL-10 also were found in response to placentarial infections (table 2). This result differs somewhat with those reported elsewhere [14]. IL-10 is an immunoregulatory cyto-
kine that can potentially alleviate many of the damaging effects on the fetus that are exerted by increased levels of proinflam-
matory cytokines [17, 18]. In the present study, a direct as-

<table>
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<th>Table 3. The relationship between the percentage of monocytes and lymphocytes in the culture and the amount of cytokines and chemokines produced.</th>
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<td>Cytokine</td>
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<td>MIP-1β</td>
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NOTE. IL, interleukin; IFN, interferon; TNF, tumor necrosis factor.; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; RANTES, regulated upon activation normal T expressed and secreted. After adjusting for age and malaria-infection status, by use of a general estimating equation model. 

a Univariate analysis.

b Multivariate analysis.

c Other researchers have reported an increase in mRNA expression of IL-8 in placentarial malaria [15]. Elevated concentrations of IL-8 have been associated with peripheral parasitemias in adults [37]. In the present study, however, levels of IL-8 in plasma and in supernatants of maternal placental WBCs were higher in malaria-positive women than in noninfected women, but the difference was not statistically significant (table 2). Since the proportion of neutrophils declined slightly in the intervillous space of women with placentarial malaria (data not shown), the role of IL-8 in placentarial malaria remains unclear. No differences in levels of RANTES were observed, but it is possible that a small number of residual platelets might have remained in the plasma and released RANTES during storage. Therefore, a difference in levels of RANTES could have been missed, although this is unlikely. The above results demonstrate that levels of several β-chemokines and of the α-chemokine IP-10 are increased during placentarial malaria and may contribute to the accumulation of inflammatory-type leukocytes in the placenta.

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sociation between placental levels of TNF-α and IL-10 was observed (P < .0001). On the other hand, levels of the anti-inflammatory cytokines IL-4 and TGF-β, which is postulated to be an important modulator in parasitic infections [40], did not differ significantly between malaria-infected women and noninfected women (table 2). It is relevant to note that all the women in the study had successful pregnancies—that is, all gave birth to live infants, of whom >82% had normal birth weights (data not shown). The number of women with poor pregnancy outcomes (i.e., low birth weight or premature delivery) was too small to have an effect on the results. Although high levels of TNF-α are associated with abortions [41, 42], the concomitant increase in levels of IL-10 and TNF-α may provide a negative feedback mechanism that successfully regulates the inflammatory process at the fetal-maternal interface [43] during normal pregnancies.

In the present study, samples from individual women and in vitro–cultured supernatants were used to evaluate a number of different cytokines and chemokines. A potential concern with this type of multiple comparisons is the magnitude of type I error generated. It is difficult to make statistical adjustments, since many of the factors studied are not independent variables—that is, if levels of 1 β-chemokine are elevated, levels of other β-chemokines are likely to be elevated. Furthermore, a simultaneous increase in coregulated proinflammatory cytokines is expected to be associated with a decrease in a variety of anti-inflammatory cytokines. Thus, making adjustments for multiple comparisons, without increasing type II errors, is difficult, from a purely statistical standpoint [44]. Accordingly, we have not made such adjustments. On the basis of data from similar studies and the overall consistency of the data obtained in the present study, our interpretation of the results is consistent with what is known about the basic biology of placental malaria. Since the results obtained in the present study are in good agreement with those of previous studies of women without HIV, changes associated with placental malaria in Cameroonian women should be similar to those of women in other parts of Africa.

In summary, our results are consistent with the following sequence of events. Sequestration of *P. falciparum* parasites in the placenta elicits the secretion of the β-chemokines MCP-1, MIP-1α, and MIP-1β by trafficking mononuclear cells. These chemokines, in turn, recruit additional macrophages to the site. Macrophages phagocytose parasite and hemoglobin pigment and release TNF-α. Fetal trophoblasts respond to parasites by increasing IFN-γ production, with additional IFN-γ being produced by maternal lymphocytes. The increase in levels of inflammatory cytokines in the local environment aids in eliminating the parasite but is closely regulated by IL-10, to help prevent the termination of pregnancy. Thus, in successful pregnancies, the chemokine and cytokine balance in the placenta is bidirectional, with both fetal and maternal cells responding to *P. falciparum* sequestration.

Acknowledgments

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References


