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Elizabeth T. Abrams; ... et. al

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Host Response to Malaria During Pregnancy: Placental Monocyte Recruitment Is Associated with Elevated β Chemokine Expression[1]

Elizabeth T. Abrams,* Heidi Brown,[§] Stephen W. Chensue,[†] Gareth D. H. Turner,[§] Eyob Tadesse,[¶] Valentino M. Lema,[¶] Malcolm E. Molyneux,[‖#] Rosemary Rochford,[‡] Steven R. Meshnick,[2] and Stephen J. Rogerson[‖††]**

Malaria during pregnancy is associated with poor birth outcomes, particularly low birth weight. Recently, monocyte infiltration into the placental intervillous space has been identified as a key risk factor for low birth weight. However, the malaria-induced chemokines involved in recruiting and activating placental monocytes have not been identified. In this study, we determined which chemokines are elevated during placental malaria infection and the association between chemokine expression and placental monocyte infiltration. Placental malaria infection was associated with elevations in mRNA expression of three β chemokines, macrophage-inflammatory protein 1 (MIP-1) α (CCL3), monocyte chemoattractant protein 1 (MCP-1; CCL2), and I-309 (CCL1), and one α chemokine, IL-8 (CXCL8); all correlated with monocyte density in the placental intervillous space. Placental plasma concentrations of MIP-1α and IL-8 were increased in women with placental malaria and were associated with placental monocyte infiltration. By immunohistochemistry, we localized placental chemokine production in malaria-infected placentas: some but not all hemozoin-laden maternal macrophages produced MIP-1β and MCP-1, and fetal stromal cells produced MCP-1. In sum, local placental production of chemokines is increased in malaria, and may be an important trigger for monocyte accumulation in the placenta. ***The Journal of Immunology*****, 2003, 170: 2759–2764.**

Plasmodium falciparum malaria infection during pregnancy is associated with poor birth outcomes, including low birth weight (LBW)[3] due to preterm delivery and intrauterine growth retardation, particularly among primigravidae (1). During pregnancy, malaria parasites sequester in the placenta (2). The mechanisms leading to poor birth outcomes are not clear, but placental parasite infection is associated with local immune responses, including elevated proinflammatory cytokine levels (3, 4) and monocyte infiltration into the placental intervillous space (5).

Placental monocyte infiltration may be a crucial mediator of poor birth outcome. In published studies, ~25% malaria-infected women have placental monocyte infiltration at delivery (2, 6, 7), and placental monocyte density has been identified as a significant predictor of LBW (5–7). Placental monocytes may be the source of the placental proinflammatory cytokines that have also been associated with LBW (3, 4).

Monocyte recruitment to sites of inflammation is mediated by chemoattractant proteins including chemokines, members of a superfamily of inducible, proinflammatory mediators that bind a large subfamily of G protein-coupled receptors (8, 9). Chemokines appear to play key roles in both normal and abnormal pregnancies (10). First trimester and term placental explants secrete the β chemokines macrophage-inflammatory protein 1 (MIP-1) α, MIP-1β, and RANTES in amounts that vary between individuals and over gestation (11). Monocyte chemoattractant protein 1 (MCP-1) and MIP-1α expression are increased in chorioamnionitis (12, 13).

The β or CC chemokines, MIP-1α, MIP-1β, RANTES, and MCP-1, are potent monocyte chemoattractants (14); MCP-1 and the α or CXC chemokine IL-8 have been implicated in monocyte adhesion (15). In this study, we examined chemokine mRNA expression and protein secretion in the placenta and used immunohistochemistry to distinguish whether maternal or fetal placental compartments produced these chemokines. This study had two major objectives: 1) to determine which chemokines are elevated during placental malaria infection and 2) to determine the association between chemokine expression and placental monocyte infiltration.

Materials and Methods

Subjects

Pregnant women attending the Labor Ward, Queen Elizabeth Central Hospital (Blantyre, Malawi) were recruited from April 1998 to November 2000 as part of studies on the pathogenesis of LBW following malaria during pregnancy (16). Placental blood-thick films stained with Field's stain were examined for *P. falciparum* malaria; parasitemias were determined as described elsewhere (16).

Departments of *Anthropology, [†]Pathology, and [‡]Epidemiology, University of Michigan, Ann Arbor, MI 48104; [§]Nuffield Department of Clinical Laboratory Sciences, John Radcliffe Hospital, Oxford, United Kingdom; [¶]Department of Obstetrics and Gynaecology and ‖Malawi-Liverpool-Wellcome Trust Clinical Research Programme, College of Medicine, University of Malawi, Blantyre, Malawi; [#]School of Tropical Medicine, University of Liverpool, Liverpool, United Kingdom; **Department of Epidemiology, University of North Carolina, Chapel Hill, NC 27514; and [††]Department of Medicine, University of Melbourne, Royal Melbourne Hospital, Australia

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[2] Address correspondence and reprint requests to Dr. Steven R. Meshnick, Departments of Epidemiology and Microbiology and Immunology, University of North Carolina, 2101 C McGavran/Greenberg Hall, Chapel Hill, NC 27599-7435. E-mail address: meshnick@email.unc.edu

[3] Abbreviations used in this paper: LBW, low birth weight; MIP-1, macrophage inflammatory protein 1; MCP-1, monocyte chemoattractant protein 1; Ltn: lymphotactin; IP-10, IFN-γ-inducible protein 10; RPA, ribonuclease protection assay.

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HIV status

Subjects who consented to HIV testing were given pretest counseling after delivery and provided with outpatient appointments to obtain results and further counseling. HIV status was determined by Serocard rapid test for HIV-1 and 2 (Trinity Biotech, Dublin, Ireland) and was confirmed by HIV-1 and 2 ELISA (Ortho-Clinical Diagnostics, Neckargemund, Germany), Vironostika HIV Uni-Form II (Organon Teknika, Boxtel, The Netherlands), or Determine (Abbott Laboratories, Amadora, Portugal).

Sampling procedure

Placental blood was collected into lithium heparin or EDTA by incising the cleaned maternal surface of the placenta and aspirating blood welling from the incision with a sterile pipette. Samples were separated within 1 h. Plasma was stored at −20 or −70°C. Placental biopsies from a healthy pericentric area (~1 cm from the cord on the maternal side) were placed into 10% neutral buffered Formalin. Samples (0.5 cm^3) were flash frozen in liquid nitrogen, with and without OCT (BDH/Merck, Modderfontein, South Africa), and stored at −70°C.

Placental examination

Slides were examined by observers blind to other patient data as described previously (7). Using a systematic method of sampling, 500 intervillous blood cells were counted using a ×100 objective and oil immersion, and classified as uninfected erythrocytes, infected erythrocytes, lymphocytes, polymorphonuclear cells, or monocyte-macrophages. Monocyte count ≥ 5% of all intervillous cells (infected erythrocytes, uninfected erythrocytes, and white blood cells; representing enrichment of $>10^3$ compared with peripheral blood) was defined as high.

Ribonuclease protection assay (RPA)

mRNA from 60 placental biopsies was extracted and stored as described elsewhere (4). Samples were selected on the basis of placental histology: 30 malaria-infected women, of which 16 had high (≥5%) monocyte infiltration and 14 had low (<5%) monocyte infiltration, and 30 malaria-uninfected women. RPAs were performed as described elsewhere (4, 17) using a chemokine-specific probe set (hCK-5; BD PharMingen, San Diego, CA) containing riboprobes specific for human lymphotactin (Ltn; XCL1), RANTES (CCL5), MIP-1α, MIP-1β, MCP-1, I-309 (CCL1), IFN-γ-inducible protein 10 (IP-10; CXCL10), IL-8, and rpL32. Probe bands were visualized by autoradiography and quantified with the Storm PhosphorImager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA). Values were normalized as a percentage of internal housekeeping gene (L32) expression.

ELISA

ELISAs were performed on placental plasma samples from 515 women; not all cytokines were measured in all women due to sample availability. MIP-1α and MIP-1β were measured using Quantikine sandwich ELISA kits (R&D Systems, Abingdon, Oxon, U.K.) according to the manufacturer's instructions. MCP-1 and IL-8 were measured using pairs of capture and biotinylated detection Abs against each cytokine (R&D Systems), with nonspecific binding blocked by Blocker Blotto in TBS (Pierce, Rockford, IL) and color developed by SigmaFast *o*-phenylenediamine dihydrochloride tablets (Sigma-Aldrich, St. Louis, MO). Assay sample sizes were as follows: 152 (MIP-1α), 134 (MIP-1β), 346 (MCP-1), and 345 (IL-8) women. Limits of detection were 10 (MIP-1α), 11 (MIP-1β), 3 (MCP-1), and 30 (IL-8) pg/ml.

Immunohistochemistry

From the samples used for the RPAs, three samples from three groups (uninfected women, malaria-infected women with low placental monocyte density, and malaria-infected women with high placental monocyte density) were selected for immunohistochemical analysis. Rabbit primary Abs (donated by Dr. S. Kunkel, University of Michigan, Ann Arbor, MI) were column purified with the Affi-Gel Protein A MAPS II kit (Bio-Rad, Hercules, CA) according to the manufacturer's instructions, then washed in PBS in an Amicon Centriprep Centrifugal Filter (Millipore, Bedford, MA) three times for 15 min each at 2650 rpm. Placental slides were prepared, fixed, and stored according to a previously published protocol (4). Slides were stained as described previously (18) with minor modifications: nonspecific binding was blocked with 100 μl of 1 mg/ml (MCP-1) or 5 mg/ml (MIP-1α, MIP-1β) human γ-globulin (Sigma-Aldrich) with 17% avidin (Avidin/Biotin Blocking kit; Vector Laboratories, Burlingame, CA). All slides were scored by S.W.C.

Ethical approval and consent

This study was approved by the College of Medicine Research Committee (University of Malawi, Blantyre, Malawi) and the Institutional Review Boards of the University of Michigan (Ann Arbor, MI) and the University of North Carolina (Chapel Hill, NC). Separate consents in the local language were administered for blood and data collection and for HIV testing.

Statistical analyses

Analyses were performed using SPSS version 10 (SPSS, Chicago, IL). mRNA ratios, plasma levels, and monocyte counts were (log + 1) transformed before analysis. Pearson's χ^2 test was used to evaluate group differences in HIV status and two-sample *t* tests were used to compare the groups in terms of age and parity. Independent sample *t* tests were used to evaluate the unadjusted effect of parasitemia (none vs any) on chemokine mRNA and plasma levels. Three-way ANOVAs were used to adjust the effects of placental parasitemia on chemokine mRNA and plasma values for parity (primigravid vs multigravid) and HIV status. The interaction of parasitemia and parity was also included. Multivariate regression was used to compare the variation in monocyte levels accounted for by chemokine mRNA expression and plasma levels in malaria-infected vs uninfected women. Spearman's rho (r_s) was used to correlate chemokine mRNA expression and plasma levels and birth weight. The 5% significance level was used to determine significance. Bonferroni's method was used as a more conservative measure of significance for multiple comparisons.

Results

Patient characteristics

Samples from 537 total women were analyzed in this study. Of these, ELISAs were performed on 515 women; samples from 60 women were selected for RPA analysis. Gravidity was available for 536 of 537 women. Of 145 women in the study with placental malaria, 95 (66%) were primigravid compared with 162 (41%) of the 391 women without placental malaria. Since malaria is more common in first pregnancies (19, 20), it is not surprising that malaria-infected women were more likely to be primigravid (t = 4.932, $p < 0.001$) and significantly younger (t = 5.723, $p < 0.001$). In the RPA subgroup, malaria-infected women were also significantly more likely to be primigravid than uninfected women, although age did not differ between the groups (data not shown).

HIV testing was optional in the study and thus self-selection for testing may have biased the results. In the study population, HIV status was available for 384 of 537 women. Overall, 22 (31%) of 72 malaria-infected women were HIV positive, as were 89 of 311 (29%) of malaria-uninfected women ($\chi^2 = 0.107$, NS). In the RPA subgroup, HIV status was available for 57 of 60 women. Five (20%) of 25 malaria-infected women were HIV positive, as were 6 (19%) of 32 malaria-uninfected women ($\chi^2 = 0.14$, NS). Thus, there were no differences in prevalence of HIV infection between malaria-infected and uninfected groups.

Malaria-associated elevations in MIP-1α, MCP-1, I-309, and IL-8 mRNA expression are associated with increased placental monocyte infiltration

To determine the effect of placental malaria infection on chemokine levels, we examined the association between malaria infection and chemokine mRNA expression and then determined which chemokines were associated with placental monocyte infiltration. HIV status and parity (primigravid vs multigravid) were examined as possible confounders. MIP-1α, MCP-1, I-309, and IL-8 mRNA expression, but not MIP-1β, RANTES, Ltn, or IP-10 expression, were significantly elevated in malaria-infected women. These associations remained significant after adjusting for parity and HIV status (Table I).

In malaria-infected women, there was a strong relationship between placental monocyte density and the relative quantity of mRNA coding for MIP-1α (t = 4.634, $p < 0.001$), MCP-1 (t = 4.620, $p < 0.001$), I-309 (t = 3.425, $p = 0.002$), and IL-8 (t =

Table I. *Effects of malaria (peripheral and/or placental) on chemokine mRNA expression*

Chemokine	Median[a] (Interquartile Range)		Parasitemia Unadjusted	Parasitemia Adjusted for Parity and HIV Status
	Malaria infected ($n = 30$)	Uninfected ($n = 30$)		
MIP-1α	22.58 (16.09–37.12)	10.82 (7.48–20.97)	$t = 3.333$ $p = 0.002$[c]	$F = 9.591$ $p = 0.003$[c]
MIP-1β	5.44 (4.00–9.37)	3.11 (1.99–4.92)	$t = 1.782$ $p = 0.085$	$F = 2.589$ $p = 0.114$
MCP-1	24.66 (16.92–31.06)	14.14 (8.98–23.46)	$t = 2.247$ $p = 0.028$	$F = 4.659$ $p = 0.036$
I-309	6.29 (4.37–10.78)	3.08 (2.04–6.26)[b]	$t = 2.384$ $p = 0.020$	$F = 4.463$ $p = 0.040$
RANTES	4.24 (3.34–5.21)	3.98 (2.87–5.84)	$t = 0.220$ $p = 0.827$	$F = 1.355$ $p = 0.250$
IL-8	19.86 (12.21–29.71)[b]	9.75 (5.62–16.41)	$t = 3.332$ $p = 0.002$[c]	$F = 9.203$ $p = 0.004$[c]
IP-10	3.20 (2.42–4.21)	2.66 (1.74–3.56)	$t = 0.600$ $p = 0.551$	$F = 0.000$ $p = 0.994$
Ltn	2.70 (1.52–3.06)	1.73 (1.35–2.57)[b]	$t = 0.864$ $p = 0.391$	$F = 1.254$ $p = 0.268$

[a] Log (% L32 expression).
[b] $n = 29$.
[c] Significant after Bonferroni's adjustment for multiple comparisons.

2.796, $p = 0.010$). The relationship between MIP-1α, MCP-1, I-309, and IL-8 chemokine mRNA expression and monocyte density was significantly different between malaria-infected and uninfected women (Fig. 1). MIP-1β mRNA expression was associated with monocyte infiltration ($t = 2.324$, $p = 0.029$), but there was no difference in the association between MIP-1β mRNA expression and monocyte density in malaria-infected and uninfected women (Fig. 1). RANTES, IP-10, and Ltn mRNA expression were not associated with monocyte infiltration, and IL-8 mRNA expression was not associated with neutrophil density (data not shown).

In plasma, malaria elevates MIP-1α and IL-8, which are associated with placental monocyte density

MIP-1α, MIP-1β, MCP-1, and IL-8 levels were measured in placental plasma (Table II). Because of sample depletion, not all chemokines were measured in all women. MIP-1α, MIP-1β, and IL-8

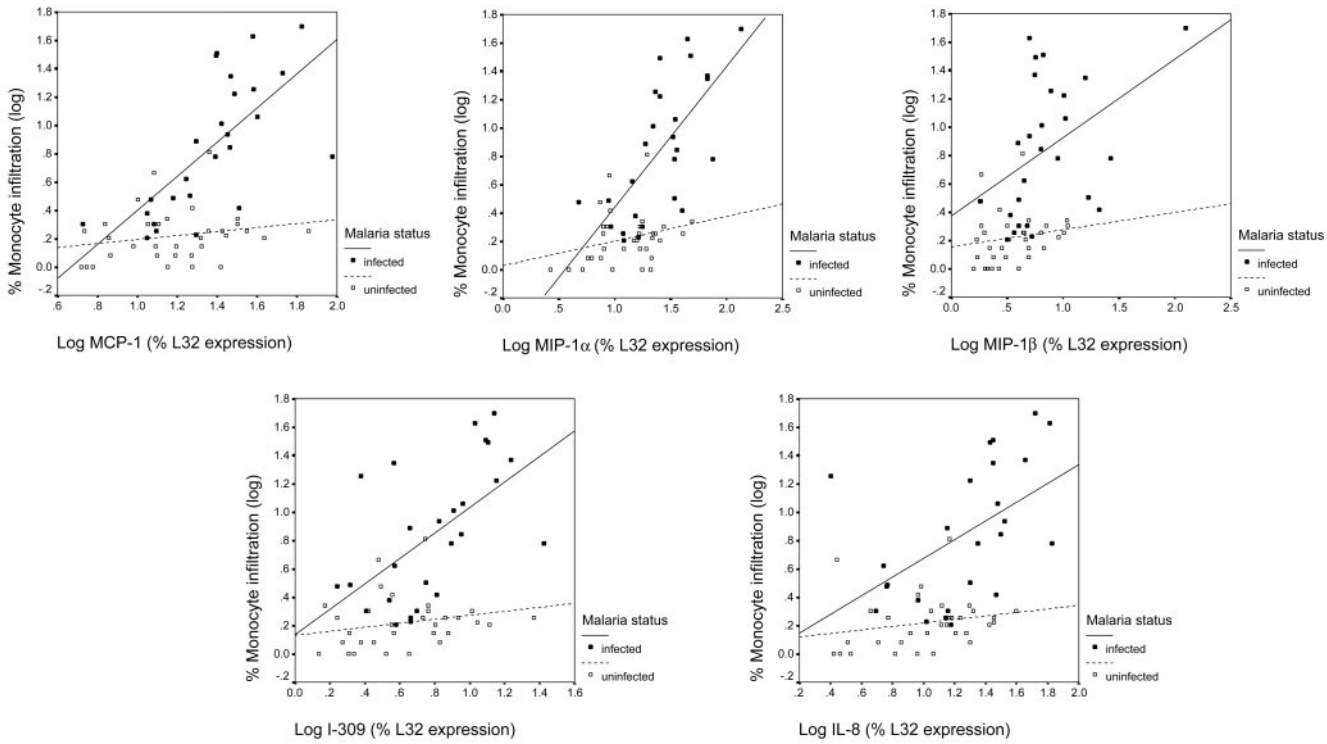

FIGURE 1. Correlation of placental MIP-1α, MIP-1β, MCP-1, I-309, and IL-8 chemokine mRNA expression with monocyte infiltration into the intervillous space in malaria-infected and uninfected women. Graphs show the log of the percentage of cells in placental intervillous space that are monocytes vs relative chemokine mRNA expression in malaria-infected ($n = 30$) and uninfected ($n = 30$) women. By multiple regression, the malaria-infected vs uninfected slopes are significantly different for MIP-1α ($t = 3.511$, $p = 0.001$), MCP-1 ($t = 4.038$, $p < 0.001$), I-309 ($t = 2.876$, $p = 0.006$), and IL-8 ($t = 2.189$, $p = 0.033$), but not for MIP-1β ($t = 1.521$, NS).

Table II. *Effect of malaria (peripheral and/or placental) on chemokine protein expression*

Chemokine	Median[a] (Interquartile Range)		Parasitemia Unadjusted	Parasitemia Adjusted for Parity and HIV Status
	Malaria infected	Uninfected		
MIP-1α	2.00 (1.47–2.74)	1.35 (0.83–1.75)	$t = 5.909$	$F = 11.901$
	$n = 93$	$n = 179$	$p < 0.001$[b]	$p = 0.001$[b]
MIP-1β	2.31 (2.08–2.87)	2.06 (1.91–2.28)	$t = 3.473$	$F = 3.087$
	$n = 87$	$n = 158$	$p = 0.001$[b]	$p = 0.082$
MCP-1	1.34 (0.83–1.59)	1.20 (0.40–1.59)	$t = 1.261$	$F = 2.767$
	$n = 67$	$n = 308$	$p = 0.208$	$p = 0.097$
IL-8	2.17 (1.52–2.65)	1.84 (1.39–2.30)	$t = 3.178$	$F = 7.138$
	$n = 66$	$n = 308$	$p = 0.002$[b]	$p = 0.008$[b]

[a] Log (picograms per milliliter).
[b] Significant after Bonferroni's adjustment for multiple comparisons.

were significantly elevated in placental plasma from malaria-infected women, but only MIP-1α and IL-8 remained significantly associated after adjusting for parity and HIV status.

Both plasma MIP-1α and IL-8, the chemokines elevated in placental plasma of malaria-infected women, were significantly associated with placental monocyte density by univariate analysis (MIP-1α: $t = 6.235$, $p < 0.001$; IL-8: $t = 2.256$, $p = 0.025$). Although HIV infection was significantly associated with plasma IL-8 levels in malaria-infected women, it did not significantly explain variance in placental monocyte infiltration.

Chemokines and outcomes

There was no correlation between expression of chemokine mRNA and birth weight, perhaps due to relatively small group numbers. Of the chemokines examined by ELISA, placental plasma IL-8 and MIP-1β were significantly correlated with lower birth weight (IL-8: $r_s = -0.169$, $p = 0.001$; MIP-1β: $r_s = -0.150$, $p = 0.020$), but MIP-1α and MCP-1 were not (data not shown).

Localization of placental chemokine production

To identify the placental compartment producing the chemokines MCP-1, MIP-1α, and MIP-1β, immunohistochemical staining was performed on three placental samples from each of the three RPA groups: malaria-infected women with high monocyte infiltration (group A), malaria-infected women with low placental monocyte infiltration (group B), and uninfected women (group C). Results are summarized in Table III. In group B, moderately strong staining by anti-MCP-1 mAbs was found almost exclusively within fetal villous cells (Fig. 2*a*). In group A, anti-MCP-1 mAbs stained stromal cells within the chorionic villi and some but not all hemozoin-laden macrophages (Fig. 2*b*). Presence of hemozoin in macrophages is indicative of parasite phagocytosis. Rarely, stained villous cells were seen in uninfected women. Anti-MIP-1β mAb staining was uniformly weak (+) in the chorionic villi of all three groups and increased in intensity in hemozoin-laden maternal macrophages across the groups, from 0 in group C to +/++ in group A women (Fig. 2*c*). Anti-MIP-1α mAbs showed weak staining in both compartments across all three groups of women.

Discussion

We identified chemokines associated with malaria infection during pregnancy and examined their relationship to monocyte infiltration into the placental intervillous spaces. We found that placental malaria is associated with increased expression of mRNA coding for MIP-1α, MIP-1β, MCP-1, and I-309, β chemokines that target monocytes, and IL-8, an α chemokine involved in monocyte adhesion (Ref. 15; Table I). Placental plasma levels of MIP-1α and IL-8 were also significantly higher in malaria-infected than uninfected women (Table II). In malaria-infected women, MIP-1α, MIP-1β, MCP-1, I-309, and IL-8 mRNA expression significantly explained variation in placental monocyte density; for all but MIP-1β, the relationship between chemokine expression and monocyte density was significantly different in malaria-infected vs uninfected women (Fig. 1). These differences remained significant after adjusting for parity and HIV status. In both malaria-infected and uninfected placentas examined immunohistochemically, MIP-1β was produced by fetal villous cells and MIP-1α staining was weak, despite strong mRNA expression in malaria-infected placentas. Both of these observations are consistent with the findings of Moussa et al. (11), who demonstrated MIP-1β production by Hofbauer cells but were unable to detect significant staining for MIP-1α in trophoblasts with commercially available Abs. In malaria-infected placentas, MIP-1β and MCP-1 were produced by some but not all hemozoin-laden maternal macrophages, the same

Table III. *Summary of MCP-1, MIP-1α, and MIP-1β staining intensity within the fetal chorionic villous cells and maternal intervillous macrophages*[a]

Infection Status	MCP-1		MIP-1α		MIP-1β	
	Fetal villous cells	Maternal macrophages	Fetal villous cells	Maternal macrophages	Fetal villous cells	Maternal macrophages
Group A Malaria-infected, high monocyte count	+++/++++	+/++	0/+	0/+	+	+/++
Group B Malaria-infected, low monocyte count	++/+++	0/+	0/+	0/+	+	+
Group C Uninfected	+/++	0	0/+	0	+	0

[a] Three women per group. Scale: 0 to ++++; "/" indicates a score between the two numbers.

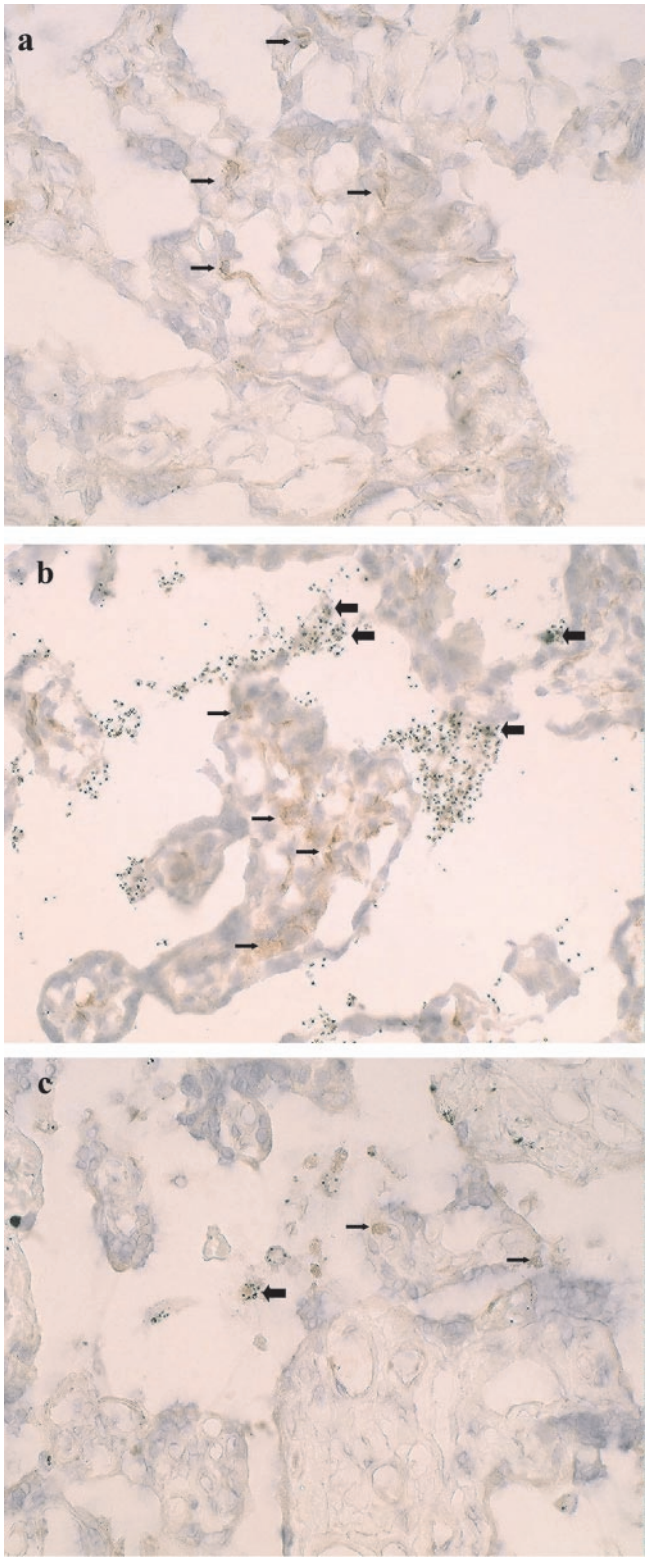

FIGURE 2. Localization of chemokines in malaria-infected placentas by immunohistochemistry. Anti-MCP-1 staining of placentas with low monocytes (*a*) and high monocytes (*b*) and anti-MIP-1β staining of placenta with high monocytes (*c*). Small arrows indicate stained stromal cells and large arrows indicate stained hemozoin-laden macrophages.

pattern previously demonstrated for IL-8 (4); MCP-1 production was strongest, however, in the stromal cells within the chorionic villi. MIP-1β, MCP-1, and IL-8 appear to be triggered by phagocytosis of malaria parasites and may play early roles in placental monocyte recruitment; MCP-1 may also be a fetal signal to recruit monocytes.

Because placental immune responses can only be examined at delivery, the sequence of events in human pregnancy must be inferred. Three groups of observations may shed light on this process. First, *P. falciparum*-infected erythrocytes release GPI, which activates macrophages and endothelial cells, inducing expression of adhesion molecules and production of iNOS, TNF-α, and IL-1β (21, 22). Macrophages secrete β chemokines including MIP-1α, MIP-1β, MCP-1, and RANTES in response to these cytokines and to another protozoan GPI (23). Placental macrophage (and possibly trophoblast (11)) activation and chemokine secretion may be mediated in part by local release of malarial GPI by sequestered parasites. Activated cells producing more MIP-1β and MCP-1 (Fig. 2) may in turn attract further monocytes to the placenta. Second, placental malaria is accompanied by fibrin deposition (2). By immunohistochemistry, more extensive lattices of fibrin surround inflammatory cells (Ref. 24 and S. Rogerson, unpublished observations). Interactions of fibrin(ogen) with macrophages promote adhesion and stimulate macrophage production of β chemokines including MIP-1α, MIP-1β, MIP-2, and MCP-1, but not RANTES (25). T cell activation-3, the murine homologue of I-309, activates monocytes and induces adhesion to fibrin (26). Fibrin may play a role in placental monocyte accumulation and activation. Third, malaria during pregnancy is associated with reduced uteroplacental blood flow, suggesting that hypoxia plays a role in placental malaria (27). A differential effect of placental malaria on MCP-1 and IL-8 mRNA, but not RANTES and IP-10 (Table I), mirrors the findings of Galindo et al. (28), examining dermal fibroblast responses to hypoxia. Hirani et al. (29) recently showed that hypoxia increased IL-8 expression but decreased β chemokine expression by blood monocyte-derived macrophages. Different tissues respond differently to hypoxia and further examination of the importance of hypoxia in placental malaria is warranted.

The strong association between elevated IL-8 mRNA expression and placental monocyte density (Fig. 1) may be important in the etiology of LBW. In this study, placental plasma IL-8 was the only chemokine correlated with birth weight; Moormann et al. (4) also identified significant elevations of IL-8 in malaria-infected women with LBW, especially due to intrauterine growth retardation, which is associated with placental monocyte density rather than parasite counts (6). Phagocytic monocytes or macrophages may have roles in both production (4) and as targets of IL-8.

Apart from placental plasma IL-8 and MIP-β, weak or no associations were found between chemokine mRNA or protein and pregnancy complications. Sample size restrictions may be partly responsible. In endemic countries, malaria infection is an important and preventable cause of LBW (1), which is associated with placental monocyte infiltrates (5–7). LBW results from complex interactions of factors over the course of pregnancy. Chemokines may be indirectly critical to this process, by attracting monocytes to the placenta and/or facilitating their adhesion. Activated monocytes and macrophages, through production of cytokines such as TNF-α (3, 4) or by other mechanisms, may be the effectors of the immune processes described.

The up-regulation of β chemokines in malaria-infected placentas may have implications for vertical transmission of HIV. Because malaria up-regulates CCR5 expression on maternal placental macrophages (18) and CCR5 is the main secondary receptor implicated in HIV vertical transmission (30), malaria infection during pregnancy has the potential to facilitate transmission. Monocyte infiltration into the placenta might also create a reservoir for HIV. However, because β chemokines that employ the CCR5 receptor, like MIP-1α and MIP-1β, inhibit HIV viral envelope binding (31),

an elevation in β-chemokines during malaria-infected pregnancy may in fact decrease the risk of vertical transmission. Thus, more work on the interaction between malaria and HIV during pregnancy is needed.

Monocyte infiltration into the intervillous space of malaria-infected placentas is a key risk factor for LBW. This is the first study to identify the chemokines associated with placental malaria infection and its accompanying monocyte infiltration. Elevations in mRNA expression of MIP-1α, MIP-1β, MCP-1, I-309, and IL-8 were associated with placental malaria and with monocyte density. The first four target monocytes and thus may be key players in monocyte infiltration and activation in malaria-infected placentas. The particular pattern of chemokine expression seen may result from the interaction of inflammation, hypoxia, and coagulation changes associated with host response to malaria. IL-8 is produced by monocytes but targets neutrophils, suggesting that its presence is probably the result of monocytic infiltration rather than its cause. Like IL-8 (4), both placental MIP-1β and MCP-1 are expressed in hemozoin-laden maternal macrophages, suggesting that they could function as an early signal in monocyte recruitment. The infiltrating monocytes could then produce most of the other chemokines. MCP-1 also appears to be a signal from fetal cells (stromal fibroblasts or Hofbauer cells) to recruit monocytes. In sum, increased placental chemokine production may be an important trigger for local monocyte accumulation in the placenta.

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