Hemoglobin Differentially Regulates Proinflammatory Cytokine Production in Human Immunodeficiency Virus-Seropositive and -Seronegative Women with Placental Malaria

Julie M. Moore, Sujittra Chaisavaneeyakorn, Douglas J. Perkins, Caroline Othoro, Juliana Otieno, Bernard L. Nahlen, Ya Ping Shi, and Venkatachalam Udhayakumar

Center for Tropical and Emerging Global Diseases and Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Public Health Service, U.S. Department of Health and Human Services, Atlanta; Georgia; Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, Thailand; Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania; Vector Biology and Control Research Center, Kenya Medical Research Institute, and New Nyanza Provincial General Hospital, Kisumu, Kenya; and Roll Back Malaria Program, World Health Organization, Geneva, Switzerland

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Pregnant women are at an increased risk for malarial infection. Plasmodium falciparum accumulates in the placenta and is associated with dysregulated immune function and poor birth outcomes. Malarial pigment (hemoglobin) also accumulates in the placenta and may modulate local immune function. In this study, the impact of hemoglobin on cytokine production by intervillous blood mononuclear cells from malaria-infected placentas was investigated. There was a dose-dependent, suppressive effect of hemoglobin on production of gamma interferon (IFN-γ), with less of an effect on tumor necrosis factor alpha (TNF-α) and interleukin-10, in human immunodeficiency virus-seronegative (HIV−) women. In contrast, IFN-γ and TNF-α production tended to increase in HIV-seropositive women with increasing hemoglobin levels. Production patterns of cytokines, especially IFN-γ in HIV− women, followed different trends as a function of parasite density and hemoglobin level. The findings suggest that the influences of hemoglobin accumulation and high-density parasitemia on placental cytokine production are not equivalent and may involve different mechanisms, all of which may operate differently in the context of HIV infection. Cytokine production dysregulated by accumulation of hemoglobin or high-density parasitemia may induce pathology and impair protective immunity in HIV-infected and -uninfected women.

Hemoglobin, or malarial pigment, is a heme polymer that is produced as a by-product of intraerythrocytic hemoglobin catabolism by plasmodial parasites (reviewed in reference 1). Because this polymer is indigestible, it accumulates in host phagocytic cells that have engulfed whole parasites or cell-free parasite material (reviewed in reference 1). Accumulation of hemoglobin-laden phagocytes in spleen, liver, bone marrow, and placenta has been reported (24; reviewed in reference 1). The association between hemoglobin-laden phagocytes and disease severity (19) has recently attracted increased attention to the functional significance of hemoglobin in dysregulating malarial immunity. Acquisition of hemoglobin by monocytes/macrophages has been associated with increased cytokine secretion (21, 28) and suppression of antigen presentation (26). During pregnancy, Plasmodium falciparum-parasitized erythrocytes accumulate in the (maternal) intervillous blood (IVB) spaces of the placenta (13). Hemoglobin-laden phagocytes and hemoglobin trapped in fibrin are common histologic features of placental malaria (PM) (4). Because hemoglobin is indigestible, its presence in the placenta has been used to categorize the intensity and longevity of PM infection (4). Although placental hemoglobin load, as assessed by biochemical and fluorometric means, is not associated with poor fetal outcomes (12, 33), some (25, 36) but not all (14) studies examining hemoglobin deposition by histopathology have noted significant associations with reduced birth weight. Furthermore, one study found strong, independent associations between high levels of placental tumor necrosis factor alpha (TNF-α) expression (derived mainly from hemoglobin-laden maternal macrophages) and both increased hemoglobin concentration and intrauterine growth retardation (17). Thus, the immunologic impact of hemoglobin in the placenta is potentially significant and needs to be further explored, in terms of both protective immunity and immunopathogenesis. In the present study, the impact of naturally accumulated hemoglobin on the ability of in vitro-cultured IVB mononuclear cells (IVBMC) from women with active PM infections to secrete proinflammatory (gamma interferon [IFN-γ] and TNF-α) and anti-inflammatory (interleukin-10 [IL-10]) cytokines was assessed. In addition, the impact of human immunodeficiency virus (HIV) infection, which has been shown in several epidemiological studies to render
women more susceptible to PM (30) and to impair antigen-specific cytokine responses by IVBMC (15), on IVBMC responses in the presence of hemozoin was assessed.

**MATERIALS AND METHODS**

**Study site, participants, and samples.** The study site and patient population have been described in detail elsewhere (15, 16) and are summarized here. The study design and use of human subjects were approved by the Institutional Review Boards of the University of Georgia, the Centers for Disease Control and Prevention, and the Kenya Medical Research Institute. All participants provided written consent. After receiving pretest HIV counseling, antenatal clinic attendees donated fingerstick blood; sera were screened for the presence of HIV-specific antibodies by the use of two rapid, commercially available tests (15). Women with discordant results were excluded. Only apparently healthy individuals with no known infections or diseases other than asymptomatic malaria and HIV infection (non-AIDS) and with uncomplicated labor and singleton, vaginal, inpatient deliveries were enrolled. Clinical characteristics of the 81 participants are summarized in Table 1.

Placentas were collected at the time of delivery, handled aseptically, and processed for IVBMC as described elsewhere (15, 16). Whole blood was collected from a shallow incision in the maternal surface of the placenta. Plasma was processed for IVBMC as described elsewhere (15, 16). Whole blood was collected from a shallow incision in the maternal surface of the placenta. Plasma was obtained by centrifugation and stored at −80°C until testing. Malarial parasitemia statuses and hemozoin scores (HS) were determined from a thick smear of IVB. Only those placentas with positive malaria smear results were included in the present study. The numbers of parasitized erythrocytes and hemozoin-laden phagocytes per 300 white blood cells were determined. Parasite density (PD) was calculated under the assumption that each microfilter of blood contains 8,000 leukocytes, which is an assumption of parasite load, because actual leukocyte counts differ between individuals. Groups were defined as having low PD (<1,000 parasitized erythrocytes per μl of blood), intermediate PD (1,000 to 10,000 erythrocytes per μl of blood), or high PD (>10,000 erythrocytes per μl of blood). Hemozoin was scored on a scale of 0 to 4, with 0 indicating that no samples had an HS greater than 3.

**TABLE 1. Patient characteristics**

<table>
<thead>
<tr>
<th>Patient characteristic</th>
<th>HIV negative (n = 52)</th>
<th>Value for patient group</th>
<th>HIV positive (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr) G1/G2 (n = 42)≥G3 (n = 10)</td>
<td>G1/G2 (n = 20)≥G3 (n = 9)</td>
<td></td>
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<tr>
<td>LDH (μA)</td>
<td>5.8 ± 15.8 (0.5; 0.03–81.1)</td>
<td>5.4 ± 8.1 (1.0; 0.3–25.4)</td>
<td>5.6 ± 12.2 (0.7; 0.05–49.3)</td>
</tr>
<tr>
<td>HS</td>
<td>1.0 ± 0.5 (1; 0–2)</td>
<td>1.1 ± 0.7 (1; 0–3)</td>
<td>1.2 ± 0.6 (1; 0–2)</td>
</tr>
</tbody>
</table>

a Placental PDs were determined from a thick blood film and are expressed as the number of parasites (10^3)/μl of whole blood. Differences in PDs and placental HS between groups are not statistically significant (P > 0.05). The age difference between the HIV+ multigravid group and the other groups is statistically significant (P = 0.0197). Statistics were derived by the use of the SAS general linear models procedure.

b n, sample size. G1/G2, primigravid and secundigravid group; ≥G3, multigravid group. The values are presented as arithmetic means ± standard deviations (median; range). Age data were unavailable for two HIV+ G1/G2 women.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>HIV negative (n = 52)</td>
<td>HIV positive (n = 29)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>20.1 ± 3.1 (20; 16–28)</td>
</tr>
<tr>
<td>PD (10^3/μl)</td>
<td>5.8 ± 15.8 (0.5; 0.03–81.1)</td>
</tr>
<tr>
<td>HS</td>
<td>1.0 ± 0.5 (1; 0–2)</td>
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**RESULTS**

Influences of HIV infection and hemozoin on IVBMC cytokine responses from malaria-infected placentas. Table 1 summarizes the clinical status of the participants, who were stratified by HIV serostatus and gravidity. PDs and HS did not differ significantly between the groups. The HIV-seropositive (HIV+) multigravid group was significantly older than the other groups.

It was previously demonstrated that HIV infection induces impaired antigen-specific cytokine responses by IVBMC (15). Thus, the cytokine data were examined as a function of HIV serostatus and HS. As shown in Fig. 1, the impact of hemozoin on IVBMC cytokine production varied as a function of HIV serostatus, with the most significant impact being observed in production of IFN-γ (Fig. 1A to E).

In the HIV-seronegative (HIV−) group, there was a trend toward decreased production of IFN-γ with increasing HS under all stimulation conditions. The decrease in spontaneous and mitogen (PHA and CD3)-induced production of IFN-γ as the HS increased was statistically significant (Fig. 1A to C) (results for the group with an HS of 0 [HS0] versus those for the HS1 group, P < 0.031 for all). The IFN-γ response following stimulation with MalAg was low relative to those to the other stimulants and was completely abolished in the presence of hemozoin (Fig. 1E) (HS0 group results versus HS1 group results, P = 0.0273).

In contrast, secretion of IFN-γ by IVBMC from HIV+ women following mitogenic stimulation increased with HS, although in the absence of hemozoin (i.e., HS0), it was low relative to that secreted from HIV− women (for CD3, P = 0.0143) (Fig. 1C). CD3 responses in the HIV+ HS≥2 group were >8-fold higher than those in the HIV− HS0 group, and PHA-stimulated production was significantly higher in the HIV+ HS≥2 group than in the HIV− HS≥2 group (P = 0.0285) (Fig. 1B). While PPD-specific IFN-γ responses of HIV+ patients were lower for those in the HS1 group than for those in the HS0 group, the responses partially recovered in the presence of a high HS (Fig. 1D). IFN-γ responses to...
FIG. 1. Cytokine production by IVBMC from malaria-infected placentas as a function of HIV infection status and HS. IFN-γ (A to E), TNF-α (F to J), and IL-10 (K to O) were measured in culture supernatants of IVBMC and categorized by HS (see Materials and Methods for definitions). Open circles represent HIV− samples, and closed circles represent HIV+ samples. Medians are indicated by open diamonds joined by a dotted line (HIV−) and open hexagons joined by a solid line (HIV+). (A, F, and K) Spontaneously produced cytokine levels; (B, G, and L) PHA-
MalAg in HIV+ samples were below the assay detection limits in the HS0 and HS1 groups (Fig. 1E). None of the differences between the HIV+ groups with different HS achieved statistical significance, perhaps due to small sample sizes.

Examination of IFN-γ levels in a limited number of placental plasma samples revealed similar patterns, particularly for HIV+ women. There was a trend toward increasing IFN-γ levels with increasing HS in HIV+ women (with an HS of 0, IFN-γ level of 0 pg/ml [interquartile range, 0 to 0; n = 3 women]; with an HS of 1, 58 pg/ml [interquartile range, 0 to 77; n = 5]; and with an HS of ≥2, 105 pg/ml [interquartile range, 0 to 148; n = 4]) with generally low responses in HIV− women (with an HS of 0, 0 pg/ml [interquartile range, 0 to 10; n = 3]; with an HS of 1, 0 pg/ml [interquartile range, 0 to 92; n = 5]; and with an HS of ≥2, 6 pg/ml [interquartile range, 3 to 9; n = 2]).

Modest, progressive reductions in TNF-α for spontaneous and mitogen-stimulated responses were observed with increasing HS in IVBMC from HIV− women (Fig. 1F to H). However, there was a slight increase in the PPD-stimulated response in the HS≥2 group relative to those of the other HS groups (Fig. 1I). TNF-α production by IVBMC from HIV+ women was dose dependently increased in the presence of hemozoin, being highest in the HS≥2 group under all stimulation conditions except MalAg stimulation (Fig. 1F to J). Regardless of HS and HIV status, TNF-α was undetectable following MalAg stimulation (Fig. 1J). None of the observed differences for TNF-α production were statistically significant.

In contrast to the effect on proinflammatory cytokines, the impact of hemozoin accumulation on IL-10 production by IVBMC from both HIV+ and HIV− women was minimal (Fig. 1K to O), although sample sizes in some groups were very small. As with IFN-γ production, IL-10 production in response to MalAg stimulation was low and decreased with increasing HS in HIV− women (Fig. 1O), but this reduction was not statistically significant.

Influences of HIV infection and PD on IVBMC cytokine responses from malaria-infected placentas. Cytokine and chemokine levels in placental plasma have been shown to positively correlate with placental PD (5, 24). To determine the extent of the PD impact on cytokine secretion by IVBMC, the data were analyzed for the three categories of PD: low, intermediate, and high (see Materials and Methods). As shown in Fig. 2, cytokine responses categorized by PD for HIV− women tended to follow different patterns than when the data were analyzed as a function of HS (as in Fig. 1). Whereas the highest responses for IFN-γ and TNF-α were generally observed in the absence of hemozoin (i.e., HS0) (Fig. 1), responses for these two cytokines were generally highest with intermediate and high PDs (Fig. 2A to E and F to J, respectively). For TNF-α, secretion was highest for those with intermediate PDs for all conditions except MalAg stimulation (compared to those with low PDs, P of <0.05 for spontaneous and PHA- and PPD-stimulated responses and P of 0.08 for CD3-stimulated responses) (Fig. 2F to J). This same pattern was also evident for PHA-stimulated IFN-γ production (those with low PDs versus those with intermediate PDs, P = 0.09) (Fig. 2B). Following CD3 and PPD stimulation, IFN-γ responses among those samples with high PDs were the highest, but the differences were not statistically significant. IL-10 production also tended to increase with PDs of >1,000/μl, although the differences were quite subtle (Fig. 2K to O). Spontaneous and PHA- and PPD-stimulated IL-10 responses were all highest for those with intermediate PDs (compared to those with low PDs, 0.05 < P < 0.1). Nonetheless, in general, the patterns of IL-10 production did not change dramatically as a function of HS or PD for HIV− women.

In samples from HIV+ women, TNF-α responses were very similar in the HS and PD analyses. However, following PHA and CD3 stimulation, IFN-γ production tended to decrease under the condition of high PDs (Fig. 2B and C), whereas it was elevated in the presence of hemozoin (Fig. 1B and C). Spontaneous and PPD-stimulated secretion changed minimally as a function of PD, although production increased slightly in those with high PDs (Fig. 2A and D). This result is in contrast to the analysis based on HS, in which responses to PPD were higher in the absence of hemozoin (Fig. 1D). IL-10 responses in the HIV+ groups followed the same pattern with increasing PDs as that observed with increasing HS.

Influences of gravidity, hemozoin, and PD on IVBMC cytokine responses from malaria-infected placentas. Gravidity has been shown to influence the cytokine responsiveness of IVBMC, particularly in the absence of PM (16). HIV appears to substantially override this gravidity dependence, rendering women of all gravidities highly susceptible to PM (15, 30). In light of those observations, analysis of cytokine data as a function of gravidity and either HS or PD was performed with samples from HIV− women only; sample size limitations precluded this analysis for HIV+ women. The patterns of cytokine production as a function of HS in HIV− women overall (Fig. 1) were evident in both gravidity groups. Although sample sizes for the multigravid groups were small, minimizing statistical power, there were no statistically significant differences between the gravidity groups as a function of HS (data not shown). The influences of PD were, likewise, similar between the gravidity groups for IFN-γ and TNF-α. Again, sample sizes for multigravidae were low, and no statistically significant differences were found. With low-density parasitemia, multigravidae tended to produce more IL-10 than did primigravidae and secundigravidae; however, this result was significant only for responses to PHA (for the multigravid group with PDs of <1,000/μl, the PHA level was 539 pg/ml [interquartile range, 165 to 584; n = 3] versus the level for the primigravid and secundigravid group with PDs of <1,000/μl, 103 pg/ml [inter-
FIG. 2. Cytokine production by IVBMC from malaria-infected placentas as a function of HIV infection status and placental PD. IFN-γ (A to E), TNF-α (F to J), and IL-10 (K to O) were measured in culture supernatants of IVBMC and categorized by PDs (see Materials and Methods for definitions). Symbols are as defined in the legend to Fig. 1. (A, F, and K) Spontaneously produced cytokine levels; (B, G, and L) PHA-stimulated cytokine levels; (C, H, and M) anti-CD3 monoclonal antibody (CD3)-stimulated cytokine levels; (D, I, and N) PPD-stimulated cytokine levels; (E, J, and O) MalAg-stimulated cytokine levels. Among HIV⁺ women, levels of spontaneously produced and PHA- and PPD-stimulated TNF-α in the HS1 group were significantly higher than those in the HS0 group. *, P < 0.03; **, P < 0.05; ***, P < 0.01.
quartile range, 30 to 193; \( n = 21 \), \( P = 0.0402 \). Only the primigravid and secundigravid group experienced increases in IL-10 production with increasing PDs, as indicated by comparing data for those with low PDs to data for those with intermediate PDs (for medium alone, 26 pg/ml [interquartile range, 0 to 194; \( n = 22 \)] versus 143 pg/ml [interquartile range, 95 to 229; \( n = 12 \), \( P = 0.0387 \)) (for PHA, 103 pg/ml [interquartile range, 30 to 193; \( n = 21 \)] versus 273 pg/ml [interquartile range, 161 to 586; \( n = 11 \), \( P = 0.0182 \)) (for PPD, 47 pg/ml [interquartile range, 1 to 167; \( n = 22 \)] versus 178 pg/ml [interquartile range, 99 to 212; \( n = 11 \), \( P = 0.0434 \)] and by comparing data for those with low PDs to those with high PDs (for PHA, 103 pg/ml [interquartile range, 30 to 193; \( n = 21 \)] versus 393 pg/ml [interquartile range, 172 to 712; \( n = 4 \), \( P = 0.0434 \)]).

**DISCUSSION**

The presence of hemozoin in phagocytic cells is a well-documented characteristic of malarial infection and is suggested to be an indicator of severity of infection (19). In the infected placenta, accumulation of hemozoin-laden cells and free hemozoin in intervillous fibrin deposits are defining features of acute and chronic PM infection (4). In this study, we examined cytokine production by IVBMC collected from women with active PM infection and categorized the samples according to the level of hemozoin-laden phagocytes and PD. We observed that secretions of IFN-\( \gamma \), TNF-\( \alpha \), and IL-10 were differentially affected by hemozoin, with the most profound effect being a significant inverse relationship between production of IFN-\( \gamma \) and HS in HIV-\( \sim \) women. In HIV-\( \sim \) women, an opposite pattern for IFN-\( \gamma \) was evident: under mitogen stimulation conditions, secretion of this cytokine increased with increasing HS. Interestingly, different patterns were observed when the data were analyzed as a function of PD, suggesting that high-density parasitemia and high levels of hemozoin have different effects on IVBMC IFN-\( \gamma \) responsiveness.

IFN-\( \gamma \) is produced predominantly by T cells and NK cells and not by phagocytic cells; thus, it is unlikely that reduced secretion in HIV-\( \sim \) women with high HS is a direct effect of hemozoin accumulation in phagocytes. However, the functions of phagocytes as accessory cells, including the reducing of major histocompatibility complex class II and intercellular adhesion molecule 1 expression, are affected by engulfment of hemozoin (26). Also, phagocytosis of hemozoin and \( \beta \)-hematin (synthetic malarial pigment) by mouse peritoneal macrophages interfered with late-stage antigen processing and inhibited T-cell IL-2 production (27). A defect in antigen presentation by hemozoin-laden macrophages could explain the reduction in MalAg IFN-\( \gamma \) responses by HIV-\( \sim \) IVBMC (and the trend toward lower responses to PPD) but not the changes in spontaneous or mitogen-stimulated responses. Scorza et al. (27) suggested that a soluble, macrophage-derived suppressive factor (independent of nitric oxide or prostaglandin) which can suppress T-cell function is released by hemozoin-laden cells. Synergistic action of a suppressive factor and antigen presentation defects could explain the reduced IFN-\( \gamma \) responses of unstimulated and mitogen- and antigen-stimulated IVBMC in the presence of hemozoin.

Paradoxically, phagocytosis of hemozoin and \( \beta \)-hematin by human monocytes/macrophages has been shown to increase the production of cytokines and chemokines (TNF-\( \alpha \), IL-1\( \beta \), and macrophage inflammatory proteins 1\( \alpha \) and 1\( \beta \)) (21, 28). However, similar to the present results, reductions of prostaglandin E\(_2\), IL-10, and TNF-\( \alpha \) production by HIV-\( \sim \) IVBMC in the presence of high levels of hemozoin were recently demonstrated (20). These differences may be related to the time dependence of hemozoin influences on phagocyte function (27). TNF-\( \alpha \) production by human monocytes was shown to decline after a peak at 8 h post-hemozoin introduction (21). Similarly, murine RAW264.7 cells produced steadily decreasing amounts of TNF-\( \alpha \) following 72 h of incubation with hemozoin (28). Thus, if the HS is assumed to be an indicator of chronicity of PM, meaning that increased HS indicates longer exposure of IVBMC to hemozoin, then the substantial reductions in IFN-\( \gamma \) and the tendency toward slight reductions in TNF-\( \alpha \) with increasing HS may reflect a time-dependent suppressive effect of hemozoin on production of some cytokines in the placenta of HIV-\( \sim \) women.

Analysis of cytokine production as a function of PD revealed that PD and hemozoin loading of monocytes/macrophages do not have the same influences on production of IFN-\( \gamma \), TNF-\( \alpha \), and IL-10 by IVBMC. This fact was especially true for IFN-\( \gamma \) and TNF-\( \alpha \) production by IVBMC from HIV-\( \sim \) women, in whom patterns of cytokine secretion followed different trends with increasing PDs and HS. This result is not unexpected, as these two phenomena, ascending parasitemia and heavy hemozoin loading, are temporally distinct. Accumulation of high levels of hemozoin results from chronic infection and requires some time to develop, whereas high-density parasitemia can occur early in infection and can be found in the absence of heavy hemozoin deposition. Thus, the data presented here suggest that whereas acute parasitemia may, at least during the ascending phase (with intermediate PDs), result in increased immune stimulation and secretion of cytokines, particularly proinflammatory cytokines, such as IFN-\( \gamma \) and TNF-\( \alpha \), the accumulation of hemozoin over time ultimately leads to suppression of immune function. Consistent with this finding, a peak in cytokine response at intermediate PDs with lower responses at high PDs was observed for all three cytokines in HIV-\( \sim \) women (under two of five stimulation conditions for IFN-\( \gamma \), four of five for TNF-\( \alpha \), and three of five for IL-10), lending further support to the concept that hemozoin can suppress cytokine production.

It deserves mention that regardless of HIV status, HS, or PD, responses to MalAg were poor. Reduced responses to MalAg during acute infection (22) in malaria-exposed pregnant women compared to those in nonpregnant women (7, 8, 23) have been reported, with cytokine responses to mitogens in some cases being relatively preserved (7, 22, 23). The mechanisms by which this outcome occurs are likely to be complex, perhaps involving parasite-induced cytokine dysregulation (2) and disruption of antigen presentation or dendritic cell function (34) and, in pregnant women, hormones (3, 35). It will be necessary to perform intensive functional and mechanistic studies to identify the important players and their respective roles in malaria-associated immune modulation.
Cytokine responses by IVBMC are gravity dependent, although this characteristic is marked only in uninfected placentas (16). Consistent with this fact, examination of cytokine production by IVBMC from infected placentas stratified by gravity and HS revealed no significant gravity-based differences. This was also true for PD, with the exception of IL-10, for which production was higher in multigravidas with low PDs. Production by primigravidas and secundigravidas did increase significantly, however, with increasing PDs. It has been reported that high-level parasitemia is associated with elevated levels of IL-10 in placentas, as well as with anemia and preterm delivery (32), which tend to occur more frequently in malaria-exposed primigravidas and secundigravidas (9–11, 18, 29, 31). How IL-10 contributes to the manifestation of these poor pregnancy outcomes and how they are influenced by PDs and hemozoin accumulation in the placenta still require intensive study.

As has been demonstrated previously (15), HIV infection significantly influences cytokine production by IVBMC. The data presented here show that the impact of hemozoin-laden cells on IVBMC production of IFN-γ, and to a lesser extent, TNF-α, is influenced by HIV infection. Although most of the HIV-based differences observed did not reach statistical significance in this study, there are evident trends that beg interpretation and further study. Under mitogen stimulation conditions, IFN-γ and TNF-α decreased with increasing HS in HIV+ samples but increased in HIV− samples. It is known that HIV infection induces proinflammatory cytokine dysregulation, a defect that is associated with chronic activation of monocytes/macrophages (6). Thus, enhanced production of IFN-γ and TNF-α by IVBMC from HIV+ women may reflect this “primed,” dysregulated state in which increased hemozoin stimulates increased cytokine production. Alternatively, HIV infection may disrupt the suppressive factor whose secretion has been proposed to be induced by hemozoin (27). Finally, it is important to note that the HIV+ women included in this study were apparently healthy and had not yet manifested symptoms of AIDS. Ongoing studies (D. J. Perkins et al., unpublished data) demonstrate that the immunologic responses to hemozoin differ markedly in both humans and nonhuman primates as HIV and SIV disease progress to AIDS. Thus, the effects of HIV infection on cytokine production observed here are likely to be subtle relative to the profound immune disruption and dysregulation that are the hallmarks of late-stage HIV infection and AIDS.

In conclusion, we have demonstrated here that intense accumulation of hemozoin-laden cells in IVB has a marked suppressive effect on IFN-γ production by IVBMC, with more subtle effects on TNF-α and IL-10. Concurrent infection with HIV appears to alter the impact of hemozoin on IVBMC cytokine production. The hemozoin-associated suppression seen in HIV+ women is not observed with intermediate PDs, suggesting that parasitemia and hemozoin loading of phagocytic cells have differential, perhaps temporally distinct, effects on maternal immune function in the placenta. Because IFN-γ is thought to be a critical factor in mediating protection against PM (15, 16), accumulation of hemozoin-laden cells in the placenta, although a necessary by-product of parasitized erythrocyte clearance, may affect the development and maintenance of the protective, cell-mediated immune responses that are required to control PM. In contrast, by stimulating high levels of production of IFN-γ and TNF-α in HIV+ women, hemozoin may disrupt the fine balance of cytokines required for protective immune responses to malaria. Further functional studies are required to assess in more detail the impact of hemozoin on antimalarial immunity and HIV pathogenesis in the placenta.

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