Macrophage migration inhibitory factor (MIF) may play a role in immune responses to malaria during pregnancy by virtue of its ability to activate macrophages and to overcome the immunosuppressive effect of glucocorticoids. The present study investigated whether plasma MIF levels are altered in pregnant women with placental malaria (PM) and/or human immunodeficiency virus (HIV) infection. For the first time it is demonstrated that MIF levels in the intervillous blood (IVB) plasma were significantly elevated, compared with that in both peripheral plasma (∼500-fold) and cord plasma (4.6-fold; \( P < .01 \)). IVB mononuclear cells also produced significantly higher levels of MIF, compared with that of peripheral blood mononuclear cells. PM was associated with increased levels of MIF in the IVB plasma (\( P < .02 \)). Primigravid and secundigravid women had significantly higher levels of MIF in their IVB plasma than did multigravid women (\( P < .05 \)). HIV infection did not significantly alter MIF levels in any site examined.

In areas where malaria is endemic, pregnant women are at an increased risk for acquiring Plasmodium falciparum infection, compared with that of their nonpregnant counterparts. Their infection often presents with an accumulation of parasitized erythrocytes in the maternal placental intervillous blood (IVB), a condition referred to as placental malaria (PM), which contributes to both maternal anemia and low infant birth weight in endemic areas with intense malaria transmission. The frequency and severity of PM decrease over successive, malaria-exposed pregnancies, which suggests that specific immunity develops after several exposures to PM [1].

The immunologic mechanisms that confer this immunity remain poorly understood. Our laboratory has used perfused intervillous blood mononuclear cells (IVBMC) from human placenta to study cell-mediated immune mechanisms in protection against PM [2]. Consistent with other studies of immune control of malarial infection [3, 4], we have shown that protection against PM is associated with an interferon (IFN)–γ response [2] and that increased susceptibility to PM in human immunodeficiency virus (HIV)–infected women is associated with impaired IFN-γ and interleukin (IL)–12 responses [5, 6].

It is evident from histological observations that macrophages in the intervillous space of the placenta are the major source of MIF. In addition, MIF is produced by murine fetal macrophages, which are important players in macrophage activation, and these cells are activated by IFN-γ. However, it is not clear whether IFN-γ and tumor necrosis factor (TNF)–α, which are important players in macrophage activation, would be sufficient to activate macrophages during pregnancy, because of the elevated levels of immunosuppressive...
hormones, such as glucocorticoids (e.g., cortisol) [8]. Glucocorticoids have been shown to suppress IFN-γ– and TNF-α–mediated activation and phagocytic activity of macrophages [9]. In addition, elevated levels of cortisol found in pregnant women have been implicated in the potential loss of immunity to malaria during pregnancy [8]. Therefore, we speculate that a cytokine with the ability to overcome the immunosuppressive effect of glucocorticoids may play an important role in modulating protective immunity during pregnancy.

Macrophage migration inhibitory factor (MIF) is the only cytokine known thus far to overcome the immunosuppressive effect of glucocorticoids on IFN-γ– and TNF-α–mediated activation of macrophages (reviewed in [10, 11]). MIF is released after the activation of macrophages by various proinflammatory stimuli, such as lipopolysaccharides, toxic shock syndrome toxin 1, TNF-α, and IFN-γ [10]. It also is involved in the activation of macrophages and the killing of intracellular parasites [12]. Recently, MIF has been shown to be induced in response to P. chabaudi infection in mice and has been implicated in the pathogenesis of malaria anemia [13].

In light of its ability to activate macrophages and to circumvent glucocorticoid-mediated immunosuppression, we hypothesized that MIF plays a pivotal role in immunity to PM. To test this hypothesis, we first investigated expression of MIF levels in women infected with PM and/or HIV.

Materials and Methods

Study site, participants, and samples. Pregnant women attending the antenatal clinic and delivery ward in the New Nyanza Provincial General Hospital in Kisumu, western Kenya, were recruited for this study. Kisumu experiences intense perennial transmission of P. falciparum malaria, with 2 peak transmission periods occurring from November to December and May to July, respectively.

Blood samples and placentas were obtained from women who participated in a cohort study to assess the impact of PM on mother-to-child HIV transmission [5]. Thick blood films were used to assess for P. falciparum parasitemia in the IVB and peripheral blood [2]. The HIV status was determined by simultaneously testing with 2 HIV rapid test kits, and only those who tested positive by both tests were considered to be HIV infected (HIV⁺) [2, 5]. Only healthy women (in both HIV⁻ and HIV-uninfected [HIV⁻] groups) with uncomplicated labor and singleton, vaginal deliveries were included in this study. Women who were likely to have AIDS were excluded.

Collection of plasma samples. IVB was collected by making an incision on the maternal side (basal plate) of the placenta. At the time of delivery, ∼500 μL of fingerprick blood was collected from participating women. About 5 mL of venous blood also was collected from 83 women who visited the antenatal clinic. All blood samples were collected in EDTA-containing tubes, with the plasma separated within a few hours, and were frozen in liquid nitrogen.

Measurement of MIF. A double-sandwich ELISA was used to measure MIF levels, as described elsewhere [2]. In brief, MIF was captured by a mouse monoclonal anti-human MIF antibody (clone 12302.2) and was detected with a biotinylated goat anti-human MIF antibody (both from R&D Systems). MIF concentrations were determined against a curve of known value standards included in each assay plate.

Statistical analyses. Unless otherwise indicated, women were grouped according to their infection status: HIV⁻/PM uninfected (PM⁻), HIV⁻/PM infected (PM⁺), HIV⁺/PM⁻, and HIV⁺/PM⁺. SAS (version 8.0; SAS Institute) statistical software package was used for data analysis. Comparisons of matched pairs were done by use of the nonparametric sign test. The nonparametric, Wilcoxon rank sum test was used to assess for differences between the 2 groups. Multiple groups were compared by use of the Kruskal-Wallis test; when comparison of multiple groups by means of Kruskal-Wallis yielded a significant result, the permutation test (SAS Proc Multtest) was used to compare mean differences of the ranks and to adjust the P value for multiple testing. P ≤ .05 was considered to be statistically significant.

Results

MIF levels in IVB and peripheral and cord plasma. MIF levels in paired IVB plasma and peripheral plasma collected randomly from 9 women at the time of delivery were compared. The mean concentration of MIF was ∼500-fold higher in the IVB plasma samples (1136 ng/mL) than in the paired peripheral plasma (2.1 ng/mL; P < .01). MIF levels in a group of 98 unpaired IVB plasma samples collected from placentae (at delivery) with 83 peripheral plasma samples collected during an antenatal visit in the last trimester of pregnancy also were compared. The results showed that the unmatched IVB plasma (289.9 ng/mL) had higher levels of MIF, compared with that in the peripheral plasma (0.8 ng/mL; P < .0001). MIF levels in the IVB plasma (169 ng/mL) also were higher (4.6-fold), compared with that of paired cord plasma samples (36 ng/mL; P < .01). Together, these results show that MIF levels are highly elevated in the IVB plasma, compared with that in peripheral or cord plasma.

MIF levels in different gravidities. We compared the mean concentrations of MIF in primigravid, secundigravid, and multigravid women. Primigravid and secundigravid women had significantly higher levels of MIF in the IVB plasma than did multigravid women (P < .05; figure 1A). In contrast, there were no significant differences in the mean concentrations of MIF in the cord and peripheral plasma of the different gravidity groups (P > .05; figure 1B, 1C, 1D).

IVB but not peripheral MIF levels are associated with PM. We also determined whether MIF levels are altered because of PM or peripheral P. falciparum infection status in both HIV⁺ and HIV⁻ women. IVB plasma MIF levels were significantly elevated in HIV⁺/PM⁻ women, compared with that in HIV⁺/PM⁺ women and HIV⁻/PM⁻ women (P < .02; figure 2A). In HIV⁻/PM⁻ women, the IVB plasma levels were similar to that of HIV⁺/PM⁺ women, which indicates that HIV infection did not significantly alter the levels of MIF. HIV⁺/PM⁻ women had
significantly elevated levels of IVB plasma, compared with that in HIV+/PM− women (P < .002), but not with that in HIV+/PM+ women (P > .05).

Unlike in the placenta, peripheral plasma MIF levels did not significantly differ because of peripheral malaria or HIV infection status (figure 2B). In cord plasma, MIF levels were significantly elevated in HIV+/PM+ mothers, compared with that in HIV-/PM− and HIV+/PM− mothers (P < .02). On the contrary, cord plasma from HIV+/PM− mothers did not have higher levels of MIF, compared with that in HIV-/PM− or HIV+/PM+ mothers (figure 2C).

In vitro production of MIF. We also compared MIF levels in IVBMC and peripheral blood mononuclear cells (PBMC) culture supernatants. Supernatants from unstimulated IVBMC cultures contained 29 ng/mL of MIF, which is ~10-fold higher than that found in PBMC cultures (2.3 ng/mL; P < .0001). The level of MIF did not significantly differ by infection status for both IVBMC and PBMC cultures (data not shown).

Discussion

Our findings in this study show for the first time that MIF is present at very high levels in the IVB plasma, compared with that in both peripheral and cord plasma. Although the immunologic significance of this finding remains to be investigated, we hypothesize that MIF may play a physiologically important role in regulating immune responses during pregnancy, especially in IVB.

Histologic studies have consistently shown that malaria-infected placentas have high numbers of macrophages loaded with malarial pigment, which indicates that these cells play a critical role in the clearance of these parasites [7]. MIF may play a critical role in activating the macrophages to clear the malaria parasites. Indeed, recent studies have shown that MIF is effective in activating macrophages to kill intracellular parasites such as Leishmania major [12]. The steroid hormones, estrogen, progesterone, and cortisol, all of which may be immunomodulatory, are elevated during pregnancy; cortisol, especially, has been reported to suppress macrophage function [9] and has been implicated in the loss of immunity to malaria during pregnancy [8]. We speculate that MIF, by its ability to activate macrophages and to overcome the immunosuppressive effect of glucocorticoid hormones such as cortisol, may play an important role, along with IFN-γ and/or TNF-α, in activating macrophages to clear and kill malaria parasites in the placenta. Because MIF can inhibit the random migration of macrophages in vitro [10], we suggest that increased MIF levels found in PM+ mothers may help to retain the macrophages that accumulate in the placenta to clear...
HIV infection. Indeed, we have found that the number of macrophages in IVBMC was at least 2-fold higher in malaria-infected than in malaria-uninfected placentas (J.M.M. and V.U., unpublished data).

Importantly, the elevated MIF concentrations in the PM* women were a local phenomenon of the placentas. The in vitro data in the current study showed that IVBMC produced MIF levels 10-fold higher than that of PBMC, which suggests that these cells may serve as an important source of the elevated quantity of MIF present in the IVB. However, immunocytochemical studies will be necessary to confirm the source, since MIF has been reported in the human endometrium and decidua of first-trimester placentas [14], placental villi, cytotrophoblasts, and in the trophoblastic cell islands [15]. Therefore, MIF has been implicated in implantation and other reproductive functions [14, 15].

It is not clear why MIF levels were significantly higher in the primigravid and secundigravid women, compared with those in multigravid women. From previous studies, it is known that cortisols levels are higher in primigravid than in multigravid women [8]. Since glucocorticoids also can induce MIF production, there is the intriguing possibility that elevated MIF levels in primigravid women may be related to the higher levels of corticoids found in those women than in multigravid women [8]. Insufficient plasma volume precluded addressing this question in this study.

We have shown in previous studies that HIV infection is associated with the down-regulation of IFN-γ and IL-12 responses in the IVBMC [5–6]. However, it is evident from this investigation that HIV infection did not significantly affect the production of MIF, which suggests that an HIV-associated increase in the susceptibility to PM is not due to lack of an MIF response.

In summary, we have shown that MIF concentration is highly elevated in the IVB plasma, compared with that in the peripheral plasma, and that PM infection further increased this difference. MIF levels were not altered in HIV-infected pregnant women. On the basis of these findings, we suggest that MIF may play an important role in immune responses to infectious agents, such as malaria, especially in the IVB.

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