Immunity to Placental Malaria. III. Impairment of Interleukin (IL)–12, not IL-18, and Interferon-Inducible Protein–10 Responses in the Placental Intervillous Blood of Human Immunodeficiency Virus/Malaria–Coinfected Women

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Pregnant women are highly susceptible to malaria, and human immunodeficiency virus (HIV)–infected pregnant women are at higher risk for peripheral and placental malaria (PM) than their HIV-seronegative (HIV−) counterparts [1–3]. Although the biological basis for the increased susceptibility to PM during pregnancy is not completely understood, we recently showed that interferon (IFN)–γ production by maternal placental (intervillous) blood mononuclear cells (IVBMC) is associated with protection against PM [4] and that this response is impaired in HIV-positive (HIV+) pregnant women [5]. The underlying mechanisms for this loss, particularly with regard to the cytokines associated with IFN-γ production, are not known.

Interleukin (IL)–12 is produced by activated macrophages, antigen-presenting cells, B lymphocytes, and neutrophils (reviewed in [6]). IL-12 is critical for inducing CD4 T lymphocytes to produce IFN-γ and for activation of antiparasitic mechanisms in NK cells and macrophages (reviewed in [6]). IL-18, which is produced by antigen-presenting cells and some nonhematopoietic cells, is another important cytokine involved in inducing IFN-γ production in T and NK cells, both independently and in synergy with IL-12 (reviewed in [7]). IL-12 and IL-18 levels are altered in HIV-infected persons [8, 9]; however, whether production of these 2 cytokines by IVBMC is altered in pregnant women who are infected with HIV and/or organisms causing malaria is not known.
IFN-inducible protein (IP)–10 is a CXC chemokine produced by a variety of leukocyte subsets in response to IFN-γ stimulation (reviewed in [10]). This chemokine provides a potent signal for migration of activated T lymphocytes and NK cells [10], which are critical for cell-mediated immunity. Although no role in protection against malaria has been described for IP-10, IP-10 is a potential candidate for recruitment of effector T and NK cells into placentas with malarial infection. Because IFN-γ expression by IVBMC is reduced in HIV+ and PM-positive (PM+) women, determining whether a reduction in local IP-10 expression may be associated with an increased susceptibility to PM in HIV+ women is of interest. Therefore, to further characterize the regulation of cytokine responsiveness by IVBMC in women with HIV infection and malaria, we investigated the in vitro expression patterns of IL-12, IL-18, and IP-10 by placent al IVBMC from HIV+/PM-negative (PM−), HIV+/PM+, HIV−/PM−, and HIV−/PM+ women.

Subjects, Materials, and Methods

Study site, participants, and samples. Pregnant women who delivered in the New Nyanza Provincial General Hospital in Kisumu (in western Kenya) were recruited for this study. There is intense perennial transmission of Plasmodium falciparum malaria in Kisumu, with 2 peak transmission periods each year, from November through December and from May through July.

Blood samples and placentas were obtained from women who participated in a cohort study to assess the impact of PM on mother-to-child transmission of HIV-1, as described elsewhere [5]. Thick blood films were used to assess P. falciparum parasitemia in the inter vival blood [4]. The parasite density was calculated using the assumption that each micro liter of blood contains 8000 leukocytes. This is only an approximation; the actual leukocyte count varies from person to person. HIV status was determined by simultaneous whole blood films were used to assess P. falciparum parasitemia in the int ival blood [4]. The parasite density was calculated using the assumption that each microliter of blood contains 8000 leukocytes. This is only an approximation; the actual leukocyte count varies from person to person. HIV status was determined by simultaneous testing with 2 HIV rapid test kits, as described elsewhere [4, 5]. Women with discordant HIV test results and those likely to have AIDS were excluded. Only healthy women (in both the HIV+ and the HIV− groups) with uncomplicated labor and singleton vaginal deliveries were included in this study. Figure 1 further summarizes patient characteristics.

Culture conditions. Placental perfusion, IVBMC preparation, and culture conditions have been described elsewhere [4]. Cells were cultured for 48 h at a concentration of 10⁶ cells/mL at 37°C in an atmosphere of 6% CO₂. After this incubation period, supernatants from cells cultured in complete medium (RPMI 1640 medium containing 5% fetal bovine serum, 5% AB-positive pooled human serum, 1mM L-glutamine, and 1mM penicillin-streptomycin) [4] alone (which served as controls) and from cells in cultures stimulated by phytohemagglutinin (PHA; Sigma; final concentration, 10 μg/mL) and purified protein derivative (PPD; Evans Medical; final concentration, 100 U/mL) were collected. Supernatants were stored at −80°C until testing.

ELISA measurement of cytokine and chemokine levels in supernatants. Double-antibody sandwich ELISAs were performed as described elsewhere [4]. IL-12 was detected by OptEIA kit (PharMingen). IL-18 was detected with a capture mouse anti–human IL-18 monoclonal antibody (Mab) and a biotinylated goat anti–human IL-18 MAb (R&D Systems). IP-10 was detected with a capture mouse anti–human IP-10 MAb and a biotinylated mouse anti–human IP-10 MAb from PharMingen.

Statistical analyses. We used a statistical software package for data analysis (SAS, version 6.0; SAS Institute). Comparisons of multiple groups were done with the Kruskal-Wallis test, for which \( P < .05 \) was considered to be significant. We used the nonparametric Wilcoxon rank sum test (WRS) to assess differences between individual groups. Significance was set at \( P < .0125 \) for WRS.

Results

IL-12 production by IVBMC. We compared the IL-12 production in the IVBMC of HIV+ and HIV− mothers with and without PM. Mean IL-12 responses from different groups are shown in figure 1A. Constitutive PHA- and PPD-stimulated IL-12 production was significantly higher in cultures of IVBMC from HIV−/PM− women than in cultures of IVBMC from HIV+/PM+ women (\( P < .01 \) for all stimuli). Levels of IL-12 in unstimulated, PHA-stimulated, and PPD-stimulated cultures were lower for HIV+/PM− women than for HIV−/PM− women (\( P > .05 \)) and HIV+/PM+ women (\( P < .005 \) for all stimuli, by WRS). The production of IL-12 was further impaired in HIV+/PM+ women, compared with that in HIV−/PM− (\( P < .01 \) in the PHA and PPD groups, by WRS) and HIV−/PM+ women (\( P < .005 \) in the PHA and PPD groups, by WRS). Among HIV+ women, PM− women had lower IL-12 levels than PM+ women; the difference was marginally significant for PPD stimulation (\( P = .02 \) for PPD and \( P = .05 \) for PHA, by WRS). Together, these data show substantial impairment of IL-12 responses in the IVBMC of HIV+ mothers.

IL-18 production by IVBMC. Production of IL-18 by both IVBMC and peripheral blood mononuclear cells was not enhanced by PHA and PPD stimulation at more than the constitutive level (figure 1B). Nonetheless, some notable differences among the infected groups were seen in the capacity of these cells to produce this cytokine. IL-18 production levels in HIV−/PM− and HIV−/PM+ women were similar. IVBMC from HIV+/PM− women showed ∼2-fold less production of IL-18, compared with HIV−/PM+ women. However, the mean concentration of IL-18 production by IVBMC from HIV+/PM+ women was higher than that from the other groups. None of these differences reached statistical significance.

IP-10 production by IVBMC. IVBMC from women with either HIV infection or PM tended to produce lower levels of IP-10 than cells from uninfected women (figure 1C). Compared with the HIV−/PM+ group, supernatants from IVBMC cultures of HIV−/PM− women and HIV+/PM− women had 3–4-fold and 2–3-fold lower levels of IP-10, respectively, under all stimulation conditions (\( P > .05 \)). The spontaneous IP-10 production by IVBMC from HIV−/PM+ women was significantly higher than that in all other groups (\( P < .01 \), by WRS). The IP-10 response to PHA stimulation in HIV+/PM+ women was signifi-
Cytokine production as a function of CD4 T cell levels. To assess whether reduced peripheral blood CD4 T lymphocyte counts influence IL-12, IL-18, or IP-10 production by IVBMC in HIV-infected women, the cytokine data for HIV+ women were stratified according to CD4 cell count, with a cutoff of 500 cells/mL. This analysis showed that IL-12 production was reduced to similar levels in the groups with high and with low CD4 cell counts (table 1). In contrast, IL-18 levels were 2-3-fold lower in the group with low CD4 cell counts than in the group with high CD4 cell counts, under all stimulation conditions. Levels of IP-10 production were lower, especially in PHA-stimulated culture supernatants, in IVBMC from mothers with low CD4 cell counts than in IVBMC from mothers with high CD4 cell counts. However, these differences in IL-18 and IP-10 levels were not statistically significant.

Discussion

In a previous investigation, we showed that IFN-γ responsiveness, which is associated with protection against PM [4], is substantially reduced in HIV-infected women [5]. The current
and IFN-γ against malaria [6], these data suggest that impairment of IL-12 responses in IVBMC from HIV+ women was more severe than that observed for IFN-γ in our previous study [5]. Given the important role of IL-12 in regulating IFN-γ production and its role in mediating protective immunity against malaria [6], these data suggest that impairment of IL-12 and IFN-γ responses may contribute to the loss of protection against PM infection in HIV-infected women. However, the basis for this down-regulation of IL-12, which is produced by antigen-presenting cells, must be determined. Likewise, further studies will be required to confirm whether the impaired IL-12 response observed in this study directly contributes to the down-regulation of malaria antigen–specific IFN-γ responses in the IVBMC of HIV-infected women.

Unlike production of IL-12, IL-18 production by IVBMC in HIV+ women was not significantly suppressed, suggesting that the reduction in the malaria antigen–induced IFN-γ production in the IVBMC of HIV+ mothers may be independent of IL-18. However, notably, IL-18 production was reduced in HIV+ women with low CD4 T cell counts, a pattern similar to that seen with IFN-γ [5]. This complex pattern of expression suggests that IL-18 production by IVBMC may be an indicator of other immunologic events in these women. IL-18 mRNA is produced in a precursor form and must be proteolytically cleaved to produce functionally active IL-18. Caspase, an enzyme that is involved in cleaving the IL-18 protein [11], is produced during the apoptotic process. We hypothesize that the elevated production of IL-18 that we observed in women coinfected with HIV and malaria may be a by-product of increased apoptosis, exacerbated by chronic, malaria antigen–induced immune stimulation.

The status of IP-10 responsiveness in persons with HIV infection and malaria has not been extensively studied. However, it has been proposed that elevated levels of IP-10 found in the cerebrospinal fluid (CSF) of HIV+ patients with neurologic disorders are responsible for the increased white blood cell count in the CSF of these patients [12]. In this study, IP-10 expression was elevated in HIV+/PM+ women but not in HIV+/PM women. These data suggest that PM alters the balance of IP-10 production by IVBMC in HIV+/PM+ women. Further immunohistochemical studies of placentas are needed to determine the role of IP-10 in the migration of immune cells and malarial pathogenesis.

### Table 1. Changes in intervillous blood mononuclear cell (IVBMC) cytokine production as a function of peripheral blood CD4 T cell count in human immunodeficiency virus–seropositive women.

<table>
<thead>
<tr>
<th>Culture, CD4 T cell count in cells/mL</th>
<th>IVBMC cytokine production, pg/mL</th>
<th>IL-12</th>
<th>IL-18</th>
<th>IP-10</th>
</tr>
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<tbody>
<tr>
<td>Medium only</td>
<td></td>
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<tr>
<td>&lt;500</td>
<td>1.5 ± 0.8 (9)</td>
<td>20.6 ± 9.4 (9)</td>
<td>16.4 ± 4.8 (9)</td>
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</tr>
<tr>
<td>&gt;500</td>
<td>2.1 ± 1.4 (13)</td>
<td>65 ± 22.3 (13)</td>
<td>23.4 ± 7.3 (13)</td>
<td></td>
</tr>
<tr>
<td>PHA stimulated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;500</td>
<td>1.7 ± 0.7 (9)</td>
<td>20.3 ± 8.1 (9)</td>
<td>58.1 ± 20.4 (9)</td>
<td></td>
</tr>
<tr>
<td>&gt;500</td>
<td>0.7 ± 0.4 (13)</td>
<td>56.1 ± 19.4 (13)</td>
<td>150.6 ± 44.3 (13)</td>
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<tr>
<td>PPD stimulated</td>
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<tr>
<td>&lt;500</td>
<td>1.9 ± 0.7 (9)</td>
<td>26.5 ± 12.4 (9)</td>
<td>28.2 ± 7.1 (9)</td>
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<tr>
<td>&gt;500</td>
<td>0.9 ± 0.5 (12)</td>
<td>62.4 ± 22.4 (12)</td>
<td>40.2 ± 11.5 (12)</td>
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**NOTE.** Data are arithmetic mean ± SE of cytokine levels of women with <500 and >500 CD4 cells/mL of blood. Values in parentheses are the total no. of subjects from whom samples were tested. CD4 T lymphocyte counts in peripheral blood were determined 1 month postpartum by flow cytometry, as described elsewhere [5]. IL, interleukin; IP, interferon-inducible protein; PHA, phytohemagglutinin; PPD, purified protein derivative.
References


