# CD16$^{+}$ Monocyte Subset Preferentially Harbors HIV-1 and Is Expanded in Pregnant Malawian Women with *Plasmodium falciparum* Malaria and HIV-1 Infection

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**In a cross-sectional study, monocyte subsets in placental, cord, and maternal peripheral blood from pregnant Malawian women with human immunodeficiency virus (HIV)–1 infection and/or malaria were analyzed. HIV-uninfected Malawian women had higher baseline proportions of CD16$^{+}$ monocytes than those reported for healthy adults in developed countries. Malaria was associated with an increase in the proportion of CD16$^{+}$ monocytes that was significant in women coinfected with HIV-1. CD16$^{+}$ monocytes expressed higher CCR5 levels than did CD14$^{hi}$/CD16$^{-}$ monocytes and were significantly more likely to harbor HIV-1. These data suggest a role for CD16$^{+}$ monocytes in the pathogenesis of maternal malaria and HIV-1 infections.**

*Plasmodium falciparum* malaria and HIV-1 infection are serious public health concerns for pregnant women in sub-Saharan Africa. Pregnant women are at a greater risk of *P. falciparum* infection than are nonpregnant women, with up to 50% of pregnant women infected in areas of high transmission [1]. Women of childbearing age are also at a high risk of HIV infection; as a consequence, coinfection with both pathogens is common in the context of pregnancy. HIV-1 infection is associated with higher prevalence and intensity of malaria in pregnancy (reviewed in [2]); conversely, malaria may augment HIV-1 loads in pregnant women [3]. The impact of each pathogen in the context of coinfection needs to be evaluated.

Peripheral blood monocytes are a heterogeneous population of cells of varying stages of differentiation and activation that are important cellular reservoirs for HIV-1. The majority of monocytes express high levels of CD14 (a component of the lipopolysaccharide receptor complex) but do not express CD16 (a low-affinity receptor for human immunoglobulin G [Fc$\gamma$RIII]). These cells (hereafter termed "CD14$^{hi}$/CD16$^{-}$ monocytes") make up most peripheral blood monocytes. Conversely, ~10% of monocytes express CD16 and variable levels of CD14 [4] (hereafter termed "CD16$^{+}$ monocytes"). CD16$^{+}$ monocytes also express more CCR5 (the coreceptor for cellular entry of R5 strains of HIV-1) than do CD14$^{hi}$/CD16$^{-}$ monocytes [5]. In response to HIV-1 infection, the CD16$^{+}$ monocyte subset may expand, particularly in patients with advanced disease or HIV dementia [6]. Our recent study of a cohort of Australian homosexual HIV-positive men [7] showed that monocyte subsets were not significantly altered in individuals with HIV-1 infection receiving antiretroviral therapy but that the proportion of CD16$^{+}$ monocytes was increased in therapy-naive patients. Thus, the prevalence of this monocyte subset may be a marker for disease severity and/or virological control. By contrast, to our knowledge, there have been no studies addressing monocyte subsets in response to malaria or malaria/HIV coinfection.

In the present study, we investigated the effect of *P. falciparum* and HIV-1 coinfection on CD14$^{hi}$/CD16$^{-}$ and CD16$^{+}$ monocyte subsets in maternal peripheral, placental, and cord blood from pregnant Malawian women. In addition, we compared HIV-1 infection in the CD14$^{hi}$/CD16$^{-}$ and CD16$^{+}$ monocyte subsets isolated from their peripheral blood.

***Subjects and methods.*** Participants were women in the late third trimester of pregnancy attending Queen Elizabeth Central

Received 30 November 2006; accepted 19 January 2007; electronically published 16 May 2007.

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**The Journal of Infectious Diseases 2007;196:38–42**
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0022-1899/2007/19601-0008$15.00
DOI: 10.1086/518443

Potential conflicts of interest: none reported.

Financial support: National Institutes of Health (NIH; grants AI 49084, NIH/Fogarty TW 00908, and Fogarty International Research Collaboration Award TW05927-01); Wellcome Trust (Senior Overseas Fellowship to S.J.R.); Australian National Health and Medical Research Council (Principal Research Fellowship to S.M.C.).

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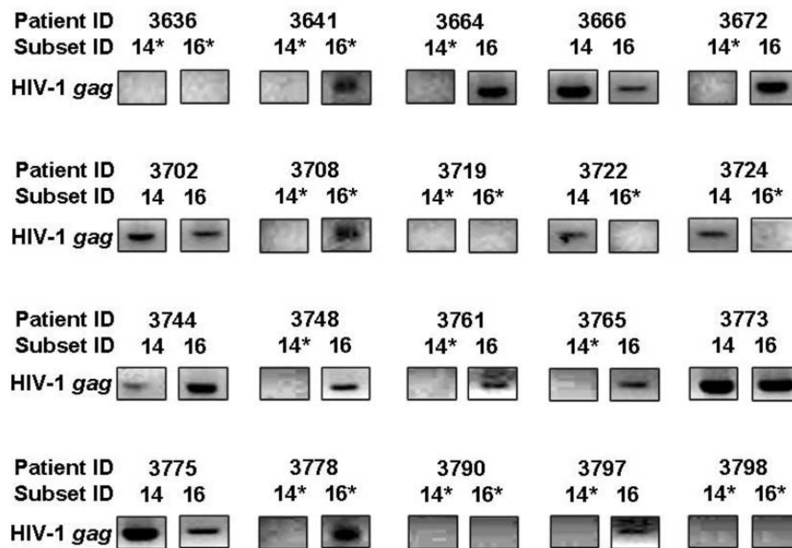


**Figure 1.** Polymerase chain reaction (PCR) analysis for the detection of HIV-1 DNA in CD14$^{hi}$/CD16$^{-}$ and CD16$^{+}$ monocyte subsets isolated from peripheral blood of 20 HIV-infected pregnant women (see table 1). DNA was extracted from monocytes and standardized for cell numbers by real-time PCR for the CCR5 gene. HIV-1 DNA was detected using primers that amplify a highly conserved region of *gag.* Samples that are denoted by an asterisk showed weak or absent first-round PCR product and were retested using a nested PCR. Samples that were clearly positive for HIV-1 DNA in the first-round PCR were not subjected to nested PCR. The contrast of the photograph of some gel bands has been adjusted only to highlight faint bands for the purposes of publication. Negative control samples (cell lysis buffer only) were also included in all PCR assays and were always negative for contaminating viral DNA (data not shown). 14, CD14$^{hi}$/CD16$^{-}$ monocytes; 16, CD16$^{+}$ monocytes.

Hospital, Blantyre, Malawi. Women in active labor, with hypertension, or with altered consciousness were excluded. Samples were collected, after informed consent had been obtained, between January 2003 and April 2004. Postpartum samples were collected at follow-up, scheduled 12 weeks after delivery. Maternal HIV-1 status was established by 2 concordant HIV-1 rapid tests (Determine [Abbott Laboratories] and Serocard [Trinity BioTech]). Maternal, placental, and cord blood malaria status was determined by microscopic analysis of Giemsa-stained thick blood films.

Blood was collected in standard $K_2$EDTA collection tubes, stored at room temperature, and processed within 24 h of collection. Placental blood was collected by making an incision on the maternal face of the placenta ~1 cm from the cord insertion and collecting blood that welled from the incision. This procedure results in a mixture of intervillous (i.e., maternal placental) monocytes and fetal blood monocytes from within the villous tree. Cord blood was collected by cord venipuncture. Samples were prepared for flow-cytometric analysis of CD14, CD16, and CCR5 using a whole-blood staining method [8]. Monocytes were initially gated using light-scatter characteristics, then further gated using CD14 and CD16 expression into 3 subsets: CD14$^{hi}$/CD16$^{-}$, CD14$^{hi}$/CD16$^{+}$, and CD14$^{lo}$/CD16$^{+}$. The percentage of monocytes expressing CCR5 and CCR5 mean fluorescence intensity (MFI) within each subset was determined. At least 2000 events in the CD16$^{+}$ monocyte gate were collected. CCR5 expression was corrected for background fluorescence of cells stained with isotype controls.

CD14$^{hi}$/CD16$^{-}$ and CD16$^{+}$ monocyte subsets were isolated from 9 mL of peripheral blood from 20 HIV-infected pregnant women (7 coinfected with malaria) using antibody-coated magnetic beads. Peripheral blood mononuclear cells were depleted of T cells using anti-CD3–coated magnetic beads (CD3 Dynabeads; Dynal Biotech), and monocytes were isolated by negative selection using a MACS Monocyte Isolation Kit II (Miltenyi Biotec). CD16$^{+}$ monocytes were positively enriched from total monocytes (CD16 microbeads; Miltenyi Biotec), whereas the negative fraction represented CD14$^{hi}$/CD16$^{-}$ monocytes. CD14$^{hi}$/CD16$^{-}$ and CD16$^{+}$ monocytes were resuspended in 500 $\mu$L of DNA stabilization buffer (Buffer AS1; Qiagen) and stored at −70°C for up to 20 weeks before they were shipped to Melbourne, Australia, at room temperature for analysis. CD14$^{hi}$/CD16$^{-}$ and CD16$^{+}$ monocyte cell numbers from each donor were standardized by real-time polymerase chain reaction (PCR) amplification of the CCR5 gene using primers LK46 and LK47 and beacon LK155 [9]. HIV-1 DNA was detected by conventional PCR. Samples were amplified for 40 cycles using primers A2 (sense, 5′-GGGGGACATCAAGCAGCCATGCAAATG-3′) and B2 (antisense, 5′-ACTCCCTGACATGCTGTCATCATTT-CTTC-3′). Negative or faintly positive samples were subsequently subjected to nested PCR using primers C2 (sense, 5′-CAGGAACTACTAGTA-3′) and D2 (antisense, 5′-GAATG-



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**Table 1. Characteristics of HIV-infected pregnant women from which CD14$^{hi}$/CD16$^{-}$ and CD16$^{+}$ monocyte subsets were examined for HIV-1 infection.**

| Patient no. | CD4$^{+}$ cell count, cells/$\mu$L | HIV-1 load, copies/mL | Detection of HIV-1 in monocytes: CD14$^{hi}$/CD16$^{-}$ | Detection of HIV-1 in monocytes: CD16$^{+}$ | Maternal malaria status in blood, parasites/$\mu$L of blood |
|---|---|---|---|---|---|
| 3636 | 343 | 192,296 | − | − | 60 |
| 3641 | 544 | 23,870 | − | + | 8310 |
| 3664 | 536 | 31,570 | − | + | 8190 |
| 3666 | 137 | 31,912 | + | + | 0 |
| 3672 | NA | <400 | − | + | 0 |
| 3702 | 402 | 128,261 | + | + | 0 |
| 3708 | 449 | NA | − | + | 0 |
| 3719 | NA | NA | − | − | 3240 |
| 3722 | 225 | NA | + | − | 750 |
| 3724 | 451 | NA | + | − | 1140 |
| 3744 | 378 | 267,829 | + | + | 0 |
| 3748 | 418 | NA | − | + | 8940 |
| 3761 | 734 | 210,987 | − | + | 0 |
| 3765 | 528 | NA | − | + | 0 |
| 3773 | NA | 29,500 | + | + | 0 |
| 3775 | 381 | NA | + | + | 0 |
| 3778 | 274 | 647 | − | + | 31,770 |
| 3790 | 302 | 595,833 | − | − | 2580 |
| 3797 | 184 | 50,667 | − | + | 0 |
| 3798 | 61 | 50,644 | − | − | 0 |

**NOTE.** In donors for whom HIV-1 DNA was detected in monocytes, it was significantly more likely to be detected in CD16$^{+}$ than in CD14$^{hi}$/CD16$^{-}$ monocytes ($P = .02$, Fisher's exact test). +, present; −, absent; NA, data not available.

TATAGCCCTA-3′). Primers showed no base-pair mismatches with the consensus sequence of HIV-1 subtype C, the predominant subtype in Malawi.

Analyses were performed using GraphPad Prism software (version 4.0; GraphPad Software). Multiple groups were analyzed using 1-way analysis of variance (ANOVA) and pairwise comparisons using the Mann-Whitney *U* test, with Bonferroni's correction to adjust significance levels for multiple comparisons where appropriate. The proportion of samples of CD14$^{hi}$/CD16$^{-}$ and CD16$^{+}$ monocytes from which HIV-1 DNA could be amplified was compared using Fisher's exact test.

***Results.*** Participating women ranged in age from 17 to 36 years (mean, 22.2 years) and had gravidity between 1 and 8 (mean, 2.5). Four groups were considered: HIV-1/malaria negative (H$^{-}$/M$^{-}$; $n = 25$), HIV-1 negative/malaria positive (H$^{-}$/M$^{+}$; $n = 21$), HIV-1 positive/malaria negative (H$^{+}$/M$^{-}$; $n = 24$), and HIV-1/malaria positive (H$^{+}$/M$^{+}$; $n = 8$). Women in the H$^{-}$/M$^{+}$ group were significantly younger and of lower gravidity ($P < .05$ for each comparison) than women in the other 3 groups. Mean axillary temperature did not differ significantly between the 4 groups. Parasitemia did not differ significantly between the H$^{-}$/M$^{+}$ (median, 6090 malaria parasites/$\mu$L; interquartile range [IQR], 78,840 malaria parasites/$\mu$L) and H$^{+}$/M$^{+}$ (median, 6225 malaria parasites/$\mu$L; IQR, 18,225 malaria parasites/$\mu$L) groups ($P = .73$). Neither CD4$^{+}$ cell counts (median, 343 [IQR, 262] cells/$\mu$L vs. median, 299 [IQR, 308] cells/$\mu$L) ($P = .38$) nor viral load (median, 40,707 [IQR, 166,938] copies/mL vs. median, 50,666 [IQR, 117,151] copies/mL) ($P = .85$) differed significantly between H$^{+}$/M$^{+}$ and H$^{+}$/M$^{-}$ women; however, this small substudy was inadequately powered to show significant differences for these parameters.

Malaria was associated with a higher proportion of CD16$^{+}$ monocytes in peripheral blood. Analysis of the 4 groups by 1-way ANOVA showed a significant difference in median values ($P = .0012$, Kruskal-Wallis test). When pairwise comparisons were used, the proportion of CD16$^{+}$ monocytes was significantly higher in the H$^{+}$/M$^{+}$ group (median, 46.64%; IQR, 19.14%) than in the H$^{+}$/M$^{-}$ group (median, 22.56%; IQR, 12.99%) ($P = .0006$), but there was only a trend toward an expansion in the H$^{-}$/M$^{+}$ group (median, 33.08%; IQR, 15.97%), compared with the H$^{-}$/M$^{-}$ group (median, 27.39%; IQR, 27.54%) ($P = .158$). The proportion of CD16$^{+}$ monocytes was not significantly altered in the H$^{+}$/M$^{-}$ group, compared with the H$^{-}$/M$^{-}$ group ($P = .171$).

Of the women who provided blood postpartum ($n = 19$), none had malaria and 10 were HIV-1 infected. There was no difference in the proportion of CD16$^{+}$ monocytes (1) between HIV-1–infected (median, 23.96%; IQR, 11.40%) and HIV-1–uninfected (median, 20.23%; IQR, 10.02%) ($P = .24$) postpartum women or (2) in these women compared to the corresponding cohorts examined during pregnancy (H$^{-}$/M$^{-}$, $P = .20$; H$^{+}$/M$^{-}$, $P = .58$).

The proportion of CD16$^{+}$ monocytes in placental and cord blood did not differ significantly between women with and without peripheral malaria parasitemia or HIV-1 infection but was significantly lower in umbilical cord blood (median, 11.51%; IQR, 11.44%) than in maternal peripheral blood (median, 28.63%; IQR, 23.43%) or placental blood (median, 21.22%; IQR, 23.03%) ($P < .0001$ and $P = .0002$, respectively).

CD16$^{+}$ monocytes may be further subdivided into CD14$^{hi}$ and CD14$^{lo}$ cells. In peripheral blood, expansion of the CD16$^{+}$ monocyte subset was specifically due to CD14$^{lo}$/CD16$^{+}$ monocytes. The proportion of CD14$^{hi}$/CD16$^{+}$ monocytes within the 4 defined groups of women was not significantly different as analyzed by 1-way ANOVA, whereas, by contrast, the proportion of CD14$^{lo}$/CD16$^{+}$ monocytes was ($P = .62$ and $P = .0062$, respectively, Kruskal-Wallis test). There was a significantly higher proportion of CD14$^{lo}$/CD16$^{+}$ monocytes in the H$^{+}$/M$^{+}$ group (median, 19.66%; IQR, 18.94%) than in the H$^{-}$/M$^{-}$ group (median, 6.22%; IQR, 11.76%) and the H$^{+}$/M$^{-}$ group (median, 8.72%; IQR, 8.42%) ($P = .002$ and $P = .0009$, re-

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spectively). Similarly, there was a trend toward elevated $CD14^{lo}$/$CD16^{+}$ monocytes in the $H^{-}/M^{+}$ group (median, 11.72%; IQR, 17.76%), compared with the $H^{-}/M^{-}$ group ($P = .11$).

$CD16^{+}$ monocytes expressed significantly higher levels of CCR5 than did $CD14^{hi}/CD16^{-}$ monocytes in the cohort of 19 nonpregnant (postpartum) women (median MFI, 3.22 and 0.33, respectively; $P = .0004$), which confirmed the results of previous studies [5]. In the context of pregnancy, $CD16^{+}$ monocytes also expressed significantly higher levels of CCR5 than did $CD14^{hi}/CD16^{-}$ monocytes in maternal peripheral blood (median MFI, 4.39 and 0.00, respectively; $P < .0001$ [$n = 74$]), placental blood (median MFI, 2.77 and 0.00, respectively; $P < .0001$ [$n = 41$]), and cord blood (median MFI, 2.30 and 0.05, respectively; $P < .0001$ [$n = 42$]). Further analysis of maternal peripheral blood samples showed that CCR5 expression was significantly higher on $CD14^{hi}/CD16^{+}$ monocytes (median MFI, 5.35; IQR, 7.16) than on $CD14^{lo}/CD16^{+}$ monocytes (median, 2.51; IQR, 5.75) ($P = .0039$).

HIV-1 DNA was analyzed in $CD14^{hi}/CD16^{-}$ and $CD16^{+}$ monocytes isolated from peripheral blood of HIV-infected pregnant Malawian women ($n = 20$) and was detected by either single-round or nested PCR in 16 of the samples (figure 1). Of these, HIV-1 DNA was exclusively found in $CD16^{+}$ monocytes from 9 of 16 donors, exclusively in $CD14^{hi}/CD16^{-}$ monocytes from 2 of 16 donors, and in both subsets from 5 of 16 donors. $CD16^{+}$ monocytes were more likely to harbor HIV-1 infection than were $CD14^{hi}/CD16^{-}$ monocytes ($P = .02$). There was no correlation between HIV-1 detection and $CD4^{+}$ cell count or plasma HIV-1 load (table 1). Although numbers analyzed were low, we observed that patients in whom HIV-1 DNA was found exclusively in the $CD16^{+}$ monocytes had higher peripheral blood parasitemia (mean $\pm$ SE, $6360 \pm 3450$ parasites/$\mu$L; $n = 9$) than patients in whom HIV-1 DNA was found in both monocyte subsets (none of whom had detectable parasitemia; $n = 5$) or exclusively in the $CD16^{-}$ compartment (mean, 945 parasites/$\mu$L; range, 195; $n = 2$).

***Discussion.*** $CD16^{+}$ monocytes make up ~10% of circulating monocytes in healthy nonpregnant adults in developed countries [10], and this proportion increases during pregnancy [11]. We found higher proportions in the peripheral blood of apparently healthy pregnant and postpartum Malawian women (median, 27.4% and 20.2%, respectively), which suggests that this monocyte subset is expanded irrespective of pregnancy status, compared with that in individuals in developed countries. This may be attributable to the greater prevalence of infectious diseases in Malawi and is consistent with elevated CD16 expression on monocytes in the context of acute infections during pregnancy [11]. A major finding of our study is the expansion of $CD16^{+}$ monocytes in pregnant women with peripheral malaria parasitemia, which was highest in women coinfected with HIV-1 and was due specifically to the expansion of $CD14^{lo}/CD16^{+}$ monocytes. $CD16^{+}$ monocytes in malaria-infected pregnant women may contribute to elevated plasma levels of tumor necrosis factor–$\alpha$ [12] and the consequent pathogenic sequelae observed in gestational malaria. We did not observe significantly higher proportions of $CD16^{+}$ monocytes in pregnant HIV-1–infected, compared with HIV-1–negative, Malawian women, possibly because of their elevated baseline levels of $CD16^{+}$ monocytes.

$CD16^{+}$ monocytes expressed higher levels of CCR5, a coreceptor for HIV-1 entry and fusion, than did $CD14^{hi}/CD16^{-}$ monocytes in peripheral, placental, and cord blood. This monocyte subset is more mature than the $CD14^{hi}/CD16^{-}$ subset; with the additional observation of the higher expression of CCR5, these features may explain our finding that $CD16^{+}$ monocytes were preferentially infected with HIV-1. We have previously shown that monocytes contain multiply spliced HIV-1 RNA and that, therefore, the HIV-1 genome is replication competent within these cells [13]. Infiltration of monocytes into intervillous spaces of the placenta is common in malaria-infected women and correlates with low birth weight and maternal anemia [14, 15]. We hypothesize that CCR5-expressing $CD16^{+}$ monocytes are responsible for this infiltration. Trafficking of HIV-1–infected $CD16^{+}$ monocytes into the placenta may potentially play a role in intrauterine mother-to-child transmission.

## Acknowledgments

We thank Dr. S. White and Jenny Lewis for generous statistical advice, and especially the patients who participated in the study.

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