CONCISE COMMUNICATION

Dichotomous Effects of *Plasmodium falciparum* Antigens on Expression of Human Immunodeficiency Virus (HIV) Coreceptors and on Infectability of CD4 Cells by HIV

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Many microbial coinfections accelerate the progression of human immunodeficiency virus (HIV) disease. Coinfections of *Plasmodium falciparum* malaria and HIV-1 are common; however, past studies of the effects of *P. falciparum* malaria on HIV-1 infection have shown little effect. The present study found that *P. falciparum* antigens (PF-Ags) variably regulate the expression of HIV-1 coreceptors and modulate the infectability of CD4 cells by HIV-1. Shortly after PF-Ag stimulation, CCR5 expression was down-regulated, but CXCR4 expression was modestly up-regulated. Subsequently, CCR5 expression on CD4 cells was induced. Infectability of PF-Ag–stimulated peripheral blood mononuclear cells (PBMC) by R5 HIV-1 was decreased, regardless of the duration of PF-Ag stimulation or CCR5 expression levels. In contrast, X4 HIV-1 replication was enhanced briefly in PBMC stimulated with PF-Ags but was inhibited with longer stimulation. Decreased HIV-1 infectability resulted, in part, from endogenous production of interferon-γ. These results may explain why malaria previously did not appear to accelerate HIV-1 disease progression.

Microbial coinfections have been associated with increased efficiency of transmission of human immunodeficiency virus (HIV) type 1 or accelerated HIV disease progression (reviewed in [1]). These pathogens influence HIV-1 replication directly or through immune activation [1]. We previously demonstrated that several microbial pathogens, including human T lymphotropic virus type I [2], herpes simplex virus [3], and bacteria [4], variably modulate HIV-1 replication, depending on virus strain or infected cell type. Of note, although such microbial stimulation usually favors infection with X4 HIV-1, which emerges in the advanced stage of HIV-1 disease and is associated with poor prognosis, it has a dichotomous effect on infection with R5 HIV-1, which is transmitted almost exclusively during primary infection. Thus, the roles of microbial pathogens in the pathogenesis of HIV disease do not appear to be straightforward and warrant further investigation.

HIV-1 infection is endemic in many African and Asian countries where *Plasmodium falciparum* malaria is also prevalent. Protection against malaria requires cell-mediated and humoral immunity, which has waned in HIV-infected persons. Furthermore, *P. falciparum* malaria itself causes both immunosuppression (i.e., lymphopenia) and perturbation of cytokine networks (i.e., induction of proinflammatory cytokine production), both of which may influence the pathogenesis of HIV disease (reviewed in [5]). Therefore, how these 2 pathogens interact with each other is of serious concern.

One in vitro study suggested that *P. falciparum* antigens (PF-Ags) induce HIV-1 replication [6]; however, a number of epidemiologic and clinical studies have found no role for malaria in accelerating the rate of HIV-1 disease progression [7–9]. These studies indicate that *P. falciparum* infection may have multiple effects on HIV infection, offering both protection against and facilitation for HIV disease progression. In the present study, we investigated in vitro effects of PF-Ags on HIV-1 infection of peripheral blood mononuclear cells (PBMC), as well as expression of CCR5 and CXCR4, which are major coreceptors for HIV-1 entry.

**Materials and Methods**

**PF-Ags, reagents, cells, and viruses.** *P. falciparum* schizont-enriched antigens were prepared from human group O erythrocytes, as described elsewhere [10]. In brief, synchronized *P. falciparum*
cultures in erythrocytes, which reached ∼8% parasitemia and consisted of ∼30% mature schizonts and 70% ring forms, were harvested (data not shown). The schizont-infected erythrocytes were subjected to 3 freeze-thaw cycles, were inactivated by incubation at 56°C for 60 min, and were clarified by centrifugation. The supernatant was used as PF-Ags for subsequent experiments. Uninfected human group O erythrocytes were used for the preparation of control antigen (C-Ag). Recombinant interferon (IFN)–γ and anti–IFN-γ were purchased from R&D Systems.

PBMC and monocyte-derived macrophages were isolated from healthy volunteers, as described elsewhere [2–4], and were either not stimulated or stimulated with PF-Ags (at 1:2000 dilution) or C-Ag (at 1:2000 dilution) for the indicated periods. NL4-3 and NL4-3/ADA molecular clone stocks were propagated, as described elsewhere [2–4].

Infection assays and single-round viral replication assays. We suspended ∼2 × 10⁶ cells in the original culture supernatant at a 1:1 ratio and infected them with NL4-3 (X4) or NL4-3/ADA (R5) at an MOI of 0.01–0.05. After 3 h of incubation, the infected cells were washed 3 times and were resuspended in fresh media mixed with the original culture supernatant at a 1:1 ratio. About half of the culture supernatants were collected every 3 or 4 days and were replaced with fresh complete medium. HIV-1 titers were determined by reverse-transcriptase assay, as described elsewhere [2–4]. Single-round viral replication assays were done by using replication-incompetent luciferase reporter molecular clone NL4-3-luc-R E supplemented with Env glycoprotein from R5 HIV-1 ADA or X4 HIV-1 HXB2, as described elsewhere [2–4].

Flow cytometric analysis and ELISAs. Flow cytometry (FACScan; Becton Dickinson Immunocytometry) was done by using the following antibodies: fluorescein isothiocyanate–conjugated anti-CD4, phycoerythrin (PE)–conjugated anti-CCR5 (2D7), and PE-conjugated anti-CXCR4 (12G5; PharMingen). Cytokine concentrations were measured with commercial ELISA kits (R&D Systems).

Results

Effects of PF-Ags on expression of CCR5 and CXCR4 on CD4 cells. To investigate the effects of PF-Ag stimulation on HIV-1 coreceptor expression, PBMC that had been treated with PF-Ags for ≤5 days were stained with CD4 and CCR5 or with CXCR4 and then were analyzed by use of flow cytometry. CCR5 expression on CD4 cells was modestly down-regulated after 12 h of stimulation with PF-Ags (figure 1A); however, prolonged PF-Ag stimulation induced CCR5 expression (figure 1B). Induction of CCR5 expression usually required ≥4 days (data not shown). In contrast, PF-Ag stimulation had minimal effect on CXCR4 expression after an initial up-regulation of expression (figure 1A and 1B).

Effects of PF-Ags on infectability of CD4 cells by R5 or X4 HIV-1. Because HIV-1 coreceptor expression levels correlate well with HIV-1 cell infectability, we next investigated the HIV-1 infectability of PF-Ag–stimulated cells. In general, PBMC that had been treated with PF-Ags for 12 h were resistant to R5 HIV-1 infection but supported X4 HIV-1 replication (figure 2A), which suggests that the levels of expression of CCR5 or CXCR4 influenced infectability by virus by using the respective coreceptor. However, replication of R5 or X4 HIV-1 was limited in PBMC that had been treated with PF-Ags for 5 days, compared with that in untreated PBMC (figure 2B), although the former expressed more CCR5 and similar levels of CXCR4 to the latter (figure 1B). Reduced infectability was confirmed by single-round viral replication assays that reflected replication efficiency from viral entry to viral transcription (data not shown).

Role of IFN-γ in infectability of PF-Ag–stimulated PBMC by HIV-1. Then we investigated what factor(s) influenced HIV-1 coreceptor expression and the reduced infectability of cells after
Figure 2. Effects of Plasmodium falciparum antigens (PF-Ags) on human immunodeficiency virus (HIV)-1 replication. Peripheral blood mononuclear cells (PBMC) were stimulated with control antigen (C-Ag) or PF-Ags for 12 h (A) or 5 days (B) and were infected with HIV-1 NL4-3/ADA (R5) or HIV-1 NL4-3 (X4). Results are representative of 5 experiments. C, PBMC treated with C-Ag or PF-Ags in the presence or absence of anti-interferon (IFN)-γ (0.1 μg/mL) for 5 days were infected with HIV-1 NL4-3/ADA (R5). Two other experiments were repeated with similar results. D, PBMC not treated or treated with recombinant human IFN-γ (10 ng/mL) for 5 days were infected with HIV-1 NL4-3/ADA (R5). Two more experiments had similar results.

prolonged stimulation with PF-Ags. Previous studies showed that IFN-γ increases expression of CCR5 [11, 12] but also suppresses HIV-1 replication [13]. Since P. falciparum infection induces IFN-γ production [14], we hypothesized that IFN-γ plays a critical role in HIV-1 coreceptor expression and infectability by HIV-1 of PF-Ag–stimulated cells.

To prove our hypothesis, we stimulated PBMC with PF-Ags in the presence or absence of anti–IFN-γ antibody. Neutralization of IFN-γ markedly reduced PF-Ag effects on CCR5 expression and infectability (figure 1C, figure 2C). Furthermore, stimulation with IFN-γ, instead of PF-Ags, similarly enhanced CCR5 expression and rendered cells resistant to HIV-1 infection (figure 1D, figure 2D), which supports our hypothesis.

Discussion

Our results demonstrate dichotomous effects of in vitro P. falciparum infection on HIV-1 replication. Similar to bacterial cell wall components (e.g., lipopolysaccharide, lipoteichoic acid, and lipoarabinomannan) [2], PF-Ag stimulation by itself appears to modestly down-regulate CCR5 expression and to up-regulate CXCR4 expression. However, it also induces production of IFN-γ, which contrarily up-regulates CCR5 expression and inhibits HIV-1 replication. Under such circumstance, infectability of PF-Ag–stimulated PBMC by R5 HIV-1 or X4 HIV-1 was generally reduced, depending on the balance of opposing effects.

Previous studies show that a number of microbial infections enhance HIV-1 replication or accelerate progression of HIV-1 disease (reviewed in [1]—with exceptions that include malaria [7–9]). The present study suggests that P. falciparum infection does not necessarily act as a cofactor for HIV-1 disease progression, because it lacks the ability to induce IFN-γ production. On the basis of our previous study results [2] and those of the present study, it is likely that PF-Ags and bacterial cell wall components have similar capabilities of regulating HIV-1
coreceptor expression and of inducing IFN-γ production. However, although prolonged (months to years) and extensive (≥10⁶ parasites/mL) malaria parasitemia, which is probably critical for producing IFN-γ, is common [15], bacteremia is not as lengthy and heavy in most bacterial infections. *P. falciparum* is also much more complex than bacteria, having many more genes and antigens expressed. This may account for the variable responses seen clinically, since the sum total of the immune response in a given patient may vary by differential responses to various plasmodial antigens. Further delineation of the interaction between HIV-1 and malaria will be important for a better understanding of the pathogenesis of HIV-1 disease and for better treatment of coinfected patients.

Acknowledgments

We thank S. Chiyoda, H. Okuda, and K. Deguchi (Nagasaki Red Cross Blood Center) for blood samples and M. Yokoyama for excellent technical assistance.

References