Relationship between Levels of Inflammatory Cytokines in the Genital Tract and CD4$^+$ Cell Counts in Women with Acute HIV-1 Infection

Lisa M. Bebell,[1,2] Jo-Ann Passmore,[3] Carolyn Williamson,[1,3] Koleka Mlisana,[1] Itua Iriogbe,[1] Francois van Loggerenberg,[1] Quarraisha Abdool Karim,[1,2] and Salim Abdool Karim[1,2]

[1]Centre for the AIDS Programme of Research in South Africa, University of KwaZulu Natal, Durban, and [3]Institute of Infectious Diseases and Molecular Medicine, Division of Medical Virology, University of Cape Town, Cape Town, South Africa; and [2]Columbia University, New York, New York

Inflammatory responses at mucosal surfaces after human immunodeficiency virus type 1 (HIV-1) transmission may influence disease outcome. We evaluated levels of interleukin (IL)–1β, IL-6, tumor necrosis factor–α, IL-8, IL-10, and IL-12 in genital tract and plasma specimens from 44 women with acute HIV infection and 29 HIV-negative control women (13 of whom were women in the acute HIV infection cohort who had preinfection samples available for analysis). Women with acute HIV infection had significantly elevated levels of IL-6, IL-10, and IL-12 in genital tract specimens and elevated levels of IL-1β, IL-8, and IL-10 in plasma specimens, compared with HIV-negative control women. Levels of IL-1β, IL-6, and IL-8 in cervicovaginal specimens from women with acute HIV infection showed a significant inverse correlation with systemic CD4$^+$ cell counts, suggesting that mucosal inflammation is associated with low CD4$^+$ cell counts during acute HIV infection.

The majority of the world's human immunodeficiency virus type 1 (HIV-1) infections now occur in women. Immune responses in the female genital tract during the acute phase of HIV infection may be an important determinant of both early control of viral replication and long-term progression of disease [1]. Despite the fact that, during sexual transmission, a woman's initial exposure to HIV-1 occurs at mucosal surfaces in the genital tract, little is known about HIV-1–specific immune responses at this site early after infection [2]. Importantly, rapid and extensive depletion of mucosal and systemic CD4$^+$ T cells occurs during the first few weeks of infection [1]. Evidence from both HIV and simian immunodeficiency virus (SIV) infections suggests a more substantial impact of the virus on the mucosal immune system than its systemic counterpart [1]. Because equilibrium between viral replication, immune control, and CD4$^+$ T cell depletion is set early during HIV infection, information on the composition, timing, and duration of early mucosal cytokine responses, especially at the site of virus entry and initial replication, could substantially contribute to understanding the immunopathology of AIDS [3].

Recent studies involving nonhuman primates have shown that SIV establishes infection systemically before robust adaptive immune responses are detected in blood, indicating that early innate immune responses may therefore be important in preventing widespread dissemination of virus [4]. In many viral illnesses, a robust immune response is crucial for effective control of virus replication and spread. In HIV and SIV infections, however, certain responses may have very undesirable outcomes. In macaques, for example, the earliest cytokine responses during SIV infection favor immune activation, which facilitates rather than controls viral replication and spread by recruiting the virus' target cells to the sites of viral replication [3]. Multiple studies of both HIV and SIV infection show a correlation between increased degrees of T cell activation and increased rates of disease progression [5–7].

In this study, we report on levels of inflammatory cytokines in a cohort of woman both before HIV acquisition and during the acute phase of HIV infection. We measured levels of cytokines in genital tract secretions (obtained via cervicovaginal lavage [CVL]) and plasma samples at these 2 time points and compared them with levels in samples from a control group of HIV-uninfected women. The relationship between genital tract and plasma levels of inflammatory cytokines during acute infection and clinical markers of disease progression (i.e., systemic CD4$^+$ cell counts and virus loads) are examined.

Subjects and methods. Forty-four women with heterosexually acquired HIV-1 infection were recruited <3 months after infection from a longitudinal HIV-negative cohort into the CA-

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Reprints or correspondence: Dr. Jo-Ann Passmore, Institute of Infectious Diseases and Molecular Medicine, University of Cape Town Medical School, Anzio Rd., Observatory, 7925 Cape Town, South Africa (Jo-ann.Passmore@uct.ac.za).

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PRISA 002 Acute Infection Study in Durban, South Africa. All 44 women had tested negative for HIV antibody during their previous monthly visit. The estimated date of HIV infection was calculated as the midpoint between the last negative result and the first positive result of HIV antibody tests; or as 14 days where the women were HIV RNA positive and HIV antibody negative. CVL and plasma samples were obtained from all 44 women.

Two groups of control CVL and plasma specimens were created. The first group consisted of samples obtained before HIV infection from 13 of the 44 women from the acute HIV infection cohort. The second control group consisted of samples from 16 women in the longitudinal HIV-negative cohort who had not become infected during follow-up. These 16 women were matched to the 44 women with acute HIV infection on the basis of age, hormone contraception use, and prevalence of sexually transmitted infections (STIs).

Cervical swabs and blood specimens were tested for *Chlamydia trachomatis, Neisseria gonorrhea, Mycoplasma genitalium,* agents of bacterial vaginosis, herpes simplex virus type 2 (HSV-2), *Trichomonas vaginalis,* and *Treponema pallidum. C. trachomatis, N. gonorrhea,* and *M. genitalium* were assessed by polymerase chain reaction (PCR), agents of bacterial vaginosis by Gram stain, HSV-2 by a combination of IgG serological tests and PCR of vulvovaginal swab specimens, *T. vaginalis* by Diamond's culture and PCR, and *T. pallidum* by a combination of the ImmuntrepTPHA test, the Macro-Vue RPR Card test, and PCR.

Gynecologic examination was performed with collection of CVL specimens on enrollment. The cervix was bathed with sterile saline (10 mL), which was allowed to pool in the posterior fornix, where it was then aspirated into a plastic bulb pipette. Fluid was dispensed into a sterile container and centrifuged, and the supernatant was stored at −80°C. Collection of the CVL sample was postponed until the following week if the participant was menstruating. None of the CVL samples had visible blood contamination. Blood collection was performed by venipuncture into acetate citrate dextran vacutainer tubes. Plasma was stored at −80°C.

We investigated the effect of acute HIV infection on the inflammatory cytokine milieu at the genital mucosa and compared this to systemic inflammation at the same time point. The human inflammation cytometric bead array system (BD Biosciences) was used to measure the concentrations of interleukin (IL)–1β, IL-6, tumor necrosis factor (TNF)–α, IL-8, IL-10, and IL-12 p70 in CVL and plasma specimens. The upper limit of detection for the assay was 5000 pg/mL for all cytokines. The lower limits of detection were 3.6 pg/mL for IL-8, 7.2 pg/mL for IL-1β, 2.5 pg/mL for IL-6, 3.3 pg/mL for IL-10, 3.7 pg/mL for TNF-α, and 1.9 pg/mL for IL-12. Samples with cytokine levels that were less than the assay's lower limit of detection were assigned values that were halfway between the lower limit of detection and zero.

The Mann-Whitney *U* test was used for independent sample comparisons, and the Spearman rank correlation test was used to determine covariance between variables. *P* values of $<.05$ were considered statistically significant.

This study was approved by ethics committees at the University of KwaZulu-Natal and the University of Cape Town, and all participants provided informed consent.

Results. A total of 44 women who had recently become infected with HIV-1 by heterosexual contact were included in this study. HIV infection for all women was in the acute phase, with samples collected a median of 44 days (range, 15–89 days) after infection onset. At this time, the participants had a mean $CD4^+$ cell count of 547 cells/μL (range, 201–1124 cells/μL) and a mean virus load of 319,131 copies/mL (range, 547–5,510,000 copies/mL). Two women were pregnant at the time of infection. We found no significant differences between inflammatory cytokine profiles in their CVL and plasma specimens and profiles in specimens from the 42 HIV-positive women who were not pregnant (data not shown).

Levels of IL-1β, IL-6, TNF-α, IL-8, IL-10, and IL-12 p70 in CVL and plasma samples obtained from women with acute HIV infection were compared with levels in samples from HIV-negative control specimens (figure 1). Thirteen of the control specimens were from women in the acute HIV infection cohort for whom preinfection samples were available, whereas 16 of the HIV-negative control samples were from HIV-negative women from the longitudinal HIV-negative cohort. Because inflammatory cytokine levels in the 13 specimens obtained before HIV acquisition did not differ from levels in specimens from the 16 women in the HIV-negative cohort (data not shown), data from all HIV-negative control samples are grouped in all analyses. Concentrations of IL-6 ($P = .04$), IL-10 ($P = .004$), and IL-12 p70 ($P = .04$) in CVL samples from women with acute HIV infection were significantly greater than those in HIV-negative CVL samples. Although inflammatory cytokine levels in plasma specimens were significantly lower than those in CVL specimens ($P < .001$), we observed a significant increase in levels of several inflammatory cytokines in plasma from women with acute HIV infection, compared with levels in HIV-negative plasma samples ($P = .004$ for IL-1β, $P < .0001$ for IL-8, and $P = .0002$ for IL-10).

To investigate the impact of concurrent STI and bacterial vaginosis on cytokine responses during acute HIV infection, we compared inflammatory cytokine levels in women without concomitant STI or bacterial vaginosis with levels in women with concomitant bacterial vaginosis or $\geq$1 STI. Twenty-one (47.7%) of 44 women with acute HIV infection were coinfected with $\geq$1 STI, whereas 34 (77.3%) of 44 had bacterial vaginosis during the acute phase of HIV infection. In the genital tract, levels of IL-12, IL-8, TNF-α, IL-10, and IL-1β did not differ significantly between women with no STIs and those with $\geq$1 STI. Levels of IL-6 in CVL specimens, however, were significantly elevated in

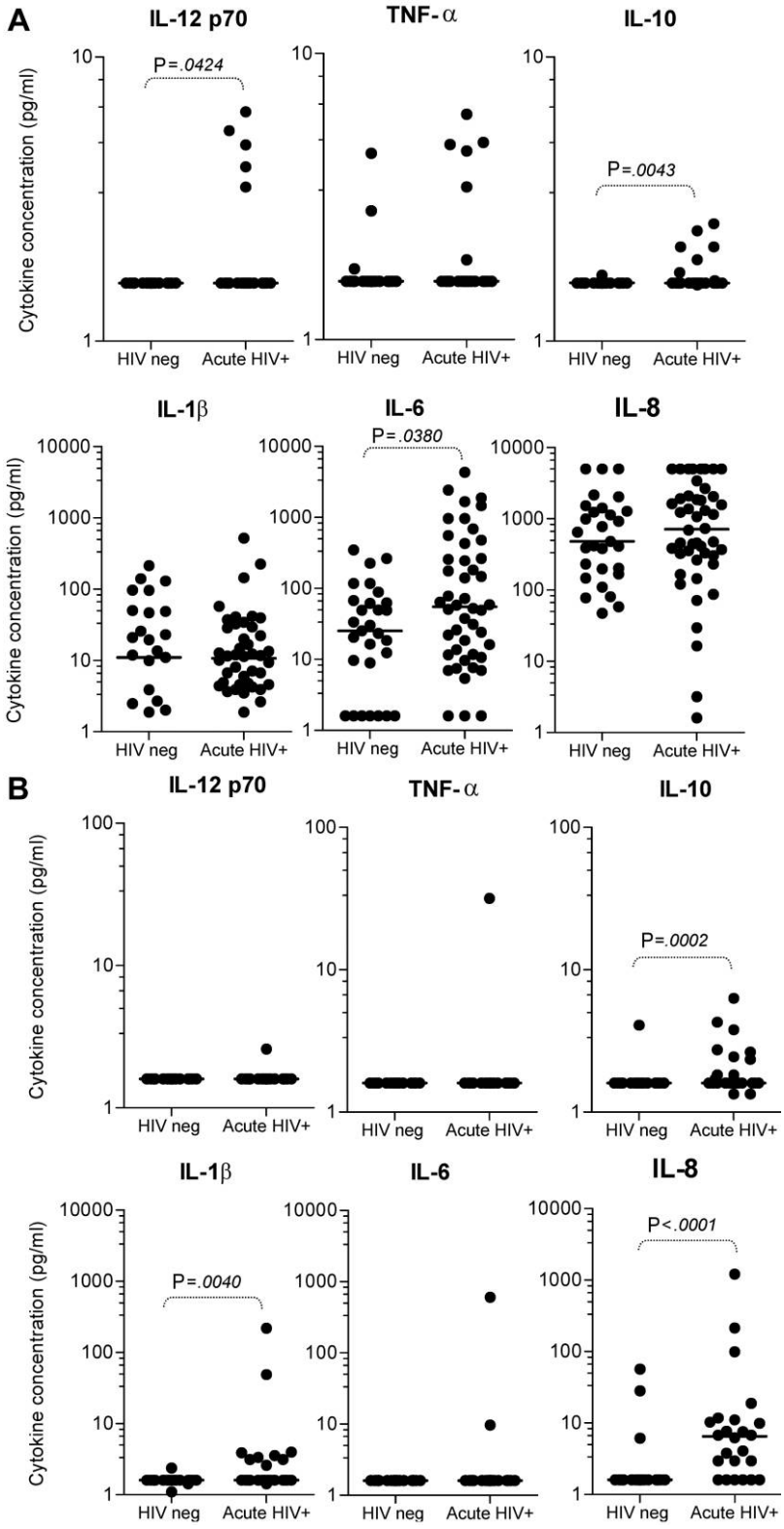

Figure 1. Comparison of inflammatory cytokine concentrations in cervicovaginal lavage *(A)* and plasma *(B)* samples from 44 women with acute HIV infection (acute HIV$^+$) and 29 women without HIV infection (HIV neg; see "Subjects and methods" for a description of the HIV-negative group). Values below the lower limit of detection of the assay are reported as the midpoint between lower limit of detection and zero. Differences between groups were calculated using Mann-Whitney *U* test. *P* values of $<.05$ were considered significant. *Dots,* cytokine levels for each study subject; *horizontal lines,* median values for each group.

women coinfected with ⩾1 STI, compared with women with HIV infection only ($P = .006$). In plasma, the presence of ⩾1 concomitant STI had no effect on inflammatory cytokine levels during acute HIV infection (data not shown). In women with concomitant bacterial vaginosis, we similarly found that IL-12, IL-8, TNF-α, IL-10, and IL-1β levels in CVL and plasma samples did not change significantly (data not shown). IL-6 levels in CVL specimens from women with acute HIV infection and bacterial vaginosis, however, were 7-fold higher than those for women with acute HIV infection and no bacterial vaginosis or STI ($P = .02$). Because bacterial vaginosis, ⩾1 concomitant STI, and acute HIV-1 infection all resulted in significantly elevated IL-6 levels in CVL (figure 1), the contribution of each agent in driving production of this cytokine is not clear.

Finally, we evaluated the impact of inflammatory cytokine production and clinical indicators of HIV disease progression (i.e., virus load and systemic $CD4^+$ cell count during acute HIV infection). We observed no association between levels of inflammatory cytokines in either plasma specimens (data not shown) or CVL specimens (figure 2) and viremia in women during acute HIV-1 infection. However, when genital tract inflammation and $CD4^+$ cell counts were compared, we observed a significant inverse correlation between systemic $CD4^+$ cell counts and IL-1β levels (r = -0.35; $P = .02$), IL-6 levels (r = -0.32; $P = .05$), and IL-8 levels (r = -0.32; $P = .04$), indicating that enhanced inflammation at the cervix is associated with more-severe $CD4^+$ T cell depletion during these early time points (figure 2).

Discussion. Cytokine profiles and mucosal inflammation in the genital tract during acute HIV infection are associated with lower $CD4^+$ cell counts and may thereby impact disease progression. We found that elevated levels of IL-1 β, IL-6, and IL-8 in the genital tract correlated with lower systemic $CD4^+$ cell counts during acute HIV infection. It would be interesting to evaluate whether genital inflammation was similarly associated with the extent of $CD4^+$ cell destruction in the mucosa; however, mucosal $CD4^+$ cell depletion was not measured in this study.

We found that levels of IL-10, IL-6, and IL-12 inflammatory cytokines in the genital compartments of women with acute HIV infection were significantly higher than those for HIV-negative women. Although IL-10 levels were also significantly increased in plasma samples from HIV-positive women, so too were IL-8 and IL-1β levels.

Recent research has shown that, during acute infection, HIV pathogenesis is focused in the gut and gastrointestinal-associated lymphoid tissue (GALT), where $CD4^+$ T cells become massively depleted [8–10] and inflammatory cytokines and chemokines are upregulated [11]. Studies of virus-host interactions in the gut have demonstrated that immune activation is emerging as a potentially important factor in the destruction of host $CD4^+$ T cells in the GALT. We show here that local genital tract inflammation is associated with systemic $CD4^+$ T cell depletion.

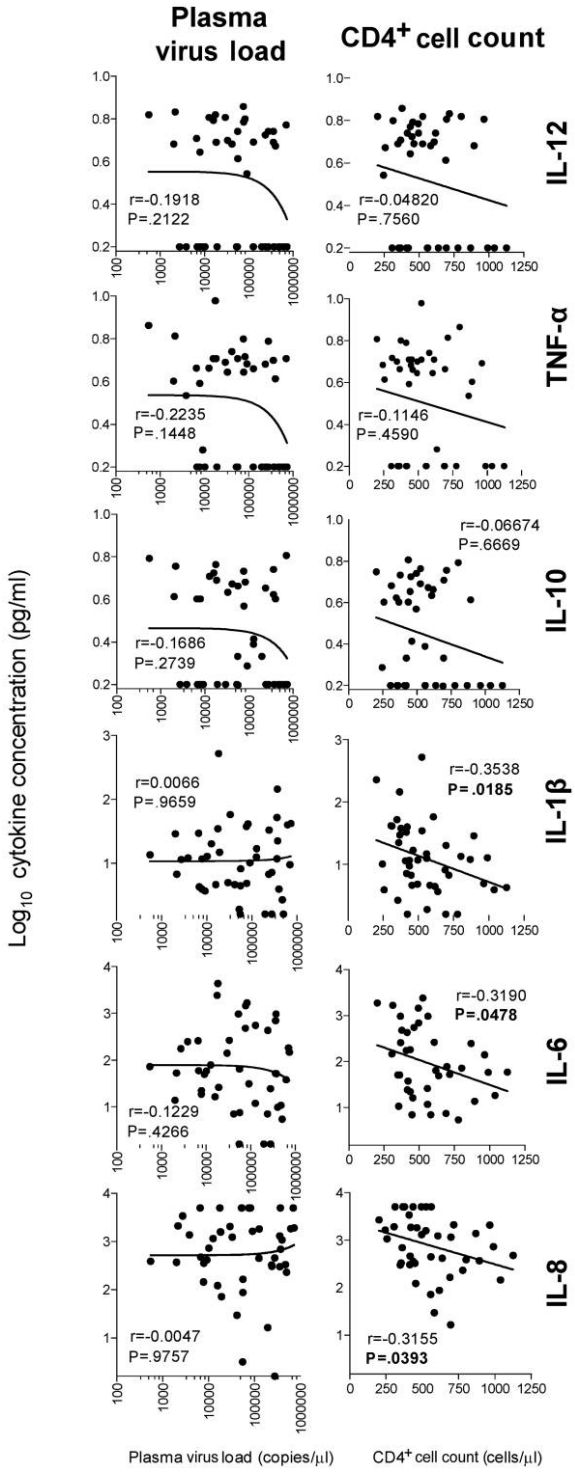

Figure 2. Comparison of inflammatory cytokine concentrations, plasma virus load, and systemic $CD4^+$ cell counts in cervicovaginal lavage samples from 44 women with acute HIV infection. *Dots,* individuals' virus loads and $CD4^+$ cell counts, by cytokine level. Values below the lower limit of detection of the assay are reported as the midpoint between lower limit of detection and zero. Correlations were calculated using the Spearman rank correlation test. *P* values of $<.05$ were considered significant. *Dots,* virus loads and $CD4^+$ cell counts, by cytokine level, for each study subject; *solid line,* linear regression for each comparison and the r and *P* values.

We anticipate that inflammation of the female genital tract may similarly undermine effective control of HIV after transmission.

Several studies have shown that bacterial vaginosis and certain STIs increase secretion of cervical inflammatory mediators both in the absence and the presence of HIV infection [12, 13]. Furthermore, STIs increase susceptibility to HIV infection and HIV shedding in HIV-infected individuals [12–14]. Our data show that acute HIV infection is associated with elevated cytokine levels regardless of the concomitant presence of STIs. The presence of bacterial vaginosis or STIs at the first visit following HIV acquisition had little or no impact in the genital tract on all cytokines assessed, apart from IL-6.

We have shown here that increased genital inflammation is an important feature of acute HIV infection and that this local inflammation is significantly associated with lower systemic $CD4^+$ cell counts. It would be of interest to determine whether these genital and systemic cytokine profiles during acute HIV infection influence local proliferation of HIV in the genital tract and, in turn, systemic viral replication and disease progression.

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