Acute Sexually Transmitted Infections Increase Human Immunodeficiency Virus Type 1 Plasma Viremia, Increase Plasma Type 2 Cytokines, and Decrease CD4 Cell Counts

Aggrey O. Anzala,1,2 J. Neil Simonsen,1,2 Joshua Kimani,2 T. Blake Ball,3 Nico J. D. Nagelkerke,1,2 John Rutherford,1 Elizabeth N. Ngugi,1 Job J. Bwayo,2 and Francis A. Plummer1,2

In Kenya, the median incubation time to AIDS in seroconverting sex workers is 4 years; this incubation time is specific to female sex workers. We studied the influence of acute sexually transmitted infections (STIs) on several immunologic parameters in 32 human immunodeficiency virus type 1 (HIV-1)-positive and 10 HIV-1-negative women sex workers who were followed for 1–5 months. Plasma cytokines, soluble cytokine receptors, CD4 and CD8 T cell counts, and HIV-1 plasma viremia were quantitated before, during, and after episodes of STI. Increases in interleukin (IL)–4, IL-6, IL-10, soluble tumor necrosis factor (TNF)–α, and viremia and a decline in CD4+ T cell counts occurred during gonococcal cervicitis and returned to baseline after treatment. Increases in viremia correlated with increased IL-4 and decreased IL-6 concentrations. Similar changes were seen among women with acute pelvic inflammatory disease. Acute bacterial STI resulted in increased HIV-1 viremia. This may be mediated through increased inflammatory cytokines or through modulation of immune responses that control HIV-1 viremia.

One important feature of human immunodeficiency virus type 1 (HIV-1) infection is the variable duration from initial infection with HIV-1 to the development of AIDS (the incubation period) or clinical latency. Some individuals infected with HIV remain asymptomatic for years before developing severe immune dysfunction and AIDS, whereas others progress to AIDS very rapidly after HIV-1 infection [1—6]. The factors underlying the variability in rates of disease progression are unknown, but they may be related to the host’s immune response, differences in the pathogenicity of HIV-1 variants, or the operation of cofactors, such as infection [7, 8]. Study of the role of other infections in accelerating HIV-1 disease progression has proven difficult because HIV-1-related immune deficiency alters susceptibility to many infectious diseases, obscuring causal relationships.

We have previously observed that the median incubation time from HIV-1 infection to Centers for Disease Control and Prevention (CDC) stage 4 HIV-1 infection in women employed as sex workers in a lower socioeconomic area of Nairobi, Kenya, is 4 years [3]. Other studies among sex workers in developing countries have indicated similar incubation times [9]. This incubation time is markedly shorter than are incubation times reported from North America and Europe, which are 8–10 years [10] in the absence of antiretroviral therapy. There are several possible explanations for the more rapid progression to AIDS observed in Kenyan sex workers. Because studies from North America and Europe predominantly involved white men, it may be that the difference is related to genetic or environmental factors specific to Africans and/or factors specific to women (such as hormonal contraception and pregnancy). The women in the Kenya cohort are sex workers with an extremely high risk of all sexually transmitted infections (STIs); consequently, multiple HIV-1 infections or repeated infections with other STIs could accelerate HIV-1 disease progression.

In support of this possibility, more frequent condom use among Kenyan sex workers was correlated with a reduced risk of progression to CDC stage 4 disease [3], suggesting that events prevented by condoms are involved in more rapid disease progression. We also compared the rate of decline of CD4 T cells among women employed as sex workers and HIV-1—positive women from the general population in Nairobi by use of Markov models [11]. The first transition time to a CD4 count of <200 cells/mm³ for sex workers was consistent with the observed...
median incubation time to clinical disease, whereas the first transition time for women from the general Kenyan population was similar to that described in cohorts from industrialized countries [10].

Analysis of these data indicates that the rapid HIV-1 disease progression observed in Kenyan sex workers is specific for women. Further, they suggest that sex and environmental and genetic factors specific to Africans are of lesser importance, as are differences in the pathogenicity of HIV-1 variants prevalent in Kenya. It is now recognized that immunologic control of HIV-1 infection is one of the key determinants of the occurrence of disease. The appearance of cytotoxic T lymphocytes to HIV-1 correlates with initial control of viremia [12], and cytotoxic T lymphocytes and type 1 T helper cell responses to HIV-1 antigens correlate with long-term nonprogression to AIDS [13]. Because sex workers in the Pumwani cohort experience a rapid progression to AIDS, which is correlated with an enormous incidence of STIs [14] and is slowed by more frequent condom use, we conducted a study to determine whether acute STIs influence the immunologic and virologic parameters that are potentially related to accelerated disease progression.

Materials and Methods

Study subjects. Women enrolled in the Pumwani sex worker cohort, a cohort based in a lower socioeconomic district of Nairobi, Kenya, participated in this study. These women are part of a large open cohort involved in the study of the epidemiology and prevention of STIs, including HIV-1, which has been described elsewhere [15].

Study design. A subset of 32 HIV–1–positive and 10 HIV–1–negative women from the Pumwani sex-worker cohort were enrolled in this study. Women were seen prospectively over a period of 1–5 months during episodes of acute STI and ≥14 d after treatment of infections or clinical resolution of symptoms. At an early visit, women were examined for evidence of STIs and for specimens that would allow diagnosis of STIs, determination of plasma cytokines, and quantitation of plasma viremia; we also obtained CD4 and CD8 T cell counts. Women who had evidence of an STI at the initial visit were treated for the infection and were asked to return 14 d after treatment for further evaluation. Women who had no evidence of an STI were scheduled for follow-up 1 month after the initial visit and were to return if symptoms of an STI became manifest. At each clinic visit, a complete physical examination, including a pelvic examination, was performed, and specimens were collected for STI diagnosis, plasma cytokine determination, plasma HIV-1 quantitation, and CD4 and CD8 T cell determinations. Women who had evidence of systemic illness (herpes zoster, respiratory tract infection, fever, diarrhea, etc.) were excluded from the study. No women in the study were receiving antiretroviral treatment.

HIV testing. All women in the study were tested for the presence of HIV-1 antibodies by use of commercial enzyme immunoassays (to detect HIV, IAF Biochem and Enzygnost HIV-1/2 EIA; Boehringer, Montreal, Canada). All HIV–1–positive findings were confirmed by a third enzyme immunoassay, Recombigen HIV-1/2 EIA (Cambridge Biotech, Worcester, MA). Immunoblots were used to resolve any discrepancies between immunoassays.

Plasma cytokine assays. Plasma samples for cytokine quantification were obtained from the women at enrollment and at each subsequent follow-up visit. Blood samples were collected in plastic Vacutainer tubes with EDTA (Becton Dickinson Vacutainer Systems, Mississauga, Canada). Specimens were transported to the laboratory within 3 h of collection. Plasma was separated by centrifugation at 400 g for 10 min at 4°C. Contaminating platelets were removed from the plasma by centrifugation for 5 min at 10,000 g. The plasma was divided into aliquots in 500-μL vials, stored at −70°C, and thawed once before analysis. Tumor necrosis factor (TNF)-α, soluble TNF-α receptor type II (sTNF-α-RII), interferon (IFN)–γ, and interleukin (IL)–2, IL-4, IL-6, and IL-10 were measured in plasma by use of commercial enzyme immunoassays (Quintikine, R&D Systems, Minneapolis, MN). Duplicate determinations were performed for each plasma sample.

CD4 and CD8 lymphocyte counts. Blood samples for CD4 and CD8 lymphocyte counts were obtained at each clinic visit. Lymphocyte subsets were measured in whole blood by the FACScan flow cytometry system (Becton Dickinson, Mississauga, Canada).

Diagnosis of STIs. At each visit, specimens were obtained to test for Neisseria gonorrhoeae, Chlamydia trachomatis, and Treponema pallidum (in the presence of genital ulcerations). Specimens were processed as described elsewhere [16]. The diagnosis of acute pelvic inflammatory disease (PID) was clinical. Clinical diagnosis of PID has a high sensitivity and specificity in populations with a high prevalence of gonococcal and chlamydial cervicitis [17]. The diagnosis of genital herpes was made in the presence of the typical vesicles of genital herpes. No diagnostic testing for herpes simplex virus types 1 or 2 was performed. Syphilis serology was performed by a screening rapid plasma reagent test, and confirmation of positive screening results was performed by Treponema pallidum hemagglutination.

Quantitation of HIV-1 plasma viremia. HIV-1 RNA in human plasma was measured by a reverse-transcriptase (RT) polymerase chain reaction (PCR) method by use of coamplification of an internal synthetic RNA standard to quantitate unknown samples. The RNA standard was generated from a cloned 172-bp HIV-1 nef region sequence into which a 34-bp adaptor was inserted. The synthetic internal RNA standard and the wild-type RNA RT-PCR products differed in length by virtue of the adapter sequence inserted in the standard. The synthetic RNA standard was shown to coamplify with an efficiency equivalent to that of wild-type HIV-1. Known amounts of synthetic RNA were added to test samples containing unknown amounts of wild-type HIV-1 and were coamplified with radiolabeled primers. This procedure allowed quantitation of wild-type HIV-1 RNA over a range of 10⁴–10⁶ RNA copies [18].

RNA extraction was performed on 2 mL of human plasma with the Macro-Fast Track Total RNA Isolation Kit (Invitrogen, Carlsbad, CA). The RNA obtained was made into aliquots, and six 10-fold dilutions of the synthetic RNA standard (10⁴–10⁶) copies were added to the aliquot specimens containing unknown wild-type RNA. These dilutions were reverse transcribed and coamplified with radiolabeled primers. The PCR products were resolved on 3% agarose gel, and the resulting bands were analyzed. At equivalent levels of incorporation of radiolabeled primer, the ratio of standard
RNA to wild-type RNA was considered to be 1. The quantity of input wild-type RNA was then extrapolated, and the number of HIV-1 RNA molecules was derived. Plasma viremia was also quantitated on 10 unselected plasma specimens from study subjects with a commercial kit (Amplicor, Roche Molecular Diagnostics, Mississauga, Canada). As shown in figure 1, there was an excellent correlation between results with the nef RT-PCR and the results obtained with the commercial kit (correlation coefficient, .89; P < .009).

**Data analysis.** Differences in plasma HIV-1 RNA copy number, T cell subset counts, and plasma cytokine concentration, at times with a given STI and at times when no STIs were present, were compared by paired-sample t tests. Thus, each individual served as her own control. Mean values were used for individuals who had >1 visit with the STI in question or >1 visit with no STI. These analyses, with the exception of PID, excluded times when individuals had STIs other than the one in question. Pearson correlation was used for examining the relationship between HIV-1 RNA copy number, T cell subset counts, and plasma cytokine concentrations.

**Results**

**Characteristics of the study population.** Forty-two women followed in the Pumwani sex worker cohort, 32 of whom were HIV-1 positive and 10 of whom were HIV-1 negative, were enrolled in this study and followed prospectively for 1–5 months. All women were in general good health and had no evidence of systemic illness. Genital herpes was summarized as the characteristics of the 42 women studied, disaggregated by HIV-1 infection status.

**Plasma HIV-1 viremia and acute STIs.** HIV-1–infected women were studied by measuring plasma HIV-1 RNA copies at the time they presented with an acute episode of STI and at ≥14 d after STI treatment had been provided or after symptoms had resolved. HIV-1 viremia was quantitated by RT-PCR of HIV-1 RNA in plasma. The number of plasma HIV-1 RNA copies was quantitated at times when participants had no STIs and during episodes of gonococcal cervicitis, acute PID, and genital herpes. Although data were collected for H. ducreyi (3 episodes) and C. trachomatis infection (3 episodes) and syphilis, these are not presented because of sample size or, in the case of syphilis, because the timing of syphilis seroconversion was not precisely known. Each woman served as her own control, and 8 women who experienced no acute STIs during the follow-up period served as an additional comparison group. Twenty-six HIV-1–positive women had ≥1 gonococcal infections during the study. Twenty-three of them experienced an absolute increase in plasma viremia during episodes of acute gonococcal cervicitis. As shown in figure 2 and table 2, plasma HIV-1 RNA copy number was significantly greater during gonococcal infection. After treatment of gonorrhea, plasma viremia appeared to return to baseline, in that among women with acute gonococcal cervicitis, there were no statistically significant differences between plasma HIV-1 RNA copy number for the first and last visits at which no STIs were detected (data not shown).

PID is caused primarily by N. gonorrhoeae and C. trachomatis in this population. We examined the effect of PID on plasma HIV-1 viremia. Of the 14 HIV-1–positive women who experienced PID during the study, 12 showed an increase in plasma HIV-1 viremia at the time of presentation with acute PID (table 2). As shown in table 2, the plasma HIV-1 RNA copy number was significantly greater during episodes of acute PID. In a subset analysis of 3 women who had episodes of gonococcal infection without PID and episodes of gonococcal infection associated with PID, there were no differences in plasma HIV-1 viremia. Six HIV-1–positive women were clinically diagnosed with genital herpes recurrences. All 6 women

| Table 1. Characteristics of 42 women, members of the Pumwani sex worker cohort from Nairobi, Kenya. |
|-----------------|-----------------|-----------------|
| Characteristic  | HIV-1+ (mean ± SD) | HIV-1+ (mean ± SD) |
| Age, years      | 34.2 ± 6.3       | 31.9 ± 18.5     |
| Duration of prostitution, years | 9.3 ± 3.8       | 7.5 ± 4.9       |
| Years of HIV-1 infection (n = 32) | 5.0 ± 3.7       |                  |
| Mean CD4 T cell count | 1021 ± 230      | 332 ± 250       |
| Mean CD8 T cell count | 833 ± 225       | 1126 ± 579      |
| Episodes of gonococcal infection (n = 30) | 0.4 ± 0.52      | 1.03 ± 0.7      |
| Episodes of genital herpes (n = 7) | 0.1 ± 0.32      | 0.22 ± 0.5      |
| Episodes of Haeimophilus ducreyi infection (n = 3) | 0.14 ± 0.35    |                  |
| Episodes of acute PID (n = 19) | 0.5 ± 0.53      | 0.5 ± 0.62      |
| Episodes of Chlamydia trachomatis infection (n = 3) | 0.09 ± 0.3     |                  |
| Episodes of positive syphilis serology (n = 7) | 0.3 ± 0.48      | 0.13 ± 0.34     |
| Study visits    | 2.3 ± 0.67       | 3.0 ± 0.67      |

NOTE. HIV-1, human immunodeficiency virus type 1; PID, pelvic inflammatory disease.
compared. Plasma viremia (table 2; figure 2) when first and last visits
in the follow-up period, there was an overall slight decline in
STIs.

HIV-1 RNA copies was greater during acute genital herpes
recurrences, but the difference did not reach statistical signifi-
cance (table 2).

Among 3 HIV-1-positive women who had no STIs during
the follow-up period, there was an overall slight decline in
plasma viremia (table 2; figure 2) when first and last visits were
compared.

Changes in CD4 and CD8 T cell counts. To evaluate the
impact of STIs on CD4 lymphocyte counts, we determined the
CD4 lymphocyte count in the study subjects at the time the
subject presented with an acute STI and at ≥14 d after treat-
ment or resolution of symptoms. Acute gonococcal infection
resulted in very large and statistically significant increases
in IL-4, IL-6, IL-10 (table 2; figures 3–5), and sTNF-α-R1II in

Plasma cytokines and acute STIs. As a measure of the effect
of acute STIs on immune regulatory factors, we sought to de-
termine the pattern of cytokines secreted by HIV-1–infected
individuals with acute episodes of STIs. Measurements of
plasma cytokines were performed in HIV-1–infected individuals
at presentation with an acute episode of STI and 14 d after
treatment or resolution of symptoms. Acute gonococcal infec-
tion resulted in very large and statistically significant increases
in IL-4, IL-6, IL-10 (table 2; figures 3–5), and sTNF-α-R1II in

Table 2. Changes in plasma cytokine concentration, plasma viremia,
and CD4 and CD8 T cell counts among women with acute sexually
transmitted infections and during first and last study visits among
women with no episodes of sexually transmitted infection.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Measures in women with no STI</th>
<th>Measures in women during STI†</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC (n = 26)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td>1.1 ± 5.4</td>
<td>8.4 ± 13.4</td>
<td>.008</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.3 ± 2.6</td>
<td>7.7 ± 9.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>IL-10</td>
<td>1.0 ± 2.6</td>
<td>6.2 ± 6.6</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.3 ± 2.1</td>
<td>1.4 ± 3.9</td>
<td>.18</td>
</tr>
<tr>
<td>sTNFr</td>
<td>2399 ± 786</td>
<td>3348 ± 980</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CD4*</td>
<td>372 ± 300</td>
<td>302 ± 218</td>
<td>.02</td>
</tr>
<tr>
<td>CD8*</td>
<td>1235 ± 707</td>
<td>1048 ± 476</td>
<td>.06</td>
</tr>
<tr>
<td>HIV</td>
<td>128,557 ± 121,474</td>
<td>186,211 ± 166,615</td>
<td>.001</td>
</tr>
<tr>
<td>Acute PID (n = 14)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td>2.1 ± 8.0</td>
<td>7.3 ± 12.6</td>
<td>.13</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.66 ± 2.0</td>
<td>6.6 ± 8.2</td>
<td>.02</td>
</tr>
<tr>
<td>IL-10</td>
<td>0</td>
<td>5.6 ± 7.0</td>
<td>.01</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0</td>
<td>1.1 ± 3.4</td>
<td>.16</td>
</tr>
<tr>
<td>sTNFr</td>
<td>2274 ± 841</td>
<td>3011 ± 1057</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CD4*</td>
<td>383 ± 336</td>
<td>371 ± 336</td>
<td>.77</td>
</tr>
<tr>
<td>CD8*</td>
<td>1213 ± 594</td>
<td>1109 ± 436</td>
<td>.21</td>
</tr>
<tr>
<td>HIV</td>
<td>132,929 ± 122,738</td>
<td>196,536 ± 79,898</td>
<td>.03</td>
</tr>
<tr>
<td>GH (n = 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td>3.1 ± 7.5</td>
<td>5.6 ± 13.7</td>
<td>.73</td>
</tr>
<tr>
<td>IL-6</td>
<td>2.8 ± 3.4</td>
<td>2.0 ± 3.4</td>
<td>.58</td>
</tr>
<tr>
<td>IL-10</td>
<td>3.7 ± 4.1</td>
<td>1.8 ± 4.3</td>
<td>.55</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.2 ± 2.9</td>
<td>0</td>
<td>.36</td>
</tr>
<tr>
<td>sTNFr</td>
<td>2223 ± 1123</td>
<td>1961 ± 749</td>
<td>.26</td>
</tr>
<tr>
<td>CD4*</td>
<td>197 ± 149</td>
<td>195 ± 126</td>
<td>.9</td>
</tr>
<tr>
<td>CD8*</td>
<td>871 ± 413</td>
<td>980 ± 613</td>
<td>.39</td>
</tr>
<tr>
<td>HIV</td>
<td>120,167 ± 89,035</td>
<td>162,500 ± 126,368</td>
<td>.08</td>
</tr>
<tr>
<td>No STI (n = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>0</td>
<td>1.03 ± 1.8</td>
<td>.42</td>
</tr>
<tr>
<td>IL-10</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>sTNFr</td>
<td>1730 ± 708</td>
<td>1729 ± 748</td>
<td>.991</td>
</tr>
<tr>
<td>CD4*</td>
<td>203 ± 84</td>
<td>193 ± 81</td>
<td>.58</td>
</tr>
<tr>
<td>CD8*</td>
<td>790 ± 151</td>
<td>770 ± 115</td>
<td>.82</td>
</tr>
<tr>
<td>HIV</td>
<td>61,506 ± 5508</td>
<td>53,906 ± 4000</td>
<td>.18</td>
</tr>
</tbody>
</table>

NOTE. Interferon-γ and interleukin-2 were not detectable. STI, sexually
transmitted infection; GC, gonococcal cervicitis; IL, interleukin; TNF, tumor
necrosis factor; sTNFr, soluble tumor necrosis factor receptor; HIV, human
immunodeficiency virus; PID, pelvic inflammatory disease; GH, genital herpes
recurrence.

* Data are mean ± SD for levels of cytokines (pg/mL), T cells (cells/mm 3),
or HIV (copies/mL).

had an increased in plasma viremia, and the number of plasma
HIV-1 RNA copies was greater during acute genital herpes
recurrences, but the difference did not reach statistical signifi-
cance (table 2).

Among 3 HIV-1-positive women who had no STIs during
the follow-up period, there was an overall slight decline in
plasma viremia (table 2; figure 2) when first and last visits were
compared.

Changes in CD4 and CD8 T cell counts. To evaluate the
impact of STIs on CD4 lymphocyte counts, we determined the
CD4 lymphocyte count in the study subjects at the time the
subject presented with an acute STI and at ≥14 d after treat-
ment or resolution of symptoms (for PID and genital herpes).
Among HIV-1–positive women, a statistically significant de-
cline in CD4 cell counts was observed during episodes of
gonococcal cervicitis (mean ± SD, 372 ± 300 cells/mm 3 vs.
302 ± 218 cells/mm 3; P < .02; table 2). Overall, CD4 cell counts
declined in 15 of 26 women during acute gonococcal cervicitis.
CD8 cell counts also declined during acute gonococcal cervi-
citis, but this finding was not statistically significant. No sta-
tistically significant changes in either CD4 or CD8 cell counts
were observed among women with acute PID or genital herpes.
In the small group of women (n = 3) who experienced both
gonococcal cervicitis and gonococcal infection with PID, there
were no differences in CD4 or CD8 cell counts when episodes
of gonococcal PID were compared with gonococcal cervicitis.
In HIV-1–negative women with acute gonococcal infection,
there was a decline in mean CD4 and CD8 cell counts during
gonococcal cervicitis, although this was not statistically signifi-
cant (data not shown). CD4 and CD8 cell counts returned to
baseline after treatment for gonococcal cervicitis. Among 8
women with no acute STI episodes, no statistically significant
changes in CD4 or CD8 cell counts were observed.

Figure 2. Levels of human immunodeficiency virus RNA in plasma
before, after, and during gonococcal infection and for the first and last
visit in women with no episodes of sexually transmitted infections
(STIs).
8 of 30, 19 of 30, 13 of 30, and 27 of 30 women, respectively (figures 3–5), and in the group overall (table 2). In most instances, these cytokines were at the lower limits of detection in the absence of infection. IFN-γ and IL-2 were undetectable in all assays performed.

Comparable results were observed for changes in plasma cytokines during acute PID, although only changes in IL-6, IL-10, and sTNF-α-RII were statistically significant (table 2). Among 3 women who experienced both gonococcal cervicitis and gonococcal infection associated with PID as separate episodes, there were no differences in plasma cytokine concentration during gonococcal infection with PID. No changes were observed in plasma cytokine concentration during episodes of genital herpes. Four women who were not infected with HIV-1 had gonococcal infections. Among them, IL-4, IL-6, and IL-10 became detectable during infection in a single individual. There was a statistically significant increase in sTNF-α-RII in these 4 HIV-1–negative women (data not shown).

Correlations between plasma viremia, CD4 cell counts, and plasma cytokines. The absolute changes in plasma viremia, CD4 cell counts, and plasma cytokines were compared, using the Pearson correlation coefficient, among women with gonococcal infection. Increasing plasma viremia was associated with increased concentrations of plasma IL-4 and decreased concentrations of plasma IL-6 but not with other cytokines. Linear-regression analysis confirmed an independent association between static or reduced levels of IL-6 and increased plasma viremia. There was no correlation between changes in CD4 or CD8 cell counts and plasma viremia, nor were there correlations between changes in individual cytokine concentrations and CD4 or CD8 cell counts. Analysis of women with PID and genital herpes showed no correlation between plasma cytokine concentration and plasma viremia or CD4 and CD8 T cell counts.

Discussion

This study takes advantage of a natural “challenge” experiment during which women who work as sex workers experience acute STIs, either as a result of new infections or through reactivation of persistent infections. The longitudinal study design circumvented the problem of confounding by the effect of HIV-1–induced immune deficiency on susceptibility to infections, thereby permitting the examination of the effect of STIs on factors potentially related to accelerated rates of HIV-1 disease progression. The principal findings are that plasma HIV-1 RNA copy number and Th2 cytokines increase during episodes of gonococcal infection, whereas CD4 cell counts decline. Similar changes in plasma viremia and plasma cytokines, but not CD4 and CD8 T cell counts, were observed among women experiencing acute PID. Women with recurrences of genital herpes showed a trend toward increased plasma viremia, but no decreases in other parameters were observed.

Women who experienced no STIs during the study showed no changes in any of these parameters. Gonococcal infection and acute PID resulted in significant but transient increases in plasma HIV-1 RNA levels. There have been several previous studies of HIV-1 plasma viremia during acute infections with other organisms [19]. The present study is unique in that we had the opportunity to observe women before, during, and after...
infection and during subsequent infection with the same organism. These data strongly suggest that gonococcal infection causes increases in plasma viremia; the suggestion is of interest, because gonococcal infection is largely limited to the mucosa of the genital tract.

Recent studies in men did not show any change in plasma viremia during gonococcal infection [20]. This suggests that the systemic effects of genital tract infection may substantially differ between men and women. Alternatively, increases in plasma viremia observed in this study may have occurred because these women have experienced many gonococcal infections in the past and therefore may have enhanced immune responses to gonococcal infection. The level of HIV-1 plasma viremia in an individual reflects a balance of many different factors. These include the number of HIV-1-infected cells, the transcription of the HIV-1 genome, the level of cellular activation, and the nature of humoral and cellular immune responses to HIV-1. Cellular transcription factors are modulated by cellular activation through the action of inflammatory mediators and cytokines, and the potential role of cytokines as a mechanism directly underlying the increase in plasma viremia may be important. Direct activation of HIV-1 replication through the action of bacterial products could also explain the increased plasma viremia during gonococcal infection and PID.

One of the major components of the outer membrane, lipooligosaccharide (LOS), of gram-negative bacteria has been shown to stimulate HIV replication in vitro. The up-regulation of HIV-1 transcription by LOS appears to function through the activation of NF-κB. The lack of association between changes in plasma viremia and the severity of gonococcal infection (development of PID) would not favor a direct activation of HIV-1 replication by LOS, because the number of bacteria and thus the amount of LOS should be related to the severity of gonococcal infection. The changes in CD4 and CD8 T cell counts and plasma cytokine concentrations with gonococcal infection and PID, but not with genital herpes, suggest that the mechanisms resulting in increased plasma viremia in these conditions may be fundamentally different.

CD4 T cell counts have been the principal marker used to assess risk of progression to AIDS. The declines in CD4 T cells observed with gonococcal cervicitis in this study suggest that repeated episodes of bacterial STIs could increase the rate of development of immunodeficiency during HIV-1 infection. Declines in CD4 T cell number during gonococcal infection could occur through several mechanisms. In this study, the number of HIV-1-infected cells was not directly assessed. It seems likely that the number of HIV-1-infected cells increased in parallel with plasma viremia during episodes of acute STI, thus, direct cellular killing by HIV-1 could explain declines in CD4 cell count. Enhanced induction of apoptosis in HIV-1-infected CD4 T cells, perhaps through gp120, could also account for this finding [21]. Cytokines have also been associated with the induction of apoptosis in HIV-1-infected cells. The lack of association of declines in CD4 cell count with PID and genital herpes suggests that the mechanism resulting in decreased CD4 counts is not induced in these infections.

We examined cytokine levels in plasma as an indicator for how acute STIs influenced the overall cytokine milieu within an individual. The complex interactions between cytokines and immune effector cells take place in lymphoid tissue, and measurement of plasma cytokines is a surrogate of what is transpiring in these tissues; however, the transient increases observed during acute STIs, with a return to baseline after infection and the failure to observe similar changes in individuals with no STIs, argue for the validity of the observations. Acute gonococcal cervicitis resulted in dramatic increases in the plasma concentration of IL-4, IL-6, and IL-10, as well as increases in the inflammatory cytokine TNF-α soluble receptor. Similar changes were observed with PID (including some of the women with gonococcal infection) but not with genital herpes.

Increases in plasma cytokines have not been consistently detected in plasma during mucosal infections previously; however, proinflammatory cytokines are readily detected during systemic infections. IL-4 is not usually detected in plasma; however, it is secreted by T cells that are plentiful in peripheral blood [22]. Perhaps the women in this study are hyperresponsive to gonococcal antigens because of frequent exposure, resulting in high levels of IL-4 production during infection. Although we did not detect type 1 cytokines in these studies, this could be a reflection of the cytokines’ transient life in plasma, because they are detectable only in overwhelming infections, such as Ebola.
hemorrhagic fever. The altered cytokine production observed in this study represents a potential mechanism whereby acute STIs increase plasma viremia and decrease CD4 counts.

Several cytokines, including IL-1, IL-6, and TNF-α, have been shown to stimulate HIV-1 replication, contribute to HIV-1 pathogenesis, and possibly hasten progression to AIDS [23, 24]. IL-10 has also been shown to markedly increase HIV-1 replication in the presence of TNF-α [25]. However, in the present study, increased plasma viremia was correlated with increased plasma IL-4 and decreased plasma IL-6 despite elevated levels of IL-6 being associated with gonococcal infection. As IL-4 has no known direct effect on HIV-1 replication [26], and as decreased levels of IL-6 correlated with increased viremia, it seems improbable that the increase in HIV-1 plasma viremia was a direct result of increased cytokine concentration.

Another possible explanation is that increases in production of IL-4 and other type 2 cytokines induced by gonococcal infection resulted in changes in the immune response to HIV-1. IL-4 down-regulates type 1 cellular immune responses, which are strongly correlated with better outcome of HIV-1 infection. Furthermore, IL-4 has been shown in vitro to result in decreased CD8 T cell responses to HIV-1 [27]. If IL-4 is responsible for the increase in plasma viremia in this study through down-regulation of protective immune responses, it would be an example of the immune response to one infection shifting the immune response to a second infection. If this hypothesis is correct, its corollary is that other types of antigenic challenge may be able to augment HIV-1-specific responses and reduce viremia. Further studies of how other infections modulate HIV-1 viremia and how antigen-specific responses change during acute infections would be of interest.

The significance of these observations for AIDS pathogenesis lies in the fact that an increase in plasma viremia perturbs the steady state of HIV-1 replication. The precise mechanisms underlying the progressive immune deficiency that is the hallmark of HIV-1 infection is unknown, although the rate of viral replication and the virus burden play a significant role. HIV-1 virus load and CD4 T cell counts are highly correlated with immune dysfunction and HIV-1 disease stage. Although plasma viremia returned to baseline levels after resolution of disease in this study, the epidemiologic observation of extremely rapid HIV-1 disease progression in this cohort suggests that the cumulative effect of repeated STIs may accelerate the development of HIV-1-related disease. This may offer a relatively inexpensive strategy for prolonging survival among HIV-1–infected individuals, and it certainly provides an additional rationale for HIV-1–infected individuals to adopt safer sexual behaviors.

Acknowledgments

We thank the women of Pumwani for their participation in this study. We are grateful for the secretarial assistance of Carol Sigurdson.

References


