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Stable CD4 Expression and Local Immune Activation in the Ectocervical Mucosa of HIV-Infected Women

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Studies using genital tissue samples from HIV-infected women might provide important information about HIV susceptibility and transmission. In this study, ectocervical biopsies were obtained from 20 HIV-seropositive (HIV+) Kenyan female sex workers (FSW) and 20 HIV-seronegative lower risk (HIV− LR) women. To control for the impact of sex work, 20 HIV− FSW were also recruited. Immune molecules were assayed in situ by immunohistochemistry and for mRNA expression by quantitative PCR. The HIV+ women were reportedly infected for a median of 3 y (1–21 y), with a median viral load of 11,735 copies/ml (20–648,000 copies/ml). These women had significantly lower CD4 blood cell counts than the HIV− LR women but comparable levels of CD4 expression in ectocervix. Whereas cellular markers were similar between the HIV+ group and the HIV− LR women, the HIV-binding molecules CCR5, dendritic cell–specific intercellular adhesion molecule-3–grabbing nonintegrin, and mannose receptor as well as the inflammatory markers CD69, IFN-γ, IL-6, and IL-22 were significantly upregulated in the HIV+ group. As compared with the HIV− FSW women, the HIV+ women had significantly upregulated levels of CD4, CD3, CCR5, Langerin, dendritic cell–specific intercellular adhesion molecule-3–grabbing nonintegrin, and mannose receptor as well as inflammatory cytokines. The CD4 cell depletion previously seen in the gut mucosa of HIV-infected individuals was thus not observed in the ectocervical mucosa. Stable CD4 cell expression and local immune activation in the lower female genital tract may promote viral replication and genital shedding and increase the risk of sexual HIV transmission. The Journal of Immunology, 2013, 191: 3948–3954.

E
ven in the early phase of acute HIV infection, the gastrointestional tract suffers from substantial immunologic dysfunction and structural epithelial damage (1, 2). These impairments include mucosal depletion of CD4+ T cells, local hyperactivation/inflammation, exhaustion of intestinal macrophage phagocytic function, and an altered gut microbiome (reviewed in Ref. 3). As a result of these events, microbial products are translocated from the gastrointestinal tract to the systemic circulation, and this mechanism has been proposed as a major driver of the chronic immune activation associated with disease progression (4).

Although progressive depletion of CD4+ T lymphocytes from blood, lymphoid organs, and gastrointestinal tissues is a hallmark of HIV pathogenesis, little is known about epithelial and submucosal HIV target cells in the genital tract mucosa or their fate during disease progression.

The CD4 molecules are expressed on epithelial and submucosal cells in both the upper and lower genital tracts of healthy women (5–7), and these cells include T lymphocytes, macrophages, dendritic cells (DCs), and Langerhans cells (8–10). The cells are all heterogeneous with regard to HIV susceptibility, which is critically dependent on the presence of the major HIV coreceptor CCR5 (7, 11–14). Also, other classes of HIV-binding molecules, including the C-type lectin receptors (CLRls), have implications for HIV susceptibility and are present on mucosal macrophages and subpopulations of DCs. The CLRls Langerin, dendritic cell–specific intercellular adhesion molecule-3–grabbing nonintegrin (DC-SIGN), and mannose receptor (MR) were all found in the genital tract of healthy women (8–10). Langerin was expressed mainly on CD1a+ Langerhans cells in the epithelium of ectocervix, whereas DC-SIGN and MR were expressed on submucosal DCs and macrophages (9). From a functional point of view, these receptors may have very different impacts on HIV susceptibility. That is, the binding of Langerin to HIV may be followed by internalization and degradation of the viral particle, at least in noninflamed tissue in the context of low-virus inocula (15); however, the binding of virus to DC-SIGN and MR can enhance viral spread to adjacent target cells (16, 17). An increased number of mucosal HIV target cells can theoretically influence genital viral load and thereby sexual and vertical HIV transmission. Many questions about the expression and distribution of these HIV-binding receptors in genital tissues of chronically HIV-infected individuals are, to our knowledge, unanswered.

In the female genital tract, sexually transmitted infections (STIs), bacterial vaginosis, and sex hormones as well as sexual exposure to...
semen, another microbiome can result in immune activation and inflammation (reviewed in Ref. 18). Increased levels of genital tract proinflammatory cytokines can promote viral replication in the mucosa and thereby recruit immune target cells leading to increased HIV shedding (19–21). When an HIV-infected sexual partner is involved, HIV particles in the seminal fluid and the fluid itself may also cause genital epithelial damage and further inflammation (22, 23). This immune activation can result in influx of HIV target cells and upregulation of CD4, CCR5, and CLR expression on selected cell populations in the genital tissue. Genital inflammation may increase HIV acquisition and heighten the plasma viral load’s set point (19, 20). In this study, in a unique collection of genital tissue samples from HIV-infected women, we assessed the distribution of the HIV-binding receptors CD4, CCR5, Langerin, DC-SIGN, and MR. By using a panel of proinflammatory molecules, we further defined the immune activation status in the ectocervical mucosa for a comparison between HIV-infected and -uninfected women.

Materials and Methods

Ethical approval

This study was reviewed and approved by the research ethics boards at Kenyatta National Hospital (Nairobi, Kenya), the Karolinska Institutet (Stockholm, Sweden) and the University of Manitoba (Winnipeg, Manitoba, Canada). All study participants provided written informed consent.

Study populations and procedures

HIV-seropositive (HIV+) and HIV-seronegative female sex workers (HIV− FSW) were recruited through the dedicated Majengo Sex Worker Clinic in the Pumwani area of Nairobi, Kenya (24, 25). HIV− lower risk control women (HIV− LR) (n = 20) with no history of sex work and only one sexual partner for the last 6 mo before this study were recruited through a Maternal Health Clinic based at the Pumwani Maternity Hospital (25, 26). General inclusion criteria were age at least 18 y, uterus and cervix present, not actively menstruating, no symptomatic or clinically apparent cervical inflammation, willingness to undergo ectocervical biopsy collection, and to abstain from vaginal sex during a healing period of 2 wk. Specific inclusion criteria for HIV+ and HIV− FSW women were 1) a reported minimum of five clients per day at the last formal resurvey visit 6 mo earlier and 2) currently active in sex work. In addition, HIV− FSW had all been enrolled in the Majengo Sex Worker cohort for a minimum of 3 y. Inclusion criteria for HIV+ women were as follows: 1) being antiretroviral treatment naive, 2) no prior history of AIDS-defining illnesses, and 3) no acute health issues. HIV+ and HIV− LR women were recruited from sex-worker control groups of women with each other, the statistical method (30). Ct values for target cDNA were computed using the comparative threshold (Ct) method (30). Ct values within the HIV+ and HIV− LR women. Because the HIV+ women were recruited from a sex-worker cohort, an additional group of HIV− women were recruited from the same cohort (HIV− FSW) to control for factors such as STIs and number of sex partners. Thus, because it was not the main aim of this study to compare the two HIV− control groups of women with each other, the statistical methods of choice were for comparison of two groups. Specifically, Fisher’s exact test for categorical variables (such as menstrual cycle stage; the use of hormonal contraception; condom use; the performance of vaginal douching; and the presence of PSA, bacterial vaginosis, and STIs) and Mann–Whitney U test for continuous variables (such as age, number of pregnancies, years in sex work, and all immune markers of interest). Spearman’s rank correlation coefficient test was used to assess correlations. All tests were two-sided with α < 0.05 considered significant.

Results

Study population

The HIV+ women were reportedly infected for a median of 3 y (1–21 y), with a median viral load of 11,735 copies/ml (20–648,000 copies/ml) at time of sample collection. These HIV+ women reported a median of 13 y in sex work (range, 2–26) and a median of 29 clients/week (range, 2–150), which was statistically similar to the HIV− FSW control women (6 y in sex work; range, 4–24; 41 clients/week; range, 9–115). None of the HIV− LR control women reported a history in sex work, and their maximum number of sex partners over the preceding 6 mo was no more than 1 (thus meeting the established recruitment criteria). Further, the HIV+ and HIV− FSW women reported a similar average of 50% casual and 50% repeat clients with whom they described a high frequency of
Condom use (data not shown). However, inconsistent condom use was the rule with their regular partners (husband, boyfriend, or lover). Inconsistent condom use with regular partners was also common in the HIV+ LR group of women (Table I).

The HIV+ women did not differ significantly to either control group regarding age, number of pregnancies, stage of menstrual cycle or the use of hormonal contraception (Table I). The proportion of progesterone-based hormonal contraception was similar in the HIV+ women (2/5, 40%) and both the HIV− LR (4/6, 67%) and the HIV− FSW (7/10, 70%) individuals (p = 0.6 and 0.3, respectively). Sexual activity during menses, detectable levels of PSA in addition to the prevalence of bacterial vaginosis, Candida, Chlamydia trachomatis, Neisseria gonorrhoea, and syphilis were also similar across the groups. Positive HIV-2 serostatus and the performance of vaginal douching were more common in HIV+ versus HIV− LR women (p < 0.0001, both) but not in HIV+ versus HIV− FSW women (Table I).

Table I. Enrollment characteristics of study population at date of biopsy

<table>
<thead>
<tr>
<th></th>
<th>HIV+ *</th>
<th>HIV− LR **</th>
<th>HIV− FSW ***</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Median or No. (Range or %)</td>
<td>Median or No. (Range or %)</td>
<td>Median or No. (Range or %)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>42 (24–58)</td>
<td>38 (24–47)</td>
<td>42 (27–51)</td>
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<tr>
<td>Pregnancies*</td>
<td>3 (0–6)</td>
<td>3 (0–9)</td>
<td>3 (1–13)</td>
<td>NS</td>
</tr>
<tr>
<td>Menstrual cycle stage</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Follicular</td>
<td>3 (15%)</td>
<td>2 (10%)</td>
<td>2 (10%)</td>
<td>NS</td>
</tr>
<tr>
<td>Periiovulatory</td>
<td>3 (15%)</td>
<td>1 (5%)</td>
<td>5 (25%)</td>
<td>NS</td>
</tr>
<tr>
<td>Luteal</td>
<td>8 (40%)</td>
<td>7 (35%)</td>
<td>5 (25%)</td>
<td>NS</td>
</tr>
<tr>
<td>Not applicable’</td>
<td>6 (30%)</td>
<td>8 (40%)</td>
<td>12 (60%)</td>
<td>NS</td>
</tr>
<tr>
<td>Hormonal contraception</td>
<td>5 (25%)</td>
<td>6 (30%)</td>
<td>10 (50%)</td>
<td>NS</td>
</tr>
<tr>
<td>Bacterial vaginosis</td>
<td>3 (15%)</td>
<td>3 (15%)</td>
<td>3 (15%)</td>
<td>NS</td>
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<tr>
<td>Candida</td>
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<td>2 (10%)</td>
<td>2 (10%)</td>
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<tr>
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<tr>
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<td>0 (0%)</td>
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<tr>
<td>Syphilis seropositive</td>
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<td>1 (5%)</td>
<td>3 (15%)</td>
<td>NS</td>
</tr>
<tr>
<td>HSV-2 seropositive</td>
<td>20 (100%)</td>
<td>9 (45%)</td>
<td>18 (90%)</td>
<td>i, i**</td>
</tr>
<tr>
<td>Condom use &lt;50%**</td>
<td>7 (35%)</td>
<td>14 (70%)</td>
<td>10 (50%)</td>
<td>NS</td>
</tr>
<tr>
<td>Sex during menses</td>
<td>3 (15%)</td>
<td>0 (0%)</td>
<td>3 (15%)</td>
<td>NS</td>
</tr>
<tr>
<td>PSA-positive*</td>
<td>1 (5%)</td>
<td>1 (5%)</td>
<td>1 (5%)</td>
<td>NS</td>
</tr>
<tr>
<td>Vaginal douching’</td>
<td>20 (100%)</td>
<td>11 (55%)</td>
<td>20 (100%)</td>
<td>j,****</td>
</tr>
</tbody>
</table>

HIV+ FSW (n = 20).

HIV− LR individuals in a reported monogamous relationship for the past 6 months (n = 20).

HIV− FSW (n = 20).

Pregnancies including abortions.

Subjects with amenorrhea due to long-term hormonal treatment or menopause.

Hormonal contraception includes oral contraception, Depo-Provera, and Norplant.

The self-reported use of condoms with regular partners defined as husband, boyfriend, or lover.

PSA detected in CVS >1 ng/ml.

Any douching performed by inserting water or water and soup in the vagina.

p Value for HIV+ vs. HIV-LR.

**p < 0.001, Fisher exact test, ***p < 0.01, Fisher exact test.

NA, Not applicable.

Reduced CD4+ cell count in blood but stable mRNA and protein expression of CD4 in the ectocervix of HIV+ women

As expected from the natural history of chronic HIV infection, the CD4+ blood cell counts were lower in HIV+ versus HIV− LR women (median, 493 versus 870 cells/μl; range, 121–1373; and 546–1151 cells/μl, respectively) (p = 0.004). We then assessed the CD4 expression in ectocervical tissue samples and demonstrated that, in contrast to blood values, both the CD4 mRNA expression and the CD4+ cell numbers in those tissue were similar in the HIV+ and the HIV− LR group (relative quantification [RQ] CD4 mRNA; median, 0.008 for both groups, p = 0.9; CD4+ cells/100 μm² tissue; median, 0.04 for both groups, p = 0.5) (Fig. 1A, 1B). The CD4+ blood cell counts neither correlated significantly with the CD4 mRNA expression nor the quantified tissue CD4+ cell numbers for either of the study groups (HIV+: CD4 blood cell counts versus CD4 mRNA, p = 0.7; CD4 blood cell counts versus tissue CD4+ cell numbers: 0.9) (HIV− LR: CD4 blood cell counts versus CD4 mRNA, p = 0.1; CD4 blood cell counts versus tissue CD4+ cell numbers, p = 0.9).

Neither did the CD4 mRNA expression correlate significantly with the quantified tissue CD4+ cell numbers in any of the groups (data not shown).

To control for the impact of sex work (i.e., number of sex partners), HSV-2 seropositivity and vaginal douching on the local immune environment of the genital tract, the HIV+ group was also compared with the HIV− FSW group. The HIV+ women had both higher CD4 mRNA expression as well as tissue-residing CD4 cell numbers as compared with the HIV− FSW women (Fig. 1A, 1B) (medians CD4 mRNA expression and CD4 cell counts HIV+ FSW, 0.005 and 0.017; p = 0.010 and p < 0.0001, respectively). In fact, the HIV− FSW group also had a significantly lower expression of both CD4 mRNA expression and cell counts versus HIV− LR: p = 0.030 and p < 0.0001, respectively.

The CD4 molecule can be expressed on T cells, macrophages, DCs, and also Langerhans cells. In this study, CD4 was present in both the epithelium and the submucosa of the ectocervix of all study participants (Fig. 1C–E). To roughly estimate whether a specific cell type was expressed any differently in the ectocervix of HIV+ versus either the HIV− LR or the HIV− FSW control group of women, all samples were analyzed for mRNA expression of CD3 (in this study defined as T cells) (median, 0.007, 0.004, and 0.004, respectively), CD68 (in this study defined as macrophages) (median, 0.064, 0.07, and 0.059, respectively), CD1a (in this study defined as Langerhans cells) (median, 0.016, 0.021, and 0.017, respectively), and CD11c (in this study defined as DCs) (median, 0.003, 0.003, and 0.001, respectively). No significant differences were detected except a higher expression of CD3 mRNA versus HIV− FSW (p = 0.03) (Fig. 1F).
Using immunohistochemical analysis (Fig. 2B). HIV+ women had more CCR5- and MR-positive cells/100 µm² tissue (median, 0.021 and 0.044) than HIV− FSW women (median, 0.009 and 0.021) and (p = 0.011 and p < 0.0001).

CCR5+ and Langerin+ cells were present in the ectocervical epithelium and the lamina propria compartments of all three groups of women. In addition, DC-SIGN− and MR-expressing cells were detected in the lamina propria and occasionally in mucosal duct/vessel formations (Fig. 2C).

**Upregulated expression of cellular activation markers and HIV receptors in the ectocervix of HIV+ women**

Immune activation at mucosal sites upregulates the cellular marker CD69 as well as CCR5 and CLRs. Therefore, the mRNA expression of these markers was assessed and compared in ectocervical tissue biopsies from HIV+ women and the two control groups (Fig. 2A). HIV+ had significantly higher mRNA expression of CD69 (median, 0.028), CCR5 (median, 0.009), and MR (median, 0.0005) versus the HIV− LR group (median, 0.017, 0.003, and 0.0001, respectively) (p = 0.009, p ≤ 0.0001, and p = 0.007, respectively), but similar expression levels of DC-SIGN and Langerin (median, 0.0009 and 0.049; and p = 0.17 and p = 0.31, respectively). Furthermore, the mRNA expression of all markers, except CD69, was significantly higher in the HIV+ versus the HIV− FSW group (CCR5, p = 0.002; Langerin, p = 0.035; DC-SIGN, p = 0.001; and MR, p = 0.001).

To complement the foregoing measurements with qPCR, the HIV-binding molecules CCR5 and the CLRs were assessed by using immunohistochemical analysis (Fig. 2B). HIV+ women had more DC-SIGN− and MR-positive cells/100 µm² tissue (median, 0.019 and 0.044) versus HIV− LR women (median, 0.010 and 0.021) and (p = 0.043 and p < 0.0001). Also, HIV+ women had more CCR5- and MR-positive cells/100 µm² tissue (median, 0.021 and 0.044) than HIV− FSW women (median, 0.009 and 0.021) and (p = 0.011 and p < 0.0001).

**Upregulated expression of cytokines in the ectocervix of HIV+ women**

A panel of proinflammatory cytokines that are upregulated in mucosal tissue upon immune activation was selected in this study for assessment of inflammation in the ectocervical tissue samples (Fig. 3). The mRNA expression of the cytokines IFN-γ, IL-6, and IL-22 was higher in HIV+ women (median, 0.002, 0.0003, and 0.0003, respectively) than in the HIV− LR group (median, 0.0003, 0.00006, and 0.00006, respectively) (p < 0.0001, p = 0.008, and p = 0.049, respectively), and a trend was noted toward higher levels of IFN-α (median, 0.017 versus 0.001; p = 0.063). There was, however, no difference in the levels of TNF-α (median, 0.006 versus 0.007; p = 0.19) or IL-17 (median, 0.0004 for both groups; p = 0.30) among these two groups. When compared with HIV− FSW women, HIV+ individuals had significantly higher mRNA expression of IFN-γ.
p, 0.0001), TNF-α (p = 0.022), IL-6 (p = 0.007), IL-17 (p = 0.044), and IL-22 (p = 0.005) but similar levels of IFN-α (p = 0.17).

Discussion

Our data presented in this paper document the expression of immune markers in genital tissues of HIV+ women and show that, although their blood CD4 cell numbers were lower, their ectocervical CD4 cell numbers were stable. Furthermore, local immune activation in the ectocervix of the HIV+ women was shown by upregulated cellular markers and cytokine expression. Many studies have described CD4 cell subpopulations and inflammatory markers in cervical mononuclear cell preparations and in cervi-

FIGURE 2. Expression of immune activation markers and HIV receptors in ectocervical tissues of the study participants. (A) RQ of mRNA expression is shown for CD69, CCR5, Langerin, DC-SIGN and MR in the ectocervical tissue samples from HIV+ (red boxes), HIV- LR (green boxes), and HIV- FSW (blue boxes) study groups. The Ct values for the selected markers were normalized to UBC by using the 2−ΔΔCT equation. y-axis: RQ (UBC = 1). *p < 0.05, **p < 0.01, and ***p < 0.001 with Mann–Whitney U test. (B) CCR5-, Langerin-, DC-SIGN–, and MR-expressing cells, respectively, were quantified by immunohistochemical analysis in one ectocervical tissue section per individual (median, 2.8 × 10^6 μm^2; range, 2.5–3 × 10^6 μm^2), including the entire height of the epithelium and all lamina propria within 500 μm from the basal membrane. Values representing HIV+ (red boxes), HIV− LR (green boxes), and HIV− FSW (blue boxes) study groups are depicted. y-axis: number of positive cells/100 μm^2 tissue. *p < 0.05 and ***p < 0.001 with Mann–Whitney U test. (C) Bright-field images of human ectocervical tissues stained with hematoxylin (in blue) for visualization of cell nuclei and DAB (in brown) for the HIV receptors CCR5, Langerin, DC-SIGN, and MR in HIV+ (top row), HIV− LR (middle row), and HIV− FSW (bottom row) women. The images were collected with a ×10/0.30 objective; scale bar, 100 μm.

FIGURE 3. Expression of proinflammatory cytokines in ectocervical tissues of the study participants. RQ of mRNA expression is shown for IFN-α, IFN-γ, TNF-α, IL-6, IL-17, and IL-22 in the ectocervical tissue samples from HIV+ (red boxes), HIV− LR (green boxes), and HIV− FSW (blue boxes) study groups. The Ct values for the selected markers were normalized to UBC by using the 2−ΔΔCT equation. y-axis: RQ (UBC = 1). *p < 0.05, **p < 0.01, and ***p < 0.001 with Mann–Whitney U test.
comparable with HIV and MR were expressed only on submucosal cells in the HIV+ both the ectocervical epithelium and submucosa, whereas DC-SIGN, and MR, which are often upregulated as a result of pathogen-induced proinflammatory cytokine responses in mucosal tissue. The HIV+ women had a greater mRNA expression and increased numbers of CCR5+, DC-SIGN−, and MR-positive cells as compared with at least one of the control groups. The mRNA expression did not exactly match the protein expression in tissues (HIV+ also had a greater mRNA of Langerin versus HIV− FSW), but this could be expected because mRNA can be translated to different extents during various inflammatory conditions. As determined by assessment in situ, CCR5 and Langerin were present in both the ectocervical epithelium and submucosa, whereas DC-SIGN and MR were expressed only on submucosal cells in the HIV+ women, confirming previous reports for HIV-uninfected women (9). To further account for the effect of sex work per se on the critical CD4 and CCR5 expression levels, the estimated number of unprotected sexual acts over the past years were analyzed for the HIV− FSW group, but no correlations emerged (data not shown).

Acute and chronic HIV infections in humans are associated with a marked immune activation in the intestinal mucosa and in genital secretions (4, 33). To further explore the possible immune activation state within actual ectocervical tissues of HIV+ individuals, mRNA quantitative analysis was performed on numerous activation markers and cytokines. The amounts of the activation marker CD69 and the cytokines IFN-γ, IL-6, and IL-22 were significantly higher in HIV+ women versus HIV− LR women. The HIV+ women also had higher expression of a number of cytokines as compared with the HIV− FSW group. Women included in the HIV− FSW group had been enrolled in the sex worker cohort for at least 3 y, and some of them thus met the criterion of being relatively HIV resistant (24). A low immune activation state can be associated with resistance to heterosexual HIV transmission in the present cohort, as previously identified in blood, cervical mononuclear cells, and cervicovaginal secretions (36–38). Thus, the relatively low CD4 cell counts and immune activation status that we observed at the genital tissue level for some of the HIV− FSW women may reflect and represent this rare phenotype of HIV-resistant individuals. Whether this could be an inherent or induced immune status, or not representative of other activation markers, is not known. Nevertheless, the upregulated inflammatory parameters observed in the HIV+ group was not merely interpreted as a result of sex work and possible HIV exposure itself because the HIV− FSW control group had a comparable demographic profile including medical and sexual history.

The number of activated CD4+ T cells, as assessed in cervical mononuclear cell preparations, is increased during mucosal inflammation and may promote HIV replication (39), and the genital HIV RNA level itself is an independent predictor of sexual transmission (40). A recent report on cytobrush-derived cervical cells from HIV+ women suggested that genital CD4 T cell activation was associated with CD4 T cell depletion (41). However, although the proportion of CD3 T cells expressing CD4 was reduced, the total number of inflammatory cells may have been increased in these individuals as clearly shown for HIV-infected women in a previous study on cervical cytobrush samples (33). In our present study, it is thus unclear whether there was a drop in actual CD4 T cell numbers or just a shift in the ratio of CD4 and CD8 T cells. We have indeed observed a significant influx of CD8+ T cells in cytobrush-derived cervical cells, as well as of foreskin-derived cells, in HIV+ individuals (CROI 2013 [http://www.ret-roconference.org/], abstracts #349: Impact of Early HIV Infection on CD4+ T Cell Subsets in the Female Genital Tract, McKinnon, L., Nyanga, B., Izulla, P., Kwampona, J., Kimani, M., Mugo, N., et al. and #350: The Impact of HIV Infection on Foreskin T Cell Frequency and Function: Rakai, Uganda, Procter, J., Gray, R., Kigozi, G., Nalugoda, F., Galiwango, R. M., Hirbod, T., et al.). Ideally, both flow cytometry and in situ methods should be combined in a single study to conclusively answer this question. The immune activation seen in cervical cell preparations (33, 41), and in the current study assessing ectocervical tissue samples, suggests that it could be important not only to use antiretroviral drugs that penetrate the genital tract mucosa but also to explore topical anti-inflammatory compounds to lower genital HIV shedding in HIV+ women.

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We thank the study participants and the staff at the Kenyan AIDS Control Project at the University of Nairobi, especially the staff of the Majengo/Pumwani clinics. We also thank Dr. Angela Muliro for clinical assistance, Dr. Qingsheng Li for technical expertise, and Pernilla Petersson for technical assistance.

Disclosures
The authors have no financial conflicts of interest.
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