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Positive Association between HIV RNA and IL-6 in the Genital Tract of Rwandan Women

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Abstract

Infections and inflammation in the genital tract can influence HIV expression or HIV susceptibility. The goal of this study was to determine if significant relationships exist between cytokines and HIV in genital tract secretions from 57 HIV-seropositive Rwandan women. Genital tract secretions were obtained by cervicovaginal lavage (CVL). Ten different cytokines in CVL were measured by multiplex cytometric bead arrays. HIV RNA in CVL and plasma were measured by quantitative PCR. In univariate analysis, genital tract HIV RNA was significantly associated with plasma HIV RNA and several of the cytokines, while in multivariate analysis, genital tract HIV RNA was significantly associated only with plasma HIV RNA and IL-6. This association of IL-6 with HIV RNA levels suggests that IL-6 is an indicator for conditions that induce HIV expression and that IL-6 may contribute to induction of HIV expression in the genital tract.

HIV IS TRANSMITTED predominantly through heterosexual contact and higher levels of HIV in genital secretions are believed to increase the risk of transmission.[1] Strong correlations between levels of HIV in genital secretions and plasma HIV levels have been shown and heterosexual transmission is increased in the setting of higher plasma HIV levels.[2–4] Inflammation or infections are known to increase HIV levels locally in the genital tract and promote HIV transmission. Thus, genital shedding of HIV has been observed in women who have no detectable plasma virus[1] and women with altered vaginal flora, sexually transmitted infections (STI), or genital tract inflammation have higher levels of genital HIV shedding than women without these conditions.[5–7] Treatment of STI reduces HIV-1 shedding.[8,9] While these studies suggest that local inflammation caused by genital infections and/or immune responses to infections increases HIV shedding, the specific immune/inflammatory mediators that are associated with genital HIV shedding have not been well characterized. Identification of such mediators could lead to their use as markers of risk or as targets for preventing HIV transmission. To identify such mediators, we analyzed the relationship between levels of HIV RNA and a panel of 10 cytokines and chemokines in genital secretions from 57 Rwandan women.

The Rwanda Women's Inter-association Study and Assessment (RWISA) is an observational prospective cohort study investigating the effectiveness and toxicity of antiretroviral therapy (ART) and comorbidities in HIV-infected Rwandan women. Between May 15 and November 15, 2005, women were enrolled from community-based contacts. Informed consent was obtained, in Kinyarwanda, in accordance with protocols approved by the Rwandan Ethics Committee and the Institutional Review Board of Montefiore Medical Center, Bronx NY. At each study visit historical information, blood, and genital samples were collected.

Vaginal swabs were collected from the women for wet mount and gram stain for Nugent evaluation.[10] Subsequently, cervicovaginal lavage (CVL) was performed by irrigation of the cervix with 10 ml of nonbacteriostatic sterile saline, followed by aspiration from the posterior fornix. CVL was held on ice until processing, (within 3 h of collection). CVL was gently vortexed to evenly distribute cells before aliquoting into 1-ml portions and freezing at –70°C. Upon thawing, 0.4 ml of CVL was used for HIV RNA measurement while the remaining sample was centrifuged for 30 min at $13{,}000 \times g$. The pellet was used for DNA isolation for quantification of bacteria,[5] while the supernatant material was immunoassayed for cytokines and *Trichomonas vaginalis*.

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TABLE 1. PATIENT CHARACTERISTICS AND QUANTITATIVE GENITAL ORGANISM AND CYTOKINE LEVELS

	Mean ± SD	*Median*	*Range*
Age, years	35.8 ± 6.5	35	25–59
Plasma HIV RNA, $\log_{10}$ copies/ml plasma	3.77 ± 1.48	4.16	1.60–6.29
CVL HIV RNA, $\log_{10}$ copies/ml CVL	1.69 ± 1.76	1.98	0–4.54
CD4 cells/mm^3 blood	271 ± 149	264	52–778
IL-12p70, pg/ml CVL	13 ± 6	14	0–24
TNF-α, pg/ml CVL	6 ± 5	5	0–35
IL-10, pg/ml CVL	8 ± 3	8	0–16
IL-6, pg/ml CVL	71 ± 144	14	0–831
IL-1β, pg/ml CVL	352 ± 837	69	0–4713
IL-8, pg/ml CVL	2695 ± 2947	1525	53–10,000[a]
IP-10, pg/ml CVL	787 ± 1298	217	0–5000[a]
MCP-1, pg/ml CVL	60 ± 112	18	0–547
MIG, pg/ml CVL	503 ± 938	196	0–5000[a]
RANTES, pg/ml CVL	108 ± 342	11	0–1900
Nugent score (0–10 scale)	4.9 ± 2.3	5	0–10

[a]Three of the samples had the maximal detectable IL-8 (10,000 pg/ml), three samples had maximal IP-10 (5000 pg/ml), and one sample had maximal MIG (5000 pg/ml).

HIV RNA in plasma was measured by COBAS TaqMan HIV-1 Test or Amplicor HIV-1 Monitor test v1.5 (both from Roche Diagnostic Corporation, Indianapolis, IN). HIV RNA in all CVL samples was quantified using the Amplicor test with a lower limit of detection of 400 copies/ml.

CVL samples clarified by centrifugation were added to Cytometric Bead Array kits (BD Biosciences, San Jose, CA). The Human Inflammation kit [interleukin (IL)-8, IL-1β, IL-6, IL-10, IL-12, and tumor necrosis factor (TNF)-a] and the Human Chemokine kit (IL-8, RANTES, MIG, MCP1, and IP10) were assayed by FACSCaliber (Becton Dickinson, San Jose, CA). Since IL-8 was measured in two different kits, IL-8 levels from only the inflammation kit were used for analysis. *T. vaginalis* p65 antigen was measured in CVL according to a previously published method[11] using a monoclonal capture antibody to p65 (Biodesign International, Saco, ME), a polyclonal rabbit anti-p65 detection antibody (HyTest Ltd., Turku, Finland), and recombinant p65 (HyTest) as a standard. The sensitivity and specificity of this test are reported as 89% and 97%, respectively.[11]

For statistical analysis, a censored linear regression model was used in the analyses. The cytokine measures, the genital HIV RNA, and the plasma virus load were all $\log_{10}$ transformed in the univariate and multivariate analysis as well as in the correlation analysis.

The median age of the subjects was 35 years (range 25–59) (Table 1). At the time of sample collection, none of the subjects was pregnant, none was menstruating, and four were postmenopausal. The median CD4 cell count was 264/μl (range 52–778), the median plasma HIV RNA was 4.16 $\log_{10}$ copies/ml (range 1.60–6.29), and the median genital tract HIV RNA was 1.98 (range <400–4.54). Five participants were found to be using or had been exposed to antiretroviral drugs at the time that CVL samples were obtained.

Wet mount examination for *T. vaginalis* showed five positive, 51 negative, and one undetermined. ELISA of CVL supernatant for *T. vaginalis* p65 confirmed three of the positives by wet mount and found an additional five. Wet mount examination for clue cells showed two positive and 55 negative. Nugent scoring of gram stains showed 12 subjects with

TABLE 2. SIGNIFICANT UNIVARIATE AND MULTIVARIATE REGRESSION ASSOCIATIONS WITH CERVICOVAGINAL LAVAGE HIV RNA LEVELS CONTROLLING FOR PLASMA HIV LEVEL[a]

	Univariate model		*Multivariate model*	
	Regression coefficient (95% CI)	p	*Regression coefficient (95% CI)*	p
CD4 cells/mm^3 blood	−3.7 (−6.6, −0.85)	0.01		
IL-10, pg/ml CVL	−2.7 (−5.10, −0.32)	0.03		
IL-6, pg/ml CVL	1.04 (0.58, 1.49)	<0.0001	1.04 (0.58, 1.49)	<0.0001
IL-1β, pg/ml CVL	0.71 (0.32, 1.10)	0.0004		
IL-8, pg/ml CVL	0.98 (0.50, 1.45)	<0.0001		
IP-10, pg/ml CVL	1.64 (0.51, 2.76)	0.004		
MCP-1, pg/ml CVL	1.87 (0.50, 3.23)	0.007		
MIG, pg/ml CVL	2.00 (0.82,3.18)	0.0009		
RANTES, pg/ml CVL	2.02 (0.95, 3.09)	0.0002		

[a]All data were log transformed for analysis. Univariate and multivariate analyses were performed controlling for plasma HIV RNA levels.

BV-like flora (score 7–10), 13 with normal flora (score 0–3), 30 with intermediate flora (score 4–6), and two with missing slides.

In analysis of cytokines in CVL samples, IL-8 was present at relatively higher levels than other cytokines (median 1525 pg/ml, Table 1) and was detected in all samples. MIG (median 196) and IP-10 (median 217) were found at intermediate levels and detected in all but three and four samples, respectively. IL-6, IL-1β, MCP-1, IL-12p70, and RANTES were found at somewhat lower levels (medians of 14, 69, 18, 11, and 14, respectively), but were detected in the majority of samples. TNF-a and IL-10 were not detected or detected at very low levels ($<$12 pg/ml) in all but one sample each.

The association of genital tract HIV RNA levels with each of the variables was assessed. Plasma HIV RNA levels were significantly associated with genital tract HIV RNA levels ($p < 0.0001$; coefficient 1.26; 95% confidence interval 0.79, 1.74). In univariate analysis, controlling only for plasma virus levels, genital tract HIV RNA levels were significantly negatively associated with CD4 and IL-10 and positively associated with IL-6, IL-1β, IL-8, ICP10, MCP-1, MIG, and RANTES (Table 2). Other variables were not associated with genital tract HIV levels (other cytokines, Nugent score, and *T. vaginalis* positivity). In a multivariate model, only IL-6 and plasma HIV RNA were significantly associated with genital tract HIV RNA levels.

Since five of the subjects had potential exposure to ART at the time samples were obtained, which could affect genital tract or plasma HIV levels, the data were reanalyzed after their exclusion. Reanalysis showed again that IL-6 ($p < 0.0004$; coefficient 0.85; 95% confidence interval 0.38, 1.32) and plasma HIV RNA ($p < 0.0001$; coefficient 1.71; 95% confidence interval 1.07, 2.45) were significantly associated with genital tract HIV RNA levels.

To determine if IL-6 levels were associated with infections or inflammation, IL-6 levels in *Trichomonas*-positive and -negative subjects were compared as well as in subjects with Nugent 0-3 versus Nugent 7-10. There was no significant difference in IL-6 between the groups. However, the subjects with absent, rare, or few neutrophils observed on Nugent slides had a median IL-6 of 12 pg/ml while subjects with moderate or many neutrophils had a median IL-6 level of 23 pg/ml ($p = 0.059$, Mann–Whitney).

In this study we found significant univariate associations between genital tract HIV RNA levels and a panel of cytokines, but a multivariate model indicated that only IL-6 was independently associated with genital tract HIV RNA. The cross-sectional nature of the study does not allow inference of a cause and effect relationship between IL-6 and HIV RNA shedding. However, previous *in vitro* studies show that proinflammatory cytokines including IL-1, TNF-α, and IL-6 can induce HIV expression in cells.[13] IL-6 has been shown to induce HIV replication by both transcriptional and post-transcriptional mechanisms and can also induce expression of other proinflammatory mediators.[14] A study of women treated for cervical intraepithelial lesions showed that after treatment, there were increased levels of genital HIV, TNF-α, IL-6, and other activation markers in CVL, while plasma HIV levels were not altered.[7] A previous study that also assessed the relationship between IL-6 and HIV in vaginal secretions of women found no significant association.[15] However, this relationship could have been obscured by the use of antiretroviral drugs and in fact an association between antiretrovirals and IL-6 was observed. In the current study, only five women used antiretroviral drugs and exclusion of those women from analysis did not change the relationship between IL-6 and HIV in CVL.

A recent study showed that intravaginal inoculation of imiquimod, a TLR7 agonist, increased susceptibility to SIV transmission in macaques. Imiquimod also increased peak SIV plasma loads in infected animals, and increased proinflammatory cytokine expression, including IL-6, in the vaginal mucosa,[16] but genital SIV levels were not measured in that study. Several groups have proposed measuring cytokines in genital samples to predict the inflammatory effect of microbicides. *In vitro* exposure of epithelial cell monolayers to certain microbicides induces production of IL-6.[17]

A limitation of this study was that we did not have data regarding *Chlamydia* or gonorrhea infection. Such information might have permitted the identification of the infections that contributed to increased levels of cytokines. During menses in HIV-postive women, several cytokines including IL-1β, IL-6, IL-8, and RANTES are reported elevated in vaginal washings.[18] In the current study, none of the CVL samples was collected during menses.

Measuring cytokines in the genital tract has been used as a marker of inflammation and also as a window that may provide insight into pathways that affect HIV expression in the genital tract. The current study suggests that measuring IL-6 is useful in these situations.

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