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Longitudinal Assessment of Systemic and Genital Tract Inflammatory Markers and Endogenous Genital Tract *E. coli* Inhibitory Activity in HIV-Infected and Uninfected Women

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Abstract

Problem—Stability over time of systemic and mucosal immunity and their associations with bacterial vaginosis (BV) and HIV-specific parameters were assessed.

Method of Study—Immune mediators and HIV viral load in plasma and cervicovaginal lavage (CVL), *E. coli* inhibition, and Nugent score were measured at 3 semiannual visits among 94 participants in the Women's Interagency HIV Study. Mixed models identified factors associated with immune mediators.

Results—There was higher *E. coli* inhibition and lower inflammation over time in the genital tract and systemically. BV was consistently associated with higher CVL inflammatory mediators and lower CVL *E. coli* inhibition. HIV-infected women with higher CD4 counts had lower systemic and genital inflammatory mediators and genital HIV shedding was associated with higher CVL inflammatory mediators. Use of antiretroviral therapy (ART) was associated with lower plasma and CVL mediators but higher *E. coli* inhibition.

Conclusions—HIV and BV are linked to inflammation and ART may be associated with improved vaginal health.

Keywords

bacterial vaginosis; female genital tract; HIV; inflammation; mucosal immunity

Introduction

Many systemic inflammatory markers are elevated in HIV-infected individuals and are strongly predictive of morbidity and mortality¹. Inflammation may persist even in those receiving antiretroviral therapy (ART)², possibly due to chronic antigen exposure and immune activation. It is unknown if the genital tract is a site of chronic inflammation as has been described for the gut among HIV-infected individuals³.

Factors other than HIV may contribute to systemic and/or genital inflammation in women, including age, smoking, other persistent viral infections, and the vaginal microbiome. Smoking is associated with higher plasma concentrations of proinflammatory mediators⁴. Bacterial vaginosis (BV), diagnosed by Nugent score ≥ 7 , has been linked to an increase in vaginal concentrations of proinflammatory cytokines in healthy and HIV-infected women^{5, 6}. Among healthy women, those with BV had elevated genital levels of interleukin (IL)-1 α and IL-1 β ⁷. Additionally, in both HIV-infected and HIV-uninfected women, BV was significantly associated with an increase in genital tract IL-1 β and a decrease in secretory leukocyte protease inhibitor (SLPI) compared to women without BV⁸. Thus, when controlling for BV, women with and without HIV might not differ with respect to vaginal cytokine concentrations.

The biological impact of changes in individual cytokines and chemokines is complex as cytokines can have both pro- or anti-inflammatory activity and chemokines may enhance or inhibit HIV. A complementary approach to assess mucosal immunity is to include a functional assay such as the ability of genital secretions to inhibit bacteria and viruses. Prior studies have shown that genital tract secretions provide activity *ex vivo* against *Escherichia coli* (*E. coli*)⁹, herpes simplex virus (HSV)^{10, 11}, and HIV^{12–14}. Endogenous *E. coli* inhibitory activity may be mediated by cytokines, chemokines, antimicrobial peptides, immunoglobulins, host proteolytic enzymes, and molecules secreted by vaginal microbiota. High *E. coli* inhibitory activity is associated with a lactobacillus-dominant vaginal microbiome in HIV-uninfected women¹⁵ and reduced activity is associated with BV⁹. Lower activity was observed in HIV-infected compared to HIV-uninfected women, indicating a link between HIV and *E. coli* inhibitory activity^{16, 17}.

We previously reported cross-sectional results from the Women's Interagency HIV Study (WIHS) Visit 33 in 2011¹⁷ and found that HIV-infected women with high plasma viral load (PVL) had higher levels of genital proinflammatory mediators compared to HIV-infected women with suppressed PVL or to HIV-uninfected controls¹⁷. Specifically, the HIV-infected women with high PVL had higher concentrations of cervicovaginal lavage (CVL) proinflammatory mediators IL-1 α , IL-1 β , MIP-1 α , MIP-1 β and RANTES. High PVL was associated with higher CVL IL-1 β and RANTES, higher Nugent score, and smoking. After adjusting for PVL, HIV genital tract shedding was significantly associated with higher CVL IL-6, IL-1 β , MIP-1 α and RANTES and lower plasma MIP-1 β ¹⁷. Interestingly, plasma cytokines and chemokines, which in prior studies of mostly men were increased in the setting of HIV infection^{18, 19}, were not elevated in our cross-sectional study of women. In fact, plasma MIP-1 β and RANTES were significantly lower in HIV-infected compared to

HIV-uninfected women¹⁷, suggesting that HIV may not induce chronic systemic inflammation in women.

To further understand these associations, we prospectively studied participants in the WIHS to test if soluble immune mediators and *E. coli* inhibitory activity are stable over time and associated with age, smoking, BV, and HIV-specific parameters, such as CD4 count, viral load, and ART use. We selected mediators that have been associated with genital inflammation (IL-1 α , IL-1 β , IL-6, IL-8, IL-12p70, macrophage inhibitory protein (MIP)-1 α , MIP-1 β , regulated on activation, normal T cell expressed and excreted [RANTES], and human neutrophil peptides 1–3 [HNP1–3]) or that have potential anti-inflammatory effects (IL-1 receptor antagonist [IL-1ra] and SLPI)^{17, 20, 21}. We also measured systemic inflammatory biomarkers such as MIP-1 β , RANTES, and added tumor necrosis factor (TNF), C-reactive protein (CRP) and D-dimer, which were not included in the previous cross-sectional study. The *E. coli* assay was selected because it may serve as a biomarker of a healthy mucosal immune environment and the inhibitory activity in CVL is stable after prolonged storage at –80°C¹⁵.

We report here the findings from the longitudinal study, with the *a priori* designated groups of HIV-uninfected, HIV-infected with undetectable PVL (UPVL) and HIV-infected with detectable PVL (DPVL).

Methods

Participants and Sample Collection

Clinical data and specimens were obtained from the WIHS, an ongoing prospective study of HIV-infected and at-risk HIV-uninfected women enrolled through 6 clinical consortia. WIHS data collection and methods have been previously described²². Written informed consent was obtained and the IRB at each of 4 participating WIHS institutions approved the study. At semiannual visits interim history is obtained, blood is collected for CD4 cell count and quantitative HIV-1 RNA, and a pelvic examination is performed. Women are asked to abstain from sex for 48 hours prior to each study visit. ART use is self-reported at each visit. Swabs are obtained from the lateral vaginal wall for Nugent score and wet mount microscopy. CVL is performed with 10 mL of sterile, non-bacteriostatic saline, divided into aliquots and stored at –80°C.

For the present study plasma, vaginal swabs, and CVL were collected from 94 women between 2009–2011 from WIHS visits 31–33. Each participant in this study had 6–12 months of follow-up. Participant selection was based on PVL from a recent WIHS visit and analyses were based on results from the participant's enrollment into this sub-study at Visit 31 or 32 (referred to as baseline for these analyses) divided into 3 groups: (1) HIV-uninfected; (2) HIV-infected with UPVL; and (3) HIV-infected with DPVL. Plasma and CVL were shipped on dry ice to Albert Einstein College of Medicine. Slides were prepared using vaginal swabs, which were also shipped and subsequently gram stained for determination of Nugent score. PVL was quantified using a nucleic acid amplification test with a lower limit of detection (LLOD) of 48 copies/mL (COBAS AmpliPrep/COBAS Taqman HIV-1 test, Roche, Branchburg, NJ). HSV serostatus was determined in plasma for

all subjects not previously identified as seropositive using HerpeSelect 1 and 2 Immunoblot IgG (Focus Diagnostic Cypress, CA). CD4 count was determined using standard flow cytometric methods (NIH/NIAID Flow Cytometry Quality Assessment Program).

Measurement of Plasma and CVL Immune Mediators

Total CVL protein concentration was determined using a MicroBCA assay (Thermo Fisher Scientific, Rockport, IL). CVL concentrations of IL-1 α , IL-1 β , IL-6, IL-8, IL-12p70, IL-1ra, MIP-1 α , MIP-1 β , RANTES, and plasma MIP-1 β and RANTES were quantified using a multiplex proteome array with beads from Millipore using a Luminex 100 instrument (Luminex, Austin, TX) and analyzed using StarStation software (Applied Cytometry Systems, Sacramento, CA). Plasma D-dimer (Beckmann Coulter, Brea, CA) and CRP (Sekisui Diagnostics, Lexington, MA) were measured by turbidimetric immunoassay on an AU400 autoanalyzer. Enzyme-linked immunosorbent assays were used to quantify CVL SLPI (R&D Systems, Inc., Minneapolis, MN), HNP1–3 (Hycult Biotechnology, Uden, the Netherlands), and plasma TNF (American Laboratory Products Company, Salem, NH).

Samples with values below the LLOD were given a numerical value of half the LLOD. The LLOD were as follows: IL-1 α , 3.5 pg/ml; IL-1 β , 0.4 pg/ml; IL-6, 0.3 pg/ml; IL-8, 0.2 pg/ml; IL-12p70, 0.4 pg/ml; IL-1ra, 2.9 pg/ml; MIP-1 α , 3.5 pg/ml; MIP-1 β , 4.5 pg/ml; RANTES, 1 pg/ml; SLPI, 62.5 pg/ml; HNP1–3, 156 pg/ml; D-dimer, 0.2 μ g/ml; CRP, 0.01 mg/l; and TNF, 3 pg/ml. The upper limit of detection (ULOD) for IL-1ra was 10,000 pg/ml.

CVL *E. coli* Inhibitory Activity

CVL activity against *E. coli* was measured as previously described²³. *E. coli* (ATCC strain 4382627) was grown overnight to stationary phase and $\sim 10^9$ colony forming units (cfu)/mL were mixed with CVL or control buffer (20 mM/L of potassium phosphate, 60 mM/L of sodium chloride, 0.2 mg/mL of albumin; pH 4.5) and incubated for 2 hours at 37°C. The mixtures were further diluted in buffer to yield 800–1000 colonies on control plates then plated on agar with trypticase soy broth. Colonies were counted after an overnight incubation at 37°C. Results are reported as the percent reduction in number of cfu relative to control plates.

CVL Viral Load

CVL HIV-1 RNA levels were determined by centrifuging CVL at 700g and quantifying HIV-1 RNA in supernatants using the Abbott m2000 HIV-1 RealTime System (Abbott Molecular, Des Plaines, IL) with a LLOD of 40 copies/mL.

Statistical Analyses

Differences in subject characteristics and immune mediators at the baseline visit were compared using chi-square or Fisher's exact tests for categorical variables and one-way ANOVA, Kruskal-Wallis tests, or Tobit models as appropriate for continuous variables. For HIV-specific characteristics, comparisons were made between HIV-infected with UPVL and DPVL.

Mixed models with a random intercept were used to identify factors associated with immune mediators (outcomes) accounting for repeated measurements taken from the same woman over time. For CVL HNP1–3, CVL SLPI, plasma MIP-1 β , plasma RANTES, and plasma CRP, where all measurements were above the LLOD, β coefficients were obtained from linear mixed models with a random intercept and represent the average difference for each unit of the mediator being analyzed accounting for the other covariates in the model. For CVL IL-1 α , CVL IL-1 β , CVL IL-8, CVL MIP-1 α , CVL RANTES, plasma D-dimer, and plasma TNF, where no more than 25% of measurements were below the LLOD at any visit, β coefficients were obtained from Tobit models with a random intercept. For CVL IL-12p70, CVLIL-6, and CVL MIP-1 β , where more than 25% of measurements were below the LLOD at any visit, mediators were converted to 3-level variables: 0 if undetectable, 1 if detectable but less than the median of the detectable values, and 2 if greater than or equal to the median. CVL IL-1ra, which had more than 25% of measurements above the ULOD at any visit, was converted to a 3-level variable: 0 if less than the median, 1 if greater than the median but less than the ULOD, and 2 if greater than the ULOD. Percent *E. coli* inhibition was converted to a 3-level variable based on tertiles at each visit; 0 if less than the first tertile; 1 if greater than the first tertile but less than the second tertile; 2 if greater than or equal to the second tertile. Nugent scores were categorized as 0 if 0–3 (normal flora), 1 if 4–6 (intermediate flora) or 2 if ≥ 7 (consistent with BV)²⁴. Odds Ratios (ORs) were then estimated from generalized linear mixed models (GLMMs) with a random intercept and a cumulative logit link function. ORs from these GLMMs can be interpreted as the odds of being in group 2 compared to groups 0 and 1 combined (or alternatively, the odds of being in groups 1 and 2 combined versus being in group 0). HIV status, visit number, Nugent score, age and smoking were forced into all mixed models. Sexually transmitted infections (other than HSV and *Trichomonas vaginalis*), vaginal candidiasis, antibiotic and antifungal treatment, and the menstrual cycle/menopausal status, were not considered in any analyses. Separate analyses were performed limited to HIV-infected women to additionally examine effects of ART, CD4 count, PVL, and CVL viral load on immune mediators. Due to sample size limitations, HIV-specific parameters were only retained in the final model if they achieved statistical significance. Smoking, Nugent score, ART, CD4 count, PVL, and CVL viral load were analyzed as time-varying covariates in all models. All analyses were performed using SAS version 9.4 (Cary, NC), without adjustment for multiple comparisons, and a p value <0.05 was considered statistically significant.

Results

Participant Characteristics

Of 94 participants (40 HIV+UPVL, 34 HIV+DPVL, and 20 HIV-uninfected), 79 (84%) had data for 3 visits and 15 (16%) had data for 2 visits. There were no significant differences at baseline between the groups for age, race/ethnicity, education, risk category, hormonal contraceptive use, douching, HSV serostatus, or *Trichomonas vaginalis* (Table I).

At baseline, HIV+UPVL were less likely to be current smokers ($p=0.03$) and HIV+DPVL had higher Nugent scores ($p<0.001$) (Table I). There was no significant difference in the HIV-infected groups with respect to ART use at the baseline visit and 86.5% reported

consistent ART use. Similarly, those not on ART during the study period were also consistent in their non-ART use.

Differences in Soluble Immunity among All Women at Baseline

Immune mediators at the baseline visit differed significantly between the groups for CVL IL-1 α , IL-1 β and IL-8, and plasma RANTES, D-dimer, and TNF (Figure 1). Compared to HIV-uninfected women, HIV+DPVL had higher CVL IL-1 α and IL-1 β , higher plasma D-dimer and TNF, and lower plasma RANTES (Figure 1). There was a trend toward a difference in *E. coli* inhibition with the least activity observed in HIV+DPVL (Table I).

Differences in Soluble Immunity among All Women over Time

Changes in Immune Mediators and *E. coli* Activity over Time—While most plasma and CVL mediators were relatively stable over time among all women, in multivariable models adjusting for HIV status, Nugent score, smoking and age (Table II), there was overall a profile of lower inflammation over time in both the genital tract and systemically, with decreases in the proinflammatory mediators CVL IL-1 α , CVL MIP-1 α , and plasma RANTES, an increase in the anti-inflammatory mediator CVL IL-1ra (which may sometimes be proinflammatory), a decrease in the anti-inflammatory mediator CVL SLPI, and higher *E. coli* inhibition (Table II and Figure 2). For example, CVL IL-1 α levels were significantly lower at visits 33 ($\beta=-0.26$, $p=0.009$) and 32 ($\beta=-0.22$, $p=0.003$) compared to visit 31. The odds of CVL IL-1ra being above the ULOD were significantly higher at visit 32 (OR=2.00, 95% Confidence Interval (CI): 1.03–3.88) compared to visit 31. CVL *E. coli* inhibitory activity also improved over time: the odds of being in the top tertile of *E. coli* inhibitory activity was 2 to 3-fold higher at visits 33 (OR=2.77, 95% CI: 1.44–5.34) and 32 (OR=2.24, 95% CI: 1.18–4.25) compared to visit 31 (Table II).

Effects of HIV, Nugent score and Smoking on Immune Mediators and *E. coli* Activity—HIV+DPVL had consistently higher CVL IL-1 α ($\beta=0.57$, $p=0.003$), plasma TNF ($\beta=0.31$, $p<0.001$), but lower plasma RANTES ($\beta=-0.65$, $p<0.001$) compared to HIV-uninfected women (Table II). Women with BV had consistently higher CVL IL-1 α ($\beta=0.43$, $p<0.001$), CVL IL-1 β ($\beta=0.97$, $p<0.001$), CVL IL-12p70 (OR=3.04, 95% CI: 1.57–5.88), CVL MIP-1 α ($\beta=0.37$, $p<0.01$), plasma RANTES ($\beta=0.13$, $p=0.003$), plasma TNF ($\beta=0.10$, $p=0.012$), and lower CVL *E. coli* inhibition (OR=0.37, 95% CI: 0.19–0.71) compared to women with normal Nugent scores (Table II). Of note, Nugent score worsened over time with the odds of having BV being 2 to 3-fold higher at visits 33 (OR 3.11, 95% CI: 1.36–7.10) and 32 (OR 2.59, 95% CI: 1.16–5.75) compared to visit 31 independent of HIV status, smoking, and age (Figure 2). Current smoking was associated with higher CVL IL-1ra (OR=2.43, 95% CI: 1.07–5.51) and plasma TNF ($\beta=0.12$, $p=0.02$), but lower CVL MIP-1 α ($\beta=-0.20$, $p=0.029$) (Table II).

The effects of HIV, Nugent score, smoking and age on immune mediators and *E. coli* activity were constant over the 3 visits given the absence of statistically significant interactions between visits and these variables. CVL IL-6, IL-8, MIP-1 β , HNP1–3, and plasma CRP and MIP-1 β are not shown in Table II as they did not vary over time and there were no effects of HIV, Nugent score, smoking and age on these mediators.

Differences in Soluble Immunity among HIV-Infected Women Over Time

Changes in Immune Mediators and *E. coli* Activity Over Time—In analyses restricted to HIV-infected women, plasma and CVL mediators were relatively stable over time except for an increase in CVL MIP-1 β (OR=3.68, 95% CI: 1.37–9.86). *E. coli* activity improved over time with significantly greater inhibition at the later visits. Systemically, plasma RANTES decreased significantly over time (Table III).

Effects of HIV and Nugent score on Immune Mediators and *E. coli* Activity—In multivariate models (Table III), women with less immune suppression as indicated by higher CD4 counts (per 100 cells) had consistently lower concentrations of inflammatory mediators in both the genital tract and systemically: CVL IL-1 α (β =−0.04, p =0.039), CVL IL-8 (β =−0.05, p =0.026), CVL RANTES (β =−0.06, p =0.009), plasma D-dimer (β =−0.03, p =0.013), and plasma TNF (β =−0.02, p =0.008). Women with a lack of virologic suppression (DPVL) did not have higher concentrations of inflammatory mediators but had lower concentrations of CVL IL-8 (β =−0.59, p =0.004), CVL MIP-1 α (β =−0.33, p =0.024), CVL HNP1–3 (β =−0.28, p =0.026), and plasma TNF (β =0.20, p <0.001). In contrast, detectable viral load in the genital tract was consistently associated with higher CVL proinflammatory mediators: CVL IL-1 β (β =0.58, p =0.005), CVL IL-8 (β =0.58, p =0.003), CVL IL-12p70 (OR=2.57, 95% CI: 1.20–5.52), CVL MIP-1 α (β =0.57, p <0.001), CVL MIP-1 β (OR=2.83, 95% CI: 1.13–7.08), CVL RANTES (β =0.69, p <0.001), and CVL HNP1–3 (β =0.42, p =0.002).

Women on ART had consistently lower CVL MIP-1 β (OR=0.33, 95% CI: 0.14–0.79) and plasma D-dimer (β =−0.22, p =0.023), but higher CVL *E. coli* inhibition (OR=2.37, 95% CI: 1.20–4.66), and higher plasma MIP-1 β (β =0.18, p =0.001) compared to those not on ART.

In general, higher Nugent scores in HIV-infected women were associated with worse genital tract health with consistently higher CVL IL-1 α , CVL IL-1 β , CVL IL-12p70, and CVL MIP-1 α and lower CVL *E. coli* inhibition (OR=0.35, 95% CI: 0.17–0.70) compared to those with a normal Nugent score. BV was also associated with higher plasma RANTES (β =0.13, p =0.016) and plasma TNF (β =0.09, p =0.038).

The effects of the variables described above were constant over time. CVL IL-6, IL-1 α , SLPI, and plasma CRP are not shown in Table III as they did not vary over time and there were no significant effects of any of the covariates on these mediators.

Discussion

We demonstrated that select genital tract immune measures, including *E. coli* inhibitory activity, varied over time, with most changing in a favorable direction (lower proinflammatory activity, higher anti-inflammatory activity, and greater *E. coli* inhibition) with the exception of SLPI. These differences were independent of changes in Nugent score, smoking, or age in all women and differences in *E. coli* inhibitory activity and plasma RANTES remained significant even after adjustment for HIV-specific factors.

Similar to our cross-sectional study¹⁷, uncontrolled HIV was consistently associated with worse genital tract health, with higher concentrations of CVL IL-1 α , CVL RANTES and

lower CVL *E. coli* activity compared to HIV-uninfected women. Importantly, these findings were consistent over time and for CVL IL-1 α and *E. coli* activity were independent of Nugent score. Similar to prior studies, we found that women with HIV and DPVL had higher plasma TNF compared to HIV-uninfected women^{25, 26}. These systemic effects were also consistent over time. Among HIV-infected women, detectable genital tract viral load predicted higher levels of many proinflammatory mediators. Better immune function with higher CD4 counts was associated with less inflammation both systemically and in the genital tract.

In general, ART use had a consistently favorable effect in the genital tract, with lower CVL MIP-1 β , higher CVL *E. coli* activity, and lower levels of plasma D-dimer, suggesting that ART may restore some changes in the mucosal and systemic immune environments. The association of ART use with higher CVL *E. coli* inhibition may be explained by a direct antibacterial effect²⁷ or by favorable effects of ART on protective vaginal lactobacillus species as demonstrated by the finding that *Lactobacillus jensenii* was associated with lower risk of genital shedding in women using ART²⁸. Higher *E. coli* inhibition found here suggests that restoring some aspects of vaginal health in HIV-infected women is a previously unmeasured benefit of ART.

Similar to prior studies, BV was significantly associated with an increase in genital inflammation^{6, 8} and with less functional health as indicated by lower CVL *E. coli* activity. Therapy with oral metronidazole has been shown to be associated with reduced CVL IL-8, IL-1 β and RANTES⁶ in HIV-infected women with BV, suggesting that treatment is beneficial in reducing genital inflammation. The effect of BV treatment on systemic proinflammatory mediators is unknown, but our finding of higher systemic proinflammatory mediators in women with BV suggests such studies would be informative. Interestingly, we found a proinflammatory association of smoking systemically (plasma TNF), but an anti-inflammatory association in the genital tract (CVL IL-1 α). This finding requires further study.

Unlike prior studies in which men predominated², we did not find a consistent pattern of higher systemic proinflammatory mediators in HIV-infected women, and found similar levels of plasma proinflammatory mediators in HIV-infected compared to HIV-negative women. However, higher CD4 counts were associated with lower plasma D-dimer and TNF, and the systemic effects of ART were mixed, with lower D-dimer, no effect on RANTES, and higher MIP-1 β . Larger longitudinal studies are needed to identify factors that trigger mucosal inflammation and loss in *E. coli* inhibitory activity, and to ascertain patterns of systemic inflammation in HIV-infected women with or without ART, which may differ from those in men.

There are several study limitations, which could potentially account for changes observed over time in immune mediators and CVL *E. coli* inhibition. Prior research has shown that high *E. coli* inhibitory activity is associated with a predominance of *Lactobacillus crispatus*²⁹ and proteomic studies identified Lactobacillus surface proteins as contributing to *E. coli* inhibitory activity in CVL¹⁵. Although we found that Nugent score worsened over time in all women despite improvement in *E. coli* inhibitory activity, healthy vaginal

lactobacillus species were not quantified and could have potentially contributed to the observed improvement in inhibitory activity. Effects of semen and hormones, which were not accounted for in these analyses, could contribute to variability in immune mediators. Unprotected sex has been linked to decreases in inflammatory mediators and antimicrobial peptides³⁰. In addition, concentrations of cytokines, chemokines, and antimicrobials in CVL have been shown to drop midcycle^{31, 32} and the loss of estrogen after menopause may result in the decline of antimicrobial concentrations in vaginal secretions³³. This study also did not include data on human papillomavirus and other sexually transmitted infections.

There were differences in sample storage time and batch effects, however, the storage time was equally distributed among the groups. Therefore, while there may be degradation of some mediators with storage at -80°C it would likely be equal across the groups and unlikely to have a large impact on the results. In addition, while the mediators were not all run on the same plate, good reproducibility of several cytokine concentrations, including IL-1 β , IL-6, and IL-8 has been previously shown across 3 laboratories³⁴. Lastly, we tested many associations, but chose not to adjust for multiple testing because this study is exploratory.

Despite these potential limitations, this study found that HIV-infected women with DPVL had higher proinflammatory mediators in the genital tract and a trend towards lower *E. coli* inhibitory activity compared to HIV-uninfected women, and that these effects in HIV-infected women were mitigated by ART use. Additionally, genital shedding among HIV-infected women was associated with higher proinflammatory mediators. These findings demonstrate that HIV and BV are linked to systemic and genital tract inflammation in women and ART use may be associated with favorable changes in the mucosal immune environment.

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Figure 1.

Mucosal and plasma immune mediators that differed significantly at baseline between HIV-uninfected (HIV-), HIV-infected with undetectable PVL (HIV+UPVL), and HIV-infected with detectable PVL (HIV+DPVL). Bar graphs showing the log-transformed concentrations of CVL and plasma mediators (mean $\pm$ standard error) with pairwise comparisons (* p<0.05, ** p<0.01, *** p<0.001).

Figure 2.

Trends in immune mediators, Nugent score and *E. coli* inhibition in all women using estimates from mixed models. The models for immune mediators and *E. coli* inhibition were adjusted for HIV status, Nugent score, smoking, and age, whereas the model for Nugent score was adjusted for HIV status, smoking, and age. β estimates are shown for the $\log_{10}$ transformation of immune mediators and odds ratios (OR) are shown for Nugent score and % *E. coli* inhibition. All trends shown are significant with $p < 0.05$.

Table I

Demographics and participant characteristics at baseline (visit 31 or 32)

	HIV-uninfected (n=20)	HIV+UPVL (n=40)	HIV+DPVL (n=34)	p value
Age (years) mean $\pm$ SD	47.0 $\pm$ 9.7	50.1 $\pm$ 7.4	46.6 $\pm$ 6.6	0.11
Race/Ethnicity				0.06
White	0 (0.0)	3 (7.5)	3 (8.8)	
Black	8 (40.0)	28 (70.0)	17 (50.0)	
Hispanic	11 (55.0)	9 (22.5)	12 (35.3)	
Other	1 (5.0)	0 (0.0)	2 (5.9)	
Education				0.98
< high school	10 (50.0)	21 (52.5)	17 (51.5)	
high school	10 (50.0)	19 (47.5)	16 (48.5)	
Risk Category				0.43
IVDU	2 (10.5)	8 (20.0)	6 (17.7)	
Heterosexual	6 (31.6)	20 (50.0)	13 (38.2)	
Transfusion	1 (5.3)	0 (0)	1 (2.9)	
No identifiable risk factor	10 (52.6)	12 (30.0)	14 (41.2)	
Current Smoking	13 (65)	14 (35)	21 (61.8)	0.03
Hormonal contraceptive use	1 (5.0)	0	1 (2.9)	0.41
Douching in past 2 days	0	0	1 (2.9)	0.41
HSV-1 seropositive				>0.99
Positive	14 (70.0)	22 (68.8)	23 (69.7)	
Negative	6 (30.0)	10 (31.2)	10 (30.3)	
HSV-2 seropositive				0.20
Positive	15 (75.0)	29 (90.6)	31 (81.2)	
Negative	5 (25.0)	3 (9.4)	3 (8.8)	
<i>Trichomonas vaginalis</i>	1 (5.0)	1 (2.5)	3 (8.8)	0.49
Nugent score	0 [0, 0.75]	1 [0.7.5]	8 [4, 8.5]	<0.001
0–3	18 (90.0)	20 (60.6)	7 (23.3)	
4–6	0 (0)	3 (9.1)	5 (16.7)	
7–10	2 (10.0)	10 (30.3)	18 (60.0)	
% <i>E. coli</i> inhibition	56.4 [7.0, 83.3]	47.6 [4.0, 74.0]	1.0 [–13.0, 52.0]	0.10
Antiretroviral therapy use	NA	29 (72.5)	21 (61.8)	0.33
CD4 count (cells/ μ l)	NA	541 [452, 731]	202 [103, 339]	<0.001
PVL copies/ml	NA	NA	67,300 [730, 193,000]	NA
CVL Viral Load				<0.001
undetectable	NA	23 (95.8)	15 (55.6)	
detectable	NA	1 (4.2)	12 (44.4)	

Categorical variables reported as n (%) and continuous variables reported as median [25th%, 75th%], unless otherwise noted

DPVL=detectable plasma viral load, UPVL=undetectable plasma viral load, IVDU=intravenous drug use, HSV=herpes simplex virus, PVL=plasma viral load, CVL=cervicovaginal lavage, SD=standard deviation

Table II

Multivariate analysis of factors associated with CVL and plasma immune mediators over 3 visits in all women

	Cervicovaginal Lavage (CVL)								Plasma		
	Pro-inflammatory				Anti-inflammatory				Proinflammatory		
	IL-1 α (pg/ml)	IL-1 β (pg/ml)	IL-12p70 (pg/ml)	MIP-1 α (pg/ml)	RANTES (pg/ml)	SLPI (pg/ml)	IL-1ra (pg/ml)	% <i>E. coli</i> inhibition	RANTES (pg/ml)	D-dimer (μ g/ml)	TNF (pg/ml)
Visit											
33	-0.26** (0.09)	-0.20 (0.13)	0.92 (0.49-1.73)	0.06 (0.11)	-0.15 (0.15)	-0.36*** (0.09)	1.41 (0.72-2.74)	2.77** (1.44-5.34)	-0.09** (0.03)	0.02 (0.06)	-0.03 (0.03)
32	-0.22** (0.08)	-0.20 (0.13)	0.74 (0.41-1.37)	-0.29** (0.11)	0.02 (0.14)	-0.18* (0.09)	2.00* (1.03-3.88)	2.24* (1.18-4.25)	-0.10*** (0.03)	0.04 (0.06)	-0.02 (0.03)
31 (reference)											
HIV Status											
HIV+DPVL	0.57** (0.19)	0.08 (0.25)	1.13 (0.49-2.59)	-0.001 (0.13)	0.48 ^a (0.26)	-0.22 (0.14)	1.80 (0.57-5.75)	0.59 (0.26-1.34)	-0.65*** (0.18)	0.20 (0.13)	0.31*** (0.08)
HIV+UPVL	0.33 ^a (0.18)	0.04 (0.24)	1.04 (0.47-2.31)	0.01 (0.12)	0.11 (0.25)	0.04 (0.13)	2.32 (0.75-7.14)	0.95 (0.44-2.07)	-0.43* (0.18)	0.06 (0.13)	0.02 (0.08)
HIV-(reference)											
Nugent Score											
7-10	0.43*** (0.11)	0.97*** (0.17)	3.04** (1.57-5.88)	0.37*** (0.11)	0.12 (0.18)	0.02 (0.10)	1.04 (0.47-2.32)	0.37** (0.19-0.71)	0.13** (0.04)	0.04 (0.08)	0.10* (0.04)
4-6	0.10 (0.13)	0.61** (0.20)	2.00 ^a (0.89-4.50)	0.62*** (0.13)	0.19 (0.21)	-0.14 (0.12)	1.70 (0.63-4.57)	0.42* (0.18-0.96)	-0.01 (0.04)	0.04 (0.09)	0.08 ^a (0.04)
0-3 (reference)											
Current Smoking (Yes vs No)	0.07 (0.13)	-0.10 (0.17)	0.84 (0.46-1.52)	-0.20* (0.09)	-0.13 (0.18)	0.05 (0.10)	2.43* (1.07-5.51)	1.03 (0.57-1.85)	0.08 (0.07)	-0.05 (0.09)	0.12* (0.05)
Age (per 5 years)	-0.08 ^a (0.04)	-0.02 (0.06)	1.10 (0.91-1.33)	0.06* (0.03)	0.03 (0.06)	-0.21*** (0.03)	0.95 (0.73-1.25)	1.07 (0.88-1.29)	0.02 (0.04)	0.004 (0.03)	0.05** (0.02)

Each column represents a separate mixed model with the mediator as the outcome.

Odds Ratios (95% Confidence Interval) are reported for CVL IL-12p70, CVL IL-1ra and % *E. coli* inhibition. β (standard error) are reported for the log10 transformation of all other outcome variables^a0.05 < p < 0.1;

* p < 0.05;

DPVL = detectable plasma viral load; UPVL=undetectable plasma viral load

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;100.0 > d

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Table III

Multivariate analysis of factors associated with CVL and plasma immune mediators over 3 visits in HIV-infected women

	Cervicovaginal Lavage							Plasma					
	IL-1 α (pg/ml)	IL-1 β (pg/ml)	IL-8 (pg/ml)	IL-12p70	MIP-1 α (pg/ml)	MIP-1 β	RANTES (pg/ml)	HNPI-3 (pg/ml)	% <i>E. coli</i> inhibition	MIP-1 β (pg/ml)	RANTES (pg/ml)	D-dimer (μ g/ml)	TNF (pg/ml)
Visit													
33	-0.17 ^a (0.10)	-0.13 (0.19)	-0.01 (0.16)	0.72 (0.31–1.67)	0.20 (0.14)	1.68 (0.63–4.49)	-0.03 (0.17)	0.13 (0.12)	3.39 ^{**} (1.55–7.42)	0.02 (0.03)	-0.09 ^{**} (0.03)	0.02 (0.07)	-0.02 (0.03)
32	-0.10 (0.09)	-0.28 (0.19)	-0.16 (0.16)	0.45 ^a (0.20–1.04)	-0.24 ^a (0.14)	3.68 [*] (1.37–9.86)	-0.03 (0.17)	-0.01 (0.12)	3.19 ^{**} (1.48–6.85)	0.03 (0.03)	-0.09 ^{**} (0.03)	0.03 (0.07)	0.002 (0.03)
31 (reference)													
Nugent Score													
7–10	0.33 ^{**} (0.12)	0.81 ^{***} (0.20)	0.31 ^a (0.17)	2.47 [*] (1.16–5.27)	0.30 [*] (0.14)	1.01 (0.41–2.50)	0.21 (0.18)	0.15 (0.12)	0.35 ^{**} (0.17–0.70)	-0.08 ^a (0.04)	0.13 [*] (0.05)	-0.04 (0.09)	0.09 [*] (0.04)
4–6	0.07 (0.14)	0.54 [*] (0.24)	0.41 [*] (0.21)	2.19 ^a (0.86–5.58)	0.44 ^{**} (0.16)	3.92 [*] (1.29–11.86)	0.58 ^{**} (0.22)	0.07 (0.14)	0.55 (0.22–1.36)	-0.04 (0.05)	-0.02 (0.05)	0.05 (0.10)	0.10 [*] (0.05)
0–3 (reference)													
Current Smoking (Yes vs No)	0.07 (0.14)	0.06 (0.19)	0.11 (0.17)	1.04 (0.54–2.03)	0.03 (0.13)	0.65 (0.29–1.48)	0.05 (0.18)	0.04 (0.11)	1.06 (0.57–1.98)	0.01 (0.05)	0.08 (0.07)	0.07 (0.09)	0.11 [*] (0.05)
Age (per 5 years)	-0.08 (0.06)	0.13 ^a (0.07)	0.08 (0.06)	1.16 (0.92–1.45)	0.18 ^{***} (0.04)	1.32 ^a (0.99–1.78)	0.06 (0.06)	0.13 ^{**} (0.04)	0.99 (0.80–1.24)	0.03 (0.02)	0.08 (0.06)	0.01 (0.03)	0.02 (0.02)
CD4 (per 100 cells)	-0.04 [*] (0.02)	--	-0.05 [*] (0.02)	--	--	--	-0.06 ^{**} (0.02)	--	--	--	--	-0.03 [*] (0.01)	-0.02 ^{**} (0.01)
Detectable PVL	--	--	-0.59 ^{**} (0.20)	--	-0.33 [*] (0.14)	--	--	-0.28 [*] (0.13)	--	--	--	--	0.20 ^{***} (0.05)
Detectable CVL VL	--	0.58 ^{**} (0.20)	0.58 ^{**} (0.19)	2.57 [*] (1.20–5.52)	0.57 ^{***} (0.16)	2.83 [*] (1.13–7.08)	0.69 ^{***} (0.18)	0.42 ^{**} (0.13)	--	--	--	--	--
ART (Yes vs No)	--	--	--	--	--	0.33 [*] (0.14–0.79)	--	--	2.37 [*] (1.20–4.66)	0.18 [*] (0.05)	--	-0.22 [*] (0.94)	--

Each column represents a separate mixed model with the mediator as the outcome.

Odds Ratios (95% Confidence Interval) are reported for CVL IL-12p70, CVL MIP-1 β and % *E. coli* inhibition. β (standard error) are reported for the log10 transformation of all other outcome variables

Due to sample size limitations, HIV-specific variables were allowed to drop out of any model if they were not statistically significant. Thus "--" indicates that variable was not retained in the specified model.

^a 0.05 < p < 0.1;

PVL = plasma viral load, CVL VL = cervicovaginal viral load, ART = antiretroviral therapy

p < 0.0001

p < 0.01
**

p < 0.05
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