Published in final edited form as:

J Acquir Immune Defic Syndr. 2016 February 1; 71(2): 155–162. doi:10.1097/QAI.0000000000000823.

Systemic Immune Activation and HIV Shedding in the Female Genital Tract

LY Spencer¹, S Christiansen², CH Wang¹, WJ Mack², M Young⁶, HD Strickler³, K Anastos³, H Minkoff⁴, M Cohen⁷, RM Greenblatt⁵, R Karim², E Operskalski¹, T Frederick¹, JD Homans¹, A Landay⁸, and A Kovacs¹

¹Maternal, Child and Adolescent Center for Infectious Diseases and Virology, Department of Pediatrics, Division of Infectious Disease, University of Southern California Keck School of Medicine, Los Angeles, CA

²Department of Preventive Medicine, University of Southern California Keck School of Medicine, Los Angeles, CA

³Departments of Medicine and Epidemiology and Population Health, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx NY

⁴Maimonides Medical Center and SUNY Downstate, Brooklyn, NY, USA

⁵University of California, San Francisco Schools of Pharmacy and Medicine, San Francisco, CA

⁶Georgetown School of Medicine, Washington DC, USA

⁷Departments of Medicine, Rush University and Cook County Bureau of Health, Chicago, Illinois

⁸Rush University Medical Center, Chicago, Illinois

Abstract

Background—Plasma HIV RNA is the most significant determinant of cervical HIV shedding. However, shedding is also associated with sexually transmitted infections (STIs) and cervical inflammation. The mechanism by which this occurs is poorly understood. There is evidence that systemic immune activation promotes viral entry, replication and HIV disease progression. We hypothesized that systemic immune activation would be associated with an increase in HIV genital shedding.

Methods—Clinical assessments, HIV RNA in plasma and genital secretions, and markers of immune activation (CD38+DR+ and CD38–DR–) on CD4+ and CD8+ T cells in blood were evaluated in 226 HIV+ women enrolled in the Women’s Interagency HIV Study. There were 569 genital evaluations of which 159 (28%) exhibited HIV RNA shedding, defined as HIV viral load > 80 copies/ml. We tested associations between immune activation and shedding using generalized estimating equations with logit link function.

Correspondence to: LaShonda Spencer, MD, Maternal, Child and Adolescent Center for Infectious Diseases and Virology, Department of Pediatrics, Division of Infectious Disease, University of Southern California Keck School of Medicine, 1000 S Fremont Ave, Unit 62, A10, Ste 10220, Alhambra, CA 91803. 626 457-5820/ 626 457-4003(fax). lspencer@usc.edu.

Presented at: Conference on Retroviruses and Opportunistic Infections 2008, Boston, MA (Poster 674, abstract T-136)

Results—In the univariate model, higher levels of CD4+ and CD8+ T cell activation in blood were significantly associated with genital tract shedding. However, in the multivariate model adjusting for plasma HIV RNA, STIs and genital tract infections, only higher levels of resting CD8+ T cells (CD38–DR–) were significantly inversely associated with HIV shedding in the genital tract (OR=0.44, 95% CI= 0.21–0.9, P= 0.02).

Conclusion—The association of systemic immune activation with genital HIV shedding is multifactorial. Systemic T cell activation is associated with genital tract shedding in univariate analysis but not when adjusting for plasma HIV RNA, STIs and genital tract infections. In addition, women with high percentage of resting T cells are less likely to have HIV shedding compared to those with lower percentages. These findings suggest that a higher percentage of resting cells, as a result of maximal viral suppression with treatment, may decrease local genital activation, HIV shedding, and transmission.

Introduction

HIV RNA is detected in the genital tract of 40–80% of HIV-infected women with detectable plasma viral loads^{1–4}. Several investigators have also shown that shedding of HIV in the genital tract can occur in 20–30% of women with low or undetectable plasma HIV RNA^{5,6}. Our previous studies and those of others have demonstrated that HIV genital tract shedding is most closely related to higher plasma HIV RNA levels¹, but is also associated with the presence of sexually transmitted infections (STI) and cervical inflammation^{7–9}, and the vaginal microbiome^{10,11}. In the era of highly active antiretroviral therapy (HAART), the likelihood of genital shedding is significantly lower in treated women^{1,12,13}. While genital shedding decreased rapidly after antiretroviral (ARV) therapy initiation in one prospective observational study, suppression of genital HIV shedding was not complete¹². We have shown that although potent ARV therapy was associated with a significant decrease in genital tract shedding, 59% of women receiving potent ARV therapy still exhibited genital tract shedding and 16% of these women had shedding even when plasma RNA was <500 (50–500) copies/mL¹.

Generalized immune activation is the hallmark of HIV infection and is associated with clinical outcome and impacts mucosal immunity. Our current study aimed to evaluate the impact of T cell activation in blood, as measured by the expression of CD38 and DR on CD4+ and CD8+ T cells, on genital tract HIV shedding. We measured HIV viral load in paired blood and cervico-vaginal lavage (CVL) samples, and blood immune activation markers by flow cytometry in a cohort of HIV-infected women. We hypothesized that systemic immune activation would be associated with an increase in HIV genital shedding.

Materials and Methods

Study population

Subjects were a subset of the 2,059 HIV-infected women enrolled in the Women's Interagency HIV Study (WIHS). Women were enrolled during 1994–1995 or 2001–2002 at 6 sites in the United States. WIHS methods and baseline cohort characteristics have been described previously¹⁴. This sub-group consists of 226 women enrolled in WIHS during

1994–1995 who had at least one quantitation of HIV in paired plasma and genital tract specimens available for evaluation from November 1994 through September 2001.

Clinical Data

Women enrolled in WIHS were monitored at 6-month intervals. At baseline (entry into the cohort) and subsequent semi-annual visits, standardized interviews collected demographic, clinical and historical data, physical and pelvic examinations were performed and clinical and research specimens were obtained, including blood, Papanicolaou (Pap) smear and genital tract specimens. The gynecologic exam consisted of visual inspection and speculum examination for lesions and discharge. CVL was collected after a lavage was performed using 10 mL of sterile, non-bacterostatic saline into the vagina and against the cervical os and then aspirating from the posterior vaginal fornix.

Laboratory Procedures

Serology—Baseline serology for herpes simplex virus 1 (HSV-1) and herpes simplex virus 2 (HSV-2) was performed with Western blot in one central laboratory (University of Washington). Syphilis testing was done at each local clinical laboratory using the rapid plasma reagin test.

Viral load determination—Plasma HIV viral load (plasma viral load) quantitation was performed in laboratories participating in the DAIDS Viral Quality Assurance (VQA) Program and who were CLIA-certified as required by DAIDS. Plasma viral load was measured real-time using isothermal nucleic acid sequence-based amplification. Initial tests used NucliSense (Organon Teknika Corp., Durham, NC, with a lower limit of detection [LLD] 80 copies/mL). After 2007, it was measured using COBAS AmpliPrep/COBAS TaqMan HIV - 1 Test (Roche Molecular Systems, Branchburg, NJ, with LLD 48 copies/mL). HIV RNA testing of CVL for all visits was performed using the NucliSens assay (LLD 80 copies/mL). HIV genital shedding was defined as HIV RNA > 80 copies/mL.

Flow cytometry—Lymphocyte subset analyses were performed real-time using standard flow cytometric clones and techniques in laboratories that were certified by the NIAID Immunology Quality Assurance Program as previously reported¹⁵. Women were evaluated for expression of activation of CD4+ and CD8+ T cells in either fresh whole blood collected in EDTA tubes using three-color flow cytometry or frozen PBMCs using three or four-color flow cytometry as reported elsewhere^{16–18}. The following fluorochrome-conjugated antibodies were used: anti- CD3, -CD4, -CD8, -HLA-DR, and -CD38 (Becton Dickinson Immunophenotyping Kit II; San Jose, CA). Flow cytometric analysis was performed using a FACSCalibur flow cytometer utilizing CELLQuest software (Becton Dickinson; San Jose, CA).

Genital Tract evaluations—CVL specimens were collected for saline and potassium hydroxide preparation for microscopy, pH, candida culture, gram stain for bacterial vaginosis (BV), swabs for gonorrhea/chlamydia nucleic acid detection test (baseline only), trichomonas by wet mount or culture, and Pap smear. Swabs for HSV culture and syphilis screening were obtained if ulcer/vesicle/fissure was present. Inflammation (leukocytes on

Pap smear) and inflammation-associated cellular changes (cellular changes found with inflammation) including basophilic cytoplasm; enlarged unevenly sized nuclei; enlarged, irregular, or multiple nucleoli were ascertained from Pap smear. Pap smears were read in a central laboratory (Kyto Diagnostics, New York, NY). Candidiasis was diagnosed by the presence of pseudohyphae or positive culture. Gram stains were interpreted for BV in a central laboratory (University of Washington) using the Nugent score criteria, with categorization as normal (0–3), intermediate (4–6), or consistent (7–10)¹⁹. Trichomoniasis was diagnosed by visualization of motile trichomonads on wet mount or with positive culture. Human papillomavirus (HPV) was identified by polymerase chain reaction in two central laboratories²⁰.

Statistical Analysis

Women in the study were classified as “shedders” if they had at least one HIV shedding visit (CVL viral load >80 copies/mL) and as “non-shedders” if they never shed HIV.

Demographic and clinical characteristics at study enrollment (baseline) were compared between HIV shedders and non-shedders by Chi-square tests. Characteristics evaluated at baseline and subsequent visits included age at visit, race/ethnicity (white, African American, Hispanic, or other), ARV therapy used since the last visit (no therapy, monotherapy, combination, or HAART), plasma viral load level (< 80, 81–1,000, 1,001–10,000, 10,001–100,000, >100,000 copies/mL), CD4 cell count (< 200, 201–350, 351–500, >500 cells/mm³), number of lifetime male sex partners, BV gram stain score (normal: 0–3, intermediate: 4–6, or consistent: 7–10), candidiasis, chlamydia, gonorrhea, HPV, HSV, syphilis, trichomonas, cervical lesions, vaginal pH (<4.5, 4.5–5.4, 5.5), inflammation and inflammation-associated cellular change.

To assess the relationship between systemic T cell activation and genital infections, generalized estimating equation models with identity link function and an exchangeable correlation matrix were used to account for multiple visits per subject. Percentages of CD4+ and CD8+ T cells in blood that were DR+CD38+ or DR–CD38– were treated as the continuous dependent variables. Adjusting for plasma viral load, the analyses were performed separately for each of the variables used to indicate genital infection in a previous study²¹. Plasma viral load was treated as a continuous independent variable and was log₁₀ transformed to reduce positive skew. Chlamydia, gonorrhea, syphilis, HSV-1 and HSV-2 were measured at baseline and were modeled as fixed variables. HPV, candidiasis, Trichomonas vaginalis, BV gram stain score, inflammation, inflammation-associated cellular changes, cervical lesions, and vaginal pH, which were obtained at each visit, were modeled as time-dependent variables.

The relationship between systemic T cell activation and HIV shedding in the genital tract was assessed using generalized estimating equations with logit link function and an exchangeable correlation matrix. The dependent variable was whether HIV shedding was present in the genital tract at a given visit. The immune markers of interest, CD38+DR+ and CD38–DR– on both CD4 and CD8 T cells, were categorized by tertiles. The longitudinal associations were first evaluated univariately (not adjusting for other covariates) and then were adjusted for HIV plasma viral load (log₁₀ transformed). The associations were also

assessed by adjusting for HIV viral load and markers of genital infections that were found significantly associated with immune activation ($P<0.05$). Vaginal pH was not included in these adjustments as it may result in over adjustment as a marker for genital infections. Associations were presented as odds ratios (OR) with 95% confidence intervals. STATA software (version 11.2) was used for all statistical analyses.

Results

Study population

The 226 HIV-positive women in this study had 569 genital evaluations with a median of 2.5 visits per women (range: 1–6 visits); women exhibited HIV genital shedding on 159 (28%) of these visits. There were few differences in demographic, virologic, behavioral, and clinical characteristics at WIHS enrollment, between the non-shedders (50.9 %, $n=115$) and shedders (49.1%, $n=111$) (Table 1). The HIV shedders tended to be younger, with a median age of 34 years vs. 37 years for non-shedders ($P=0.03$). Among the HIV shedders, African Americans accounted for 58.6%, Hispanics 20.7% and Whites 19.8%. At baseline, only 5% of all women had plasma viral load < 80 copies/mL. The shedders tended to have higher viral loads at baseline (median: 17,500copies/mL, IQR: 4,400–76,000) vs. nonshedders (median: 15,051 copies/mL, IQR: 1,400–53,000), although this was not statistically significant ($P=0.07$). The median CD4 cell count was similar in both groups, with about one-third in each group having CD4 cell counts > 500 cells/mm³. Approximately half of the women were not receiving any ARV therapy at baseline (50% vs. 40%, non-shedders vs. shedders), and only one woman in the shedder cohort was on HAART at enrollment (0.9%). The median vaginal pH was 5 for both non-shedders and shedders. Very few women had evidence of active HSV infection by culture (0.9% in both groups, data not shown), chlamydia (0% vs. 0.9%, non-shedders vs. shedders), and gonorrhea (0% in both groups) at enrollment. Shedders were more likely to have cervical inflammation (leukocytes on Pap smear) than non-shedders (20.6 % vs. 8.9 %, respectively, $P=0.02$). Other common genital infections including BV, HPV (all types), and trichomonas did not differ by group. A large percent of women in both cohorts had evidence of HSV seropositivity: HSV- 1 (79% vs. 80%, non-shedders vs. shedders), HSV- 2 (76% vs. 80%, non-shedders vs. shedders). Few women in either cohort had cervical lesions, candidiasis, syphilis or inflammation-associated cellular changes.

Immune activation markers and genital infections

Each of the immune markers was analyzed for their association with cervical inflammation and STIs (Table 2). Adjusting for plasma viral load, CD4+CD38+DR+ was associated with HSV-1 seropositivity ($\beta=3.18$, SE= 1.52, $P=0.04$) and HPV infection ($\beta=2.2$, SE=1.04, $P=0.04$). The presence of *Trichomonas vaginalis* was associated with lower levels of resting CD8+ (CD38–DR–) T cells ($\beta= -2.19$, SE=0.72, $P=0.002$) and candidiasis was associated with lower levels of resting CD4+ (CD38–DR–) T cells ($\beta= -2.8$, SE=0.97, $P= 0.004$). Of the other markers of cervical inflammation, only vaginal pH 5.5+ ($\beta= 2.58$, SE=1.04, $P=0.01$) was associated with higher levels of resting CD4+ (CD38–DR–) T cells.

Univariate and multivariate models analyzing the association between the immune markers and genital shedding are shown in Table 3. In the univariate model, high levels of T cell activation (CD38+DR+) were significantly associated with genital shedding for both CD8+ T cells (OR=3.52, 95% CI= 2.05–6.03, $P < 0.001$ for highest vs. lowest tertile) and CD4+ T cells (OR=4.31, 95% CI= 2.35–7.91, $P < 0.001$ for highest vs. lowest tertile). Conversely, more resting cells (CD38–DR–) were associated with less shedding for both CD4+ and CD8+ T cells ($P < 0.001$ for both) and these associations remained significant after adjusting for plasma viral load. However, after adjusting for plasma viral load and those significant genital co-factors measured at every visit including candidiasis, HPV, and trichomoniasis, only higher levels of resting CD8+ (CD38–DR–) T cells (OR=0.44, 95% CI= 0.21–0.9, $P = 0.02$ for highest vs. lowest tertile) was significantly inversely associated with presence of HIV shedding in the genital tract.

Discussion

This is one of the largest prospective studies to evaluate the association between systemic T cell activation and genital HIV shedding. This study has several notable findings. First, in our unadjusted model systemic T cell activation was significantly associated with genital tract shedding as supported by other studies²². However, when controlling for HIV RNA alone and with STIs/genital tract infections, the association of systemic immune activation and genital shedding was lost. Second, while we did not see an association with specific genital infections and HIV shedding at baseline, cervical inflammation was more common in the HIV shedder cohort. Third, when analyzing the association of STI, genital tract infections, cervical inflammation, and immune activation, we found that only HSV-1 seropositivity and HPV infection were associated with CD4 activation, but not CD8 activation. Our findings are consistent with other studies which have shown that subclinical or asymptomatic herpes infection and co-infection with HPV are associated with increased plasma and genital HIV viral loads in addition to systemic and/or local immune activation²⁰. Finally, and perhaps most importantly, when adjusted for plasma viral load, we found that women with higher percentages of non-activated, resting CD4 and CD8 T cells (CD38–DR–) were significantly less likely to have genital tract shedding. This held true for CD8 resting cells in our fully adjusted model which included significant genital tract infections.

Activated T cells are necessary for HIV replication and are more likely to have HIV reservoirs and replication competent virus than resting T cells. Activation promotes virus entry, provirus integration into host cell DNA, and viral RNA transcription^{23–25} and predicts adverse prognosis for HIV-infected patients^{26–28}. Our findings support results of previous studies suggesting that systemic immune activation levels may reflect local activation levels which may promote genital tract HIV replication^{22,25,29}. In one small study, investigators showed that the presence of immune activation in the cervix was independently associated with genital HIV shedding and that cervical immune activation can be predicted by immune activation in the blood²². Although we did not specifically look at activation of T cells in the genital mucosa, we did find that plasma activation levels in the unadjusted models and levels of resting T cells in the fully adjusted models were associated with shedding.

These data suggest that although levels of systemic activation and HIV replication are reflected in the genital tract, local factors such as STIs, cervical inflammation and infection may contribute to local activation of T cells and replication of HIV in the genital tract. However, when there are more resting CD8 T cells circulating, local genital tract HIV replication may be diminished which underscores the importance of achieving maximal viral suppression with ARV therapy with the aim of achieving higher levels of resting T cells.

Our study has several limitations. First, we may have underestimated the percentage of activated T cells as we defined activation as co-expression of both CD38 and HLA-DR and did not include T cells that express CD38 or HLA-DR alone. Second, our study was not designed to evaluate the impact of local genital immune activation on systemic activation. In addition, we did not sample the genital tract at the same time during the menstrual cycle, nor did we evaluate the levels of local inflammatory markers and cytokines as done in other studies^{22,30}. However, it is possible that similar to gut-associated T cell depletion and immune activation^{31–33}, HIV replication in the genital tract results in depletion of mucosal T cells and translocation of bacterial products resulting in systemic immune activation. In fact, Bull et al demonstrated that an increase in local inflammatory cytokines/chemokines and genital tract HIV shedding was associated with a depletion in CD4 T-cells (FOXP3+)³⁰ in the genital tract although it is unclear what effect this had on systemic immune activation. Alternatively, it is possible that cells that are activated prior to circulating through the female genital tract may increase local activation through secretion of cytokines and thereby HIV replication locally. In addition, protective factors within the vaginal microbiome, such as H₂O₂-producing lactobacillus, may inhibit viral replication and local immune activation³⁴ and thus systemic immune activation.

In conclusion, our study suggests that preventing or controlling systemic immune activation by suppressing HIV viral replication and/or genital inflammation and treating co-infections may decrease the risk of genital immune activation and local HIV replication, and therefore decrease the risk of HIV transmission to partners and offspring. Having higher percentages of resting T cells, which is seen with maximal viral suppression with treatment, may also decrease the likelihood of mucosal replication and in turn, transmission. Further studies to better characterize the associations and causality are warranted.

Acknowledgments

This study was funded by the National Institute of Allergy and Infectious Diseases Grant AI052065 (A.K.) (R01 and R56). Data in this manuscript were collected by the Women's Interagency HIV Study (WIHS). The contents of this publication are solely the responsibility of the authors and do not represent the official views of the National Institutes of Health (NIH). WIHS (Principal Investigators): UAB-MS WIHS (Michael Saag, Mirjam-Colette Kempf, and Deborah Konkle-Parker), U01-AI-103401; Atlanta WIHS (Ighovwerha Oforokun and Gina Wingood), U01-AI-103408; Bronx WIHS (Kathryn Anastos), U01-AI-035004; Brooklyn WIHS (Howard Minkoff and Deborah Gustafson), U01-AI-031834; Chicago WIHS (Mardge Cohen), U01-AI-034993; Metropolitan Washington WIHS (Mary Young), U01-AI-034994; Miami WIHS (Margaret Fischl and Lisa Metsch), U01-AI-103397; UNC WIHS (Adaora Adimora), U01-AI-103390; Connie Wofsy Women's HIV Study, Northern California (Ruth Greenblatt, Bradley Aouizerat, and Phyllis Tien), U01-AI-034989; WIHS Data Management and Analysis Center (Stephen Gange and Elizabeth Golub), U01-AI-042590; Southern California WIHS (Alexandra Levine and Marek Nowicki), U01-HD-032632 (WIHS I – WIHS IV). The WIHS is funded primarily by the National Institute of Allergy and Infectious Diseases (NIAID), with additional co-funding from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), the National Cancer Institute (NCI), the National Institute on Drug Abuse (NIDA), and the National Institute on Mental Health (NIMH). Targeted supplemental funding for specific projects is also provided by the National Institute of Dental and Craniofacial Research

(NIDCR), the National Institute on Alcohol Abuse and Alcoholism (NIAAA), the National Institute on Deafness and other Communication Disorders (NIDCD), and the NIH Office of Research on Women's Health. WIHS data collection is also supported by UL1-TR000004 (UCSF CTSA) and UL1-TR000454 (Atlanta CTSA).

Conflicts of Interest and Source of funding: This study was supported by grant RO1 AI052065 (A.K.) from the National Institute of Allergy and Infectious Diseases and grant R01 CA85178 (H.S) from National Cancer Institute.

Reference

1. Kovacs A, et al. Determinants of HIV-1 shedding in the genital tract of women. *Lancet*. 2001; 358:1593–1601. [PubMed: 11716886]
2. Khopkar P, Sane S, Bichare S, Verma A, Kulkarni S. Association of plasma viremia with HIV-1 RNA levels in cervicovaginal lavage secretions in HIV-1 seropositive ART naïve women from Pune, India. *J. Clin. Virol*. 2013; 58:730–732. [PubMed: 24238888]
3. Natividad-Villanueva GU, et al. Human immunodeficiency virus in plasma and cervicovaginal secretions in Filipino women. *Int J STD AIDS*. 2003; 14:826–829. [PubMed: 14678591]
4. Uvin SC, Caliendo AM. Cervicovaginal human immunodeficiency virus secretion and plasma viral load in human immunodeficiency virus-seropositive women. *Obstet Gynecol*. 1997; 90:739–743. [PubMed: 9351756]
5. Spinillo A, et al. Factors associated with nucleic acids related to human immunodeficiency virus type 1 in cervico-vaginal secretions. *BJOG*. 2001; 108:634–641. [PubMed: 11426900]
6. Debiaggi M, et al. Viral excretion in cervicovaginal secretions of HIV-1-infected women receiving antiretroviral therapy. *Eur. J. Clin. Microbiol. Infect. Dis*. 2001; 20:91–96. [PubMed: 11305478]
7. Ghys PD, et al. The associations between cervicovaginal HIV shedding, sexually transmitted diseases and immunosuppression in female sex workers in Abidjan, Côte d'Ivoire. *AIDS*. 1997; 11:F85–F93. [PubMed: 9342059]
8. Kreiss J, et al. Association between cervical inflammation and cervical shedding of human immunodeficiency virus DNA. *J. Infect. Dis*. 1994; 170:1597–1601. [PubMed: 7996003]
9. Kovacs A, et al. HIV-1 RNA in plasma and genital tract secretions in women infected with HIV-1. *J. Acquir. Immune Defic. Syndr*. 1999; 22:124–131. [PubMed: 10843525]
10. Borgdorff H, et al. Lactobacillus-dominated cervicovaginal microbiota associated with reduced HIV/STI prevalence and genital HIV viral load in African women. *ISME J*. 2014; 8:1781–1793. [PubMed: 24599071]
11. Cone RA. Vaginal microbiota and sexually transmitted infections that may influence transmission of cell-associated HIV. *J. Infect. Dis*. 2014; 210(Suppl 3):S616–S621. [PubMed: 25414415]
12. Graham SM, et al. Initiation of antiretroviral therapy leads to a rapid decline in cervical and vaginal HIV-1 shedding. *AIDS*. 2007; 21:501–507. [PubMed: 17301569]
13. Fiore JR, et al. Correlates of HIV-1 shedding in cervicovaginal secretions and effects of antiretroviral therapies. *AIDS*. 2003; 17:2169–2176. [PubMed: 14523273]
14. Barkan SE, et al. The Women's Interagency HIV Study. WIHS Collaborative Study Group. *Epidemiology*. 1998; 9:117–125. [PubMed: 9504278]
15. Nicholson J, Kidd P, Mandy F, Livnat D, Kagan J. Three-color supplement to the NIAID DAIDS guideline for flow cytometric immunophenotyping. *Cytometry*. 1996; 26:227–230. [PubMed: 8889396]
16. Landay A, et al. Correlates of immune activation marker changes in human immunodeficiency virus (HIV)-seropositive and high-risk HIV-seronegative women who use illicit drugs. *J. Infect. Dis*. 2003; 188:209–218. [PubMed: 12854075]
17. Al-Harhi L, et al. Evaluating the impact of hepatitis C virus (HCV) on highly active antiretroviral therapy-mediated immune responses in HCV/HIV-coinfected women: role of HCV on expression of primed/memory T cells. *J. Infect. Dis*. 2006; 193:1202–1210. [PubMed: 16586355]
18. Kovacs A, et al. CD8(+) T cell activation in women coinfecting with human immunodeficiency virus type 1 and hepatitis C virus. *J. Infect. Dis*. 2008; 197:1402–1407. [PubMed: 18444798]
19. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J. Clin. Microbiol*. 1991; 29:297–301. [PubMed: 1706728]

20. Palefsky JM, et al. Cervicovaginal human papillomavirus infection in human immunodeficiency virus-1 (HIV)-positive and high-risk HIV-negative women. *J. Natl. Cancer Inst.* 1999; 91:226–236. [PubMed: 10037100]
21. Homans J, et al. Permissive and Protective Factors Associated With Presence, Level, and Longitudinal Pattern of Cervicovaginal HIV Shedding. *JAIDS Journal of Acquired Immune Deficiency Syndromes.* 2012; 60:99–110. [PubMed: 22517416]
22. Jaspan HB, et al. Immune Activation in the Female Genital Tract During HIV Infection Predicts Mucosal CD4 Depletion and HIV Shedding. *J Infect Dis.* 2011; 204:1550–1556. [PubMed: 21940422]
23. Wahl SM, Orenstein JM. Immune stimulation and HIV-1 viral replication. *J. Leukoc. Biol.* 1997; 62:67–71. [PubMed: 9225995]
24. Copeland KF, Heeney JL. T helper cell activation and human retroviral pathogenesis. *Microbiol. Rev.* 1996; 60:722–742. [PubMed: 8987361]
25. Appay V, Sauce D. Immune activation and inflammation in HIV-1 infection: causes and consequences. *J. Pathol.* 2008; 214:231–241. [PubMed: 18161758]
26. Giorgi JV, et al. Elevated levels of CD38+ CD8+ T cells in HIV infection add to the prognostic value of low CD4+ T cell levels: results of 6 years of follow-up. The Los Angeles Center, Multicenter AIDS Cohort Study. *J. Acquir. Immune Defic. Syndr.* 1993; 6:904–912. [PubMed: 7686224]
27. Giorgi JV, et al. Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. *J. Infect. Dis.* 1999; 179:859–870. [PubMed: 10068581]
28. Benito JM, et al. Quantitative alterations of the functionally distinct subsets of CD4 and CD8 T lymphocytes in asymptomatic HIV infection: changes in the expression of CD45RO, CD45RA, CD11b, CD38, HLA-DR, and CD25 antigens. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 1997; 14:128–135. [PubMed: 9052721]
29. Zara F, et al. Markers of local immunity in cervico-vaginal secretions of HIV infected women: implications for HIV shedding. *Sex Transm Infect.* 2004; 80:108–112. [PubMed: 15054170]
30. Bull ME, et al. HIV-1 shedding from the female genital tract is associated with increased Th1 cytokines/chemokines that maintain tissue homeostasis and proportions of CD8+FOXP3+ T cells. *J. Acquir. Immune Defic. Syndr.* 2014; 67:357–364. [PubMed: 25202922]
31. Brenchley JM, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med.* 2006; 12:1365–1371. [PubMed: 17115046]
32. Brenchley JM, Douek DC. HIV infection and the gastrointestinal immune system. *Mucosal Immunol.* 2008; 1:23–30. [PubMed: 19079157]
33. Dandekar S. Pathogenesis of HIV in the gastrointestinal tract. *Curr HIV/AIDS Rep.* 2007; 4:10–15. [PubMed: 17338855]
34. Klebanoff SJ, Coombs RW. Viricidal effect of *Lactobacillus acidophilus* on human immunodeficiency virus type 1: possible role in heterosexual transmission. *J. Exp. Med.* 1991; 174:289–292. [PubMed: 1647436]

Table 1

Baseline demographic and clinical characteristics of study population (N=226).

Characteristic	Total No. of participants	HIV non-shedders			HIV shedders			p-value ^b
		No. of participants	No. (%) ^a	Median (IQR)	No. of participants	No. (%) ^a	Median (IQR)	
Age, years	226	115		37 (32–41)	111		34 (31–41)	0.03
<35			47 (40.9)			61 (55)		
35–40			40 (34.8)			22 (19.8)		
41+			28 (24.4)			28 (25.2)		
Self-identified race/ethnicity	226	115			111			0.44
White (Non-Hispanic)			20 (17.4)			22 (19.8)		
African-American (Non-Hispanic)			60 (52.2)			65 (58.6)		
Hispanic			32 (27.8)			23 (20.7)		
Other			3 (2.6)			1 (0.9)		
Antiretroviral therapy	226	115			111			0.32
None			58 (50.4)			44 (39.6)		
Monotherapy			34 (29.6)			40 (36)		
Combination therapy			23 (20)			26 (23.4)		
HAART			0			1 (0.9)		
Plasma HIV-1 RNA level, copies/mL	224	114		15,051 (1,400–53,000)	110		17,500 (4,400–76,000)	0.07
80			9 (7.9)			2 (1.8)		
81–1,000			14 (12.3)			11 (10)		
1,001–10,000			32 (28.1)			31 (28.2)		
10,001–100,000			49 (43)			45 (40.9)		
>100,000			10 (8.8)			21 (19.1)		
CD4 cell count, cells/mm ³	225	115		398 (256–595)	110		375 (232–598)	0.75
200			21 (18.3)			21 (19.1)		
201–350			24 (20.9)			29 (26.4)		
351–500			31 (27)			25 (22.7)		

Characteristic	HIV non-shedders				HIV shedders			p-value ^b
	Total No. of participants	No. of participants	No. (%) ^a	Median (IQR)	No. of participants	No. (%) ^a	Median (IQR)	
>500			39 (33.9)			35 (31.8)		
Number of lifetime male sex partners	226	115		15 (5–50)	111		15 (5–60)	0.46
0–6			33 (28.7)			39 (35.1)		
7–29			41 (35.7)			32 (28.8)		
30+			41 (35.7)			40 (36)		
Bacterial vaginosis Gram stain	226	111			109			0.79
Normal (0–3)			40 (36)			36 (33)		
Intermediate (4–6)			17 (15.3)			15 (13.8)		
Consistent (7–10)			54 (48.7)			58 (53.2)		
Candidiasis	219	112	11 (9.8)		107	10 (9.4)		0.91
Chlamydia	219	110	0		109	1 (0.9)		-
Gonorrhea	218	109	0		109	0		-
Human papillomavirus, all types	160	74	38 (51.4)		86	44 (51.2)		0.98
Human papillomavirus, types 16, 18, 31,	160	74	10 (13.5)		86	14 (16.3)		0.63
Syphilis	224	115	15 (13)		109	23 (21.1)		0.11
Herpes simplex virus, type 1 (serology)	219	112	88 (78.6)		107	86 (80.4)		0.74
Herpes simplex virus, type 2 (serology)	218	112	85 (75.9)		106	85 (80.2)		0.44
Cervical lesions	198	97	6 (6.2)		101	7 (6.9)		0.83
Vaginal PH	218	110		5 (4.5–5.8)	108		5 (4.5–5.5)	0.83
<4.5			17 (15.5)			20 (18.5)		
4.5–5.4			46 (41.8)			43 (39.8)		
≥5.5			47 (42.7)			45 (41.7)		
Inflammation	219	112	10 (8.9)		107	22 (20.6)		0.02

Characteristic	HIV non-shedders			HIV shedders			p-value ^b
	Total No. of participants	No. of participants	No. (%) ^a	Median (IQR)	No. of participants	No. (%) ^a	Median (IQR)
Inflammation-associated cellular change	219	112	9 (8)		107	5 (4.7)	0.31
Trichomonas vaginalis	219	112	13 (11.6)		107	17 (15.9)	0.36

NOTE. HAART, highly active antiretroviral therapy; IQR, interquartile range.

^a Percentages may not add up to 100% due to rounding.

^b P-value from chi-square test comparing proportions between HIV non-shedders and shedders.

Table 2

Association of genital infections with immune activation markers among 226 participants across 569 visits.

Variable	CD8+CD38+DR+		CD8+CD38-DR-		CD4+CD38+DR+		CD4+CD38-DR-	
	β (SE)	P-value	β (SE)	P-value	β (SE)	P-value	β (SE)	P-value
Human papillomavirus, all types								
Absent	ref		ref		ref		ref	
Present	0.35 (1.34)	0.80	-1.11 (0.84)	0.19	2.2 (1.04)	0.04	-1.30 (0.93)	0.16
Human papillomavirus (types 16, 18, 31, 33, 35)								
Absent	ref		ref		ref		ref	
Present	-0.30 (1.71)	0.86	-1.11 (0.99)	0.26	1.49 (1.65)	0.37	0.21 (1.28)	0.87
Syphilis (+RPR)								
Absent	ref		ref		ref		ref	
Present	1.17 (2.81)	0.68	-0.04 (1.98)	0.99	1.31 (2.09)	0.53	0.08 (2.72)	0.98
Herpes simplex virus, type 1 (Serology)								
Absent	ref		ref		ref		ref	
Present	-0.1 (2.25)	0.96	1.71 (1.53)	0.26	3.18 (1.52)	0.04	3.20 (2.01)	0.11
Herpes simplex virus, type 2 (Serology)								
Absent	ref		ref		ref		ref	
Present	4.74 (2.47)	0.06	-2.14 (1.6)	0.18	-0.02 (1.47)	0.99	-0.22 (2.0)	0.91
Candidiasis								
Absent	ref		ref		ref		ref	
Present	1.91 (1.29)	0.14	-1.12 (0.87)	0.20	1.71 (1.12)	0.13	-2.8 (0.97)	0.004
Trichomonas vaginalis								
Absent	ref		ref		ref		ref	
Present	2.92 (1.88)	0.12	-2.19 (0.72)	0.002	0.86 (1.82)	0.64	-0.37 (1.59)	0.81
Bacterial vaginosis Gram stain								
Normal (0-3)	ref		ref		ref		ref	

Variable	CD8+CD38+DR+			CD8+CD38-DR-			CD4+CD38+DR+			CD4+CD38-DR-		
	β (SE)	P-value		β (SE)	P-value		β (SE)	P-value		β (SE)	P-value	
Intermediate (4-6)	0.02 (1.17)	0.99		-0.22 (0.96)	0.82		0.91 (1.04)	0.38		-0.32 (0.99)	0.75	
Consistent (7-10)	-1.23 (1.16)	0.29		0.45 (0.90)	0.62		-0.15 (0.90)	0.87		1.86 (1.1)	0.09	
Inflammation												
Absent	ref			ref			ref			ref		
Present	-1.3 (1.8)	0.47		2.52 (1.89)	0.18		-0.25 (1.11)	0.82		1.72 (1.62)	0.29	
Inflammation-associated cellular changes												
Absent	ref			ref			ref			ref		
Present	1.59 (2.62)	0.55		0.59 (1.42)	0.68		1.44 (1.52)	0.35		-2.61 (1.63)	0.11	
Cervical lesions												
Absent	ref			ref			ref			ref		
Present	0.66 (2.16)	0.76		-0.31 (1.81)	0.87		0.73 (2.11)	0.73		0.59 (2.11)	0.78	
Vaginal PH												
<4.5	ref			ref			ref			ref		
4.5-5.4	-0.49 (1.17)	0.67		0.11 (0.72)	0.88		0.45 (0.76)	0.55		0.21 (0.85)	0.81	
5.5+	-0.47 (1.32)	0.72		1.25 (0.86)	0.15		0.55 (0.83)	0.51		2.58 (1.04)	0.01	

Note: Estimated from generalized estimating equations assuming a gaussian distribution with identity link function and exchangeable correlation structure. All analyses were adjusted for log10 transformed HIV viral load.

Table 3

Longitudinal association of immunological variables with HIV-1 genital shedding.

HIV-1 shedding		Unadjusted			Adjusted for HIV-1 viral load			Adjusted for HIV-1 viral load, and STI ^a		
No. of visits/ total no. of visits	%	OR (95% CI)	P	P trend	OR (95% CI)	P	P trend	OR (95% CI)	P	P trend
CD8+CD38+DR+, %										
<38	28/185	15%	Ref		Ref			Ref		
38–<56	55/191	29%	1.93 (1.13, 3.28)	0.02	<0.001	1.00 (0.57, 1.75)	>0.99	1.10 (0.55, 2.20)	0.8	0.52
56+	76/193	39%	3.52 (2.05, 6.03)	<0.001		1.29 (0.71, 2.35)	0.41	1.28 (0.59, 2.77)	0.54	
CD8+CD38–DR–, %										
<5.75	90/189	48%	Ref		Ref			Ref		
5.75–<13	41/189	22%	0.30 (0.19, 0.48)	<0.001	<0.001	0.50 (0.30, 0.82)	0.007	0.49 (0.26, 0.91)	0.02	0.02
13+	28/191	15%	0.21 (0.12, 0.37)	<0.001		0.45 (0.25, 0.80)	0.007	0.44 (0.21, 0.90)	0.02	
CD4+CD38+DR+, %										
<9	24/185	13%	Ref		Ref			Ref		
9–<16	58/192	30%	3.12 (1.76, 5.52)	<0.001	<0.001	1.81 (0.98, 3.36)	0.06	1.51 (0.70, 3.24)	0.29	0.83
16+	77/192	40%	4.31 (2.35, 7.91)	<0.001		1.67 (0.84, 3.30)	0.14	1.19 (0.53, 2.67)	0.68	
CD4+CD38–DR–, %										
<17	77/185	42%	Ref		Ref			Ref		
17–<28	53/190	28%	0.60 (0.38, 0.97)	0.04	<0.001	0.80 (0.49, 1.29)	0.36	0.80 (0.45, 1.41)	0.44	0.16
28+	29/194	15%	0.28 (0.17, 0.48)	<0.001		0.46 (0.27, 0.78)	0.004	0.64 (0.34, 1.22)	0.17	

Note: Boldface type indicates statistical significance. STI: sexually transmitted infection; OR: odds ratio; P: p-value; CI: confidence interval; Ref: reference group.

^a Adjusted models control for HIV viral load, clinically relevant STI and genital tract infection with p-values < 0.05 from Table 2 (measured at the visit when HIV shedding was evaluated, including candidiasis, human papillomavirus (all types), and trichomonas vaginalis).