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Cervical Intraepithelial Neoplasia Is Associated With Genital Tract Mucosal Inflammation

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Abstract

Background—Clinical studies demonstrate increased prevalence of human papillomavirus (HPV)-associated disease in HIV-infected individuals and an increased risk of HIV acquisition in HPV-infected individuals. The mechanisms underlying this synergy are not defined. We hypothesize that women with cervical intraepithelial neoplasia (CIN) will exhibit changes in soluble mucosal immunity that may promote HPV persistence and facilitate HIV infection.

Methods—The concentrations of immune mediators and endogenous anti-*Escherichia coli* activity in genital tract secretions collected by cervicovaginal lavage were compared in HIV-negative women with high-risk HPV-positive (HRHPV+) CIN-3 (n = 37), HRHPV+ CIN-1 (n = 12), or PAP-negative control subjects (n = 57).

Results—Compared with control subjects, women with CIN-3 or CIN-1 displayed significantly higher levels of proinflammatory cytokines including interleukin (IL)-1 α , IL-1 β , and IL-8 ($P < 0.002$) and significantly lower levels of anti-inflammatory mediators and antimicrobial peptides, including IL-1 receptor antagonist, secretory leukocyte protease inhibitor ($P < 0.01$), and human β defensins 2 and 3 ($P < 0.02$). There was no significant difference in endogenous anti-*E. coli* activity after controlling for age and sample storage time.

Conclusion—HRHPV+ CIN is characterized by changes in soluble mucosal immunity that could contribute to HPV persistence. The observed mucosal inflammation suggests a mechanism that may also contribute to the epidemiologic link between persistent HPV and HIV.

Human papillomavirus (HPV), the central etiologic agent in cervical cancer, is the most common sexually transmitted infection (STI) worldwide, with a cumulative incidence

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approaching 70% and a cross-sectional prevalence of 42.5% in US women aged 14 to 49 years.^{1,2} Annually, >300,000 US women have HPV-induced precancerous cervical intraepithelial neoplasia (CIN) or a cancer that requires treatment.³ In much of the developing world where cervical cancer rates and HIV infection are the greatest, screening and access to treatment is limited or unavailable, resulting in increased HPV-associated morbidity and mortality.

Higher rates of CIN and cervical cancer are well described among HIV-infected women.^{4,5} HPV infection may also increase the risk of acquiring HIV, as demonstrated in a prospective study conducted among 2040 HIV-negative women from Zimbabwe.⁶ These clinical findings suggest that mechanisms such as the local cellular or soluble mucosal immune responses to HPV may predispose women to HIV acquisition and interact in other ways to increase clinical morbidity. For example, HPV is associated with hyperproliferative changes, which are often characterized by a stromal infiltration of lymphocytes and macrophages, targets for HIV infection.⁷ Specifically, biopsies from high-grade CIN lesions have increased numbers of activated T cells, regulatory T cells, and macrophages compared with cervical tissue from healthy cervix.⁸

HPV and CIN may also modulate the expression and release of soluble immune mediators into genital tract secretions, an important component of innate host defense. One major class of antimicrobial peptides is defensins, which include the human neutrophil peptides 1 to 3 (HNP1–3) and the epithelial cell human β defensins (HBDs). HNP1–3, HBD-2, and HBD-3 directly inactivate HIV when incubated with virus, presumably reflecting their lectin properties and also inhibit HIV replication after reverse transcription is complete.⁹ Moreover, studies with HPV pseudovirions have shown that defensins also possess potent anti-HPV activity.¹⁰

Another major antimicrobial peptide that contributes to mucosal immunity is secretory leukocyte protease inhibitor (SLPI). SLPI is a small anti-inflammatory protein that also possesses potent anti-HIV and anti-HSV activity at physiological concentrations.^{11,12} Activity against HPV has not been reported. SLPI is secreted by several different cell types, including keratinocytes, the target of HPV infection. SLPI's dual role as a mediator of innate defense and as an anti-inflammatory suggests that decreased SLPI secretion might increase the risk of HIV acquisition. Consistent with this notion is the observation that lower levels of genital tract SLPI were detected in women with bacterial vaginosis,¹³ as well as the observation that HSV downregulates SLPI's expression.¹⁴ Lower levels of SLPI in these settings may contribute to the increased risk of HIV infection.¹⁵

A few small studies have examined the concentration of a limited number of cytokines in the genital tract in the setting of HPV infection with variable results.^{8,16,17} However, no studies have examined the complex network of cytokines, chemokines, and antimicrobial proteins secreted into the genital tract in women with high-risk HPV-positive (HRHPV+) CIN. We hypothesize that HRHPV+ CIN may induce a local inflammatory response and/or promote the loss of antimicrobial peptides, which could contribute to its persistence and promote HIV infection. We took advantage of cervicovaginal lavage (CVL) samples collected from women with HPV-associated CIN or healthy control subjects to explore this hypothesis.

MATERIALS AND METHODS

Study Subjects

Women who were enrolled in studies on oncogenic HPV-positive CIN conducted at the Montefiore Medical Center and had provided consent for additional laboratory studies related to HPV, were eligible. Inclusion criteria included CIN-1 or CIN-3 on colposcopically

directed cervical biopsy at enrollment and the presence of HPV types 16, 18, 31, 45, and/or 52, five of the most common HPV types implicated in cancer. Healthy controls were convenience samples obtained from women who had been recruited from a similar catchment population as CIN subjects, were participating in studies on genital tract mucosal immunity, and had provided consent for additional studies. All participants were HIV-negative by enzyme-linked immunosorbent assay (ELISA), had no symptoms or findings on pelvic examination of an active STI (eg, vaginal discharge or ulcerations), and were not using antibiotics at the time of sampling. In addition, control subjects had a negative Pap test. The Albert Einstein College of Medicine Institutional Review Board approved the parent studies, and all subjects provided written informed consent.

CVL Samples

CVL samples were collected by washing the cervical os and posterior vaginal wall with 10 mL of normal saline. Samples were centrifuged at 2000 rpm $\times$ 10 minutes; supernatants were divided into aliquots; and cell pellets and supernatants were stored at -80°C . Samples were collected between 2003 and 2010.

HPV Typing

HPV testing was performed on CVL cell pellets. Total DNA was extracted, and HPV DNA was detected using the L1 MY09/MY11 modified polymerase chain reaction system with AmpliTaq Gold polymerase.¹⁸ This assay includes the primers PC04/GH20 that amplify a 268-bp cellular β -globin DNA fragment, which serves as an internal control for adequacy of amplification. Amplification products were probed for the presence of any HPV DNA by Southern blot analysis with a radiolabeled generic probe mixture and subsequently typed by dot blot hybridization for HPV types 6, 11, 13, 16, 18, 26, 31 to 35, 39, 40, 42, 45, 51 to 59, 61, 62, 64, 66 to 74, 81 to 85, 89, and 97. Controls for HPV testing included water samples processed in parallel to monitor for potential contamination and positive and negative controls for amplification. A positive control for each type-specific oligonucleotide hybridization was present on each membrane.

Measurement of Immune Mediators

Concentrations of interleukin (IL)-1 α , IL-1 β , IL-6, IL-8, interferon (IFN)- α , IFN- γ , IL-1 receptor antagonist (IL-1ra), macrophage inflammatory protein (MIP)-1 α , MIP-1 β , and CCL5 (RANTES; Regulated upon Activation, Normal T-cell Expressed, and Secreted) were quantified for each sample using a multiplex proteome array with beads from Millipore (Billerica) using Luminex¹⁰⁰ and analyzed using StarStation (Applied Cytometry Systems). Total protein levels for each CVL sample were determined using a MicroBCA assay (Thermo-Scientific). Commercial ELISA kits were used to quantify levels of SLPI (R&D Systems), HNP1–3 (the ELISA does not differentiate between the 3 related peptides; Hycult Biotech), and HBDs 1, 2, and 3 (Alpha Diagnostic International). All samples were tested in duplicate.

Measurement of Endogenous Anti-*Escherichia coli* Activity

Bacteria were grown overnight in Luria-Bertani (LB) broth until they reached stationary phase, were washed, and then approximately 10^9 colony-forming units were mixed with CVL samples, genital tract buffer (20 mmol/L potassium phosphate, 60 mmol/L sodium chloride, 0.2 mg/mL albumin, pH 4.5) (negative control), or with penicillin-streptomycin (positive control) and incubated for 2 hours at 37°C . The mixtures were then diluted in genital tract buffer (to yield ~ 1000 colony-forming units on the negative control plates), mixed with overlay medium, and plated on agar enriched with trypticase soy broth. Colonies were counted using ImageQuant TL v2005 after overnight incubation at 37°C . The

percentage inhibition was determined relative to the CFU on the negative control plates. CVLs were tested in duplicate.

Statistical Analysis

The concentration of each mediator was divided by the total protein recovered in the sample and then log transformed. However, because molecules such as lactic acid may contribute to the antimicrobial activity, we did not correct the anti-*E. coli* activity for protein concentration.¹⁹ Sample values below the limit of detection (LOD) were set at the midpoint between zero and the LOD. Spearman's correlation coefficients were calculated to compare age with immune mediators. The Kruskal–Wallis test was used to determine whether there were differences between any of the mediators and race or specimen storage time. To adjust for the potential confounders of age and storage time, mixed effects multivariate analysis of covariance (allows each sample to be analyzed with all 14 mediator levels) and mixed effects analysis of covariance (ANCOVA), where mediators are analyzed individually, were used with a false discovery rate considered significant at 0.05 (Q value). Each ANCOVA went through diagnostics and was log transformed to alleviate nonnormality and heteroscedasticity. Spearman's rank-order correlation was also used to determine associations between mediators. This measure of association was used over Pearson's product-moment correlation coefficient (*r*) because the data are not normally distributed. Statistical analyses were performed using R v 2.12.2, R Studio v 0.93.75, and SAS 9.2 (SAS, Cary, NC).

RESULTS

Description of Participants

CVL samples from 106 eligible participants were evaluated: 37 from women with CIN-3, 12 from women with CIN-1, and 57 from control subjects with negative PAP results. The CIN-3 cohort (median: 28; range in years: 19 – 66) was significantly older than CIN-1 (median: 28; range in years: 20 – 52) or control subjects (median: 26; range in years: 16 – 50), and the groups also differed with respect to race and sample storage time ($P < 0.01$) (Table 1). There were also differences in the prevalence of HPV types, with HPV-16 predominating among women with CIN-3. As expected, many CIN subjects were coinfecting with >1 HRHPV type or had coinfection with low-risk HPV types.²⁰ No HPV was detected from the majority of the PAP-negative control subjects ($n = 41$, 73%). Fifteen controls had HPV DNA isolated; 1 had HPV16; 1 had HPV 31, 39, and 58; 1 had HPV 45; 2 had HPV 51; and the other 10 had low-risk HPV types detected. One sample could not be amplified (Table 1).

CIN Association With Mucosal Inflammatory Cytokines and Chemokines

The total protein recovered differed significantly with higher concentrations in women with CIN-3, supporting the need to standardize quantities of the cytokines and chemokines by dividing by the total protein before further analyses (Table 2). Kruskal–Wallis did not identify race as a significant variable across immune mediators. Similarly, there was only a modest negative correlation between age and SLPI ($\rho = -0.02$, Spearman), which was not significant when adjusting for false discovery rate ($Q = 0.34$). The mediator levels did, however, vary by storage time (Kruskal–Wallis); therefore, we adjusted for the possible confounders of age and storage time when comparing mediators.

Women with CIN-3 and CIN-1 had significantly higher levels of IL-1 α , IL-1 β , and IL-8 (pg/ μ g protein recovered) (Fig. 1 upper panels) and significantly lower levels of the anti-inflammatory protein IL-1ra (Fig. 1, lower middle) compared with control subjects (ANCOVA, $Q < 0.002$, Table 2). In addition, women with CIN-3 (but not CIN-1) also had

significantly higher levels of RANTES compared with control subjects ($Q < 0.001$) (Fig. 1, lower left), although the concentrations of the other chemokines measured, MIP-1 α and MIP-1 β , did not differ significantly (Table 2). However, the concentration of IL-6, which is typically increased in the setting of inflammation, was significantly lower in CIN-3 ($Q = 0.003$) compared with the control subjects (Fig. 1, lower right). The concentrations of IFN- γ and IFN- α were below or near the LOD in the majority of samples and did not differ between cohorts.

CIN Association With Antimicrobial Proteins

To explore the possibility that CIN is associated with alterations in antimicrobial peptides, the concentrations of defensins and SLPI were measured in CVL. Women with CIN-3 and CIN-1 had significantly lower levels of HBD-2 (Fig. 2, upper middle, $Q < 0.001$) and HBD-3 (Fig. 2 upper right, $Q < 0.02$). Women with CIN-3 also had reduced levels of HBD-1 in their CVL when compared with control subjects (Fig. 2, upper left, $Q = 0.03$). However, there were no significant differences in the concentration of HNP1–3 (Fig. 2, lower left); the latter antimicrobial peptides are expressed primarily by neutrophils and monocytes. SLPI, which has both antimicrobial and anti-inflammatory properties, was also significantly lower in CVL from women with CIN-3 and CIN-1 compared with control subjects (Fig. 2, lower middle, $Q = 0.002$).

Although measurements of individual mucosal mediators may provide insights into the mucosal immune environment, they may not reflect the complex interactions between various factors. The antimicrobial activity of CVL may provide a surrogate biomarker to evaluate host mucosal defense. Therefore, we also measured the anti-*E. coli* activity in CVL samples. We limited the study to *E. coli* activity because repeat testing of individual aliquots thawed at monthly intervals, indicating that *E. coli* activity is stable after prolonged storage, whereas the endogenous anti-HIV and anti-HSV activity is reduced after storage at -80°C for >6 months. There were no significant differences in the bactericidal activity of CVL from CIN or control subjects (Fig. 2, lower right).

To further evaluate the impact of CIN on mediators, we also conducted a multivariate analysis of covariance and found that there was a significant difference in concentrations of mediators between CIN and control subjects while adjusting for age and storage time ($P < 0.001$).

Correlations Between Mediators

Heat maps were generated from Spearman's ρ values between cytokines, chemokines, antimicrobial proteins, and anti-*E. coli* activity (Fig. 3). In CVL samples obtained from control subjects, the concentrations of IL-1 α , IL-1 β , IL-8, RANTES, and MIP-1 α , correlated strongly with one another (Spearman $\rho > 0.5$) and modestly with IL-6, MIP-1 β , and HNP1–3 ($\rho 0.3$ – 0.5). However, the strength of these correlations was diminished or absent in samples from participants with CIN-3, suggesting dysregulation of the cytokine/chemokine network. Moreover, IL-6, which correlated modestly or strongly with proinflammatory mediators such as IL-1 α ($\rho = 0.41$), IL-1 β (0.46), IL-8 (0.62), RANTES (0.49), MIP-1 α (0.71), and MIP-1 β (0.61) in the control samples, correlated strongly with HBD-1 (0.69) and SLPI (0.69) in CIN-3 samples (Supplementary Table 1, Supplemental Digital Content, online only, available at: <http://links.lww.com/OLQ/A42>).

To further explore the differences in the soluble mucosal immune environment between women with cervical dysplasia and PAP-negative control subjects, we calculated a standard Z-score to determine by how many standard deviations the concentrations of each immune mediator (or anti-*E. coli* activity) was above or below the mean for the population. CVL

from CIN-1 and CIN-3 were characterized by an increase in proinflammatory and a decrease in anti-inflammatory and antimicrobial mediators compared with healthy PAP-negative control subjects (Fig. 4).

DISCUSSION

Results of this first comprehensive study of soluble genital tract mucosal immune mediators in women with HRHPV+CIN demonstrate changes in the genital tract immune environment that could contribute to HPV persistence and to the clinical associations between HPV and HIV. The finding that these differences are also observed between control subjects and CIN-I subjects suggests these local immune events occur before HPV persistence and might be an important step in the natural history of persistent HPV infection. The temporality of these associations, however, can only be determined with a prospective study design.

The increase in proinflammatory mediators may not only promote HPV persistence but could also facilitate HIV infection through the recruitment and activation of immune cells that are targets for HIV infection.²¹ In addition, inflammatory cytokines could augment HIV replication through activation of the HIV long terminal repeats.²² The latter notion is supported by studies demonstrating a link between inflammation and cervicovaginal shedding of HIV.²³ For example, one recent study found that the genital tract concentrations of IL-1 β and IL-8 were associated with higher cervicovaginal HIV-1 RNA concentrations, even after controlling for plasma viral load and vaginal microbial cofactors²⁴; these 2 inflammatory mediators were significantly increased in the setting of CIN (Fig. 1).

Although the current study is limited by the cross-sectional design, lack of parallel biopsy samples for evaluation of gene expression, and absence of molecular testing for subclinical STI or vaginal microbiota data, the findings suggest additional mechanisms that may contribute to HPV persistence. Notably, few of the control subjects had HR HPV DNA detected, and exclusion of these subjects did not substantially alter the results; therefore, these PAP-negative controls were included in the analyses. The findings are consistent with a prior smaller study that examined a limited number of cytokines in vaginal washes in 32 women referred for colposcopy. The authors observed an increase in IL-1 β in vaginal lavage fluid in women with cervical dysplasia (n = 17).¹⁷ In vitro studies also suggest an inflammatory response to oncogenic HPV. A microarray study showed that the introduction of E6 and E7 into human keratinocytes resulted in upregulation of proinflammatory cytokines and downregulation of type-I IFN responses.²⁵ Notably, we did not detect any increased IFN response in the CVL samples, although we only tested for IFN- α and IFN- γ .

Another limitation of the current study is that subjects differed by age, race, and sample storage time. However, the differences in mediators persisted when controlling for these potential confounders. Moreover, any degradation secondary to more prolonged storage likely would have only strengthened the observed differences between CIN and control subjects as CIN subjects had higher concentrations of inflammatory mediators.

In addition to an increase in inflammatory mediators, CIN-3 and CIN-1 were also associated with decreased expression of anti-inflammatory markers (SLPI and IL-1ra) and lower levels of protective immune mediators (HBDs). A recent study found that HBD-2 was absent in HPV-transformed keratinocytes and weakly expressed in cervical precancerous lesions in comparison with normal keratinocytes, possibly contributing to an impaired immune response.²⁶ HBD-2 and HBD-3 are typically upregulated in response to inflammation or infection.²⁷ The paradoxical finding of decreased HBD-2 and HBD-3 in the setting of increased proinflammatory cytokines suggests dys-regulation of the normal cytokine-defensin network.²⁸ Dys-regulation is also suggested by the loss of correlation between

proinflammatory markers and the unanticipated gain of correlation between mediators not typically coregulated, particularly in CIN-3 samples (eg, IL-6 and SLPI) (Fig. 3).

Just as increased inflammation may facilitate HIV acquisition through recruitment or activation of immune target cells, lower levels of protective and anti-inflammatory mediators in women with CIN may render them more susceptible to HPV persistence and to HIV infection. Defensins exhibit anti-HPV activity¹⁰ and have been associated with protection against HIV acquisition in studies of highly exposed seronegative subjects.²⁹ Similarly, higher vaginal levels of SLPI in HIV-infected mothers have been associated with reduced HIV transmission during childbirth and higher salivary SLPI levels in infants with reduced HIV acquisition through breast-feeding.³⁰

This study highlights the need to expand efforts investigating the local cervical-vaginal milieu and host response to HPV in HIV-uninfected and HIV-infected women. Mucosal responses may contribute to HPV persistence and to the link between HPV and HIV. If the findings in the current study are confirmed in a larger prospective study, they may provide insights into novel strategies for prevention and vaccination against HPV and identify approaches to disrupt the synergy between these 2 viral pathogens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Hariri S, Unger ER, Sternberg M, et al. Prevalence of genital human papillomavirus among females in the United States, the national health and nutrition examination survey, 2003–2006. *J Infect Dis.* 2011; 204:566–73. [PubMed: 21791659]
2. Schiffman M, Castle PE, Jeronimo J, et al. Human papillomavirus and cervical cancer. *Lancet.* 2007; 370:890–907. [PubMed: 17826171]
3. Dunne EF, Sternberg M, Markowitz LE, et al. Human papillomavirus (HPV) 6, 11, 16, and 18 prevalence among females in the United States–National health and nutrition examination survey, 2003–2006: Opportunity to measure HPV vaccine impact? *J Infect Dis.* 2011; 204:562–565. [PubMed: 21791658]
4. Strickler HD, Burk RD, Fazzari M, et al. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus-positive women. *J Natl Cancer Inst.* 2005; 97:577–586. [PubMed: 15840880]
5. Wang C, Wright TC, Denny L, et al. Rapid rise in detection of human papillomavirus (HPV) infection soon after incident HIV infection among South African women. *J Infect Dis.* 2011; 203:479–486. [PubMed: 21216869]
6. Smith-McCune KK, Shiboski S, Chirenje MZ, et al. Type-specific cervico-vaginal human papillomavirus infection increases risk of HIV acquisition independent of other sexually transmitted infections. *PLoS One.* 2010; 5:e10094. [PubMed: 20386706]

7. Edwards RP, Kuykendall K, Crowley-Nowick P, et al. T lymphocytes infiltrating advanced grades of cervical neoplasia. CD8-positive cells are recruited to invasion. *Cancer*. 1995; 76:1411–1415. [PubMed: 8620416]
8. Kobayashi A, Greenblatt RM, Anastos K, et al. Functional attributes of mucosal immunity in cervical intraepithelial neoplasia and effects of HIV infection. *Cancer Res*. 2004; 64:6766–6774. [PubMed: 15374995]
9. Cole AM, Cole AL. Antimicrobial polypeptides are key anti-HIV-1 effector molecules of cervicovaginal host defense. *Am J Reprod Immunol*. 2008; 59:27–34. [PubMed: 18154593]
10. Buck CB, Day PM, Thompson CD, et al. Human alpha-defensins block papillomavirus infection. *Proc Natl Acad Sci U S A*. 2006; 103:1516–1521. [PubMed: 16432216]
11. McNeely TB, Dealy M, Dripps DJ, et al. Secretory leukocyte protease inhibitor: A human saliva protein exhibiting anti-human immunodeficiency virus 1 activity in vitro. *J Clin Invest*. 1995; 96:456–464. [PubMed: 7615818]
12. John M, Keller MJ, Fam EH, et al. Cervicovaginal secretions contribute to innate resistance to herpes simplex virus infection. *J Infect Dis*. 2005; 192:1731–1740. [PubMed: 16235171]
13. Novak RM, Donoval BA, Graham PJ, et al. Cervicovaginal levels of lactoferrin, secretory leukocyte protease inhibitor, and RANTES and the effects of coexisting vaginosis in human immunodeficiency virus (HIV)-seronegative women with a high risk of heterosexual acquisition of HIV infection. *Clin Vaccine Immunol*. 2007; 14:1102–1107. [PubMed: 17671228]
14. Fakioglu E, Wilson SS, Mesquita PM, et al. Herpes simplex virus downregulates secretory leukocyte protease inhibitor: A novel immune evasion mechanism. *J Virol*. 2008; 82:9337–9344. [PubMed: 18667508]
15. Low N, Chersich MF, Schmidlin K, et al. Intravaginal practices, bacterial vaginosis, and HIV infection in women: Individual participant data meta-analysis. *PLoS Med*. 2011; 8:e1000416. [PubMed: 21358808]
16. Crowley-Nowick PA, Ellenberg JH, Vermund SH, et al. Cytokine profile in genital tract secretions from female adolescents: Impact of human immunodeficiency virus, human papillomavirus, and other sexually transmitted pathogens. *J Infect Dis*. 2000; 181:939–945. [PubMed: 10720516]
17. Behbakht K, Friedman J, Heimler I, et al. Role of the vaginal microbiological ecosystem and cytokine profile in the promotion of cervical dysplasia: A case-control study. *Infect Dis Obstet Gynecol*. 2002; 10:181–186. [PubMed: 12648311]
18. Castle PE, Schiffman M, Gravitt PE, et al. Comparisons of HPV DNA detection by MY09/11 PCR methods. *J Med Virol*. 2002; 68:417–423. [PubMed: 12226831]
19. Valore EV, Park CH, Igreti SL, et al. Antimicrobial components of vaginal fluid. *Am J Obstet Gynecol*. 2002; 187:561–568. [PubMed: 12237628]
20. Chaturvedi AK, Katki HA, Hildesheim A, et al. Human papillomavirus infection with multiple types: Pattern of coinfection and risk of cervical disease. *J Infect Dis*. 2011; 203:910–920. [PubMed: 21402543]
21. Li Q, Estes JD, Schlievert PM, et al. Glycerol monolaurate prevents mucosal SIV transmission. *Nature*. 2009 published online 4 March.
22. Ferreira VH, Nazli A, Khan G, et al. Endometrial epithelial cell responses to coinfecting viral and bacterial pathogens in the genital tract can activate the HIV-1 LTR in an NF- κ B- and AP-1-dependent manner. *J Infect Dis*. 2011; 204:299–308. [PubMed: 21673042]
23. Henning TR, Kissinger P, Lacour N, et al. Elevated cervical white blood cell infiltrate is associated with genital HIV detection in a longitudinal cohort of antiretroviral therapy-adherent women. *J Infect Dis*. 2010; 202:1543–1552. [PubMed: 20925530]
24. Mitchell C, Hitti J, Paul K, et al. Cervicovaginal shedding of HIV type 1 is related to genital tract inflammation independent of changes in vaginal microbiota. *AIDS Res Hum Retroviruses*. 2011; 27:35–39. [PubMed: 20929397]
25. Nees M, Geoghegan JM, Hyman T, et al. Papillomavirus type 16 oncogenes downregulate expression of interferon-responsive genes and upregulate proliferation-associated and NF- κ B-responsive genes in cervical keratinocytes. *J Virol*. 2001; 75:4283–4296. [PubMed: 11287578]

26. Hubert P, Herman L, Maillard C, et al. Defensins induce the recruitment of dendritic cells in cervical human papillomavirus-associated (pre)neoplastic lesions formed in vitro and transplanted in vivo. *FASEB J.* 2007; 21:2765–2775. [PubMed: 17470569]
27. Pioli PA, Weaver LK, Schaefer TM, et al. Lipopolysaccharide-induced IL-1 beta production by human uterine macrophages up-regulates uterine epithelial cell expression of human beta-defensin 2. *J Immunol.* 2006; 176:6647– 6655. [PubMed: 16709823]
28. King AE, Fleming DC, Critchley HO, et al. Differential expression of the natural antimicrobials, beta-defensins 3 and 4, in human endometrium. *J Reprod Immunol.* 2003; 59:1–16. [PubMed: 12892899]
29. Zapata W, Rodriguez B, Weber J, et al. Increased levels of human beta-defensins mRNA in sexually HIV-1 exposed but uninfected individuals. *Curr HIV Res.* 2008; 6:531–538. [PubMed: 18991618]
30. Farquhar C, VanCott TC, Mbori-Ngacha DA, et al. Salivary secretory leukocyte protease inhibitor is associated with reduced transmission of human immunodeficiency virus type 1 through breast milk. *J Infect Dis.* 2002; 186:1173–1176. [PubMed: 12355371]

Figure 1.

CIN is associated with increased proinflammatory cytokines. Boxplots showing the log transformed concentrations of IL-1 α (upper left), IL-1 β (upper middle), IL-8 (upper right), IL-1RA (lower left) RANTES (lower middle), and IL-6 (lower left) after correcting for total protein recovered from CVL samples for each cohort. The line indicates median value, and the circles are outliers. The asterisks denote significant differences relative to controls.

Figure 2.

CIN is associated with lower levels of anti-inflammatory and protective immune mediators. Boxplots showing log transformed concentrations of HBD-1 (upper left), HBD-2 (upper middle), HBD-3 (upper right), HNP1-3 (lower left), and SLPI (lower middle) after correcting for total protein recovered from CVL samples for each cohort. The percentage inhibition of *E. coli* (lower right) is not corrected for protein. The line indicates median values, and the circles are outliers; asterisks indicate significant differences relative to controls.

Figure 3.

Cervical dysplasia is associated with dysregulation of soluble immune mediator networks. Each panel depicts a color-coded heat map of Spearman's correlation coefficients between soluble immune mediators and endogenous anti-*E. coli* activity (controls (A), CIN-1 (B), and CIN-3 (C)).

Figure 4.

Heat map of Z-scores for mucosal immune mediators illustrating the different patterns characterizing each cohort. The Z-score for indicated mediators was calculated, and the results are shown as a heat map, with each row representing the indicated cohort. A Z-score of zero was assigned the color black; positive Z-scores, indicative of higher levels, were assigned increasing shades of red; and negative Z-scores, indicative of lower levels, were assigned increasing shades of green.

TABLE 1

Demographics and HPV Status

	Controls n = 57 (%)	CIN-1 n = 12 (%)	CIN-3 n = 37 (%)
Age			
<25 yr	22 (38.6)	4 (33.3)	6 (16.2)
25–50 yr	34 (59.7)	7 (58.3)	21 (56.8)
>50 yr	1 (1.8)	1 (8.3)	10 (27.0)
Race			
White	23 (40.4)	0	15 (40.5)
Black	21 (22.8)	7 (58.3)	3 (8.1)
Other	13 (36.8)	5 (41.7)	19 (51.4)
Ethnicity			
Hispanic	24 (42.1)	5 (41.7)	17 (46)
Non-Hispanic	33 (57.9)	7 (58.3)	20 (54)
CVL storage time (years, median [range])	1.4 (0.8–2.7)	1.7 (0.4–3.1)	6.6 (5.6–7.4)
HPV status			
HPV 16 Positive	1 (1.8)	2 (16.7)	27 (73)
HPV 18 Positive	0	2 (16.7)	5 (13.5)
Other high-risk HPV	4 (7.1)	8 (66.7)	5 (13.5)
Low-risk HPV	10 (17.9)	0	0
No HPV	41 (73.2)	0	0
HPV risk profile			
Single high risk	2 (3.6)	4 (33.3)	21 (56.8)
Multiple high risk	0	2 (16.67)	7 (18.9)
High and low risk	3 (5.4)	6 (50.00)	9 (24.3)

TABLE 2

Concentrations of Mucosal Immune Mediators

Mediator	Median (25%, 75%)			FDR Adjusted Q Value*
	Controls	CIN-1	CIN-3	
Protein ($\mu\text{g/mL}$)	245 (148, 404)	235.2 (148.3, 358.3)	504.8 (354.8, 615.1)	0.85/<0.001
<i>E. coli</i> inhibition (%)	67 (49, 90)	59.5 (36.3, 83.3)	53 (46, 62)	0.72/0.56
HBD-1 (pg/mL)	2200 (800, 3200)	1500 (700, 2200)	1200 (660, 4600)	0.58/0.03
HBD-2 (pg/mL)	800 (800, 800)	25 (25, 340.4)	576 (190, 1490)	<0.001/<0.001
HBD-3 (pg/mL)	853 (250, 2400)	100 (100, 423)	100 (100, 374)	0.01/<0.001
HNP1-3 (ng/mL)	24 (5.8, 180)	126 (28.5, 190)	110 (28, 140)	0.17/0.98
SLPI (ng/mL)	210 (100, 500)	44 (15, 67)	47 (18, 120)	0.002/<0.001
IL-1ra (ng/mL)	780 (600, 1000)	530 (27, 138)	110 (81, 390)	<0.001/<0.001
IL-6 (pg/mL)	10 (3.2, 18.1)	2.05 (1.5, 3.28)	2.6 (2, 5.2)	0.17/0.003
IL-1 α (pg/mL)	44 (19, 83)	770 (525, 1475)	490 (310, 837)	<0.001/<0.001
IL-1 β (pg/mL)	2.9 (0.6, 9.8)	20.80 (12.35, 84.12)	127.7 (38.1, 377.6)	0.002/<0.001
IL-8 (pg/mL)	270 (90, 8700)	1950 (827.5, 5225)	4900 (1800, 10,000)	0.002/0.002
RANTES (pg/mL)	2.2 (1.1, 4.6)	2.3 (1, 11.2)	8.5 (4.6, 41)	0.24/<0.001
MIP-1 α (pg/mL)	8.3 (1.8, 13.6)	8.4 (4.7, 12.4)	17.6 (13.9, 32.3)	0.72/0.44
MIP-1 β (pg/mL)	6.3 (2.3, 15.3)	3.6 (2.3, 5.7)	12.6 (7, 24.4)	0.87/0.87

* FDR adjusted Q values comparing concentration of each mediator per μg protein for controls versus CIN-1/controls versus CIN-3, mixed effects ANCOVA controlling for age, and CVL storage time.