Genital Inflammation and the Risk of HIV Acquisition in Women

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Background. Women in Africa, especially young women, have very high human immunodeficiency virus (HIV) incidence rates that cannot be fully explained by behavioral risks. We investigated whether genital inflammation influenced HIV acquisition in this group.

Methods. Twelve selected cytokines, including 9 inflammatory cytokines and chemokines (interleukin [IL]-1α, IL-1β, IL-6, tumor necrosis factor-α, IL-8, interferon-γ inducible protein-10 [IP-10], monocyte chemoattractant protein-1, macrophage inflammatory protein [MIP]-1α, MIP-1β), hematopoietic IL-7, and granulocyte macrophage colony-stimulating factor, and regulatory IL-10 were measured prior to HIV infection in cervicovaginal lavages from 58 HIV seroconverters and 58 matched uninfected controls and in plasma from a subset of 107 of these women from the Centre for the AIDS Programme of Research in South Africa 004 tenofovir gel trial.

Results. HIV seroconversion was associated with raised genital inflammatory cytokines (including chemokines MIP-1α, MIP-1β, and IP-10). The risk of HIV acquisition was significantly higher in women with evidence of genital inflammation, defined by at least 5 of 9 inflammatory cytokines being raised (odds ratio, 3.2; 95% confidence interval, 1.3–7.9; $P = .014$). Genital cytokine concentrations were persistently raised (for about 1 year before infection), with no readily identifiable cause despite extensive investigation of several potential factors, including sexually transmitted infections and systemic cytokines.

Conclusions. Elevated genital concentrations of HIV target cell–recruiting chemokines and a genital inflammatory profile contributes to the high risk of HIV acquisition in these African women.

Keywords. HIV transmission; female genital tract; inflammation; cytokine.

Substantial progress has been made in slowing the global human immunodeficiency virus (HIV) epidemic over the past decade, with the annual number of new HIV infections declining from 3.4 million in 2001 to 2.1 million in 2013 [1]. Despite this achievement, the HIV epidemic continues to grow among young women in Africa, with HIV infection rates up to 8-fold higher than in men of the same age [2]. In South Africa, national HIV prevalence rates among pregnant women have risen from 24.8% in 2001 to 29.5% in 2012 [3]. These high HIV prevalence rates are being sustained by incidence rates as high as 9.1/100 person-years in women from KwaZulu-Natal in South Africa [4]. Reported incidence rates are particularly concerning in teenaged girls who have HIV incidence rates of up to 17.2/100 person-years [5]. Continual transmission of

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HIV infection in young women in southern Africa is one of the greatest challenges preventing the world from achieving the goal of an AIDS-free generation [6].

Multiple partners, sexual frequency, low condom use rates, sexual violence [7], and sexually transmitted infections (STIs) [8, 9] have been shown to have an impact on the risk of young African women acquiring HIV. While there is a growing understanding of factors that impact HIV acquisition, these factors, singly or in combination, are insufficient to fully explain the heightened vulnerability of young women in southern Africa.

Inflammatory cytokines such as macrophage inflammatory protein (MIP)-1α, MIP-1β, and interleukin (IL)-8 in the genital tracts of rhesus macaques were found to be essential for establishment of a productive simian immunodeficiency virus (SIV) infection following vaginal exposure [10], and STIs that cause inflammation are associated with increased susceptibility to HIV infection [11]. For these reasons, we hypothesized that an inflammatory milieu in the human female genital tract increases the risk of HIV acquisition. In macaques, these chemokines led to the recruitment of CD4+ T-cell targets at the early stages of infection, thereby facilitating SIV replication. Furthermore, topical application of the antiinflammatory agent, glycerol-monolaurate, downregulated inflammatory chemokine concentrations, inhibited inflammatory cell influx to the genital tract, and thereby prevented SIV infection in macaques [10].

The purpose of this study was to assess whether genital inflammation was associated with an increase in the risk of HIV acquisition in South African women.

METHODS

Study Design

A total of 889 HIV-uninfected women were enrolled in the Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 phase IIb, double-blind, tenofovir gel trial in rural Vulindlela and urban eThekwini in KwaZulu-Natal, South Africa [4]. Women were randomized to use either hydroxyethylcellulose placebo or tenofovir gel. Women were enrolled and followed between May 2007 and March 2010.

HIV testing was conducted monthly using 2 rapid HIV antibody tests (Abbott Determine and Unigold) and confirmed with 2 independent RNA-polymerase chain reaction (PCR) assays at least 1 week apart. Stored plasma samples from prior visits by each seroconverter were tested using PCR to identify the window period of HIV infection (RNA-PCR–positive but rapid HIV test–negative). Quarterly pelvic examinations were performed, cervicovaginal lavages (CVLs) were collected (Supplementary Material), and the presence of genital abnormalities was recorded. Serology was performed for herpes simplex virus type 2 (HSV-2) using the HSV-2 gG2 enzyme-linked immunosorbent assay (Kalon Biologicals Ltd.). *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Treponema pallidum*, *Trichomonas vaginalis*, *Haemophilus ducreyi*, and HSV-1 and -2 were assessed using real-time PCR (Supplementary Material).

Fifty-eight HIV seroconverters who had preinfection CVLs in storage that did not have visible blood contamination were included. Of the available samples for each participant, those who were closest to the estimated time of HIV infection were analyzed (median 4.5 months prior to HIV infection; interquartile range [IQR], 2.4–6.9 months). Each of the 58 cases had a negative PCR test result on the same day as the sample used for analysis or at a visit after sample collection. The 58 cases included 23 women assigned to the tenofovir gel arm of the study. HIV negative controls (n = 58) were matched on gel assignment and date of CVL collection (each had a CVL available within 7 days of the date of sample collection for each case) in order to minimize the possible effects of product use and sample storage time on cytokine concentrations.

The University of KwaZulu-Natal's and University of Cape Town's research ethics committees approved this study, and all participants provided written informed consent.

Measurement of Cytokine Concentrations

Luminex was used to measure the concentrations of 12 cytokines (IL-1α, IL-1β, IL-6, IL-7, IL-8, IL-10, granulocyte macrophage colony-stimulating factor [GM-CSF], interferon-γ inducible protein [IP]-10, monocyte chemoattractant protein [MCP]-1, MIP-1α, MIP-1β, and tumor necrosis factor [TNF]-α) in preinfection CVLs from women who later became HIV-infected (median 4.5 months preinfection [IQR, 2.4–6.9 months]), and in CVLs from women who remained HIV-uninfected during the follow-up period [4] (Supplementary Material). The 12 cytokines were selected based on their previously identified association with genital infections and/or HIV disease progression [11–13] and the fact that they could be reproducibly measured in CVL (Supplementary Table 1). To determine whether the raised cytokine profiles found in women prior to HIV infection were acute or persistent, 57 CVL samples (21 women who later acquired HIV infection and 36 women who remained uninfected) were available from a second time point at a median of 48 weeks from the first time period (range, 8–104 weeks). Cytokine concentrations were also measured in matching plasma samples from a subset of 107 women (57 cases and 50 controls) for whom plasma was available.

Statistical Analyses

Unsupervised hierarchical clustering was used to group women according to the relatedness of their cytokine expression profiles. Conditional logistic regression was used for the matched-pair case-control analysis. *P* values were adjusted for multiple comparisons using a false discovery rate step-down procedure [14]. Partial least squares discriminant analysis (PLSDA) [15] was used to determine multivariate cytokine profiles that best distinguished between cases and controls (Supplementary Material).

Table 1. Relationship Between Genital Tract Cytokines and Risk of Human Immunodeficiency Virus Infection in Women From the Centre for the AIDS Programme of Research in South Africa 004 Trial

	HIV-Negative Women (N = 58)	HIV Seroconverters (N = 58)	Pair-Matched Odds Ratio (95% Confidence Interval)	
Cytokine	% Detectable (n)	% Detectable (n)	Per Detection of Cytokine	*P* Value
MIP-1β	43.1 (25)	75.9 (44)	3.17 (1.49–6.77)	.003[a]
MIP-1α	15.5 (9)	44.8 (26)	3.09 (1.38–6.92)	.006[a]
GM-CSF	75.9 (44)	89.7 (52)	2.64 (.87–8.05)	.086
IL-7	58.6 (34)	60.3 (35)	1.06 (.52–2.15)	.874
	Median (IQR) $\log_{10}$ pg/mL	Median (IQR) $\log_{10}$ pg/mL	Per 1 $\log_{10}$ pg/mL increase	
IP-10	1.88 (0.94–2.47)	2.26 (1.54–2.86)	1.94 (1.19–3.17)	.008[a]
IL-8	2.81 (2.41–3.28)	3.18 (2.82–3.92)	1.85 (1.09–3.13)	.022
IL-1α	1.97 (1.61–2.44)	2.22 (1.71–2.74)	1.65 (.96–2.83)	.067
MCP-1	1.31 (0.82–1.68)	1.43 (0.94–1.89)	1.47 (.85–2.54)	.164
IL-10	−0.37 (−1.70–0.33)	−0.14 (−0.89–0.55)	1.21 (.91–1.60)	.197
IL-6	0.79 (0.25–1.29)	0.87 (0.50–1.41)	1.23 (.75–2.01)	.419
IL-1β	0.27 (−0.42–1.02)	0.52 (−0.32–1.27)	1.22 (.86–1.73)	.265
TNF-α	−0.68 (−1.10–0.01)	−0.62 (−1.05–0.31)	1.16 (.76–1.77)	.498

Abbreviations: GM-CSF, granulocyte macrophage colony-stimulating factor; HIV, human immunodeficiency virus; IL, interleukin; IP-10, interferon-γ inducible protein-10; IQR, interquartile range; MCP-1, monocyte chemoattractant protein-1; MIP, macrophage inflammatory protein; TNF-α, tumor necrosis factor-alpha.

[a] Statistically significant after adjusting for multiple comparisons.

RESULTS

Genital Cytokine Concentrations and HIV Acquisition

Women who acquired HIV infection (n = 58) during the CAPRISA tenofovir gel trial had higher genital concentrations of the chemotactic cytokines MIP-1α, MIP-1β, IL-8, and IP-10 prior to infection (Table 1) than matched women who remained HIV-negative (n = 58). The women who acquired HIV infection also clustered separately from women who remained uninfected in an unsupervised hierarchical analysis of the 12 cytokines (Figure 1).

Genital Inflammatory Profiles and HIV Acquisition

Of the 12 cytokines measured in this study, 9 are classified as proinflammatory or chemotactic (MIP-1α, MIP-1β, IP-10,

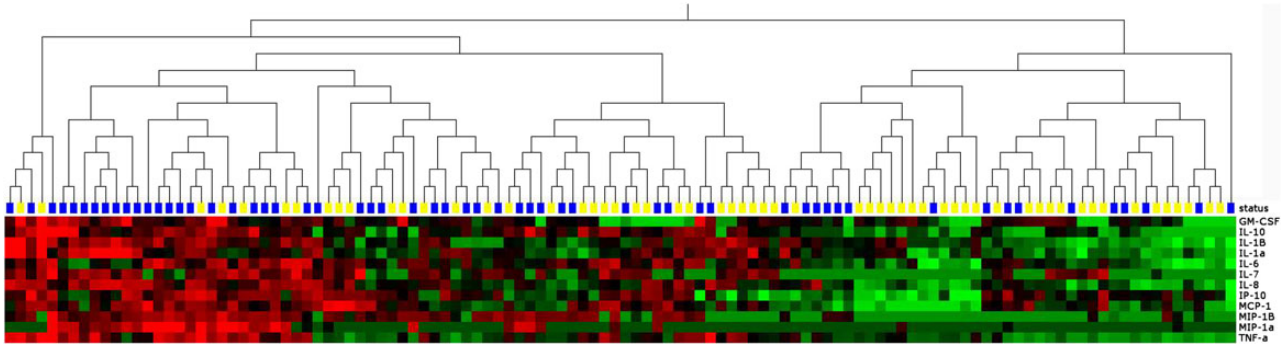

Figure 1. Unsupervised hierarchical clustering was used to visualize the variation in cytokine concentrations in individual women and to cluster women according to the similarities of their cytokine expression profiles (using Qlucore Omics Explorer). Women who later became human immunodeficiency virus (HIV)–infected (n = 58; blue blocks) had upregulated preinfection cervicovaginal lavage cytokine concentrations and tended to cluster together, while women who remained HIV-uninfected had lower cytokine concentrations and also clustered together (n = 58; yellow blocks). Cytokine concentrations are indicated using a color scale that ranges from green (low) through black to red (high). The dendrogram above the heat map illustrates degrees of relatedness between genital cytokine profiles evident within the various women. The dendrogram on the left-hand side of the heat map indicates relationships between the expression profiles of the analyzed cytokines across all of the women assessed in this study. Abbreviations: GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; IP-10, interferon-γ inducible protein-10; MCP-1, monocyte chemoattractant protein-1; MIP, macrophage inflammatory protein; TNF-a, tumor necrosis factor-alpha.

IL-8, MCP-1, IL-1α, IL-1β, IL-6, and TNF-α), while IL-10 is antiinflammatory and GM-CSF and IL-7 are hematopoietic [16–22]. The risk of HIV acquisition was significantly higher in women who had elevated genital inflammatory cytokines (odds ratio [OR], 3.2; 95% confidence interval [CI], 1.3–7.9; $P = .014$), as indicated by raised levels of at least 5 of the 9 proinflammatory or chemotactic cytokines assessed (Table 2).

The women who later became HIV-infected and those who remained negative had similar demographic characteristics (Table 3). Women who became infected had a slightly higher median number of monthly sex acts and returned used gel applicators (Table 3). The increased risk of HIV acquisition associated with genital inflammation was evident in women assigned to both tenofovir and placebo gel and did not change after adjusting for age, urban/rural residence, condom use, hormonal contraceptives, number of sex acts, number of returned used applicators, and HSV-2 serostatus (OR, 3.8; 95% CI, 1.3–11.2; $P = .016$).

Irrespective of the definition of genital inflammation applied, using various permutations of the 12 cytokines, the relationship between cytokine profiles and HIV infection risk remained evident. Women with ≥4 (OR, 2.4; 95% CI, 1.1–5.3) or ≥6 (OR, 3.0; 95% CI, 1.0–9.3) of these 9 cytokines above the 75th percentile were at increased risk of HIV infection. Furthermore, women with ≥3 (OR, 3.2; 95% CI, 1.3–7.9) or ≥4 (OR, 10.0; 95% CI, 1.3–78.1) of the 5 chemokines assessed (MIP-1α, MIP-1β, IP-10, IL-8, and MCP-1) above the 75th percentile were at increased risk of HIV acquisition.

PLSDA has previously been used to identify multivariate combinations of features that best differentiate individuals based on disease status [15]. Using this approach, we identified specific cytokine profiles that differentiated women who acquired HIV infection from those who remained uninfected. Latent variable 1 (LV1) of our PLSDA model best separated women who became infected from those who remained uninfected (Figure 2*A*). Women who later became infected had higher levels of cytokines positively loaded on LV1 (MIP-1α, MIP-1β, IP-10, IL-8, IL-1α, IL-1β, IL-6, TNF-α, IL-7, and IL-10) and comparatively lower levels of cytokines negatively loaded on LV1 (MCP-1 and GM-CSF; Figure 2*B*). A conditional logistic regression using the LV1 scores as an independent variable determined that the odds of HIV acquisition were 2.9-fold (95% CI, 1.5–5.3; $P = .0009$) higher in women with this cytokine profile. Furthermore, using this PLSDA model, MIP-1α, MIP-1β, IP-10, and IL-8 were identified as the most critical of the 12 cytokines for classifying the 2 groups (evidenced by variable importance projection [VIP] scores >1). This result using a relatively unbiased approach was in agreement with our univariate analysis of cytokines (Table 1).

Table 2. Association Between Genital Inflammation Prior to Human Immunodeficiency Virus (HIV) Infection and HIV Acquisition in Women From the Centre for the AIDS Programme of Research in South Africa 004 Trial

	All Women	
Level of Inflammation[a]	HIV+	HIV−
Genital inflammation present	19	6
Genital inflammation absent	39	52
Total	58	58
Pair-matched odds ratio (95% confidence interval)	3.2 (1.3–7.9)	
P value	.014	

Abbreviation: HIV, human immunodeficiency virus.

[a] Women who had at least 5 of the 9 inflammatory cytokines (interleukin [IL]-1α, IL-1β, tumor necrosis factor-alpha, IL-6, IL-8, interferon-γ inducible protein-10, monocyte chemoattractant protein-1, macrophage inflammatory protein [MIP]-1α, MIP-1β) above the 75th percentile concentration for each cytokine were categorized as having genital inflammation.

Table 3. Demographic, Behavioral, and Clinical Characteristics of the Women Who Acquired Human Immunodeficiency Virus (HIV) Infection and Women Who Remained HIV Negative

Demographic and Behavioral Characteristic	HIV Seroconverters (n = 58) Median (IQR)	Remained HIV-Uninfected (n = 58) Median (IQR)
Age, in years	22 (20–24)	22 (20–26)
Number of sexual partners in lifetime	2 (1–3)	2 (1–3)
Returned used applicators per month	7 (6–10)	6 (4–8)[a]
Reported sexual intercourse per month	5 (3–8)	4 (3–6)[a]
	% (n)	% (n)
Reside in rural setting	65.5 (38)	70.7 (41)
Completed high school	43.1 (25)	39.7 (23)
Gave birth previously	75.9 (44)	81.0 (47)
Married	1.7 (1)	8.6 (5)
Stable partner	94.8 (55)	89.7 (52)
Reported always using a condom during sex	34.5 (20)	27.6 (16)
Baseline contraception		
Injectable	91.4 (52)	82.8 (48)
Oral contraceptive pill	8.6 (5)	13.8 (8)
Tubal ligation/hysterectomy	0.0 (0)	3.5 (2)
Baseline genital discharge	32.8 (19)	20.7 (12)
Herpes simplex virus type-2 prevalence	64.9 (37/57[b])	51.7 (30)
Assigned to use tenofovir gel	39.7 (23)	39.7 (23)

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range.

[a] $P < .05$ on a Wilcoxon signed rank test for paired data.

[b] One woman had no sample to test.

Persistence of Raised Genital Inflammatory Cytokines

The raised genital inflammatory cytokine levels were not short-lived (Figure 3). In a longitudinal analysis, genital cytokine

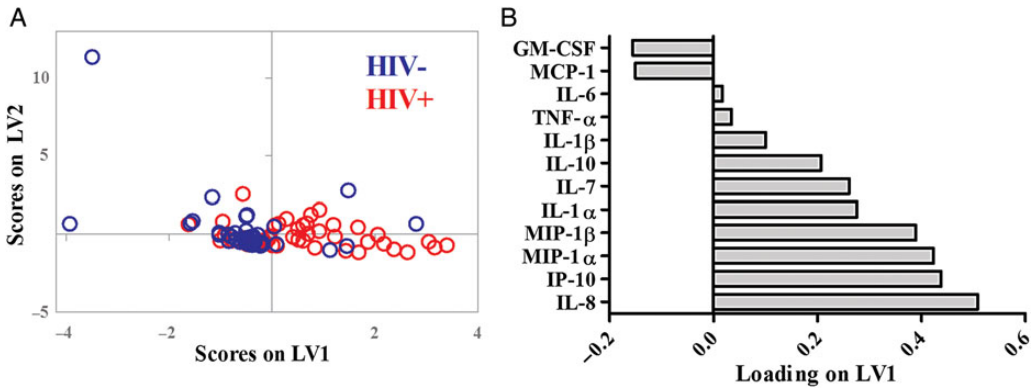

Figure 2. Identification of multivariate cytokine profiles associated with human immunodeficiency virus (HIV) infection at a later time. *A*, Partial least squares discriminant analysis model of all 12 cytokines classified individuals with 65% overall accuracy for classification and 63% accuracy for cross-validation (blue, remain uninfected; red, become infected). *B*, Latent variable 1 best separated individuals who became infected with HIV from those who remained uninfected. Cytokine loadings indicate multivariate cytokines associated with HIV infection. Since individuals who became infected with HIV clustered in the positive region of latent variable 1 (LV1) (*A*), cytokines positively loaded on LV1 (macrophage inflammatory protein [MIP]-1α, MIP-1β, interferon-γ inducible protein-10 [IP-10], interleukin [IL]-8, IL-1α, IL-1β, IL-6, tumor necrosis factor-alpha [TNF-α], IL-7, and IL-10) (*B*) are elevated in profiles of those who became infected while negative loadings (monocyte chemoattractant protein-1 [MCP-1], granulocyte macrophage colony-stimulating factor [GM-CSF]) were comparatively reduced. Logistic regression analysis indicated that higher scores on LV1 were associated with a 2.86-fold (95% confidence interval, 1.54–5.30 and $P = .0009$) higher odds of infection. Variable importance projection scores indicated that MIP-1α, MIP-1β, IL-8, and IP-10 were most important for classification of the 2 groups.

concentrations at 2 time points were examined in 21 women who later acquired HIV and in 36 women who remained uninfected (median time between sampling was 48 weeks [range, 8–104]). The correlation coefficient for the cytokine concentrations at these 2 time points was >0.3 for IL-1α, IL-1β, IL-6, TNF-α, MIP-1β, IL-7, and MCP-1 ($P < .05$ following adjustment for multiple comparisons; Figure 3), indicating that women with the highest cytokine concentrations at one time point ranked similarly at the second time point. In a matched-pair analysis (Supplementary Table 2), the concentrations of 11 of 12 cytokines did not differ significantly between the 2 time points.

Searching for Possible Causes of Raised Genital Inflammatory Cytokines

No significant associations were found between having a raised genital inflammatory profile (defined by ≥5 of 9 inflammatory cytokines above the 75th percentile) and vaginal discharge, genital ulceration, HSV-2 serostatus, contraceptive choice, intravaginal substance use, presence of a stable partner, parity, and reported sex acts per month (Table 4). Women without genital inflammation reported less frequent condom use ($P = .05$). None of the inflammatory cytokines measured in CVLs differed between women in the tenofovir and placebo arms (Figure 4).

Associations between genital and plasma cytokine concentrations were investigated in a subset of women (n = 107). Plasma cytokine concentrations were not elevated in women who had raised genital cytokines (n = 23) compared with women who did not have raised genital cytokine concentrations (n = 84; Table 4). Further, plasma and genital cytokine concentrations did not correlate significantly (data not shown).

Samples for STI testing were available for 20 of 25 women with raised genital inflammatory profiles and 68 of 91 without evidence of genital inflammation. Ten women with genital inflammation (50.0%) and 23 women without genital inflammation (33.8%) were PCR positive for *T. vaginalis*, *C. trachomatis*, *N. gonorrhoeae*, or HSV-2 ($P = .20$; Table 4). Twenty percent of the raised genital inflammatory cytokines measured in this study could be attributed to the presence of any one of the common STIs caused by these organisms. The strongest association with genital inflammation was with *T. vaginalis*. These results show that STIs (including those caused by *T. vaginalis*) could not fully explain the majority of genital inflammation that was detected in this study, suggesting that other inflammatory mediators may play a role.

Elevated IL-6, IP-10, MCP-1, and MIP-1β concentrations were significantly associated with younger age, after adjusting for discharge, STIs, and hormone contraceptive use (β-coefficient per 5 year decrease in age [*P* value]: 0.31 $\log_{10}$ pg/mL [$P = .002$], 0.43 $\log_{10}$ pg/mL [$P = .001$], 0.63 $\log_{10}$ pg/mL [$P = .002$], and 0.32 $\log_{10}$ pg/mL [$P = .041$], respectively). Age, however, did not differ among women defined as having genital inflammation compared with those who did not (Table 4).

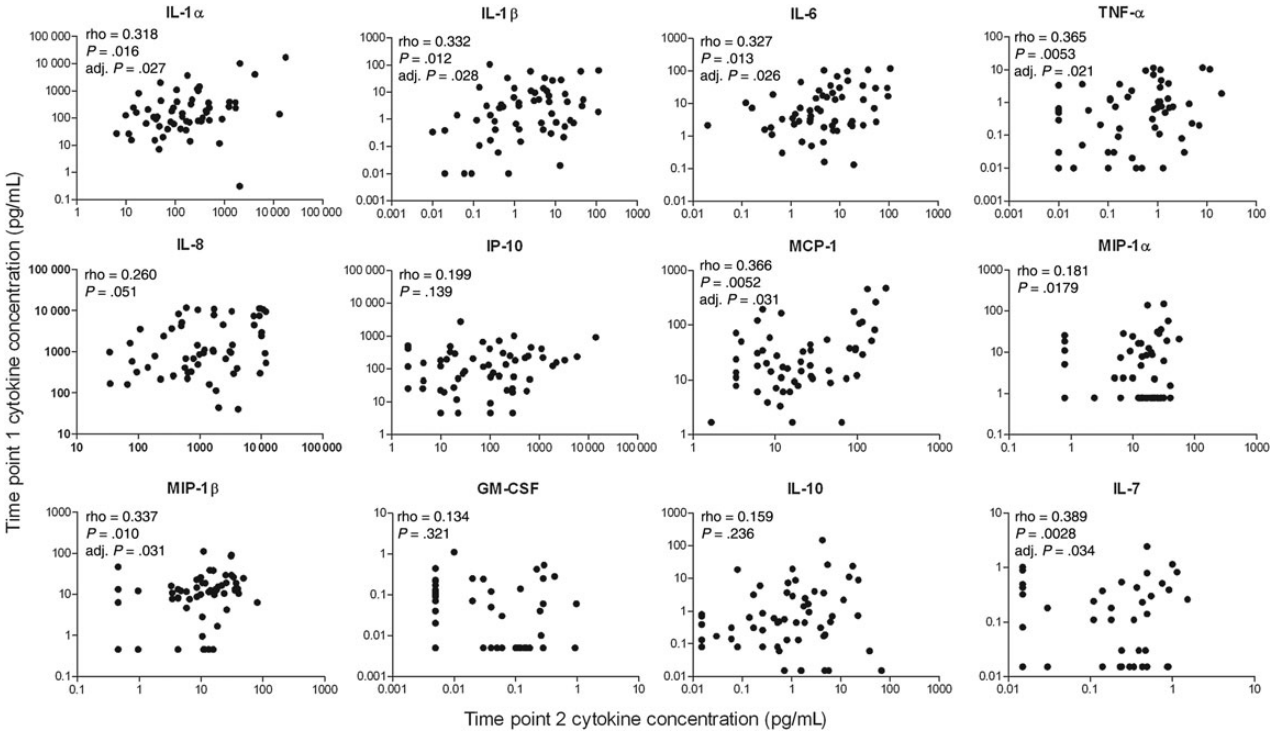

Figure 3. Spearman correlations between genital tract cytokine concentrations in the same women (n = 57) at 2 time points (median 48 weeks apart [range, 8–104]). The correlation coefficient was >0.3 ($P < .05$) for interleukin (IL)-1α, IL-1β, IL-6, tumor necrosis factor-alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1β, and IL-7, and these associations remained significant following adjustment for multiple comparisons. Abbreviations: GM-CSF, granulocyte macrophage colony-stimulating factor; IP-10, interferon-γ inducible protein-10.

DISCUSSION

Raised genital tract inflammatory cytokine concentrations were found to be an important risk factor for HIV acquisition in the women in this study. Women with a genital inflammatory profile (≥5 of 9 inflammatory cytokines elevated) were at significantly increased risk of HIV acquisition (OR, 3.2; 95% CI, 1.3–7.9). The raised cytokine concentrations were sustained for 1 year, on average, prior to infection, suggesting the presence of persistent genital inflammatory signatures.

Higher concentrations of chemotactic cytokines IP-10, MIP-1α, MIP-1β, and IL-8 were each associated with increased HIV acquisition risk and were identified as the most influential mediators to differentiate women who subsequently became HIV infected from those who remained HIV negative using PLSDA and VIP analyses. IP-10, MIP-1α, and MIP-1β are chemotactic for potential HIV target cells, including T cells, monocytes, macrophages, and dendritic cells [10, 23–25]. MIP-1α and MIP-1β, in particular, bind to the HIV coreceptor C-C chemokine receptor type 5 (CCR5) and specifically recruit CCR5+ target cells that potentially enhance HIV infection [26]. Although previous studies in exposed seronegative women and in vitro models suggested that MIP-1α and MIP-1β may protect against HIV infection by competitively inhibiting HIV binding to CCR5 [27, 28], it has been suggested that exposed seronegative women have higher genital concentrations of these chemokines compared with HIV-uninfected low-risk women because they are more likely to have STIs [29]. Furthermore, although MIP-1α and MIP-1β may competitively inhibit HIV in vitro, these models do not account for the fact that the production of these chemokines may be accompanied by upregulation of other inflammatory factors or recruitment of HIV target cells that may facilitate HIV replication in vivo. In macaques, production of these and other inflammatory cytokines was found to be essential for recruitment of CD4+ T cells needed for SIV replication [10]. IL-8, which similarly correlated with susceptibility to HIV infection in this study, has also been associated with increased HIV transmission in cervical explant tissue [30].

There was little correlation between elevation of cytokines in the genital tract and plasma. This is in keeping with other reports that genital cytokine concentrations are not related to systemic cytokine concentrations [31, 32]. Combined, the evidence points to a genital compartment factor as a likely cause of the persistent genital inflammation that predisposed these women to HIV acquisition.

Table 4. Relationship Between Genital Inflammation and Possible Behavioral, Clinical, and Systemic Causes

Demographic and Behavioral Characteristic	Genital Inflammation Present (n = 25) % (n)	Genital Inflammation Absent (n = 91) % (n)	*P* Value
Age in years (median [IQR])	22 (20–24)	22 (20–25)	.40
Assigned to use tenofovir gel	36.0 (9)	40.7 (37)	.82
Reside in rural setting	64.0 (16)	69.2 (63)	.63
Completed high school	44.0 (11)	40.7 (37)	.82
Gave birth previously	72.0 (18)	80.2 (73)	.41
Number of live births (median [IQR])	1 (0–1)	1 (1–2)	.19
Married	8.0 (2)	4.4 (4)	.61
Stable partner	92.0 (23)	92.3 (84)	1.00
Reported sexual intercourse per month (median [IQR])	5 (4–6)	4 (3–7)	.54
Number of sexual partners in lifetime (median [IQR])	2 (2–3)	2 (1–3)	.52
Reported always using a condom during sex	48.0 (12)	26.4 (24)	.05
HSV-2 status during study[a]			
Baseline positive	56.0 (14)	58.9 (53)	.50
Acquired new infection	20.0 (5)	11.1 (10)	
Remained negative	24.0 (6)	30.0 (27)	
Genital discharge at sampled visit	24.0 (6)	13.3 (12)	.23
Genital ulcer at sampled visit	0.0 (0)	2.2 (2)	1.00
Contraceptive choice at sampled visit			
Injectable (Depo-Provera or Nur-Isterate)	80.0 (20)	82.4 (75)	.73
Oral contraceptive pill	20.0 (5)	15.4 (14)	
Tubal ligation/hysterectomy	0.0 (0)	2.2 (2)	
Intravaginal insertions within 30 d of sampled visit	4.0 (1)	4.4 (4)	1.00
Intravaginal insertions at any point during trial	20.0 (5)	17.6 (16)	.77
Sexually Transmitted Infections (by Polymerase Chain Reaction)	Genital Inflammation Present (n = 20) % (n)	Genital Inflammation Absent (n = 68) % (n)	
Trichomonas vaginalis	40.0 (8)	20.6 (14)	.09
Chlamydia trachomatis	20.0 (4)	16.2 (11)	.74
Neisseria gonorrhoeae	5.0 (1)	4.4 (3)	1.00
HSV-2	5.0 (1)	2.9 (2)	.54
Any one of the above sexually transmitted infections	50.0 (10)	33.8 (23)	.20
Plasma Cytokine Concentration (pg/mL)[b]	Genital Inflammation Present (n = 23) Median (IQR)	Genital Inflammation Absent (n = 84) Median (IQR)	
IL-1β	0.07 (0.01–0.23)	0.03 (0.01–0.14)	.31
IL-1α	0.68 (0.68–0.68)	0.68 (0.68–0.68)	.34
IL-6	0.86 (0.49–1.66)	0.78 (0.21–1.91)	.47
IL-7	0.01 (0.01–0.08)	0.01 (0.01–0.33)	.99
IL-8	1.73 (0.01–3.76)	1.87 (0.01–3.46)	.88
IL-10	0.24 (0.24–8.12)	0.24 (0.24–6.02)	.85
GM-CSF	0.89 (0.52–1.93)	0.86 (0.43–1.80)	.55
TNF-α	3.72 (2.40–5.92)	3.63 (2.11–5.42)	.77
IP-10	147.04 (99.85–246.13)	166.49 (111.27–253.28)	.49
MCP-1	151.49 (122.68–218.63)	136.73 (86.62–198.16)	.44
MIP-1β	15.63 (11.79–27.67)	24.75 (16.31–31.23)	.19
MIP-1α	4.22 (0.21–8.40)	1.96 (0.21–5.52)	.14

Sampled visit refers to the visit at which genital cytokine concentrations were assessed. Intravaginal insertions refer to reported insertion of any substance and/or fingers into the genital tract.

Abbreviations: GM-CSF, granulocyte macrophage colony-stimulating factor; HSV-2, herpes simplex virus type-2; IL, interleukin; IP-10, interferon-γ inducible protein-10; IQR, interquartile range; MCP-1, monocyte chemoattractant protein-1; MIP, macrophage inflammatory protein; TNF-α, tumor necrosis factor-alpha.

[a] One woman had missing HSV-2 data.

[b] Plasma cytokine concentration data were available for a subset of 107 women (23 women who had genital inflammation and 84 women who did not have inflammation).

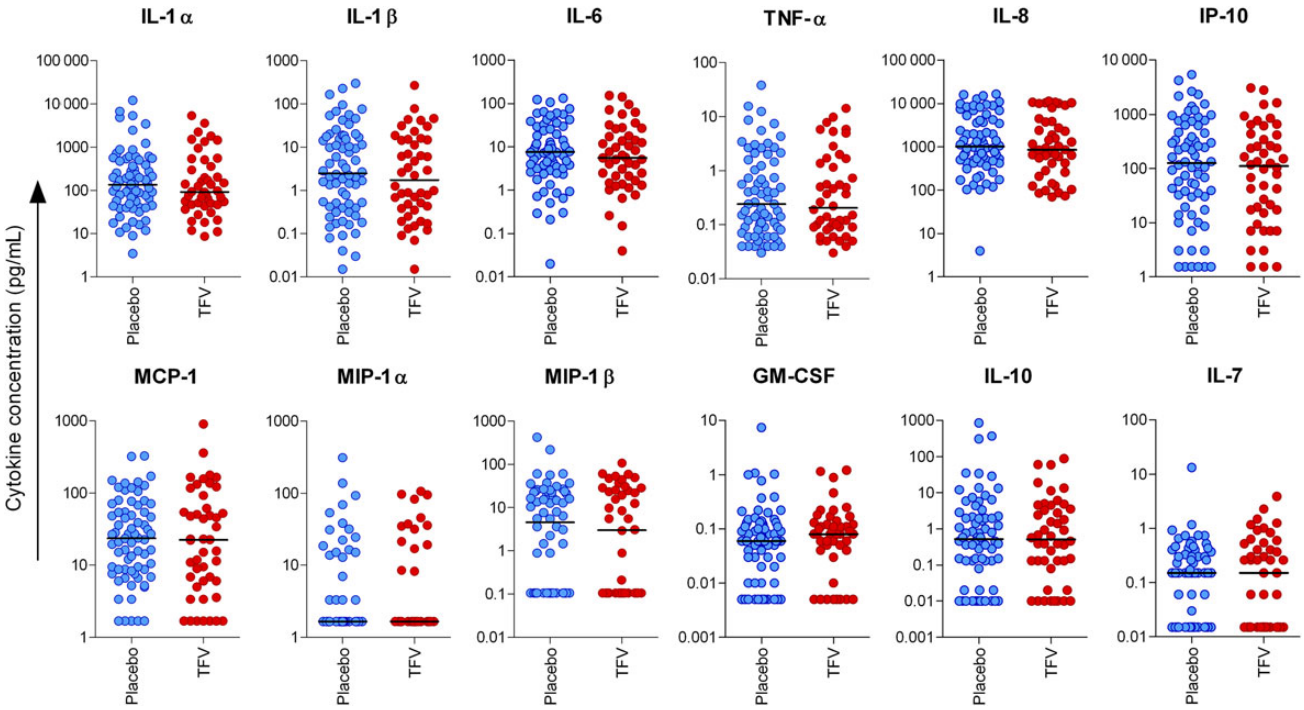

Figure 4. Cytokine concentrations in cervicovaginal lavage (CVL) samples from human immunodeficiency virus–uninfected placebo and tenofovir (TFV) gel users. CVL cytokine concentrations in women who were using placebo gel (n = 70) are indicated by blue circles; red circles indicate those of TFV gel users (n = 46). Lines indicate medians. No significant differences ($P < .05$) were found using Mann–Whitney *U* test. Abbreviations: GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; IP-10, interferon-γ inducible protein-10; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; TNF-α, tumor necrosis factor-alpha.

Since genital infections are associated with an increase in local inflammatory cytokine concentrations [11, 12], we assessed associations between inflammatory cytokines and common STIs. Half of the women with genital inflammation and a third of women without genital inflammation had evidence of an STI. Relatively few of the 116 women in this study had vaginal discharge (n = 18) or genital ulceration (n = 2). Only 6 of 25 (24%) women who had an elevated genital inflammatory profile had signs of an infection, and this did not differ significantly in women who did not have inflammation (15%). Hence, these genital conditions could not account for more than a small fraction of the women with raised genital inflammatory cytokine concentrations observed in this study.

Limitations of this study are that we were not able to screen all of the women for STIs, due to samples not being available, and we were not able to assess bacterial vaginosis as a potential cause of genital inflammation. The role of bacterial vaginosis as well as other possible causes of genital inflammation, including vaginal hygiene practices [33], exposure to seminal proteins [34], lubricants [35], hormone cycling [36], human papilloma virus [37], and genital schistosomiasis [38], are being investigated in follow-on studies. All of the women in this study were using placebo or tenofovir gel. Although there were no differences in cytokine concentrations between these 2 groups, both gels may have caused variation in cytokine levels at the individual level. It has been suggested that hydroxyethylcellulose gel, which was used as the placebo in this trial, increases certain genital cytokines [39] and may have altered genital inflammatory profiles of the women who used gel in this study.

Several studies have suggested that demographic or geographic characteristics of women may influence the genital inflammatory environment [40, 41]. White women with normal vaginal flora have been found to have higher cervical proinflammatory cytokine concentrations compared with black women [40]. In addition, the allelic distribution at commonly assayed sites in genes encoding certain proinflammatory cytokines was found to differ significantly between white and black populations [41]. Also, wide variation in genital cytokine concentrations has been observed in many studies within control groups of women who do not have any common infections, are not using hormone contraception, and do not have detectable seminal plasma in their genital secretions [11, 12, 36]. These findings suggest that the "normal" healthy immune milieu in the female genital tract differs at the population level and at the individual level and may influence susceptibility to HIV infection.

Although the cytokines assessed in this study are well-established biomarkers of cellular recruitment to the genital tract and play important roles in genital inflammation [10, 24, 35], a limitation was that we did not have matching genital tissue specimens available from women before they became infected for assessment of HIV target-cell infiltration or more in-depth mechanistic studies.

Younger women were found to have higher genital concentrations of IL-6, IP-10, MCP-1, and MIP-1β compared with older women, providing a possible reason, in addition to behavioral factors, for the higher rates of HIV infection that are seen in young South African women. Although women who later became HIV-infected reported more frequent sexual activity than those who did not become infected, the relationship between inflammation and risk of HIV infection was found to be independent of the number and frequency of sex acts. Additionally, genital cytokine concentrations were not associated with sexual activity and did not differ between tenofovir and placebo gel users. Women who did not have genital inflammation reported less frequent condom use compared with women with an inflammatory profile, which may be due to differences in risk perception between the groups.

In conclusion, susceptibility to HIV infection was associated with elevations in genital inflammatory cytokines, as a marker of genital inflammation. The increased frequency of 4 inflammatory cytokines in younger women may be an important driver of the high HIV incidence rates in young women in Africa. While a fraction of the genital inflammation may be attributed to asymptomatic STIs, the dominant cause of genital inflammation remains to be elucidated. Treatment of asymptomatic STIs and topical agents to modulate inflammation are potentially important mechanisms for reducing susceptibility to HIV infection, especially in young women, where the epidemic is most severe in Africa. These mechanisms require further investigation.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No potential conflicts of interest.

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