Genital inflammation, immune activation and risk of sexual HIV acquisition

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\textbf{Purpose of review}  
Women who have genital inflammation are at increased risk of sexual HIV infection. The purpose of this review is to evaluate the mechanisms for this relationship, causes of genital inflammation, and strategies to manage this condition.

\textbf{Recent findings}  
We have recently shown in a cohort of South African women that HIV seroconversion was associated with persistently raised genital inflammatory cytokines (including MIP-1\textalpha, MIP-1\beta, and IP-10). Elevated inflammatory cytokine concentrations may facilitate HIV infection by recruiting and activating HIV target cells and disrupting the mucosal epithelial barrier. Bacterial vaginosis and sexually transmitted infections (STIs), which are predominantly asymptomatic in women, cause lower genital tract inflammation and increased HIV acquisition risk. In Africa, where syndromic management of STIs and bacterial vaginosis is standard-of-care, the substantial burden of asymptomatic infections has likely contributed to high-HIV incidence rates.

\textbf{Summary}  
A genital inflammatory profile contributes to the high risk of HIV acquisition in African women. STIs and bacterial vaginosis are poorly managed in Africa and other developing nations and as such remain major drivers of persistent genital inflammation and HIV acquisition among these women.

\textbf{Keywords}  
cytokines, genital tract, HIV risk, inflammation, T-cell activation

\textbf{INTRODUCTION}  
Young women in sub-Saharan Africa are disproportionately affected by HIV, with infection risk of up to eight-fold higher than in men of the same age \cite{1}. Despite new HIV infections dropping from 3.4 million in 2001 to 2.0 million globally in 2014 \cite{2}, continued transmission in young women is one of the greatest challenges preventing an AIDS-free generation \cite{3}. Susceptibility to HIV infection varies considerably from person-to-person, with some women remaining uninfected despite repeated exposure \cite{4}. Bacterial vaginosis and sexually transmitted infections (STIs) \cite{5–8}, as well as other biological factors, have been shown to impact the risk of young women acquiring HIV. Genital inflammation underlies many of these risk factors, providing a unifying mechanism driving risk \cite{8,9**,10**}.

\textbf{HIV RISK AND GENITAL INFLAMMATION}  
Inflammation in the female genital tract, regardless of the cause, creates an environment that favors HIV replication and establishment of a productive infection. Women with elevated concentrations of proinflammatory cytokines, including macrophage inflammatory protein (MIP)-1\textalpha, MIP-1\beta and IP-10, in their genital tracts were found to be at increased risk of HIV acquisition \cite{9**}. Interferon gamma-induced protein 10 (IP-10) MIP-1\textalpha, and MIP-1\beta are chemotactic for HIV target cells, including T cells, macrophages, and dendritic cells \cite{11–14}.
MIP-1α and MIP-1β are also ligands for the HIV coreceptor C–C chemokine receptor 5 (CCR5) and specifically recruit CCR5⁺ target cells into tissues [15]. Proinflammatory cytokine signatures in the lower reproductive tract have been associated with increased frequencies of neutrophils, T and B cells, as well as higher levels of cellular activation [16-17]. Proinflammatory cytokines and chemokines that are involved in activation, differentiation, and recruitment of immune cells to the genital tract may increase HIV transmission as HIV replication depends on the presence of immune cell targets, the level of immune cell activation and monocyte differentiation to macrophages or dendritic cells [13,16]. In rhesus macaques, proinflammatory cytokine production following vaginal simian immunodeficiency virus (SIV) exposure resulted in recruitment of CD4⁺ T cells needed for establishment of SIV infection [11,18]. The essential role of inflammation in SIV infection was clearly demonstrated when topical application of an anti-inflammatory, glycerol-monolaurate, down-regulated chemokine concentrations, inhibited inflammatory cell influx to the genital tract, and prevented SIV infection in macaques [11].

Studies in exposed seronegative (ESN) women, who remained HIV-uninfected despite high-risk sexual activity, have improved our understanding of risk factors for HIV acquisition. In ESN women, concentrations of the CCR5-binding chemokine regulated on activation, normal T cell expressed and secreted were found to be elevated compared with low-risk controls, whereas MIP-1α and MIP-1β have been shown to competitively inhibit HIV binding to CCR5 in vitro, suggesting that these chemokines may protect against HIV infection [19,20]. However, ESN women may have higher genital chemokine concentrations compared with low-risk controls because they are more likely to have STIs [4], and in vitro models do not account for up-regulation of other inflammatory factors or recruitment of HIV target cells by these chemokines that may facilitate HIV replication in vivo. More recent ESN studies have shown that an immune quiescent phenotype in the female genital tract may account for reduced susceptibility to HIV infection in these women [21,22]. Although ESN women were found to have higher CD4⁺ T cell numbers at the cervix, fewer of these cells expressed CCR5 compared with low-risk women [22].

In addition to recruiting more target cells for HIV replication, proinflammatory cytokines induce expression of the transcription factor, nuclear factor (NF)-κB, which binds to HIV-long terminal repeat and directly upregulates HIV replication [23]. Proinflammatory cytokines may also facilitate HIV infection by disrupting tight junctions between epithelial cells, reducing the integrity of this barrier [24]. In support of this, proteomic studies have shown that women with elevated genital proinflammatory cytokine concentrations have unique protein signatures of reduced epithelial barrier function [17]. Several proteins that regulate actin cytoskeleton organization and extracellular matrix components were found to be associated with genital inflammation, suggesting that tissue remodeling occurs in women with inflammation at the expense of effective barrier function [17].

**SYSTEMIC MARKERS OF INFLAMMATION, CHEMOKINE GRADIENTS AND HIV RISK**

Blood biomarkers have also been associated with increased risk of HIV infection [25,26]. Others from our group reported that women who later became HIV-infected had higher plasma concentrations of TNF-α, IL-2, IL-7, and IL-12p70 than women who remained uninfected [25]. We found that these and other cytokines do not correlate between blood and the genital tract, suggesting that cytokine risk factors identified in blood do not predict those in the genital tract and vice versa [9,27]. Kahle et al. [26] found that elevated plasma IP-10 and IL-10 concentrations predicted HIV seroconversion in individuals in HIV discordant relationships.

ESN women had lower concentrations of HIV-target cell recruiting chemokines, including IP-10, MIP-1α, and MIP-1β, in the genital tract than blood, which may result in reduced target cell influx in the absence of a chemokine gradient to the genital tract, and thereby confer a certain degree of protection against HIV infection [21,22]. This suggests that a chemokine gradient from blood to the genital mucosa may contribute to risk for HIV infection.

The level of T-cell activation in blood appears to be important in HIV risk. CD4⁺ T-cell quiescence has shown to be protective against HIV infection in vivo [28]. Studies in European adult ESNs showed relatively lower CD38 and
Immune activation

HLA-DR-expressing CD4+ T cells in blood than persons who go on to become HIV-infected [28]. SIV-exposed sooty mangabey infants, with few peripheral and mucosal CD4+CCR5+ cells, are less likely to acquire SIV via low-dose oral challenge than their rhesus macaque counterparts [29]. We found a strong correlation between peripheral and cervical T-cell activation in HIV-uninfected women [30]. Global T-cell activation may be an important contributing factor determining HIV risk.

SEXUALLY TRANSMITTED INFECTIONS CAUSE GENITAL INFLAMMATION

STIs are major causes of inflammatory cytokine upregulation and immune cell recruitment to the genital mucosa [27,31–34]. Although inflammation can be important in STI clearance, it may also cause destruction of infected epithelial layers, allowing STI-associated microbes to access deeper tissues [35,36]. Relatively few women are able to clear an infection in the absence of treatment, with STIs often being recurrent or persistent [37,38]. In addition to reproductive complications, nonulcerative STIs have been found to influence susceptibility to HIV infection [5,8]. We have shown that Chlamydia trachomatis, Neisseria gonorrhoeae, and Mycoplasma genitalium infections were associated with increased risk of HIV acquisition [8]. Highly prevalent STIs, such as human papillomavirus (HPV) infections, have also been shown to increase risk of HIV infection [39]. Of the common STIs, we found that Chlamydia was associated with the highest genital cytokine levels, followed by gonorrhoea, herpes simplex virus-2 (HSV-2), trichomoniasis, and bacterial vaginosis [27].

In a cohort of African women, Masese et al. [40**] reported that the overall population risk for HIV infection was largely attributable to HSV-2, even in the absence of ulcers, with prevalent HSV-2 accounting for 48.3% and incident HSV-2 infections accounting for 4.5% of risk. Although HSV-2 ulcerative lesions disrupt the mucosal barrier, higher numbers of DC-SIGN+ DCs and CCR5+ CD4+ T cells are observed in the genital tracts of women who have HSV-2, even in the absence of HSV-2 shedding or genital ulceration, and subclinical inflammatory responses in the mucosa are evident for months after a reactivation event [34,41]. Other infections, including yeast (6.4%), Trichomonas vaginalis (1.1%), N. gonorrhoeae (0.9%), and nontypable cervicitis (0.7%), accounted collectively for 9% of the population attributable risk for HIV in an African cohort [40**]. Masese et al. [40**] showed that prevalent HSV-2 infections continued to be the most dominant population attributable risk (40.4–61.8% between 1998 and 2012) over time. In South Africa it is estimated that over 50% of new HIV infections in women could be attributed to STIs, bacterial vaginosis and candidiasis in 2010, with HSV-2 being the most influential infection [42].

BACTERIAL VAGINOSIS AND THE VAGINAL MICROBIOME INFLUENCE GENITAL INFLAMMATION

Bacterial vaginosis is a syndrome characterized by a displacement of healthy vaginal commensal microflora by other Gram-positive and Gram-negative bacteria [43,44]. A recent meta-analysis found that bacterial vaginosis was associated with 1.7-fold increased risk of HIV acquisition [7]. Masese et al. [40**] reported that bacterial vaginosis contributed substantially to HIV acquisition risk, with 15.1% of the overall population risk attributable to this condition and 7.5% attributable to intermediate microflora [40**]. Given the high infection risk and recurrence of bacterial vaginosis, this strong association with HIV risk has important public health implications.

Several studies from North America have defined a healthy female genital tract as one harboring predominantly Lactobacillus species (particularly L. crispatus and L. jensenii), having a pH between 3.5–4.5, having no bacterial vaginosis, candida, or other STIs [45–47], although this may not be perfectly applicable to women in Africa [47]. Recent studies from South Africa found that less than 40% of women had a vaginal microbiota dominated by Lactobacillus spp., with more than half of the women not having an easily definable predominant bacterial taxon [10**]. Commensal microorganisms are recognized as an important component of vaginal mucosal defense against STIs [48], including HIV [49–52], but the mechanisms of this protection are not well elucidated and are likely multifactorial. There are several ways by which commensal bacteria could potentially affect vaginal inflammation and HIV susceptibility. These include: lowering vaginal pH as a result of their lactic acid and H2O2 metabolites; competitive antagonism of pathogens; antimicrobial factor production [53], modulation of epithelial barrier integrity, epithelial or immune cell function [17**,54**]; generation of tolerizing cells such as Tregs [55,56]. No single bacterial strain drives all of these effects, and it is likely that more than one of these mechanisms may be at play, and may not be mutually exclusive.

In vitro experiments have demonstrated that Lactobacillus species generally induce low or no proinflammatory cytokine production by vaginal epithelial cell lines, compared with common
bacterial vaginosis-associated organisms, such as *Atopobium vaginae* or *Gardnerella vaginalis* [57,58]. Anahtar et al. [10**] found that the presence-specific combinations of noncommensal organisms (cervicotype IV defined by a high diversity of organisms, dominated by *Gardnerella* and *Prevotella* species, but also featuring *Shuttleworthia, Sneathia, Megasphaera, Mobiluncus*, and *Atopobium*) was associated with higher levels of inflammation (measured by IL-1α, IL-1β, and TNF-α concentrations) in the genital tracts of young African women. Only half of the young women in this category had Nugent scores less than 7. Some of these noncommensal bacteria individually (*Sneathia amnii, Streptococcus sanguinegens* and *Mobiluncus mulieris*) induced inflammatory responses by vaginal epithelial cell lines [10**]. These women were followed longitudinally, and changes in prevalent cervicotypes were associated with significant increases in IL-1α, IL-1β, and TNF-α, implying a causal relationship [10**]. Other studies have found that bacterial vaginosis is associated with genital proinflammatory cytokine upregulation, but also downregulation of some cytokines [27,59,60]. This is likely because of the fact that bacterial vaginosis is complex, and is not the same syndrome in every case.

Proteomic analysis of women with increasing levels of vaginal dysbiosis was able to identify several cytokines and cytokine receptors that increased with bacterial vaginosis, but also found alterations in proteins associated with mucosal barrier breakdown, including mucus and cytoskeletal alterations (decreased keratins and cornified envelope proteins) [54**]. Interestingly, Arnold et al. [17**] reported similar changes in women with increased genital inflammation, implying that bacterial vaginosis may act through these same pathways to increase susceptibility to HIV.

**HORMONAL CONTRACEPTIVES AND GENITAL INFLAMMATION**

Over 50 studies have examined the association between hormonal contraceptive use and HIV. Some studies have found no association [61], whereas others have found up to two-fold higher risk of HIV acquisition in seronegative women using any hormonal contraceptive [62]. In macaques, progesterone implants increase susceptibility to vaginal inoculation with SIV [63]. This is thought to be because of epithelial thinning, which can be reversed by pretreatment with estrogen [64,65]. Studies of the effect of hormonal contraceptive among humans on genital epithelium did not observe the thinning seen in nonhuman primate studies [66]. Cervical ectopy, or extension of the endocervical columnar epithelium onto the ectocervix, has been associated with hormonal contraceptive use [67,68]. depot medroxyprogesterone acetate (DMPA) may decrease vaginal colonization by *H₂O₂*-producing *Lactobacillus* species [66]. Conversely, DMPA has been shown in cohort studies to decrease the risk of bacterial vaginosis, but to increase the risk of other STIs, including *C. trachomatis* and HSV-2 [69,70]. On a cellular level, hormonal contraceptives have been associated with cervical and vaginal inflammation [71–73], increased genital tract cellular CCR5 expression [74–76], and T-cell and macrophage mucosal trafficking [77]. Conversely, DMPA may also have anti-inflammatory effects [74,78].

Other possible causes of genital inflammation that may influence HIV risk include vaginal hygiene practices [79], exposure to seminal proteins [80], lubricants [81], hormone cycling [59], and genital schistosomiasis [82], as well as host genetics [83].

**MANAGEMENT OF SEXUALLY TRANSMITTED INFECTIONS AND BACTERIAL VAGINOSIS TO REDUCE HIV INCIDENCE**

In resource-limited settings, STIs and bacterial vaginosis are managed syndromically, according to the presence of clinical signs and symptoms [84]. However, large proportions of women who have STIs or bacterial vaginosis are asymptomatic and are thus left untreated [8,85]. In South Africa, the implementation of syndromic management in the mid-1990s, as well as increased condom use, resulted in a decline in gonorrhoea, chancroid, and syphilis, although there has been little or no evidence of declining infection risk of other STIs and bacterial vaginosis [42]. After the introduction in South Africa, the proportion of new HIV infections attributable to curable STIs decreased from 39 to 14% between 1990 and 2010, however, the proportion of HIV infections attributable to HSV-2 increased and the contribution of bacterial vaginosis remained unchanged [42].

The results of population-wide STI treatment interventions for HIV prevention have been largely disappointing [86–90]. Two of three STI syndromic management interventions in Africa resulted in no change in HIV acquisition [91–94], suggesting that asymptomatic infections may play a significant role. We have demonstrated in South African women that asymptomatic STIs were just as inflammatory as symptomatic infections, but only 12% of women with laboratory confirmed STIs had clinical signs [8]. This suggests that women with asymptomatic infections are also at high risk of acquiring HIV.
Treatment of HSV-2 has also been found to be ineffective at reducing HIV infection rates [87,95]. Although HSV-2 suppressive therapy may reduce genital ulceration, HSV-2 may induce a persistent state of susceptibility to HIV infection because of the ongoing inflammation it causes [34]. Bacterial vaginosis may also have been a significant factor contributing to the failure of these interventions, as bacterial vaginosis has proven difficult to treat, with a recurrence rate of 50% within 6 months of antibiotic treatment [96].

CONCLUSION

Although we do not fully understand the causes of genital inflammation that is associated with high-HIV acquisition risk in women, prevalent STIs and bacterial vaginosis clearly play a major role. Syndromic diagnoses of these conditions are inadequate, with the vast majority of women asymptomatic. Current treatment strategies for HSV-2 and bacterial vaginosis are ineffective, with HSV-2 suppressive therapy associated with ongoing genital inflammation and antibiotic treatment of bacterial vaginosis having high recurrence rates. There is thus an urgent need for better strategies to manage STIs and bacterial vaginosis to reduce genital inflammation in women at high risk for HIV infection.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest
■ of outstanding interest


10. The first study to demonstrate a relationship between elevated genital inflammatory cytokine concentrations and increased risk of HIV acquisition in vivo, suggesting that genital inflammatory responses play a crucial role in establishment of HIV infection.


16. The study comprehensively describes the relationship between high-vaginal bacterial diversity and local inflammation, providing a possible cause for the genital infection observed in South African women; that was associated with HIV acquisition risk. Additionally, this work describes the unique vaginal microbiome of South African women.


27. The study describes the proteomic profile and activation of HIV target cells that are associated with female genital tract inflammation, as well as possible mechanisms for the relationship between elevated genital inflammatory cytokine concentrations and increased HIV acquisition risk.


