Published in final edited form as:
Am J Reprod Immunol. 2013 February ; 69(0 1): 61–67. doi:10.1111/aji.12044.

HIV, Sexual Violence and Special Populations: Adolescence and Pregnancy

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Abstract

The risk of male to female transmission of HIV is impacted by baseline inflammation in the female genital tract, semen viral load and seminal plasma's ability to induce specific patterns of cervical cytokine signalling and influx of immune cell populations. Disruption of the epithelial barrier during non-consensual intercourse may trigger further inflammation and initiation of cell-signalling pathways, thus facilitating transmission of HIV and expansion of local infection. Adolescent and pregnant women are at high risk for sexual violence and may exhibit alterations of genital mucosal immunity that promote immune activation, making them uniquely vulnerable to HIV acquisition.

Keywords

Adolescence; genital mucosal immunity; HIV; lactobacillus; pregnancy

Introduction

Transmission of HIV through heterosexual intercourse is relatively inefficient, with an estimated rate of male to female transmission of 0.08% per act (95% CI 0.06–0.11) and of 0.04% per act for female to male transmission (95% CI 0.01–0.14).[1] However this transmission risk is impacted by numerous host and viral variables and may be significantly increased in the setting of non-consensual sexual intercourse. Disruption of the genital epithelial barrier, anal penetration, mucosal inflammation and concomitant sexually transmitted infection (STI) may all increase the risk of HIV acquisition.[2] Higher semen viral load, which characterizes acute HIV infection, is associated with increased risk of transmission.[3–5] Transmission of sexually transmitted infections is well described in rape victims[6] and may increase both HIV infectiousness and susceptibility through disruption of mucosal barriers, target cell recruitment and activation, altered cytokine production, impaired HIV-specific cytotoxic T lymphocyte (CTL) function and enhanced HIV replication.[7,8] Adolescents and pregnant women, who are highly vulnerable to sexual violence and non-consensual sex, may be at higher risk for HIV acquisition secondary to

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Disclosures
The authors have no potential conflicts of interest to declare.

altered baseline concentrations of specific genital tract cytokines, chemokines, vaginal microbiota and antimicrobial peptides. The current review examines the genital mucosal factors that may make adolescent and pregnant women especially vulnerable to HIV acquisition in the setting of sexual violence.

The epithelial barrier and the impact of sexual intercourse

An intact genital mucosal epithelium is the first barrier to infection and is coated in protective genital tract secretions with an acidic pH. Microtrauma to this barrier may occur with consensual sex, but more severe breaches may occur with non-consensual sex,[9,10] thus facilitating viral transmission across the epithelium and infection of submucosal target cells. Disruption of the epithelial barrier may also occur in response to seminal plasma cytokines or in response to cytokines released by genital tract epithelial cells following semen exposure. Tumour necrosis factor (TNF)-α and interleukin (IL)-1, for example, may disrupt epithelial cell tight junctions. In addition, epithelial trauma is likely to activate mucosal cytokine and chemokine signalling, resulting in an influx of HIV target cells.[11] NF-κB activation following epithelial trauma may promote HIV replication through binding to the viral long terminal repeat sequence.[12]

The risk of HIV acquisition is also associated with specific properties of semen, independent of viral load. Semen buffers the protective acidity of the vagina.[13] Several seminal peptides, including fragments of prostatic acid phosphatase and semenogelins, assemble into amyloid fibrils that may enhance HIV infection by facilitating virion attachment and entry.[14,15] Seminal proteins may alter female genital tract cytokine and chemokine profiles to induce TNF-α, macrophage inflammatory protein (MIP)-3α, granulocyte macrophage-colony stimulating factor (GM-CSF), monocyte chemotactic protein (MCP-1), IL-7 and IL-8, therefore activating the cells that facilitate HIV infection;[16–19] this inflammatory effect may be exaggerated when semen is HIV infected.[17] Transforming growth factor (TGF)-β, which is highly abundant in seminal fluid, may play a key role in induction of cervical cytokine response following coitus. A recent study investigating the effects of pooled seminal fluid on immortalized and primary cervical cells found that TGF-β induced expression of GM-CSF and IL-6 from both human Ect1 cervical epithelial cells and primary cervical cells.[20] Similar responses were not observed following stimulation of cells with a selection of other cytokines found in seminal plasma. TGF-β neutralizing antibodies, receptor antagonists and signalling inhibitors diminished seminal plasma induction of GM-CSF and IL-6, but did not affect production of IL-8, MCP-1, MIP-3α or IL-1α. Thus, seminal fluid TGF-β may influence cervical immune function after coitus.

Semen also impacts genital tract immune cell populations. In a cross-sectional study of healthy, HIV uninfected women in the United Kingdom, women who reported unprotected sexual intercourse within the past 3 days ($n = 11$) had a significantly greater proportion of CD4+ T lymphocytes in endocervical cytobrushes compared with women who were abstinent for at least 3 days ($n = 20$). No significant differences in density of T lymphocyte activation markers were identified between the two groups.[21] In a more recent cross-sectional study of HIV uninfected women in Australia, biopsies were taken from the ectocervix 12 hr after unprotected vaginal coitus, after vaginal coitus with use of a condom or in the absence of coitus. A significant influx of macrophages, dendritic cells and memory T cells was observed after coitus.[19] An ongoing study of cervical intraepithelial immune cells collected by cytobrush from healthy, HIV uninfected American women (all receiving hormonal contraception) after both barrier protected and unprotected sex suggests similar findings, with a significant increase in percentage of CD3+ lymphocytes identified by flow cytometry 10–14 hr after barrier unprotected vaginal intercourse (Nakra, Buckley and

Herold, work in progress). All of these immune cell responses could increase the risk of HIV acquisition.

Special populations: adolescent and pregnant women

Adolescent women may be uniquely susceptible to HIV for several biological and behavioural reasons. Epidemiological studies in areas of high HIV prevalence, such as South Africa, have identified early sexual debut as an independent risk factor for HIV acquisition.[22] Adolescent women are highly vulnerable to sexual violence; in a recent large, cross-sectional study, 18.7% of females in the United States ages 14–17 years reported a history of sexual assault.[23] In a 2011 survey of 653 African American females ages 15–21 years who were participating in a STI prevention study, 24% of subjects reported a history of sexual abuse.[24] Subjects reported an average of 8.4 lifetime sexual partners, and only 26% reported consistent condom use in the prior 60 days. Moreover, 10.6% of subjects reported engaging in anal sexual intercourse in the prior 60 days, which has been independently associated with a greater risk of STI in adolescents.[25]

High rates of STI in adolescents[26–28] and the presence of cervical ectopy may result in a synergistic risk for HIV acquisition. The relationship between HIV acquisition and cervical ectopy is discussed in more detail by Venkatesh and Cu-Uvin in this issue of the Journal. Young women are more likely to have a greater degree of cervical ectopy or immature cervical epithelium, characterized by predominantly single-layer columnar epithelial cells. The degree of cervical ectopy may vary with tobacco and hormonal contraception or may increase in the setting of incident STI.[29] The cervical transformation zone is especially dynamic during the first years post-menarche, when ovulation is irregular, making early sexual debut an especially vulnerable interval for STI acquisition and persistence.[30–32] An increased proportion of cervical ectopy may increase STI and HIV acquisition risk by providing a larger ‘target’ with thinner epithelium. However, cervical ectopy may also impact the epithelial cytokine and chemokine network. A cross-sectional study comparing cervicovaginal lavage (CVL) concentrations of cytokines and chemokines in adolescent females in the United States with predominantly immature versus mature cervical epithelium identified significantly higher concentrations of IL-1α, IL-1β, IL-6, IL-8, MIP-1α, regulated upon activation, normal T cell expressed, and secreted (RANTES), TNF-β, IL-10, IL-12, and interferon (IFN)-γ in the group with a greater proportion of immature cervical epithelium.[33]

Alterations of mucosal antimicrobial peptides and microbiota in the genital tract may also impact susceptibility or response to genital tract pathogens in adolescent females.[33–35] In a cross-sectional analysis of genital tract secretions collected by cervicovaginal lavage (CVL) at a single time point from 20 sexually active American adolescents [mean age ± standard deviation (SD) of 16.9 ± 0.2 years] and 54 adult females (mean age ± SD of 30.7 ± 1.1 years, $P < 0.001$) who had no evidence of incident genital tract infection, CVL from adolescents had higher concentrations of lactoferrin, lysozyme and human neutrophil peptides (HNP) 1–3, as well as modest increases in IL-6 and IL-1α. These findings suggest a state of genital mucosal immune activation in the adolescents sampled, which could also represent an influx of activated HIV target cells in the genital tract and thus a greater risk of HIV acquisition. Antimicrobial peptides such as HNP 1–3, lysozyme and lactoferrin have intrinsic antimicrobial activity and may contribute to the observation that genital tract secretions have variable antimicrobial activity against herpes simplex virus (HSV), HIV and bacterial pathogens *ex vivo*.[36–44] In multiple studies of HIV uninfected adult women in the United States, Herold and Keller et al. observed that the concentrations of pro-inflammatory cytokines, lysozyme, lactoferrin and HNP 1–3 correlated with the ability of CVL to inhibit HSV plaque formation *ex vivo*.[42,43,45] Consistent with the observation that the adolescents

had higher levels of these immune mediators, adolescents also had greater anti-HSV activity relative to adults.[34] Whether this finding translates to protection against HSV or is simply a biomarker of inflammation will require further studies.

The *in vivo* protective effects of pro-inflammatory antimicrobial peptides remain unclear. For example, despite their *ex vivo* antiviral activity, higher genital HNP 1–3 levels have been independently associated with HIV acquisition, possibly reflecting an influx of HIV immune target cells.[46] HNP 1–3, lysozyme and lactoferrin are released by degranulating neutrophils recruited to the genital tract in response to chemokines, including IL-8, MIP-3α and other stimuli.[47] This cascade of immune activation, while augmenting host defence against some pathogens, could also recruit and activate HIV target cells,[11] thus conferring a greater risk of HIV acquisition. These complex findings highlight the delicate balance of mucosal immunity (Fig. 1).

While adolescents displayed increased levels of inflammatory mediators and anti-HSV activity relative to adults, they exhibited lower CVL levels of secretory leucocyte protease inhibitor (SLPI), IgG and IgA.[34] Lower concentrations of vaginal SLPI have been associated with increased risk of HIV acquisition,[48] and diminished cervicovaginal IgA could contribute to the high prevalence and recurrence rate of chlamydia observed in adolescents.[49–53] Adolescent subjects were also noted to have a significantly higher average vaginal pH relative to reproductively mature women (mean ± SD 4.8 ± 0.08 versus 4.5 ± 0.07, $P = 0.005$). A paucity of *Lactobacillus jensenii* was observed in vaginal swabs collected from adolescents, and 30–45% of adolescents were found to be colonized with bacterial vaginosis associated bacterium (BVAB) 1, 2, 3 and *Megasphaera,* organisms found to be specific markers of bacterial vaginosis (BV) in older populations of women. This observed shift towards BV-associated microbiota could represent a significant risk factor for HIV acquisition,[54] possibly by increasing concentrations of activated endocervical CD4+ T cells.[55] Thus, a net increased inflammatory state in the genital tract of sexually active adolescents may synergize with behavioural factors to increase HIV acquisition risk.

Multiple studies have described a higher risk of HIV acquisition during pregnancy, which is not fully explained by behavioural variables.[56] As with adolescents, few studies have described the genital mucosal immune environment in pregnant women. However, the available data suggest significant mucosal immune alterations in the setting of pregnancy relative to non-pregnant women, which may provide a rationale for the observed risk of HIV acquisition. Vaginal swabs collected at a single time point from 70 pregnant women in the United States at 35–37 weeks gestation had significantly higher concentrations of IL-1α, IL-1β, IL-1 receptor antagonist (ra), IL-8 and RANTES[45] relative to non-pregnant women. These higher chemokine and cytokine concentrations may reflect a heightened state of immune activation to prevent ascending infection to the foetus but may also promote an influx of activated HIV target cells. Another study comparing mucosal immune mediators in CVL from pregnant or non-pregnant women also reported higher absolute concentrations of RANTES, IL-1α and IL-1ra in pregnant subjects,[57] although protein-corrected CVL levels of IL-6, MCP-1, MIP-1α, elafin, human β defensin-2 and MIP-3α were lower in pregnant subjects. Of note, in this study, the *in vitro* ability of CVL to prevent HIV infection of TZM-bl cells was similar between pregnant and non-pregnant women, although it remains unclear if this activity translates to *in vivo* protection against HIV acquisition.

Bactericidal anti-*E. coli* activity of female genital tract secretions: harmful or protective?

The notion that the pregnant female genital tract may be modified to prevent perinatal infection is supported by the observation that vaginal swabs from pregnant women had

significantly greater bactericidal *E. coli* activity relative to swabs collected from non-pregnant women [median percentage inhibition 65% (range 17–99) versus 26.2% (−39 to 96), respectively; $P < 0.001$].[45] The bactericidal activity was inversely correlated with *E. coli* colonization, suggesting that this activity may protect against pathogens. This hypothesis is further supported by earlier studies demonstrating that women with BV exhibited reduced *E. coli* bactericidal activity[58] and by proteomic work demonstrating that, in addition to host factors, proteins specific to *Lactobacillus crispatus* and *jensenii* may contribute to the *E. coli* bactericidal activity.[59]

However, as with the anti-HSV activity of genital tract secretions, the host and/or bacterial factors that contribute to *E. coli* bactericidal activity may differ between populations, and their impact on HIV risk may be complex. The increased activity observed in pregnant women could reflect an increased net state of immune activation and thus, paradoxically, a greater risk of HIV acquisition. Two studies conducted with samples from African women suggest that increased *E. coli* bactericidal activity may be a biomarker of increased risk of HIV acquisition. In a small sub-study that took advantage of vaginal swabs collected during the HIV Prevention Trials Network 035 study, women who acquired HIV during the trial had significantly increased bactericidal *E. coli* activity pre-seroconversion than women who remained HIV seronegative, even after adjusting for multiple other variables [OR 1.25 (1.04–1.49)].[60] Similarly, higher levels of bactericidal *E. coli* activity were observed in South African women who acquired HIV during the CAPRISA 002 Acute Infection Study (R. P. Madan, J. Tugetman, L. Roberts, L. Werner, A. Grobler, S. S. Abdool Karim, J. A. Passmore, B. C. Herold, manuscript in progress). The high prevalence of BV in these cohorts suggests that mediators other than those produced by lactobacilli contributed to this activity. Thus, whether this activity serves as a biomarker of vaginal health and intact host defence or as a biomarker of inflammation and increased HIV risk (reflecting recruitment and/or activation of immune target cells) may depend on the population being studied. Larger, prospective studies are needed in diverse populations, including victims of sexual violence, to define the host and bacterial mediators of bactericidal *E. coli* activity in genital tract secretions and to elucidate the association between this activity, vaginal health and HIV acquisition risk.

Conclusions and future directions

The risk of HIV acquisition depends upon multiple, dynamic viral and host variables, which may be adversely impacted by sexual violence. Larger prospective studies that focus on the epithelial barrier, immune cell populations, concentrations of inflammatory and protective immune mediators, genital tract microbiota and the antimicrobial activity of genital tract secretions should promote the identification of factors that facilitate or prevent HIV infection. These studies are critical to informing the development of safe and effective oral and vaginal forms of HIV pre-exposure prophylaxis (PrEP) for adolescent and pregnant women, who are typically excluded from PrEP clinical trials. Defining the biological mechanisms that place specific populations at increased risk of HIV acquisition, including those that are more likely to be victims of sexual violence, such as adolescent and pregnant women, will accelerate the identification of novel prevention strategies.

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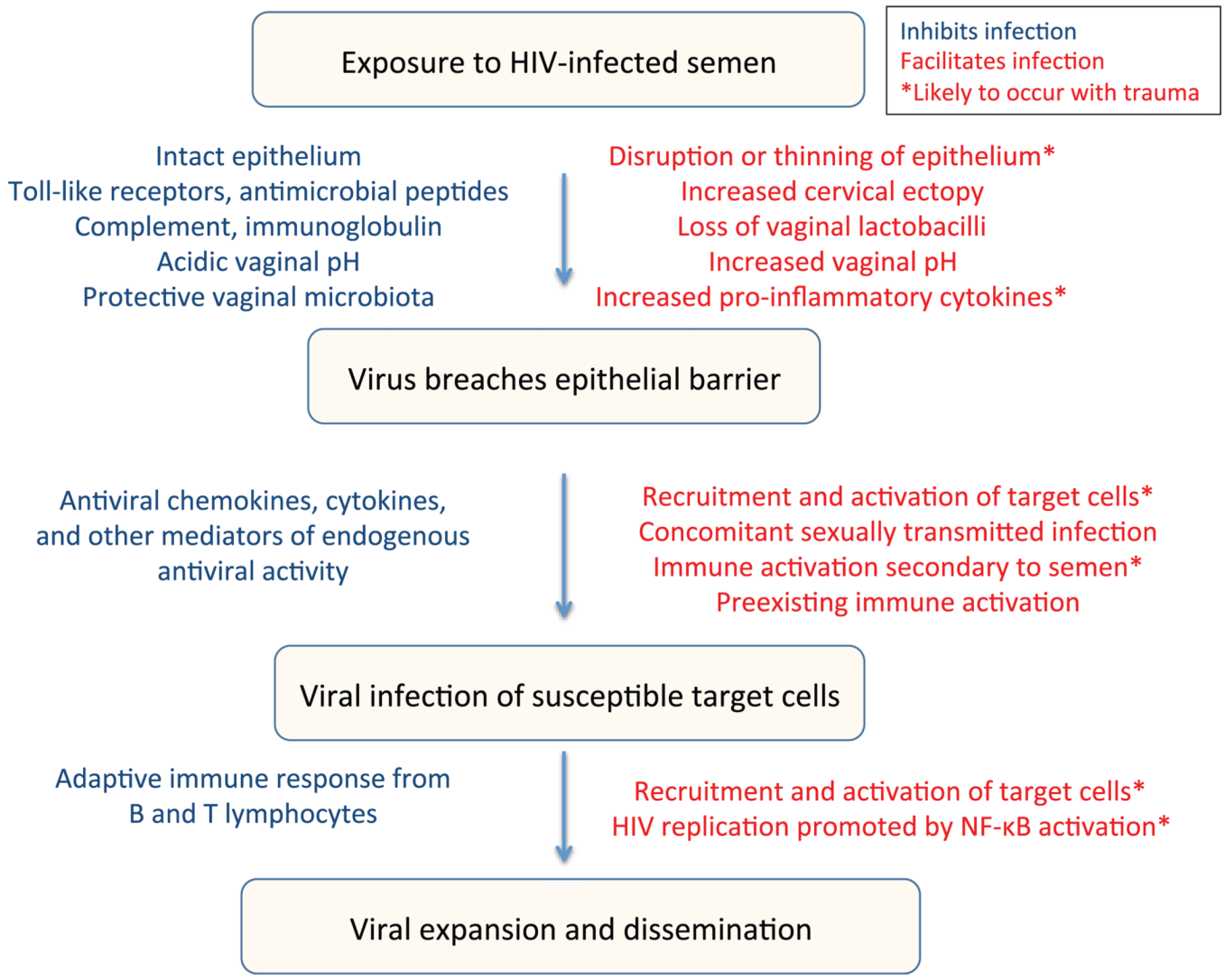

Fig. 1.
Factors in blue may inhibit HIV infection, and factors in red may facilitate infection. Asterisk denotes factors likely to occur with trauma.