Published in final edited form as:

Am J Reprod Immunol. 2011 March ; 65(3): 352–360. doi:10.1111/j.1600-0897.2010.00947.x.

Clinical Parameters Essential to Methodology and Interpretation of Mucosal Responses

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Abstract

Research aimed at putting an end to the HIV pandemic is dynamic given the marked advances in understanding of pathogenesis since its origin. Attention has shifted from systemic management of disease to a focus on the most common site of acquisition, the female genital tract. Research on the female genital tract of humans requires consideration of a number of specific clinical parameters. If such parameters are not considered when enrolling subjects into studies, it could lead to faulty data ascertainment. This article reviews important clinical characteristics to consider when conducting studies of the human female genital tract in regard to mucosal immunity and HIV disease. Important topics to consider include the method and source of sample collection, the individual patient characteristics, and in the case of recruitment of HIV-infected women, HIV disease characteristics.

Keywords

Clinical parameters; female genital tract; HIV; mucosal immunity

Introduction

The HIV pandemic continues to devastate the developing world despite millions of dollars of research aimed at fighting the disease. The vast majority of incident HIV in the world occurs as a result of heterosexual contact <http://www.cdc.gov/hiv/topics/surveillance/resources/slides/general/index.htm> 2009 (accessed September 28, 2010). When compared to other sexually transmitted infections (STI), HIV is not a particularly infectious virus. In the Rakai cohort, the likelihood of infection from an individual sex act was only 1 per 1000, suggesting that the body's natural host defenses are successful in preventing infection the vast majority of the time.¹ However, given that there are over 30 million people living with HIV in the world, these natural immune defenses are overcome with great regularity. Because the genital tract is the site of entry of HIV for the majority of infections on a global scale, research attention has begun to shift from studying transmission and acquisition systemically to the human genital tract.

Many factors need to be considered, though, when researching human genital tract mucosal immunity. There are a number of patient characteristics and exposures that could themselves

impact on genital immunity and if not considered could lead to faulty interpretation of results. This article will focus on clinical characteristics that must be considered when performing mucosal immunity research as it relates to HIV.

Method of collection/source of sample

A workshop was convened by the EUROPRISE network of scientists researching HIV-1 vaccines and microbicides in April 2009. Because there is a gap in knowledge with regard to best practices for sampling techniques and assessment of mucosal immune responses, the workshop addressed two specific areas and then summarized the results in a review article.² The major goals of the workshop were to define a consensus set of mucosal sampling methods and to determine the remaining challenges to assessing mucosal responses. The review details various collection techniques from the female genital tract that have been published in the literature. They specify which collection methods can be used to collect specimens from various sources. They report the different assays that can be performed on such specimens and point out the pros and cons of the various techniques. They also provide suggestions for normal ranges of immune globulins within various specimens. The normal values for the measurement of immune globulins, IgG and IgA, vary by approximately 100-fold based on site and method of collection within the human female genital tract. Normal average values are quite low for collection via cervicovaginal lavage, likely due to dilution effect. However, the concentration is quite high in endocervical secretions collected with the use of ophthalmic sponges. The authors conclude, though, that despite a growing body of literature on the topic, more efforts are needed to standardize both sampling methods and assays of female genital tract immunity. They stress that there is an urgent need to develop prevention strategies and that to do so, consensus standard operating procedures for testing immunity of the female lower genital tract will need to be utilized. An earlier review by Coombs et al.³ provides detailed anatomic instruction for collection of a variety of sample types.

Patient characteristics

There are a number of clinical characteristics that are known to alter genital immunity. These should be considered when planning studies that involve the genital tract with regard to mucosal immunity and prevention of or influence on HIV infection. The clinical characteristics specific to individual patients as well as those specific to HIV infection are summarized in Table I.

Menstrual Cycles

Whether the phase of the menstrual cycle impacts on genital shedding of HIV or susceptibility to HIV infection remains unclear. Data are conflicting with some studies showing an association between changes in the concentration of genital tract HIV RNA⁴ and others failing to show such an association.⁵⁻⁷ A review by Wira and Fahey⁸ points out, though, that there are many immunologic changes that occur during the course of the menstrual cycle. There are changes in migration of macrophages, B cells, neutrophils, and dendritic cells across the cycle.⁹⁻¹¹ Lactoferrin, an antiviral peptide produced by neutrophils, is depressed mid-cycle.¹² In the same study examining women across a menstrual cycle, a number of other immune mediators were depressed midcycle and returned to proliferative stage at approximately day 21.¹² Normal values at various points in the menstrual cycle have not been established and would be expected to vary by the stage of the cycle. Therefore, it is important that studies designed to examine the female genital tract immune response should consider the phase of the menstrual cycle. Possible strategies to minimize the variation owing to immune changes caused by the menstrual cycle include planning sampling during a single phase of the cycle, secretory, ovulatory, or proliferative in cycling

women. Another strategy might include sampling longitudinally across the cycle for all studied women so that such differences can be considered in analyses.

Menopause is an understudied area of reproductive immunology as it relates to risk of HIV acquisition. One aspect of menopause that is certain, however, is the change in the systemic and local hormonal milieu. There is a marked drop in estrogen levels and the loss of the cyclic hormonal changes in the lower genital tract. Several reports have shown that a number of genital immune functions are impacted by hormonal regulation as detailed earlier. While these changes are incompletely described, menopausal status must be considered when examining genital tract immunity in research studies. For example, epithelial cells from the upper tract of post-menopausal women lack the capacity to secrete antimicrobials compared to pre-menopausal women.¹³ When planning studies of response to microbicides or vaccination, investigators should decide whether to include menopausal women or whether to control for menopausal status in analyses.

Pregnancy

Pregnancy may increase the risk of HIV acquisition and is associated with marked hormonal and immunologic changes. A large, rigorous study carried out in Rakai, Uganda, found that women were at significantly increased risk of HIV acquisition during pregnancy. Data from a community cohort with longitudinal data were analyzed for the incidence rate of HIV during pregnancy and lactation, and compared to the incidence rate during periods of non-pregnancy and non-lactation. The incidence rate was 2.3 per 100 person years in pregnancy when compared to 1.1 per 100 person years in non-pregnant and lactating women. This study was rigorous because sexual behavior was recorded as part of a community, epidemiologic study. This difference in incidence rates resulted in an incident rate ratio of HIV acquisition in pregnancy of 2.16 (95% CI 1.39–3.37) after adjusting for age, marital status, education, multiple sex partners, genital ulcer disease, and condom use.¹⁴ Data remain conflicting, however, regarding the risk of HIV infection in pregnancy. Other studies also carried out in Africa failed to confirm the findings in the Rakai study.^{15,16}

The ability of the mother's body to tolerate a fetus that is not genetically identical to her has long been a topic of immunologic interest. While there are immunologic changes that occur at the maternal-fetal interface to allow the mother to tolerate her semi-allogeneic fetus, there are also major components of the lower genital tract that play an important role in immunity and modification of these may not be beneficial to the mother. The concentration of some antimicrobial peptides thought to be important in anti-HIV activity is frequently altered in pregnancy. In normal pregnancy, secretory leukocyte protease inhibitor concentrations are significantly greater than in the non-pregnant state, particularly in the cervical mucous.¹⁷ Kutteh and Franklin¹⁸ followed 36 pregnant women through pregnancy and found increasing concentrations of IL-1 β , a pro-inflammatory cytokine during the course of pregnancy. Donders et al. performed a small, prospective cohort study examining the changes in cytokine concentrations of 30 women during normal pregnancy. They found that, compared to non-pregnant women, pregnant women were less likely to have detectable IL-6 and IL-8 and that the concentrations of these molecules dipped during the second trimester. The concentrations then returned to pre-pregnancy levels in the third trimester.¹⁹

Studies aimed at enrollment of pregnant women are needed to make prevention interventions available to this highly vulnerable, important, and understudied population.

Contraception

There are a number of hormonal contraceptive formulations. These are available in a number of routes of administration, dosages, and pharmaceutical preparations. This topic is

discussed in detail in the accompanying article by Blish et al. In brief, oral contraceptives are commonly used and result in a cessation of the normal menstrual cycles by providing high enough baseline hormone levels to suppress the hypothalamic pituitary axis and prevent ovulation. There are other forms of combination hormonal contraceptives, some of which are in a patch form and others that are contained in a vaginal ring. Each of these likely has differing impacts on genital tract cell trafficking and immune function. Progesterone-containing therapies alter the cervical mucous and the uterine lining. These can be in the form of a pill, a depot injection, or a long-acting implantable rod. Intrauterine devices likely cause some amount of local inflammatory response and progesterone-containing devices work in multiple pathways. Finally, barrier contraceptive methods such as condoms and diaphragms as well as the concomitant use of spermicides may influence genital flora and genital immunity.

The impact that oral combination hormonal contraceptives have on HIV risk is an unresolved issue. Oral contraceptives upregulate cervical CCR5 receptors on CD4 T cells.²⁰ There have been human and animal data suggesting that there may be an increased risk of HIV acquisition as well as of HIV disease progression with the use of hormonal contraception.²¹⁻²³ A recent systematic review examined eight observational studies that did not find an association with HIV progression or transmission but did report the one randomized trial that found an association.²⁴ The authors concluded that while this association deserves further study, the majority of literature is reassuring. A more recent research letter by Morrison et al. reanalyzed the results of their multicenter cohort study examining this risk. They found that when using a marginal structural modeling statistical technique to limit the time-dependent confounding, there existed a significant association between HIV acquisition risk and hormonal contraceptive use among young women, in particular.²³ Given that sex hormones alter many components of genital immunity, it is likely that hormonal contraception has some impact on the innate immunity within the female genital tract. Whether this is a clinically significant impact is yet to be determined but should be considered when conducting such research.

Race

Race is known to impact many disease states over and above that which would be expected based on factors such as sociodemographic differences from comparison groups. This appears to involve a potential biologic difference between races that could account for variation in a number of disease presentations. This topic is reviewed thoroughly in an accompanying article by Kaul. Briefly, race has been shown to modify the association between bacterial vaginosis and incident STI.²⁵ One study found that certain cytokine and chemokine single-nucleotide polymorphisms were associated with ethnicity among HIV-infected individuals. The authors hypothesized that heritable variations in certain of these loci may contribute to the acquisition or progression of HIV infection.²⁶

Further, the concept of race is a complicated one. The National Institutes of Health has historically used self-identified racial categories. Individual patients frequently do not self-identify with one of these categories and thus are classified as 'other'. A newer technology uses single-nucleotide polymorphisms to create families of ethnic derivation called ancestry informative markers.²⁷ These require obtaining biologic samples and laboratory work by a reputable facility so are not used frequently. However, if race is an important component of an individual HIV risk study, consideration can be given to collection of more detailed ethnicity data.

Sexually Transmitted Infections

There exists a vast body of literature detailing the association between genital tract infections and HIV acquisition and transmission.

Much recent work has focused on herpes simplex virus-2 (HSV2) given the ulcerative and inflammatory nature of the infection and the high prevalence of the infection. If having HSV2 impacts shedding of HIV and the risk of transmission, then curbing the shedding caused by this infection alone might decrease the burden of HIV infection worldwide.

Herpes simplex virus-2 has been shown to increase viral load of HIV in both plasma and the genital tract, independent of the level of immunodeficiency.²⁸ The etiology of increased shedding of HIV in the presence of HSV appears to be immunologically mediated. Rebbapragada et al.²⁹ termed the interaction between HSV2 and HIV-1 ‘negative mucosal synergy’. While HSV suppression appears to decrease the risk of shedding HIV among women already infected with HIV, it does not appear to protect against acquisition or transmission of HIV-1.^{30,31}

Herpes simplex virus-2 is not the only infection that alters mucosal immune handling of HIV. A less noticed but still highly prevalent virus that may impact on genital shedding of HIV is human cytomegalovirus (CMV). The prevalence of CMV varies by geographical location, but after infection, it establishes lifelong latency. It can reactivate or hosts can be re-infected. A group well known for their CMV expertise recently developed a cervical explant study of CMV and HIV co-infection. They found that HIV appeared to enhance CMV in co-infected tissues which produced inflammatory cytokines. This explant model may be a useful tool for future studies examining the impact of CMV on HIV expression and vice versa.³²

Frequently encountered STI have also been implicated in altering mucosal immunity. Infection with *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are both associated with increased genital shedding of HIV among infected women.³³⁻³⁶ Other causes of genital inflammation also increase shedding of HIV, even in the absence of a known STI.^{37,38} *Neisseria gonorrhoeae* has been shown to enhance HIV infection of CD4 cells³⁹ and activated dendritic cells.⁴⁰

Human papillomavirus (HPV) is receiving renewed attention in the mucosal immunity research. After years of being considered ‘the common cold’ of STI, the development of the HPV vaccine for the prevention of cervical cancer has allowed for greater research in the area of genital mucosal immunity. Much of this research has implications for studies involving HIV or risk of HIV. High-risk HPV reactivation has been shown to occur more commonly in HIV-infected women and is associated with an increase in genital shedding of HIV.⁴¹ HIV-positive serostatus is also associated with a delay in clearance of both high- and low-risk HPV.⁴²

Disruption of the normal flora is well known to impact the delicate balance of the local genital immune system. Bacterial vaginosis has been associated with increased genital shedding of HIV RNA.^{43,44} Coleman et al.⁴⁵ confirmed the importance of vaginal flora in a prospective study of vaginal health among HIV-infected Kenyan women. Anti-retroviral naïve, HIV-infected women with normal CD4 counts had paired plasma and cervical wick samples collected for viral load measurement. Women with diminished *Lactobacillus* had a markedly increased endocervical viral load, 15.8-fold (95% CI: 2.0–123), compared to women with normal *Lactobacillus* levels ($\geq 3+$). Among women without HIV, BV has been shown to significantly increase the risk of HIV acquisition, probably as a function of disruption of natural immunity. In a large meta-analysis of 23 studies and including over

30,000 women, incident HIV was increased by BV, (relative risk = 1.6, 95% confidence interval: 1.2, 2.1).⁴⁶

Other clinical characteristics that should be considered in studies of female genital tract mucosal immunity include age, body mass index, use of alcohol or substances, recent immunizations, use of systemic drugs (steroids, antiinflammatory agents, immune modulators, chemotherapy), gynecologic procedures (hysterectomy, curettage, biopsies), and vaginal practices. Vaginal practices include the very common practice internationally of vaginal douching. A prospective cohort study of female sex workers in Kenya showed that vaginal washing was associated with an increased risk of HIV acquisition, aHR, 1.47; 95% CI, 1.02–2.13.⁴⁷ Clark et al.⁴⁸ examined the effect of douching on vaginal health among HIV-infected women. The prevalence of detectable HIV genital shedding was overall low, 27.3%, compared to that of plasma viral load, 79.8%. While not statistically significant, only 18.9% of non-douchers had genital HIV shedding while 31.9% of women who douched had shedding. Recent intercourse must be noted and a large body of work is focusing on the impact of semen on HIV transmission.⁴⁹ Further, it has been shown in both animal models and human studies that seminal plasma interferes with the anti-viral effectiveness of topical microbicides.^{50,51}

HIV disease state characteristics

The immune capability of the female genital tract may differ between HIV-infected and uninfected women. HIV-uninfected women in general should have a low risk of contracting infection from a single coital act. Those clinical characteristics noted in the above section may alter a woman's susceptibility to infection. Once a woman is infected with HIV, though, her genital immunity may be compromised. This may impact her risk of acquisition of multiple strains of HIV, or of resistant virus, and her risk of shedding HIV and thus transmission. HIV-1 has been shown to directly impair mucosal integrity in an *in vitro* model of the female genital tract allowing translocation of other pathogens.⁵²

The phase of HIV may impact immunity and thus should be considered when enrolling patients in studies. Studies examining genital immunity in people at high risk for acquisition of HIV will likely include sampling during a time of new infection in some patients. This time will include marked viremia and likely heavy genital shedding of virus. Acute infection is usually accompanied by a temporary degradation in the systemic CD4 cell count, and there is likely a similar impact in the genital tract, although this is not well characterized. Such studies also provide an opportunity for characterizing these changes if investigators are able to identify these acute infections.

It is well established that plasma HIV viral load is the most important predictor of genital tract shedding of virus.^{38,53,54} However, genital shedding of HIV can occur even in the setting of completely suppressed plasma viremia. A recent study showed that 37% of women had genital tract shedding of virus during a study visit when plasma viral load was undetectable.⁵⁵ While the sample size of this study was small, it appeared that median CD4 counts increased with decreasing frequency of genital shedding of HIV.⁵⁵ The specific relationship between systemic CD4 cell counts and genital immunity remains incompletely characterized but should be considered in studies of genital immunity.

The mode of HIV infection may also play a role in the female genital tract immunity. Women who have acquired infection via the genital tract may exhibit variable genital immunity compared to those who have acquired the disease through injection drug use. The tropism of the virus may differ and thus could result in differing ability to stimulate cytokine or chemokine responses to insults within the genital tract. Virus that utilizes CCR5 (R5) coreceptor transmits sexually more readily than does virus that is CXCR4-tropic (X4). It has

been shown that in asymptomatic, treatment-naïve women, the systemic viral tropism does not necessarily reflect the tropism of genital virus.⁵⁶ This variation in viral tropism could have an impact on immunologic responses in the genital tract.

The penetration of various antiretroviral (ART) drugs into the lower genital tract has been examined in several studies. Concentrations have been examined at various times in the dosing interval, and the cervicovaginal concentrations vary significantly from drug to drug. One study examined how quickly each drug achieved concentrations in the genital tract compared to plasma at steady state in 27 women.⁵⁷ They reported the median rank order of drugs with highest to lowest genital tract concentrations. As the authors anticipated, the commonly used nucleoside reverse transcriptase inhibitors tended to be high on the list while efavirenz was the lowest, with protease inhibitors (PIs) falling in the middle. This study confirmed findings from an earlier study of seven women.⁵⁸ Another study with a larger sample size (34) examined both drug concentrations as well as virologic response to drug.⁵⁹ The use of ART in patients is an incredibly important factor in the determination of genital immunity. As these drugs appear in measurable concentrations in the genital fluids, it is also important to note that any *in vitro* models using live virus will not perform properly if using genital fluids from women taking ART.

Although there is a strong correlation between plasma viral load and genital tract viral load, there is evidence of compartmentalization between the blood and genital tract in both men and women. Evidence of compartmentalization occurs in terms of resistance patterns.⁶⁰⁻⁶² An interesting study examined the theory that virologic failure might occur in one compartment and not another. The authors examined 14 women with detectable HIV-1 in both plasma and genital tract despite antiretroviral therapy.⁶³ Fifty-seven percent of the patients exhibited mutations conferring high-level HIV-1 drug resistance. Interestingly, in one patient, resistance mutations appeared only in the plasma while all genital variants were susceptible. It has also been shown that resistance mutations detected in the genital tract can persist for years.⁶⁴ Differences in resistance patterns as well as the possibility of resistance must be considered in studies including HIV-infected women.

Summary

The HIV pandemic continues to result in millions of deaths annually on a global scale. Despite the advent of antiretroviral therapy, the spread of the infection has not been halted. The millions of dollars of research aimed at determining the pathogenesis of HIV spread have led to marked improvements in the understanding of disease. This has brought a change in life expectancy of those diagnosed with HIV in the United States from terminal to chronic illness. It has also caused a shift in attention from the blood compartment to the genital compartment as the major point-of-entry for HIV and thus for research endeavors. The many clinical characteristics that must be considered when studying the blood compartment must be expanded when considering research work on the genital compartment. The mucosal immunity in the female genital tract differs from anywhere else in the body and is altered by exposures in addition to usual demographic characteristics. Consideration of these factors when enrolling subjects and controlling for them in analyses will minimize erroneous interpretation of results in the continuing battle against HIV.

Acknowledgments

Time preparing this manuscript was supported by 1K23HD062340-01 (Anderson-PI) and K24 AI066884 (Cu-Uvin-PI).

References

1. Gray RH, Wawer MJ, Brookmeyer R, Sewankambo NK, Serwadda D, Wabwire-Mangen F, Lutalo T, Li X, vanCott T, Quinn TC. Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda. *Lancet*. 2001; 357:1149–1153. [PubMed: 11323041]
2. Jespers V, Harandi AM, Hinkula J, Medaglini D, Grand RL, Stahl-Hennig C, Bogers W, Habib RE, Wegmann F, Fraser C, Cranage M, Shattock RJ, Spetz A-L: Assessment of mucosal immunity to HIV-1. *Expert Rev Vaccines*. 2010; 9:381–394. [PubMed: 20370549]
3. Coombs RW, Reichelderfer PS, Landay AL. Recent observations on HIV type-1 infection in the genital tract of men and women. *AIDS*. 2003; 17:455–480. [PubMed: 12598766]
4. Reichelderfer PS, Coombs RW, Wright DJ, Cohn J, Burns DN, Cu-Uvin S, Baron PA, Coheng MH, Landay AL, Beckner SK, Lewis SR, Kovacs AA, WHS 001 Study Team. Effect of menstrual cycle on HIV-1 levels in the peripheral blood and genital tract. *AIDS*. 2000; 14:2101–2107. [PubMed: 11061650]
5. Villanueva JM, Ellerbrock TV, Lennox JL, Bush TJ, Wright TC, Pratt-Palmore M, Evans-Strickfaden T, Conley LJ, Schnell C, Hart CE. The menstrual cycle does not affect human immunodeficiency virus type 1 levels in vaginal secretions. *J Infect Dis*. 2002; 185
6. Goulston C, McFarland W, Katzenstein D. Human immunodeficiency virus type 1 RNA shedding in the female genital tract. *J Infect Dis*. 1998; 177:1100. [PubMed: 9534992]
7. Mostad SB, Jackson S, Overbaugh J, Reilly M, Chohan B, Mandaliya K, Nyange P, Ndinya-Achola J, Bwayo JJ, Kreiss JK. Cervical and vaginal shedding of human immunodeficiency virus type 1-infected cells throughout the menstrual cycle. *J Infect Dis*. 1998; 178:983. [PubMed: 9806025]
8. Wira CR, Fahey JV. A new strategy to understand how HIV infects women: identification of a window of vulnerability during the menstrual cycle. *AIDS*. 2008; 22:1909–1917. [PubMed: 18784454]
9. Givan AL, White HD, Stern JE, Colby E, Gosselin EJ, Guyre PM, Wira CR. Flow cytometric analysis of leukocytes in the human female reproductive tract: comparison of fallopian tube, uterus, cervix, and vagina. *Am J Reprod Immunol*. 1997; 38:350–359. [PubMed: 9352027]
10. Smith JM, Wira CR, Fanger MW, Shen L. Human fallopian tube neutrophils—a distinct phenotype from blood neutrophils. *Am J Reprod Immunol*. 2006; 56:218–229. [PubMed: 16938110]
11. Piguet V, Steinman RM. The interaction of HIV with dendritic cells: outcomes and pathways. *Trends Immunol*. 2007; 28:503–510. [PubMed: 17950666]
12. Keller MJ, Guzman E, Hazrati E, Kasowitz A, Cheshenko N, Wallenstein S, Cole AL, Cole AM, Profy AT, Wira CR, Hogarty K, Herold BC. PRO 2000 elicits a decline in genital tract immune mediators without compromising intrinsic antimicrobial activity. *AIDS*. 2007; 21:467–476. [PubMed: 17301565]
13. Fahey JV, Wright JA, Shen L, Smith JM, Ghosh M, Rossoll RM, Wira CR. Estradiol selectively regulates innate immune function by polarized human uterine epithelial cells in culture. *Mucosal Immunol*. 2008; 1:317–325. [PubMed: 19079193]
14. Gray RH, Li X, Kigozi G, Serwadda D, Brahmabhatt H, Wabwire-Mangen F, Nalugoda F, Kiddugavu M, Sewankambo N, Quinn TC, Reynolds SJ, Wawer MJ. Increased risk of incident HIV during pregnancy in Rakai, Uganda: a prospective study. *Lancet*. 2005; 366:1182–1188. [PubMed: 16198767]
15. Morrison CS, Wang J, Van Der Pol B, Padian N, Salata RA, Richardson BA. Pregnancy and the risk of HIV-1 acquisition among women in Uganda and Zimbabwe. *AIDS*. 2007; 21:1027–1034. [PubMed: 17457097]
16. Reid SE, Dai JY, Wang J, Sichalwe BN, Akpomimie G, Cowan FM, Delany-Moretlwe S, Baeten JM, Hughes JP, Wald A, Celum C. Pregnancy, contraceptive use, and HIV acquisition in HPTN 039: relevance for HIV prevention trials among African women. *J Acquir Immune Defic Syndr*. 2010; 53:606–613. [PubMed: 19838129]
17. Helmig R, Uldbjerg N, Ohlsson K. Secretory leukocyte protease inhibitor in the cervical mucus and in the fetal membranes. *Eur J Obstet Gynecol Reprod Biol*. 1995; 59:95–101. [PubMed: 7781866]

18. Kutteh WH, Franklin RD. Quantification of immunoglobulins and cytokines in human cervical mucus during each trimester of pregnancy. *Am J Obstet Gynecol.* 2001; 184:865–874. [PubMed: 11303193]
19. Donders GG, Vereecken A, Bosmans E, Spitz B. Vaginal cytokines in normal pregnancy. *Am J Obstet Gynecol.* 2003; 189:1433–1438. [PubMed: 14634582]
20. Prakash M, Kapembwa MS, Gotch F, Patterson S. Oral contraceptive use induces upregulation of the CCR5 chemokine receptor on CD4+ T cells in the cervical epithelium of healthy women. *J Reprod Immunol.* 2002; 54:117–131. [PubMed: 11839399]
21. Baeten JM, Lavreys L, Overbaugh J. The influence of hormonal contraceptive use on HIV-1 transmission and disease progression. *Clin Infect Dis.* 2007; 45:360–369. [PubMed: 17599316]
22. Stringer E, Antonsen E. Hormonal contraception and HIV disease progression. *Clin Infect Dis.* 2008; 47:945–951. [PubMed: 18715161]
23. Morrison CS, Chen P-L, Kwok C, Richardson BA, Chipato T, Mugerwa R, Byamugisha J, Padian N, Celentano DD, Salata RA. Hormonal contraception and HIV acquisition: reanalysis using marginal structural modeling. *AIDS.* 2010; 24:1778–1781. [PubMed: 20588106]
24. Curtis KM, Nanda K, Kapp N. Safety of hormonal and intrauterine methods of contraception for women with HIV/AIDS: a systematic review. *AIDS.* 2009; 23(Suppl 1):S55–S67. [PubMed: 20081389]
25. Peipert JF, Lapane KL, Allsworth JE, Redding CA, Blume JD, Stein MD. Bacterial vaginosis, race, and sexually transmitted infections: does race modify the association? *Sex Transm Dis.* 2008; 35:363–367. [PubMed: 18360319]
26. Wang C, Song W, Lobashevsky E, Wilson CM, Douglas SD, Mytilineos J, Schoenbaum EE, Tang J, Kaslow RA. Cytokine and chemokine gene polymorphisms among ethnically diverse North Americans with HIV-1 infection. *J Acquir Immune Defic Syndr.* 2004; 35:446–454. [PubMed: 15021309]
27. Tian C, Gregersen PK, Seldin MF. Accounting for ancestry: population substructure and genome-wide association studies. *Hum Mol Genet.* 2008; 17:143–150.
28. LeGoff J, Weiss HA, Gresenguet G, Nzambi K, Frost E, Hayes RJ, Mabey DCW, Malkin J-E, Mayaud P, Belec L. Cervicovaginal HIV-1 and herpes simplex virus type 2 shedding during genital ulcer disease episodes. *AIDS.* 2007; 21:1569–1578. [PubMed: 17630552]
29. Rebbapragada A, Wachihhi C, Pettengell C, Sunderji S, Huibner S, Jaoko W, Ball B, Fowke K, Mazzulli T, Plummer FA, Kaul R. Negative mucosal synergy between Herpes simplex type 2 and HIV in the female genital tract. *AIDS.* 2007; 21:589–598. [PubMed: 17314521]
30. Celum C, Wald A, Hughes J, Sanchez J, Reid S, Delany-Moretlwe S, Cowan F, Casapia M, Ortiz A, Fuchs J, Buchbinder S, Koblin B, Zwierski S, Rose S, Wang J, Corey L, Team HP. Effect of aciclovir on HIV-1 acquisition in herpes simplex virus 2 seropositive women and men who have sex with men: a randomised, double-blind, placebo-controlled trial. *Lancet.* 2008; 371:2109–2119. [PubMed: 18572080]
31. Celum C, Wald A, Lingappa JR, Magaret AS, Wang RS, Mugo N, Mujugira A, Baeten JM, Mullins JI, Hughes JP, Bukusi EA, Cohen CR, Katabira E, Ronald A, Kiarie J, Farquhar C, Stewart GJ, Makhema J, Essex M, Were E, Fife KH, de Bruyn G, Gray GE, McIntyre JA, Manongi R, Kapiga S, Coetzee D, Allen S, Inambao M, Kayitenkore K, Karita E, Kanweka W, Delany S, Rees H, Vwalika B, Stevens W, Campbell MS, Thomas KK, Coombs RW, Morrow R, Whittington WLH, McElrath MJ, Barnes L, Ridzon R, Corey L. Acyclovir and transmission of HIV-1 from persons infected with HIV-1 and HSV-2. *N Engl J Med.* 2010; 362:427–439. [PubMed: 20089951]
32. Fox-Canale AM, Hope TJ, Martinson J, Lurain JR, Rademaker AW, Bremer JW, Landay A, Spear GT, Lurain NS. Human cytomegalovirus and human immunodeficiency virus type-1 co-infection in human cervical tissue. *Virology.* 2007; 369:55–68. [PubMed: 17716703]
33. Ghys PD, Fransen K, Diallo MO, Ettiegn-Traore V, Coulibaly I-M, Yeboue KM, Kalish ML, Maurice C, Whitaker JP, Greenberg AE, Laga M. The associations between cervicovaginal HIV shedding, sexually transmitted diseases and immunosuppression in female sex workers in Abidjan, Cote d'Ivoire. *AIDS.* 1997; 11:F85–F93. [PubMed: 9342059]

34. McClelland, RSa; Wang, CCa; Mandaliya, Kb; Overbaugh, Jc; Reiner, MTd; Panteleeff, DDc; Lavreys, Le; Ndinya-Achola, Jf; Bwayo, Jjf; Kreiss, JKae. Treatment of cervicitis is associated with decreased cervical shedding of HIV-1. *AIDS*. 2001; 15:105–110. [PubMed: 11192850]
35. Mostad SB, Overbaugh J, DeVange DM, Welch MJ, Chohan B, Mandaliya K, Nyange P, Martin JHL, Ndinya-Achola J, Bwayo JJ, Kreiss JK. Hormonal contraception, vitamin A deficiency, and other risk factors for shedding of HIV-1 infected cells from the cervix and vagina. *Lancet*. 1997; 350:922–927. [PubMed: 9314871]
36. Wang CC, McClelland RS, Reilly M, Overbaugh J, Emery SR, Mandaliya K, Chohan B, Ndinya-Achola J, Bwayo J, Kreiss JK. The effect of treatment of vaginal infections on shedding of human immunodeficiency virus type 1. *J Infect Dis*. 2001; 183:1017–1022. [PubMed: 11237825]
37. Laga M, Manoka A, Kivuvu M, Malele B, Tuliza M, Nzila N, Goeman J, Behets F, Batter V, Alary M. Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study. *AIDS*. 1993; 7:95–102. [PubMed: 8442924]
38. Anderson BL, Wang C-C, DeLong AK, Liu T, Kojic EM, Kurpewski J, Ingersoll J, Mayer K, Caliendo AM, Cu-Uvin S. Genital tract leukocytes and shedding of genital HIV type 1 RNA. *Clin Infect Dis*. 2008; 47:1216–1221. [PubMed: 18808359]
39. Ding J, Rapista A, Teleshova N, Mosoyan G, Jarvis GA, Klotman ME, Chang TL. *Neisseria gonorrhoeae* enhances HIV-1 infection of primary resting CD4+ T cells through TLR2 activation. *J Immunol*. 2010; 184:2814–2824. [PubMed: 20147631]
40. Zhang J, Li G, Bafica A, Pantelic M, Zhang P, Broxmeyer H, Liu Y, Wetzler L, He JJ, Chen T. *Neisseria gonorrhoeae* enhances infection of dendritic cells by HIV type 1. *J Immunol*. 2005; 174:7995–8002. [PubMed: 15944306]
41. Theiler RN, Farr SL, Karon JM, Paramsothy P, Viscidi R, Duerr A, Cu-Uvin S, Sobel J, Shah K, Klein RS, Jamieson DJ. High-risk human papillomavirus reactivation in human immunodeficiency virus-infected women: risk factors for cervical viral shedding. *Obstet Gynecol*. 2010; 115:1150–1158. [PubMed: 20502284]
42. Koshiol JE, Schroeder JC, Jamieson DJ, Marshall SW, Duerr A, Heilig CM, Shah KV, Klein RS, Cu-Uvin S, Schuman P, Celentano D, Smith JS. Time to clearance of human papillomavirus infection by type and human immunodeficiency virus serostatus. *Int J Cancer*. 2006; 119:1623–1629. [PubMed: 16646070]
43. Cu-Uvin S, Hogan JW, Caliendo AM, Harwell J, Mayer KH, Carpenter CC. Association between bacterial vaginosis and expression of human immunodeficiency virus type 1 RNA in the female genital tract. *Clin Infect Dis*. 2001; 33:894–896. [PubMed: 11512096]
44. Spinillo A, Debiaggi M, Zara F, Maserati R, Polatti F, De Santolo A. Factors associated with nucleic acids related to human immunodeficiency virus type 1 in cervico-vaginal secretions. *BJOG*. 2001; 108:634–641. [PubMed: 11426900]
45. Coleman JS, Hitti J, Bukusi EA, Mwachari C, Muliro A, Nguti R, Gausman R, Jensen S, Patton D, Lockhart D, Coombs R, Cohen CR. Infectious correlates of HIV-1 shedding in the female upper and lower genital tracts. *AIDS*. 2007; 21:755–759. [PubMed: 17413697]
46. Atashili J, Poole C, Ndumbe PM, Adimora AA, Smith JS. Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies. *AIDS*. 2008; 22:1493–1501. [PubMed: 18614873]
47. McClelland RS, Lavreys L, Hassan WM, Mandaliya K, Ndinya-Achola JO, Baeten JM. Vaginal washing and increased risk of HIV-1 acquisition among African women: a 10-year prospective study. *AIDS*. 2006; 20:269–273. [PubMed: 16511421]
48. Clark RA, Theall KP, Amedee AM, Kissinger PJ. Frequent douching and clinical outcomes among HIV-infected women. *Sex Transm Dis*. 2007; 34:985–990. [PubMed: 17621248]
49. Kaushic C, Ferreira VH, Kafka JK, Nazli A. HIV infection in the female genital tract: discrete influence of the local mucosal microenvironment. *Am J Reprod Immunol*. 2010; 63:566–575. [PubMed: 20384619]
50. Patel S, Hazrati E, Cheshenko N, Galen B, Yang H, Guzman E, Wang R, Herold Betsy A C, Keller Marla A J. Seminal plasma reduces the effectiveness of topical polyanionic microbicides. *J Infect Dis*. 2007; 196:1394–1402. [PubMed: 17922405]
51. Keller MJ, Mesquita PMM, Torres NM, Cho S, Shust G, Madan RP, Cohen HW, Petrie J, Ford T, Soto-Torres L, Profy AT, Herold BC. Postcoital bioavailability and antiviral activity of 0.5% PRO

- 2000 Gel: implications for future microbicide clinical trials. *PLoS ONE*. 2010; 5:e8781. [PubMed: 20107502]
52. Nazli A, Chan O, Dobson-Belaire WN, Ouellet M, Tremblay MJ, Gray-Owen SD, Arsenault AL, Kaushic C. Exposure to HIV-1 directly impairs mucosal epithelial barrier integrity allowing microbial translocation. *PLoS Pathog*. 2010; 6:e1000852. [PubMed: 20386714]
 53. Cu-Uvin S, Caliendo AM, Reinert S, Chang A, Juliano-Remollino C, Flanigan TP, Mayer KH, Carpenter CC. Effect of highly active antiretroviral therapy on cervicovaginal HIV-1 RNA. *AIDS*. 2000; 14:415–421. [PubMed: 10770544]
 54. Cu-Uvin S, Snyder B, Harwell JI, Hogan J, Chibwesa C, Hanley D, Ingersoll J, Kurpewski J, Mayer KH, Caliendo AM. Association between paired plasma and cervicovaginal lavage fluid HIV-1 RNA levels during 36 months. *J Acquir Immune Defic Syndr*. 2006; 42:584–587. [PubMed: 16837866]
 55. Cu-Uvin S, DeLong AK, Venkatesh KK, Hogan JW, Ingersoll J, Kurpewski J, De Pasquale MP, D'Aquila R, Caliendo AM. Genital tract HIV-1 RNA shedding among women with below detectable plasma viral load. *AIDS*. 2010; 24:2489–2497. [PubMed: 20736815]
 56. Andreoletti L, Skrabal K, Perrin V, Chomont N, Saragosti S, Gresenguet G, Moret H, Jacques J, Longo JdD, Matta M, Mammano F, Belec L. Genetic and phenotypic features of blood and genital viral populations of clinically asymptomatic and antiretroviral-treatment-naïve clade A human immunodeficiency virus type 1-infected women. *J Clin Microbiol*. 2007; 45:1838–1842. [PubMed: 17460054]
 57. Dumond JB, Yeh RF, Patterson KB, Corbett AH, Jung BH, Rezk NL, Bridges AS, Stewart PW, Cohen MS, Kashuba ADM. Antiretroviral drug exposure in the female genital tract: implications for oral pre- and post-exposure prophylaxis. *AIDS*. 2007; 21:1899–1907. [PubMed: 17721097]
 58. Min SS, Corbett AH, Rezk N, Cu-Uvin S, Fiscus SA, Petch L, Cohen MS, Kashuba ADM. Protease inhibitor and nonnucleoside reverse transcriptase inhibitor concentrations in the genital tract of HIV-1-infected women. *J Acquir Immune Defic Syndr*. 2004; 37:1577–1580. [PubMed: 15577412]
 59. Kwaru A, DeLong AK, Rezk N, Hogan J, Burtwell H, Chapman S, Moreira CC, Kurpewski J, Ingersoll J, Caliendo AM, Kashuba A, Cu-Uvin S. Antiretroviral drug concentrations and HIV RNA in the genital tract of HIV-infected women receiving long-term highly active antiretroviral therapy. *Clin Infect Dis*. 2008; 46:719–725. [PubMed: 18220480]
 60. Goulston C, Stevens E, Gallo D, Mullins JI, Hanson CV, Katzenstein D. Human immunodeficiency virus in plasma and genital secretions during the menstrual cycle. *J Infect Dis*. 1996; 174:858–861. [PubMed: 8843230]
 61. Overbaugh J, Anderson RJ, Ndinya-Achola JO, Kreiss JK. Distinct but related human immunodeficiency virus type 1 variant populations in genital secretions and blood. *AIDS Res Hum Retroviruses*. 1996; 12:107–115. [PubMed: 8834460]
 62. Shaheen F, Sison AV, McIntosh L, Mukhtar M, Pomerantz RJ. Analysis of HIV-1 in the cervicovaginal secretions and blood of pregnant and nonpregnant women. *J Hum Virol*. 1999; 2:154–166. [PubMed: 10413367]
 63. Kemal KS, Burger H, Mayers D, Anastos K, Foley B, Kitchen C, Huggins P, Schroeder T, Picchio G, Back S, Gao W, Meyer WA III, Weiser B. HIV-1 drug resistance in variants from the female genital tract and plasma. *J Infect Dis*. 2007; 195:535–545. [PubMed: 17230413]
 64. Newstein M, Losikoff P, Caliendo A, Ingersoll J, Kurpewski J, Hanley D, Cerezo J, Ramratnam B, Cu-Uvin S. Prevalence and persistence of nonnucleoside reverse transcriptase inhibitor mutations in the female genital tract. *J Acquir Immune Defic Syndr*. 2005; 38:364–366. [PubMed: 15735459]

Table I

Clinical characteristics to consider in studies of female genital tract immunity with regard to HIV

Patient demographic characteristics	Disease state characteristics
Age	HIV serostatus
Race	Phase of HIV infection
Body mass index	CD4 cell count
Sexually transmitted infections	Plasma viral load
Other genital tract infections	Use of antiretrovirals
Vaginal flora	Mode of HIV acquisition
Alcohol or substance use	Resistance characteristics
Recent immunization	
Pregnancy	
Phase of menstrual cycle	
Genital inflammation	
Recent douching	
Gynecologic procedures	
Recent intercourse/Semen	
Contraception	