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# Innate and Adaptive Anti-HIV Immune Responses in the Female Reproductive Tract

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## Abstract

The mucosal surface of the female reproductive tract (FRT) is the primary site of transmission for a plethora of sexually transmitted infections, including human immunodeficiency virus (HIV), that represent a significant burden upon womens' health worldwide. However, fundamental aspects of innate and adaptive immune protection against HIV infection in the FRT are poorly understood. The FRT immune system is regulated by the cyclical changes of the sex hormones estradiol and progesterone across the menstrual cycle, which as we have hypothesized, leads to the creation of a window of vulnerability during the secretory stage of the menstrual cycle, when the risk of HIV transmission is increased. The goal of this review is to summarize the multiple levels of protection against HIV infection in the FRT, the contribution of different cell types including epithelial cells, macrophages, T cells, and dendritic cells to this, and their regulation by estradiol and progesterone. Understanding the unique immune environment in the FRT will allow for the potential development of novel therapeutic interventions such as vaccines and microbicides that may reduce or prevent HIV transmission in women.

### Keywords

female reproductive tract; HIV; epithelial cells; immune cells; innate immunity; adaptive immunity; window of vulnerability

## 1. Introduction: the window of vulnerability

Women represent half of the 34 million people currently living with human immunodeficiency virus (HIV) according to the latest World Health Organization report (WHO 2011). Young women (aged 15–24) are a particularly susceptible group, accounting for 22% of all new infections, almost twice as high as young men. In sub-Saharan Africa, women represent 59% of infected individuals; a new young woman is infected every minute and the likelihood of their living with HIV is eight times higher than in men. Consequently, HIV is the main cause of death in women of reproductive age (UNAIDS 2010).



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Sexual transmission of HIV is the main route of HIV acquisition in women (NIAID 2010). While the risk of transmission with sexual intercourse is low, ranging from 1/200 to 1/2000 per sexual act, once infection occurs, HIV rapidly replicates in the mucosal tissues and CD4+ T cells are depleted from the gut within weeks of infection (Haase 2011). There is a short period of time when early immune responses at mucosal surfaces could effectively reduce susceptibility to HIV infection or abort the infection before it spreads. Even though the female reproductive tract (FRT) is the main portal of entry for HIV, early HIV infection at this mucosal surface has not been extensively studied and most of our knowledge about mucosal HIV infection and antiviral responses in humans comes from studies performed in the gastrointestinal tract.

The mucosal FRT environment needs to be evaluated in the context of its main function – reproduction. The FRT immune system is tightly regulated by cyclic changes in the sex hormones estradiol and progesterone to optimize conditions for implantation; thus, an adequate balance between immune protection against pathogens and tolerance to allogeneic sperm and semi-allogeneic fetus results in successful reproduction (Hickey *et al.* 2011). As discussed elsewhere, changes in hormone levels during the menstrual cycle result in cyclical changes in FRT innate and adaptive immune responses, as well as the immune cell populations within the upper and lower tract, which in turn modulate conditions for the opportunistic establishment of HIV infection (Wira and Fahey 2008). Based on these changes, we developed the hypothesis of a window of vulnerability that lasts about 7–10 days after ovulation during the secretory phase when HIV infection is more likely to occur because of the dampening of protective immune responses (Wira and Fahey 2008). This review summarizes the current literature on innate and adaptive immune responses in the FRT with emphasis on how modulation of these responses by sex hormones influences susceptibility to HIV infection.

## 2. HIV Tropism

The primary cellular receptor for HIV entry is CD4 (Sattentau *et al.* 1986). HIV also uses either CCR5 (Deng *et al.* 1996) or CXCR4 (Feng *et al.* 1996) as coreceptors alongside CD4. There are two main strains of HIV:

**(1)** CCR5-tropic (R5) strains that interact with the CCR5 coreceptor, and

**(2)** CXCR4-tropic (X4) strains that utilize the CXCR4 coreceptor.

In addition, there are alternative cell surface receptors, such as the C-type lectins DC-SIGN (Turville *et al.* 2001), mannose receptor (MR) (Turville *et al.* 2001), heparan sulfate proteoglycans (HSPG) (Patel *et al.* 1993) and syndecans, as well as gal ceramide (GalC) (Bhat 1992), gp340 (Stoddard *et al.* 2007) and α4β7 integrin (Arthos *et al.* 2008) amongst others that HIV also employs to enter the cell. The majority of heterosexual transmission events are due to CCR5-tropic strains of HIV. Viral infection is characterized by a genetic bottleneck as approximately 80% of new cases of sexually transmitted HIV are due to a single infectious CCR5-tropic virion. The reasons behind this phenomenon are unclear, but are currently an active area of research.

## 3. HIV and Cervico-Vaginal Secretions

Bathing the lining of the lower FRT are cervico-vaginal fluids (CVF), composed of vaginal transudate, mucus, and epithelial cell secretions (Owen and Katz 1999) from the cervix, uterus, and Fallopian tubes. CVF is known to restrict the infection of target cells by multiple pathogenic organisms including HIV and HSV-2 (Ghosh *et al.* 2010a; Shust *et al.* 2010). Both HIV-positive and HIV-negative women possess anti-HIV activity in their CVF. Anti-HIV activity in the CVF of HIV-positive women varies with disease progression (Lahey *et*

*al.* 2012), as women with lower CD4+ T cell counts have diminished protection in their CVF (Ghosh *et al.* 2010a). CVF antiviral activity against different viral strains is not uniform, with some strains of reporter cells being highly infectious and others having very low infectivity in the presence of CVF (Ghosh *et al.* 2010b). This suggests that the constituents of CVF have differential activity against HIV, potentially creating a situation where some strains of virus are more easily transmitted than others.

The CVF contains a rich cocktail of more than 20 antimicrobials, chemokines, and cytokines including CCL20, RANTES, human beta-defensin 2 (HBD2), elafin, and secretory leukocyte protease inhibitor (SLPI) (Valore *et al.* 2002; Keller *et al.* 2007; Ghosh *et al.* 2010a), which can potently reduce HIV infection of target cells *in vitro* and are associated with greater protective activity against HIV in CVF (Lahey *et al.* 2012). They exert their effects via several mechanisms:

- **(a)** By preventing binding of HIV to coreceptors expressed on HIV target cells;
- **(b)** By directly interacting with the viral membrane and subsequently destabilizing it; and
- **(c)** By altering HIV target cell signaling pathways that alter gene expression.

Epithelial cells produce several of the anti-HIV proteins that inhibit HIV infection *in vitro*. The protein complement of cellular secretions varies with cell type. For example, the anti-HIV cytokine CCL20 is present in secretions from uterine (Ghosh *et al.* 2009), but not superficial vaginal epithelial cells (Wira *et al.* 2010) suggesting that antiviral activity is not equal at each site in the FRT. Apical secretions collected from polarized endometrial epithelial cells contain potent, but variable anti-HIV activity that reduces infection of TZM-bl cells (Wira *et al.* 2011a). In contrast, there is no detectable anti-HIV activity in superficial vaginal epithelial cell secretions (Patel and Wira, unpublished observation). Unfortunately, no one has compared the protein complement of epithelial secretions, and thus their potential antiviral activity, between the different anatomical regions of the FRT epithelium.

Several of the antiviral proteins are differentially regulated by hormonal status. For example, HBD2 and SLPI concentration decreases in CVF recovered at ovulation (Keller *et al.* 2007). Both HBD2 and elafin are increased by estradiol in uterine epithelial cells (Fahey *et al.* 2008), but decreased in superficial vaginal epithelial cells (Patel and Wira, unpublished observation). Contraceptive use is associated with decreased SLPI production in uterine tissues (Li *et al.* 2008) and in the vaginal epithelium (Kumar *et al.* 2011). Whether hormonal status modulates anti-HIV activity in CVF is unknown, but urgently needs to be addressed, especially in light of recent publications suggesting that contraceptive use might be linked to increased risk of HIV acquisition (Heffron *et al.* 2012).

## 4. HIV and FRT Epithelial Cells

Despite being the first cells exposed to incoming pathogens, the responses of epithelial cells from the lower and upper FRT to HIV have not been adequately studied. Much remains to be determined about the initial interactions between HIV and epithelial cells that occur in the hours following deposition of infected semen in the vagina. Given their role as the primary sentinel cells of the FRT, the epithelial cell response to HIV will be a key event in determining whether viral transmission occurs.

Epithelial cells form a physical barrier that protects the underlying FRT tissue and its resident immune cells against potential viral and bacterial pathogens. The stratified squamous epithelium of the ectocervix and vagina is 25–50 layers thick and consists of the superficial, parabasal and basal layers (Patton *et al.* 2000). The squamo-columnar junction or

transformation zone is where the stratified epithelium of the lower FRT meets the single layer columnar epithelium of the upper FRT and is considered to be uniquely vulnerable to HIV infection because of the abundance of immune target cells (CD4+ T cells, macrophages, dendritic cells) (Pudney *et al*. 2005) and the transition in epithelial phenotype (Hladik and McElrath 2008). Given their structural differences, the lower FRT epithelium is thought to provide a more robust mechanical barrier to incoming pathogens than the upper FRT epithelium. Whether increased thickness correlates with increased protection against HIV is not definitively known. However, cervical ectopy, where the columnar epithelium of the endocervix extrudes onto the surface of the ectocervix, is associated with increased transmission risk of HIV (Moss *et al*. 1991), human papillomavirus (HPV) (Rocha-Zavaleta *et al*. 2004), *Chlamydia trachomatis* (Lee *et al*. 2006), and cytomegalovirus (CMV) (Critchlow *et al*. 1995). Intriguingly, occurrence of cervical ectopy varies with stage of reproductive development and is more prevalent in adolescent women (Rahm *et al*. 1991) as well as pregnant women (Goldacre *et al*. 1978) and women taking oral/hormonal contraceptives (Critchlow *et al*. 1995); each of these populations of women is thought to be at increased risk of HIV infection.

Epithelial thickness varies across the menstrual cycle and is under the control of estradiol. In the vagina epithelial thickness peaks at ovulation, following the surge in blood estradiol concentration (Galand *et al*. 1971). In post-menopausal women, the lack of ovarian estradiol secretion is associated with long-term thinning of the epithelium, which may be partially responsible for the increased risk of HIV infection in this population (Nilsson *et al*. 1995, Aaby *et al*. 1996). The administration of systemic or topical estrogens alleviates these symptoms. Indeed, the topical administration of estradiol in the primate vagina is associated with decreased risk of simian immunodeficiency virus (SIV) infection (Smith *et al*. 2004). In the endometrium, the thickness of the *stratum functionalis* increases through the proliferative phase along with the appearance of tortuous glands lined with columnar epithelial cells that penetrate into the tissue. The thickened endometrium is naturally shed during menstruation because of decreased levels of progesterone and estradiol. Whether these natural changes in turn degrade epithelial barrier protection and lead to increased rates of HIV transmission is unknown. The effect of hormonal contraceptive use on FRT epithelial cells is controversial. For example, administration of Depo-Provera (DMPA), a progestin-containing contraceptive in rhesus macaques leads to dramatic thinning of the vaginal epithelium that is associated with increased SIV transmission (Marx *et al*. 1996). However, the thinning effect of DMPA on the vaginal epithelium is considerably less pronounced in women (Bahamondes *et al*. 2000; Miller *et al*. 2000). Given the large numbers of women who use oral/hormonal contraceptives to regulate their reproductive function, further studies are needed to understand how these compounds alter the epithelial barrier function and innate immune protection *in vivo*.

## 5. Crossing the Epithelial Barrier

HIV must cross the epithelial barrier to infect the immune target cells present in the reproductive stromal tissues. As mentioned previously, the nature of this barrier changes between the upper and lower FRT. There are at least three potential mechanisms by which HIV and other pathogens including bacteria can access the FRT stroma:

**(1)** Via existing gaps or breaches in the epithelial barrier;

**(2)** Transcytosis through epithelial cells, and

**(3)** Paracellular movement between epithelial cells.

Breaches in the epithelium can arise in several ways:

**(a)** The effects of repeated penile entry into the vagina can result in microabrasions in the vaginal epithelium, allowing direct viral access to resident intraepithelial lymphocytes and Langerhans cells present in the basal and parabasal layers (Patton *et al.* 2000). Because of the mechanics of vaginal intercourse, however, it is unlikely that penile entry will directly disrupt the single-layer epithelium in the upper FRT.

**(b)** The introduction of foreign compounds into the FRT can sometimes disrupt the integrity of the epithelial barrier. For example, the spermicide/microbicide Nonoxynol-9, which leads to increased HIV infection, causes rapid exfoliation and loss of uterine, vaginal, and rectal epithelial cell sheets, thus exposing the underlying stroma (Krebs *et al.* 2000; Phillips *et al.* 2000; Jain *et al.* 2005; Cone *et al.* 2006). Nonoxynol-9 further alters the phenotype of regenerating uterine epithelial cells, causing them to transition from columnar to cuboidal epithelial cells (Dayal *et al.* 2003).

**(c)** Sexually transmitted pathogens such as HSV2 infect FRT epithelial cells and, as part of their lytic cycle, cause cell death and ulcerations through which viruses can penetrate into the FRT stroma (Horbul *et al.* 2011).

A second potential mechanism by which HIV can cross the epithelial barrier is transcytosis, where virus is taken up at the apical surface of epithelial cells, transported to the basolateral membrane and secreted into the stromal compartment (Bomsel 1997). Several studies using both cell lines and primary epithelial cells have demonstrated that transcytosis is exceedingly inefficient with an average of less than 0.5% of the initial cell-free viral inoculum crossing polarized epithelial monolayers *in vitro* and entering the basolateral compartment (Hocini *et al.* 2001; Bobardt *et al.* 2007). Viral uptake is an active, temperature-dependent process that relies on the expression of HIV receptors (Bobardt *et al.* 2007). However, depending on the system employed, HSPG (Bobardt *et al.* 2007), gp340 (Stoddard *et al.* 2007, 2009), MR (Hocini *et al.* 2001) and the co-receptors CCR5 and CXCR4 (Hocini *et al.* 2001) have been implicated in transcytosis demonstrating a fundamental difference between experimental systems. The question of whether cell-free or cell-associated HIV transcytose with greater efficiency is unresolved, with one study showing no difference between the two (Hocini *et al.* 2001), and another demonstrating preferential movement of cell-associated HIV (Lawrence *et al.* 2012). The virus that passes through cells is significantly less infectious than the initial viral inoculum suggesting that the intracellular environment is detrimental to viral integrity (Saidi *et al.* 2007). Intriguingly, some investigators have reported that R5 viruses are preferentially transcytosed over X4 viruses (Bobardt *et al.* 2007; Lawrence *et al.* 2012) representing a potential mechanism explaining why R5 viruses are the dominant sexually-transmitted strain *in vivo*.

The third potential mechanism by which HIV can cross the epithelium is paracellular movement. This is controlled by tight junctions that link adjacent columnar epithelial cells in the upper FRT. Tight junctions are protein complexes located at the apical membrane of epithelial cells and are composed of occludin, ZO-1, and multiple claudins amongst others, which regulate the paracellular movement of solutes, and thus create a polarized environment between the apical and basolateral surface (Matter and Balda 2003). Both R5 and X4 strains of HIV decrease transepithelial resistance (TER) in polarized monolayers of primary human endometrial epithelial cells *in vitro* by approximately 60% over 6–48 h (Nazli *et al.* 2010). Gp120 binding to host cell receptors leads to the induction of TNFα, which in turn decreases the expression of tight junction proteins. This is associated with increased translocation of bacterial and viral components across the epithelium (Nazli *et al.* 2010). However, TER is unaffected in polarized monolayers of HEC-1A cells after R5 exposure (Lawrence *et al.* 2012). Furthermore, recombinant TNFα and IL-1β are unable to

alter TER in HEC-1A cells (Lawrence *et al*. 2012), suggesting that there might be a fundamental difference between primary cells and cell lines in their response to HIV.

In the stratified epithelium of the lower FRT, superficial epithelial cells are not bound together by tight junctions (Blaskewicz *et al*. 2011) and have gaps or pores between adjacent cells that are filled with interstitial fluid and vaginal secretions. It is believed that HIV diffuses deeper into the epithelium layer via these fluid phase channels to potentially access lymphocytes in the *stratum corneum*. Parabasal and basal epithelial cells express claudins and occludins and are linked by tight junctions (Blaskewicz *et al*. 2011). Whether HIV can induce the relaxation of these protein complexes and thus reduce barrier function, as it does in the upper FRT, remains to be determined.

## 6. HIV Receptor Expression on FRT Epithelial Cells

The primary viral receptor CD4, the coreceptors CCR5 and CXCR4, and the alternative receptor GalC are expressed on fresh human endometrial epithelial cells. In contrast, CD4 and CCR5 vary in freshly isolated ectocervical epithelial cells with expression absent in superficial cells and low in parabasal and basal cells (Yeaman *et al*. 2003, 2004). CXCR4 is absent from all the epithelial layers in the ectocervix. GalC displays a different expression profile from other receptors and is highest in superficial and parabasal epithelial cells and undetectable in the basal epithelium (Yeaman *et al*. 2003, 2004). Expression of the receptors varies with the stage of the menstrual cycle (Table 1). However, whether this predisposes epithelial cells to greater interactions with HIV is unknown.

The expression profile of most alternative HIV receptors on FRT epithelial cells is relatively understudied. β1 integrin is expressed by endo- and ectocervical epithelial cells *in vivo* (Maher *et al*. 2005). Mannose receptor (MR) and DC-SIGN are absent from endometrial, endocervical, and vaginal epithelial cells (Hirbod *et al*. 2011; Kaldensjö *et al*. 2011). gp340 is present on multiple cell lines and primary epithelial cells from the FRT (Stoddard *et al*. 2007, 2009). Recent studies suggest that alternative receptors may play a pivotal role in HIV translocation and infection in epithelial cells. As noted above, receptor expression can vary dramatically between anatomical sites *in vivo*. Thus, in order to understand the potential contribution of these receptors to HIV transmission one must first elucidate their spatial and temporal expression patterns.

## 7. Epithelial Cell Infection and Transmission

The question of whether FRT epithelial cells *in vivo* are permissive to HIV infection is controversial. Given that FRT epithelial cells express known HIV receptors, it is possible for HIV to enter and infect them. However, examination of human and non-human primate FRT tissue exposed to HIV has demonstrated the presence of viral particles in small populations of leukocytes, but their total absence in epithelial cells. Several groups have tried to infect upper FRT epithelial cells *in vitro* using either primary human cells or cell lines. Unfortunately, the data from these studies are contradictory, with a spectrum of results ranging from no detectable sign of viral entry to *de novo* production of infectious virions (Tan *et al*. 1993; Furuta *et al*. 1994; Howell *et al*. 1997; Dezzutti *et al*. 2001; Asin *et al*. 2003; Wu *et al*. 2003; Asin *et al*. 2004; Berlier *et al*. 2005; Saidi *et al*. 2007). Epithelial cells also sequester virus and release it slowly over time (Wu *et al*. 2003; Saidi *et al*. 2007). This observation may explain the appearance of infectious virions in basolateral secretions in the absence of detectable proviral integration.

Primary epithelial cells and multiple cell lines (HEC-1A, CaSKi, ECC-1, RL95-2, ME-180, and SiHa) have been used as models for HIV infection. The variation in infectivity experiments probably results from the use of cell preparations that are phenotypically

different from each other. For example, Asin et al. (2003) reported that 20, 0, 6, and 5% of HEC-1A cells expressed CD4, CCR5, CXCR4, and GalC respectively. In contrast, Berlier et al. (2005) reported that 0, 0, 59, and 14% of their HEC-1A cells expressed CD4, CCR5, CXCR4, and GalC. In addition, the use of polarized versus nonpolarized epithelial cells may be a confounding factor given their different responses to identical stimuli. Another source of variation may arise from the use of different HIV strains used to infect epithelial cells. Unfortunately, most studies have only used two or three different viral strains in their protocols. Given that some strains of HIV are more infectious than others, it will be essential to expand the panel of viruses used in infection studies, and standardize the cell preparations used in order to derive clear and consistent results.

## 8. Innate Immune Responses to HIV

The majority of studies investigating the interactions between HIV and FRT epithelial cells have focused on whether epithelial cells are permissive with regard to infection, and if they can subsequently infect immune target cells. The direct innate immune response of epithelial cells to HIV, in terms of altered gene expression and protein secretion, has not been addressed in significant detail. Epithelial cells from throughout the FRT are able to respond to pathogens and pathogen ligands by modulating the expression of potentially hundreds of genes. Determining the immune response to HIV is essential to understanding the early interactions between host and pathogen and should provide valuable insight into how HIV is transmitted.

HEC-1A cells differentially respond to R5 and X4 strains of HIV. R5 HIV induces the secretion of IL-8, GRO, TNFβ, and IL-1α while X4 HIV decreases the secretion of TGFβ, IL-1α, RANTES, IL-8, and GRO (Saidi *et al.* 2007). Primary human uterine epithelial cells also upregulate apical secretion of a panel of cytokines including TNFα, IL-6, IL-10, and IL-1β in response to R5 HIV (ADA) (Nazli *et al.* 2010). In studies from our laboratory using primary human uterine epithelial cells and the ECC-1 cell line, we observed the preferential induction of the cytokines IP-10, IL-6, and TNFα in the presence of X4 HIV (IIIB), while MIP1β was selectively induced after R5 HIV (BaL) treatment (Patel and Wira, unpublished observation). In general, IIIB (X4) induces a more proinflammatory environment than BaL (R5). Intriguingly, we also observed the selective induction of the intracellular interferon-stimulated genes MxA, OAS2, PKR, and A3G in the presence of X4 (IIIB) but not R5 (BaL) virus. The expression and secretion of anti-HIV molecules such as HBD2, elafin, and CCL20 are unaltered by either virus (Patel and Wira, unpublished observation). Together, these studies demonstrate the selective induction of a unique panel of genes by different strains of HIV in primary epithelial cells. Whether this expression profile is responsible for the preferential transmission of R5 strains over X4 strains remains a tantalizing question. Unfortunately, there has been no systematic evaluation of epithelial cell responses to different strains of HIV or to identifying the underlying mechanisms regulating the differential response.

Recognizing that epithelial cell secretions can alter the phenotype of resident immune cells, and that R5 and X4 viruses induce altered protein secretion profiles by epithelial cells, it is quite likely that the epithelial response to HIV can affect the phenotype of FRT immune cells and thus their susceptibility to HIV infection. Incubation of macrophages with basolateral secretions from polarized HEC-1A monolayers apically-exposed to R5 HIV leads to their decreased secretion of MCP1, MCP2, MIP1α, MIP1β, MIP3α, and RANTES (Saidi *et al.* 2009). In contrast, secretions from X4-treated HEC-1A cells induce higher levels of MIP1β, RANTES, IP-10, I-TAC, MIG, MCP1, eotaxin 2, and IL-8 secretion by macrophages (compared with R5 HIV) (Saidi *et al.* 2009). These phenotypic changes extend to the ability of macrophages to recruit CD4+ T cells. Macrophages cultured in the presence

of R5 HIV HEC-1A secretions recruited lower numbers of CD4+ T cells than macrophages cultured in the presence of X4 HEC-1A secretions.

Given the dearth of information regarding epithelial responses to HIV, three fundamental questions remain to be answered:

**(1)** Do R5 strains in general induce epithelial responses that are different from those seen with X4 strains or are the responses unique to individual viruses in each strain?

**(2)** Do epithelial cells from throughout the FRT respond in the same manner to individual strains of HIV or are there fundamental differences that are site-specific in the FRT?

**(3)** If there are differential responses, can these alter the susceptibility of target cells in the stroma, thus increasing or decreasing the chances of successful transmission?

## 9. Immune cells susceptible to HIV infection in the lower and upper FRT

After successfully surviving the secretions, mucus, and epithelial barriers, HIV virions will encounter immune cells that are both ideal targets for viral replication and active participants in restricting viral infection. Susceptible target cells in the FRT mucosa include CD4+ T cells, macrophages, dendritic cells (DC), and Langerhans cells (LC), which can be found all along the upper and lower FRT.

### CD4+ T cells

Many studies concur that CD4+ T cells may represent the initial and main target cell for HIV replication (McKinnon and Kaul 2012). However, not all CD4+ T cells subsets are equally susceptible to HIV infection and the location and phenotype of these initial CD4+ T cell targets in the FRT remain unknown. T cells are present in the vagina, cervix, and endometrium, localized in the sub-epithelial stroma, and also located as intraepithelial lymphocytes between epithelial cells that line the lumen (Hickey *et al.* 2011). T cells constitute a main proportion of all leukocytes present in the FRT, representing about 40% of leukocytes in the upper tract and about 50% in the lower tract (Givan *et al.* 1997). Non-human primate models with rhesus macaques showed that SIV infection begins with a small focus of infected CD4+ T cells, with productive SIV infection detected in both activated and resting cells (Zhang *et al.* 1999; Li *et al.* 2005). Studies in humans using explants from vagina, ectocervix, and endocervix indicate that HIV productively infects CD4+ T cells in the genital mucosa very efficiently. Infected cells can be found in the stroma one day after viral exposure, but analysis of the distribution of virions in the initial hours after vaginal challenge demonstrated viral fusion with intraepithelial CD4+ T cells (Hladik *et al.* 2007). Since sexual transmission of HIV is established by CCR5-tropic viral strains (Keele *et al.* 2008; Salazar-Gonzalez *et al.* 2009), characterization of CD4+ T cells subsets expressing CCR5 has become a priority. Using immune cells obtained from cytobrush, a recent report found a subset of CD4+ T cells expressing CCR5 in the cervix (McKinnon *et al.* 2011). Interestingly, CCR5 expression was enhanced in endocervical CD4+ T cells from postmenopausal women relative to premenopausal controls (Meditz *et al.* 2012), suggesting a possible hormonal control of CCR5 expression. Nevertheless, molecules and markers other than CCR5 most likely influence infection of CD4+ T cells since expression of CXCR4 is high in CD4+ T cells at mucosal surfaces (McKinnon *et al.* 2011); paradoxically, initial infection has not been described to involve CXCR4-tropic strains.

### Macrophages

Approximately 10% of the leukocytes present in the FRT are macrophages (Givan *et al.* 1997). These cells are irregularly distributed among FRT tissues, with numbers in the endometrial stroma and myometrial connective tissue (Wira *et al.* 2005) higher than those seen in the endo- or ectocervix. Influx of macrophages into the endometrium is under the influence of estradiol and progesterone with their numbers increasing just prior to menstruation (Starkey *et al.* 1991). In contrast, vaginal macrophage numbers are not affected by sex hormones and remain constant throughout the menstrual cycle (Wira *et al.* 2005). Compared with gastrointestinal macrophages, vaginal macrophages display greater susceptibility to HIV infection, most likely because of higher levels of the HIV-1 receptor CD4 and co-receptors CCR5 and CXCR4 (Shen *et al.* 2009; Cassol *et al.* 2010), and resemble the monocytic phenotype found in blood with high CD14 expression. The involvement of macrophages as initial targets for HIV infection in the FRT mucosa is controversial. While some *in vitro* HIV infection studies in women demonstrated that initial infection was supported by macrophages (Collins *et al.* 2000; Greenhead *et al.* 2000; Cummins *et al.* 2007), SIV infection studies in primate models did not support these findings. Whether macrophages are involved in the initial steps of HIV infection in women as primary cells that support initial replication, or as cells that capture the virus and transmit it to T cells, remains to be elucidated (Sharova *et al.* 2005).

### Dendritic cells

Dendritic cells are potent antigen-presenting cells that generate and regulate adaptive immune T-cell responses (Banchereau and Steinman 1998; Steinman and Hemmi 2006). Hematopoietic stem cells in the bone marrow continuously produce DCs that migrate to the different mucosal surfaces of the human body, where they reside in an immature state. In the upper FRT, DCs can be found in the sub-epithelial stroma in the endometrium, while in the vagina DCs mostly reside within the epithelial layer (Iijima *et al.* 2007, 2008). In the uterine endometrium, immature CD1a+ DCs outnumber mature CD83+ DCs throughout the menstrual cycle (Schulke *et al.* 2008), while CD83+ DCs remain constant. The movement of CD1a+ DCs into the FRT is regulated by hormonal changes during the menstrual cycle with numbers increasing from the proliferative to the secretory phases and peaking at menses (Schulke *et al.* 2008).

As sentinel cells, DCs can sample antigens at the mucosal surface, which potentially makes them one of the first cells that encounters HIV (Wu and Kewalramani 2006; Randolph *et al.* 2008; Liu *et al.* 2009; Altfeld *et al.* 2011). HIV can exploit the biological properties of DCs (Cunningham *et al.* 2010; Lambotin *et al.* 2010) to spread virus from mucosal surfaces to lymph nodes, the most important site for HIV replication. Thus, interaction between DCs and HIV can be either advantageous to the host if the final outcome is induction of efficient immune responses able to control infection, or beneficial to the virus if it exploits DC function to facilitate the spread of infection. DC-SIGN expression has been demonstrated to mediate HIV transmission from DCs to CD4+ T cells *in vitro* (Chehimi *et al.* 2003; Gringhuis *et al.* 2010). However, the role of DC-SIGN+ DCs in sexual transmission *in vivo* is not so well characterized. DC-SIGN+ DCs remain relatively constant in the human endometrium throughout the proliferative and secretory stages of the menstrual cycle (Rieger *et al.* 2004), although possible changes during menses have not been explored. Previous studies from our laboratory indicate that uterine epithelial cells through secreted soluble factors modulate DC phenotype. This phenotype is characterized by decreased sensitivity to Toll-like receptor (TLR) 3 and TLR4 stimulation and reduced expression of co-stimulatory molecules and DC-SIGN (Ochiel *et al.* 2010a, 2010b). Importantly, this down-regulation in DC-SIGN was associated with a reduction in HIV trans-infection by immature DCs (Ochiel *et al.* 2010b). These results highlight the complexity of the FRT,

where not only the influence of sex hormones, but also the local microenvironment needs to be taken into account when evaluating immune responses.

In addition to their major role in triggering adaptive immune responses, DCs exert antiviral activity, which can also be modulated by sex hormones. For example, production of α-defensins by immature DC is inhibited by high doses of estradiol (Rodriguez-Garcia *et al.* 2007; Escribese *et al.* 2011). Additionally, higher production of α-defensins by immature DCs is associated with slower disease progression in HIV-infected subjects (Rodriguez-Garcia *et al.* 2010).

Plasmacytoid DCs (pDC) represent a DC subset of a different origin. pDCs are also present in the FRT and rapidly respond to viral infections secreting large amounts of Type I and Type II interferons (IFN) (Kaldensjö *et al.* 2011; Southern *et al.* 2011). Differences between men and women have been described in the responses of blood-derived pDC to HIV stimulation. For example, HIV-encoded TLR7 ligands induce a stronger IFNα response in women than in men (Meier *et al.* 2009), resulting in enhanced CD8+T cell activation. In non-primate human models, pDCs were found very early after SIV infection to produce both Type I IFN and chemokines within the infected foci of cells in the cervical mucosa (Li *et al.* 2009).

Whether these early innate immune responses effectively inhibit HIV infection or favor infection by attracting new target cells to the infected foci remains to be determined. While *in vitro* studies in humans have demonstrated potent anti-HIV activity of innate immune molecules and correlations with protection (Ghosh *et al.* 2010a; Rodriguez-Garcia *et al.* 2010; Wira *et al.* 2011b), studies of acute SIV infection in rhesus macaques show that inhibition of proinflammatory cytokine and chemokine production by 5% glycerol monolaurate prior to SIV high-dose intravaginal challenge protects from systemic viral infection (Li *et al.* 2009). The general biology and immunology of stromal DCs in the FRT constitute a major gap in our knowledge, and a better understanding is needed regarding which DC subsets are present in the FRT and what their role is in HIV infection.

### Langerhans cells

Distinct from DCs, LCs are characterized by the expression of Langerin and their location at the mucosal surface. LCs are target cells for HIV that can be productively infected *in vitro*, although it appears likely that their main role is to capture and transfer virus to susceptible cells (Wu and Kewalramani 2006). Studies using epidermal cells showed that viral up-take through Langerin, a C-type lectin, mediates internalization and degradation of the virus in Birbeck granules (de Witte *et al.* 2007). Analysis of vaginal LCs, however, indicated that viral internalization occurs shortly after HIV exposure primarily by endocytosis with intact virions persisting in the cytoplasm for days (Hladik *et al.* 2007). Furthermore, non-productively infected LCs containing virus migrate from the exposed vaginal epithelium and transfer HIV to CD4+ T cells (Ballweber *et al.* 2011). In the upper FRT, LCs coexpressing CD1a, CD11c, and Langerin are mainly located in the luminal or glandular epithelium in the endometrium, where they represent potential targets for HIV (Kaldensjö *et al.* 2011). The role that LCs from the upper FRT play in HIV infection has not yet been determined and further studies are required to elucidate their contribution to HIV transmission.

## 10. Induction of HIV-specific cellular immune responses in the FRT

Although *in vitro* studies found that adaptive immune responses can control HIV infection, *in vivo* correlates of protection against HIV infection remain to be determined (Peretz *et al.* 2012). The role of the adaptive immune response in HIV infection is thought to be too little and too late (Reynolds *et al.* 2005), since by the time an adaptive immune response is

mounted against HIV, it is too late to clear infection. A better understanding of the uniqueness of the FRT physiology and immunology is necessary to achieve induction of stronger immune responses at the site of entry that can rapidly act in response to the viral challenge.

The role that humoral immune responses play in protecting mucosal surfaces against HIV infection and the differences between these responses in the FRT and other mucosal surfaces constitute an area of active research and have been reviewed elsewhere (Mestecky *et al.* 2009,; Ackerman *et al.* 2012). Something to consider for the induction of effective cellular immune responses in the FRT is that immune control is more effectively achieved by localized memory T cells than circulating memory T cells (Iwasaki 2010), suggesting that immunization against HIV should induce memory T cell populations in the genital mucosa. Certain tissues, such as the FRT mucosa, skin or central nervous system have restricted access of circulating memory CD8+ T cells in response to localized infections (Gebhardt *et al.* 2009). Entry into these tissues is only facilitated after a subset of CD4+ T cells access the tissue, first in response to local signals, and induce the secretion of chemokines, which in turn allows the migration of other CD4+ and CD8+ memory T cells (Nakanishi *et al.* 2009).

Within one to two weeks following viral infection, effector cells (CD4+ and CD8+ T cells) die, leaving a residual population of quiescent antigen-specific memory T cells in tissues to provide a rapid response against subsequent infections (Pepper and Jenkins 2011). This retention process is partially mediated by TGF-β in non-lymphoid tissues by downregulation of CCR7 and upregulation of E-cadherin receptor CD103 (Sheridan and Lefrancois 2011). In the lower FRT, memory CD4+ or CD8+ T cell and B cell clusters appear in the submucosa and below the epithelium in the vagina after infection clearance. These cell clusters are absent in uninfected individuals, but can be detected in infected subjects for months following infection resolution (Iwasaki 2010). In the upper FRT, lymphoid aggregate structures consisting of a B cell core surrounded by CD8+ T cells and a halo of macrophages are located in the endometrium (Yeaman *et al.* 1997). CD8+ T cells are found in these lymphoid aggregates located between glands in the functionalis region, while CD4+ T cells can be found outside the aggregates, usually located in the stroma. In contrast to the lower FRT CTL whose activity remains high and independent of hormonal changes throughout the menstrual cycle (White *et al.* 1997a, 1997b), aggregates in the upper FRT are regulated by cyclical hormonal fluctuations. A reduced size of the lymphocyte aggregates is accompanied by high CTL activity during the proliferative phase of the menstrual cycle while maximal size of the lymphoid aggregates during the secretory phase is accompanied by suppression of the CTL activity (Yeaman *et al.* 1997, 2001). The demonstration that uterine lymphocyte aggregates are absent in postmenopausal women, when FRT CTL activity is significantly higher than in pre-menopausal women at any stage of the menstrual cycle (White *et al.* 1997a, 1997b), further proves the implication of sex hormones in both processes.

A major gap in our knowledge remains the characterization of HIV-specific immune responses and the T-cell repertoire in the FRT. HIV-specific T-cell responses have been described in the lower and upper FRT of chronically infected women (Musey *et al.* 2003; Shacklett and Greenblatt 2011) and also in the cervix of exposed uninfected individuals (Kaul *et al.* 2000). Several reports demonstrate a lack of correlation between peripheral blood and the mucosal tissue (White *et al.* 2001; Gumbi *et al.* 2008) highlighting the necessity of characterizing local responses since HIV-specific T-cell responses in blood are not necessarily predictive of mucosal protection.

## 11. Current state of vaginal microbicides and vaccine trials

Recent partial but hopeful success has been achieved with vaginal microbicides and vaccine trials (Opoku-Anane *et al.* 2012). Since women are primarily infected via heterosexual intercourse (70–90%), an effective vaginal/rectal microbicide represents a female-controlled protective method that will have a great impact on the prevention of HIV acquisition in women. Microbicides act by reducing susceptibility of target cells at the mucosal surface. The CAPRISA 004 trial showed that the use of 1% tenofovir gel (a reverse-transcriptase inhibitor) applied before and after sex was 39% effective in preventing HIV acquisition overall, with 54% efficacy in highly adherent users (Abdool Karim *et al.* 2010). However, a second trial testing the daily application of 1% tenofovir gel (VOICE) was stopped owing to futility and a lack of protection (van der Straten *et al.* 2012). Reasons for this disparity in tenofovir effectiveness are under investigation and include the different treatment regimens (continuous versus intermittent), user adherence to the daily application, and how sustained use of 1% tenofovir gel alters the immunobiology of the FRT. Further trials are being conducted to determine the effectiveness of tenofovir, including one with the application of 1% tenofovir gel before and after sex (FACTS 001) to try to reproduce the success of the CAPRISA 004 trial. New microbicides are also being developed and a new trial with maraviroc, which blocks CCR5, will soon be initiated (Shattock and Rosenberg 2012).

The RV144 Thai vaccine trial showed a 31% efficacy in preventing HIV acquisition (Rerks-Ngarm *et al.* 2009). The exact mechanisms of how the vaccine worked remain under investigation. Current results suggest that IgG antibodies directed against envelope proteins protect against HIV infection while IgA antibodies mitigate the effects of protective antibodies (Haynes *et al.* 2012). Protective cellular responses in peripheral blood were not found in this trial. Unfortunately, mucosal samples are unavailable since they were not part of the study design; thus, the state of the mucosal innate and adaptive immune responses as well as the characteristics of the potential HIV target cells cannot be determined at present.

Successful preventive strategies in women require a better understanding of the FRT and how immune responses are induced. Animal model studies using vaccination followed by infection demonstrate that cyclical hormonal changes influence the induction of adaptive immune responses. Strong immune responses can be generated when antigen challenge is performed at diestrus (low estradiol levels) while challenge at estrus (high estradiol levels) results in induction of tolerance (Black *et al.* 2000; Kaushic *et al.* 2000; Gockel *et al.* 2003). New vaccine trials need to take into account the biology of women and plan genital cells and secretions sampling in their design for evaluation of anti-HIV responses at the portal of entry.

## 12. Conclusions

The continued expansion and magnitude of the HIV epidemic in women require urgent efforts to find effective preventive methods. The characteristics of the HIV target cells and innate and adaptive anti-HIV responses in the FRT remain largely unknown; thus, a considerable increase in knowledge is necessary to achieve this goal. Given the complexity of interactions between HIV and the FRT, it appears likely that a combination of strategies that act at the portal of entry to protect the HIV target cells induces an effective local innate and adaptive immune response, and control of hormonal changes to reduce local HIV susceptibility will be necessary for successful prevention.

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## References


Aaby P, et al. Age of wife as a major determinant of male-to-female transmission of HIV-2 infection: a community study from rural West Africa. AIDS. 1996; 10:1585–1590. [PubMed: 8931796]

Abdool Karim Q, et al. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. Science. 2010; 329:1168–1174. [PubMed: 20643915]

Ackerman ME, et al. Emerging concepts on the role of innate immunity in the prevention and control of HIV infection. Annu. Rev. Med. 2012; 63:113–130. [PubMed: 22077718]

Altfeld M, et al. DCs and NK cells: critical effectors in the immune response to HIV-1. Nat. Rev. Immunol. 2011; 11:176–186. [PubMed: 21350578]

Asin SN, et al. Human Immunodefidency Virus Type 1 Infection of Human Uterine Epithelial Cells: Viral Shedding and Cell Contact-Mediated Infectivity. J. Infect. Dis. 2003; 187:1522–1533. [PubMed: 12721932]

Asin SN, et al. Transmission of HIV-1 by Primary Human Uterine Epithelial Cells and Stromal Fibroblasts. J. Infect. Dis. 2004; 190:236–245. [PubMed: 15216456]

Arthos J, et al. HIV-1 envelope protein binds to and signals through integrin [alpha]4[beta]7, the gut mucosal homing receptor for peripheral T cells. Nat. Immunol. 2008; 9:301–309. [PubMed: 18264102]

Bahamondes L, et al. The effect upon the human vaginal histology of the long-term use of the injectable contraceptive Depo-Provera. Contraception. 2000; 62:23–27. [PubMed: 11024225]

Ballweber L, et al. Vaginal langerhans cells nonproductively transporting HIV-1 mediate infection of T cells. J. Virol. 2011; 85:13443–13447. [PubMed: 21976645]

Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998; 392:245–252. [PubMed: 9521319]

Berlier W, et al. Selective sequestration of X4 isolates by human genital epithelial cells: Implication for virus tropism selection process during sexual transmission of HIV. J. Med. Virol. 2005; 77:465–474. [PubMed: 16254974]

Bhat S. Galactose to ceramide linkage is essential for the binding of a polyclonal antibody to galactosyl ceramide. J. Neuroimmunol. 1992; 41:105–110. [PubMed: 1460087]

Black CA, et al. Vaginal mucosa serves as an inductive site for tolerance. J. Immunol. 2000; 165:5077–5083. [PubMed: 11046038]

Blaskewicz CD, et al. Structure and function of intercellular junctions in human cervical and vaginal mucosal epithelia. Biol. Reprod. 2011; 85:97–104. [PubMed: 21471299]

Bobardt MD, et al. Cell-Free Human Immunodeficiency Virus Type 1 Transcytosis through Primary Genital Epithelial Cells. J. Virol. 2007; 81:395–405. [PubMed: 17050597]

Bomsel M. Transcytosis of infectious human immunodeficiency virus across a tight human epithelial cell line barrier. Nat. Med. 1997; 3:42–47. [PubMed: 8986739]

Cassol E, et al. Macrophage polarization and HIV-1 infection. J. Leukoc. Biol. 2010; 87:599–608. [PubMed: 20042468]

Chehimi J, et al. HIV-1 transmission and cytokine-induced expression of DC-SIGN in human monocyte-derived macrophages. J. Leukoc. Biol. 2003; 74:757–763. [PubMed: 12960240]

Collins KB, et al. Development of an in vitro organ culture model to study transmission of HIV-1 in the female genital tract. Nat. Med. 2000; 6:475–479. [PubMed: 10742159]

Cone RA, et al. Vaginal microbicides: detecting toxicities in vivo that paradoxically increase pathogen transmission. BMC. Infect. Dis. 2006; 6:90. [PubMed: 16740164]

Critchlow CW, et al. Determinants of cervical ectopia and of cervicitis: Age, oral contraception, specific cervical infection, smoking, and douching. Am. J. Obstet. Gynecol. 1995; 173:534–543. [PubMed: 7645632]

Cummins JE Jr. et al. Preclinical testing of candidate topical microbicides for anti-human immunodeficiency virus type 1 activity and tissue toxicity in a human cervical explant culture. Antimicrob. Agents Chemother. 2007; 51:1770–1779. [PubMed: 17353237]

Cunningham AL, et al. Manipulation of dendritic cell function by viruses. Curr. Opin. Microbiol. 2010; 13:524–529. [PubMed: 20598938]

Dayal MB, et al. Disruption of the upper female reproductive tract epithelium by nonoxynol-9. Contraception. 2003; 68:273–279. [PubMed: 14572891]

De Witte L, et al. Langerin is a natural barrier to HIV-1 transmission by Langerhans cells. Nat. Med. 2007; 13:367–371. [PubMed: 17334373]

Deng H, et al. Identification of a major co-receptor for primary isolates of HIV-1. Nature. 1996; 381:661–666. [PubMed: 8649511]

Dezzutti CS, et al. Cervical and Prostate Primary Epithelial Cells Are Not Productively Infected but Sequester Human Immunodeficiency Virus Type 1. J. Infect. Dis. 2001; 183:1204–1213. [PubMed: 11262202]

Escribese MM, et al. Alpha-defensins 1–3 release by dendritic cells is reduced by estrogen. Reprod. Biol. Endocrinol. 2011; 9:118. [PubMed: 21861873]

Fahey JV, et al. Estradiol selectively regulates innate immune function by polarized human uterine epithelial cells in culture. Mucosal. Immunol. 2008; 1:317–325. [PubMed: 19079193]

Feng Y, et al. HIV-1 Entry Cofactor: Functional cDNA Cloning of a Seven-Transmembrane, G Protein-Coupled Receptor. Science. 1996; 272:872–877. [PubMed: 8629022]

Furuta Y, et al. Infection of vaginal and colonic epithelial cells by the human immunodeficiency virus type 1 is neutralized by antibodies raised against conserved epitopes in the envelope glycoprotein gp120. Proc. Natl. Acad. Sci. 1994; 91:12559–12563. [PubMed: 7809077]

Galand P, et al. Effect Of Oestradiol On Cell Proliferation And Histological Changes In The Uterus And Vagina Of Mice. J. Endocrinol. 1971; 49:243–252. [PubMed: 5091234]

Gebhardt T, et al. Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. Nat. Immunol. 2009; 10:524–530. [PubMed: 19305395]

Ghosh M, et al. CCL20/MIP3α is a Novel Anti-HIV-1 Molecule of the Human Female Reproductive Tract. Am. J. Reprod. Immunol. 2009; 62:60–71. [PubMed: 19527233]

Ghosh M, et al. Anti-HIV activity in cervical-vaginal secretions from HIV-positive and -negative women correlate with innate antimicrobial levels and IgG antibodies. PLoS ONE. 2010a; 5:e11366. [PubMed: 20614007]

Ghosh M, et al. Differential susceptibility of HIV strains to innate immune factors in human cervical-vaginal secretions. Virus Adapt. and Treat. 2010b; 2010:63–71.

Givan AL, et al. 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]

Gockel CM, et al. Influence of the murine oestrous cycle on the induction of mucosal immunity. Am. J. Reprod. Immunol. 2003; 50:369–379. [PubMed: 14750696]

Goldacre MJ, et al. Epidemiology and clinical significance of cervical erosion in women attending a family planning clinic. Br Med J. 1978; 1:748–750. [PubMed: 630328]

Greenhead P, et al. Parameters of human immunodeficiency virus infection of human cervical tissue and inhibition by vaginal virucides. J. Virol. 2000; 74:5577–5586. [PubMed: 10823865]

Gringhuis SI, et al. HIV-1 exploits innate signaling by TLR8 and DC-SIGN for productive infection of dendritic cells. Nat. Immunol. 2010; 11:419–426. [PubMed: 20364151]

Gumbi PP, et al. Impact of mucosal inflammation on cervical Human Immunodeficiency Virus (HIV-1)-specific CD8 T-cell responses in the female genital tract during chronic HIV infection. J. Virol. 2008; 82:8529–8536. [PubMed: 18562528]

Haase AT. Early events in sexual transmission of HIV and SIV and opportunities for interventions. Annu. Rev. Med. 2011; 62:127–139. [PubMed: 21054171]

Haynes BF, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N. Engl. J. Med. 2012; 366:1275–1286. [PubMed: 22475592]

Heffron R, et al. Use of hormonal contraceptives and risk of HIV-1 transmission: a prospective cohort study. Lancet. Inf. Dis. 2012; 12:19–26.

Hickey DK, et al. Innate and adaptive immunity at mucosal surfaces of the female reproductive tract: stratification and integration of immune protection against the transmission of sexually transmitted infections. J. Reprod. Immunol. 2011; 88:185–194. [PubMed: 21353708]

Hirbod T, et al. In Situ Distribution of HIV-Binding CCR5 and C-Type Lectin Receptors in the Human Endocervical Mucosa. PLoS ONE. 2011; 6:e25551. [PubMed: 21984929]

Hladik F, Mcelrath MJ. Setting the stage: host invasion by HIV. Nat. Rev. Immunol. 2008; 8:447–457. [PubMed: 18469831]

Hladik F, et al. Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. Immunity. 2007; 26:257–270. [PubMed: 17306567]

Hocini H, et al. Active and Selective Transcytosis of Cell-Free Human Immunodeficiency Virus through a Tight Polarized Monolayer of Human Endometrial Cells. J. Virol. 2001; 75:5370–5374. [PubMed: 11333919]

Horbul JE, et al. Herpes Simplex Virus-Induced Epithelial Damage and Susceptibility to Human Immunodeficiency Virus Type 1 Infection in Human Cervical Organ Culture. PLoS ONE. 2011; 6:e22638. [PubMed: 21818356]

Howell A, et al. Human Immunodeficiency Virus type 1 infection of cells and tissues from the upper and lower human female reproductive tract. J. Virol. 1997; 71:3498–3506. [PubMed: 9094621]

Iijima N, et al. Vaginal epithelial dendritic cells renew from bone marrow precursors. Proc. Natl. Acad. Sci. 2007; 104:19061–19066. [PubMed: 18006657]

Iijima N, et al. Dendritic cells and B cells maximize mucosal Th1 memory response to Herpes Simplex Virus. J. Exp. Med. 2008; 205:3041–3052. [PubMed: 19047439]

Iwasaki A. Antiviral immune responses in the genital tract: clues for vaccines. Nat. Rev. Immunol. 2010; 10:699–711. [PubMed: 20829886]

Jain JK, et al. Nonoxynol-9 Induces Apoptosis of Endometrial Explants by Both Caspase-Dependent and -Independent Apoptotic Pathways. Biol. Reprod. 2005; 73:382–388. [PubMed: 15829625]

Kaldensjö T, et al. Detection of Intraepithelial and Stromal Langerin and CCR5 Positive Cells in the Human Endometrium: Potential Targets for HIV Infection. PLoS ONE. 2011; 6:e21344. [PubMed: 21738639]

Kaul R, et al. HIV-1-specific mucosal CD8+ lymphocyte responses in the cervix of HIV-1-resistant prostitutes in Nairobi. J. Immunol. 2000; 164:1602–1611. [PubMed: 10640781]

Kaushic C, et al. Effects of estradiol and progesterone on susceptibility and early immune responses to Chlamydia trachomatis infection in the female reproductive tract. Infect. Immun. 2000; 68:4207–4216. [PubMed: 10858238]

Keele BF, et al. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. Proc. Natl. Acad. Sci. 2008; 105:7552–7557. [PubMed: 18490657]

Keller MJ, et al. PRO 2000 elicits a decline in genital tract immune mediators without compromising intrinsic antimicrobial activity. AIDS. 2007; 21:467–476. [PubMed: 17301565]

Krebs FC, et al. Sodium dodecyl sulfate and C31G as microbicidal alternatives to nonoxynol 9: comparative sensitivity of primary human vaginal keratinocytes. Antimicrob. Agents Chemother. 2000; 44:1954–1960. [PubMed: 10858360]

Kumar R, et al. Compartmentalized secretory leukocyte protease inhibitor expression and hormone responses along the reproductive tract of postmenopausal women. J. Reprod. Immunol. 2011; 92:88–96. [PubMed: 21940052]

Lahey T, et al. Selective Impact of HIV Disease Progression on the Innate Immune System in the Human Female Reproductive Tract. PLoS ONE. 2012; 7:e38100. [PubMed: 22675510]

Lambotin M, et al. A look behind closed doors: interaction of persistent viruses with dendritic cells. Nat. Rev. Microbiol. 2010; 8:350–360. [PubMed: 20372157]

Lawrence P, et al. Selective transmigration of monocyte-associated HIV-1 across a human cervical monolayer and its modulation by seminal plasma. AIDS. 2012; 26:785–796. [PubMed: 22495223]

Lee V, et al. Relationship of cervical ectopy to chlamydia infection in young women. J. Fam. Plann. Reprod. Health Care. 2006; 32:104–106. [PubMed: 16824301]

Li Q, et al. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. Nature. 2005; 434:1148–1152. [PubMed: 15793562]

Li A, et al. Effect of mifepristone on the expression of endometrial secretory leukocyte protease inhibitor in new medroxyprogesterone acetate users. Fertility and Sterility. 2008; 90:872–875. [PubMed: 18155704]

Li Q, et al. Glycerol monolaurate prevents mucosal SIV transmission. Nature. 2009; 458:1034–1038. [PubMed: 19262509]

Liu K, et al. In vivo analysis of dendritic cell development and homeostasis. Science. 2009; 324:392–397. [PubMed: 19286519]

Maher D, et al. HIV binding, penetration, and primary infection in human cervicovaginal tissue. Proc. Natl. Acad. Sci. 2005; 102:11504–11509. [PubMed: 16061810]

Marx PA, et al. Progesterone implants enhance SIV vaginal transmission and early virus load. Nat. Med. 1996; 2:1084–1089. [PubMed: 8837605]

Matter K, Balda MS. Signalling to and from tight junctions. Nat. Rev. Mol. Cell Biol. 2003; 4:225–237. [PubMed: 12612641]

McKinnon LR, Kaul R. Quality and quantity: mucosal CD4+ T cells and HIV susceptibility. Curr. Opin. HIV AIDS. 2012; 7:195–202. [PubMed: 22314505]

McKinnon LR, et al. Characterization of a human cervical CD4+ T cell subset coexpressing multiple markers of HIV susceptibility. J. Immunol. 2011; 187:6032–6042. [PubMed: 22048765]

Meditz AL, et al. CCR5 expression is elevated on endocervical CD4+ T cells in healthy postmenopausal women. J. Acquir. Immune Defic. Syndr. 2012; 59:221–228. [PubMed: 22083068]

Meier A, et al. Sex differences in the Toll-like receptor-mediated response of plasmacytoid dendritic cells to HIV-1. Nat. Med. 2009; 15:955–959. [PubMed: 19597505]

Mestecky J, et al. Mucosal immunology of the genital and gastrointestinal tracts and HIV-1 infection. J. Reprod. Immunol. 2009; 83:196–200. [PubMed: 19853927]

Mestecky J, et al. Methods for evaluation of humoral immune responses in human genital tract secretions. Am. J. Reprod. Immunol. 2011; 65:361–367. [PubMed: 21087333]

Miller L, et al. Depomedroxyprogesterone-induced hypoestrogenism and changes in vaginal flora and epithelium. Obstet. Gynecol. 2000; 96:431–439. [PubMed: 10960638]

Moss GB, et al. Association of Cervical Ectopy with Heterosexual Transmission of Human Immunodeficiency Virus: Results of a Study of Couples in Nairobi, Kenya. J. Infect. Dis. 1991; 164:588–591. [PubMed: 1869844]

Musey L, et al. Ontogeny and specificities of mucosal and blood human immunodeficiency virus type 1-specific CD8(+) cytotoxic T lymphocytes. J. Virol. 2003; 77:291–300. [PubMed: 12477834]

Nakanishi Y, et al. CD8(+) T lymphocyte mobilization to virus-infected tissue requires CD4(+) T-cell help. Nature. 2009; 462:510–513. [PubMed: 19898495]

Nazli A, et al. Exposure to HIV-1 Directly Impairs Mucosal Epithelial Barrier Integrity Allowing Microbial Translocation. PLoS Pathog. 2010; 6:e1000852. [PubMed: 20386714]

NIAID. HIV infection in Women. 2010

Nilsson K, et al. The vaginal epithelium in the postmenopause — cytology, histology and pH as methods of assessment. Maturitas. 1995; 21:51–56. [PubMed: 7731384]

Ochiel DO, et al. Human uterine epithelial cell secretions regulate dendritic cell differentiation and responses to TLR ligands. J. Leukoc. Biol. 2010a; 88:435–444. [PubMed: 20385795]

Ochiel DO, et al. Uterine epithelial cell regulation of DC-SIGN expression inhibits transmitted/founder HIV-1 trans infection by immature dendritic cells. PLoS ONE. 2010b; 5:e14306. [PubMed: 21179465]

Opoku-Anane J, et al. New Success With Microbicides and Pre-Exposure Prophylaxis for Human Immunodeficiency Virus (HIV): Is Female-Controlled Prevention the Answer to the HIV Epidemic? Rev. Obstet. Gynecol. 2012; 5:50–55. [PubMed: 22582127]

Owen DH, Katz DF. A vaginal fluid simulant. Contraception. 1999; 59:91–95. [PubMed: 10361623]

Patel M, et al. Cell-surface heparan sulfate proteoglycan mediates HIV-1 infection of T-cell lines. AIDS Res. Hum. Retroviruses. 1993; 9:167–174. [PubMed: 8096145]

Patton DL, et al. Epithelial cell layer thickness and immune cell populations in the normal human vagina at different stages of the menstrual cycle. Am. J. Obstet. Gynecol. 2000; 183:967–973. [PubMed: 11035348]

Pepper M, Jenkins MK. Origins of CD4(+) effector and central memory T cells. Nat. Immunol. 2011; 12:467–471. [PubMed: 21739668]

Peretz Y, et al. Dissecting the HIV-specific immune response: a systems biology approach. Curr. Opin. HIV AIDS. 2012; 7:17–23. [PubMed: 22134339]

Phillips DM, et al. Nonoxynol-9 causes rapid exfoliation of sheets of rectal epithelium. Contraception. 2000; 62:149–154. [PubMed: 11124363]

Pudney J, et al. Immunological Microenvironments in the Human Vagina and Cervix: Mediators of Cellular Immunity Are Concentrated in the Cervical Transformation Zone. Biol Reprod. 2005; 73:1253–1263. [PubMed: 16093359]

Rahm VA, et al. Chlamydia trachomatis in sexually active teenage girls. Factors related to genital chlamydial infection: a prospective study. Genitourin. Med. 1991; 67:317–321. [PubMed: 1916795]

Randolph GJ, et al. Migration of dendritic cell subsets and their precursors. Annu. Rev. Immunol. 2008; 26:293–316. [PubMed: 18045026]

Rerks-Ngarm S, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N. Engl. J. Med. 2009; 361:2209–2220. [PubMed: 19843557]

Reynolds MR, et al. CD8+ T-lymphocyte response to major immunodominant epitopes after vaginal exposure to simian immunodeficiency virus: too late and too little. J. Virol. 2005; 79:9228–9235. [PubMed: 15994817]

Rieger L, et al. Antigen-Presenting Cells in Human Endometrium During the Menstrual Cycle Compared to Early Pregnancy. J. Soc. Gynecol. Invest. 2004; 11:488–493.

Rocha-Zavaleta L, et al. Human Papillomavirus infection and cervical ectopy. Int. J. Gyn. Obst. 2004; 85:259–266.

Rodriguez-Garcia M, et al. Human immature monocyte-derived dendritic cells produce and secrete alpha-defensins 1–3. J. Leukoc. Biol. 2007; 82:1143–1146. [PubMed: 17684038]

Rodriguez-Garcia M, et al. Increased alpha-defensins 1–3 production by dendritic cells in HIV-infected individuals is associated with slower disease progression. PLoS ONE. 2010; 5:e9436. [PubMed: 20195543]

Saidi H, et al. R5- and X4-HIV-1 use differentially the endometrial epithelial cells HEC-1A to ensure their own spread: implication for mechanisms of sexual transmission. Virology. 2007; 358:55–68. [PubMed: 16934308]

Saidi H, et al. Apical interactions of HIV type 1 with polarized HEC-1 cell monolayer modulate R5-HIV type 1 spread by submucosal macrophages. AIDS Res. Hum. Retroviruses. 2009; 25:497–509. [PubMed: 19397398]

Salazar-Gonzalez JF, et al. Genetic identity, biological phenotype, and evolutionary pathways of transmitted/founder viruses in acute and early HIV-1 infection. J. Exp. Med. 2009; 206:1273–1289. [PubMed: 19487424]

Sattentau Q, et al. Epitopes of the CD4 antigen and HIV infection. Science. 1986; 234:1120–1123. [PubMed: 2430333]

Schulke L, et al. Endometrial dendritic cell populations during the normal menstrual cycle. Human Reproduction. 2008; 23:1574–1580. [PubMed: 18285323]

Shacklett BL, Greenblatt RM. Immune responses to HIV in the female reproductive tract, immunologic parallels with the gastrointestinal tract, and research implications. Am. J. Reprod. Immunol. 2011; 65:230–241. [PubMed: 21223420]

Sharova N, et al. Macrophages archive HIV-1 virions for dissemination in trans. EMBO J. 2005; 24:2481–2489. [PubMed: 15920469]

Shattock RJ, Rosenberg Z. Microbicides: Topical Prevention against HIV. Cold Spring Harb. Perspect. Med. 2012; 2:a007385. [PubMed: 22355798]

Shen R, et al. Macrophages in Vaginal but Not Intestinal Mucosa Are Monocyte-Like and Permissive to Human Immunodeficiency Virus Type 1 Infection. J.Virol. 2009; 83:3258–3267. [PubMed: 19153236]

Sheridan BS, Lefrancois L. Regional and mucosal memory T cells. Nat. Immunol. 2011; 12:485–491. [PubMed: 21739671]

Shust GF, et al. Female Genital Tract Secretions Inhibit Herpes Simplex Virus Infection: Correlation with Soluble Mucosal Immune Mediators and Impact of Hormonal Contraception. Am. J. Reprod. Immunol. 2010; 63:110–119. [PubMed: 20015330]

Smith SM, et al. Topical estrogen protects against SIV vaginal transmission without evidence of systemic effect. AIDS. 2004; 18:1637–1643. [PubMed: 15280774]

Southern PJ, et al. Coming of age: reconstruction of heterosexual HIV-1 transmission in human ex vivo organ culture systems. Mucosal. Immunol. 2011; 4:383–396. [PubMed: 21430654]

Starkey PM, et al. Variation during the menstrual cycle of immune cell populations in human endometrium. Eur. J. Obstet. Gynecol. Reprod. Biol. 1991; 39:203–207. [PubMed: 1709605]

Steinman RM, Hemmi H. Dendritic cells: translating innate to adaptive immunity. Curr. Top. Microbiol. Immunol. 2006; 311:17–58. [PubMed: 17048704]

Stoddard E, et al. gp340 Expressed on Human Genital Epithelia Binds HIV-1 Envelope Protein and Facilitates Viral Transmission. J. Immunol. 2007; 179:3126–3132. [PubMed: 17709527]

Stoddard E, et al. gp340 Promotes Transcytosis of Human Immunodeficiency Virus Type 1 in Genital Tract-Derived Cell Lines and Primary Endocervical Tissue. J. Virol. 2009; 83:8596–8603. [PubMed: 19553331]

Tan X, et al. Productive infection of a cervical epithelial cell line with human immunodeficiency virus: implications for sexual transmission. J. Virol. 1993; 67:6447–6452. [PubMed: 8411347]

Turville SG, et al. HIV gp120 receptors on human dendritic cells. Blood. 2001; 98:2482–2488. [PubMed: 11588046]

UNAIDS. Women, girls, gender equality and HIV. 2010

Valore EV, et al. Antimicrobial components of vaginal fluid. Am. J. Obstet. Gynecol. 2002; 187:561–568. [PubMed: 12237628]

Van Der Straten A, et al. Unraveling the divergent results of pre-exposure prophylaxis trials for HIV prevention. AIDS. 2012; 26:F13–19. [PubMed: 22333749]

White HD, et al. CD3+ CD8+ CTL activity within the human female reproductive tract: influence of stage of the menstrual cycle and menopause. J. Immunol. 1997a; 158:3017–3027. [PubMed: 9058841]

White HD, et al. Mucosal immunity in the human female reproductive tract: cytotoxic T lymphocyte function in the cervix and vagina of premenopausal and postmenopausal women. Am. J. Reprod. Immunol. 1997b; 37:30–38. [PubMed: 9138451]

White HD, et al. Human immunodeficiency virus-specific and CD3-redirected cytotoxic T lymphocyte activity in the human female reproductive tract: lack of correlation between mucosa and peripheral blood. J. Infect. Dis. 2001; 183:977–983. [PubMed: 11237817]

WHO. Progress report 2011: Global HIV/AIDS response. 2011

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]

Wira CR, et al. Sex Hormone Regulation of Innate Immunity in the Female Reproductive Tract: The Role of Epithelial Cells in Balancing Reproductive Potential with Protection against Sexually Transmitted Pathogens. Am. J. Reprod. Immunol. 2010; 63:544–565. [PubMed: 20367623]

Wira CR, et al. Epithelial cell secretions from the human female reproductive tract inhibit sexually transmitted pathogens and Candida albicans but not Lactobacillus. Mucosal Immunol. 2011a; 4:335–342. [PubMed: 21048705]

Wira CR, et al. Innate immunity in the human female reproductive tract: endocrine regulation of endogenous antimicrobial protection against HIV and other sexually transmitted infections. Am. J. Reprod. Immunol. 2011b; 65:196–211. [PubMed: 21294805]

Wu L, Kewalramani VN. Dendritic-cell interactions with HIV: infection and viral dissemination. Nat. Rev. Immunol. 2006; 6:859–868. [PubMed: 17063186]

Wu Z, et al. Human Genital Epithelial Cells Capture Cell-Free Human Immunodeficiency Virus Type 1 and Transmit the Virus to CD4+ Cells: Implications for Mechanisms of Sexual Transmission. J. Infect. Dis. 2003; 188:1473–1482. [PubMed: 14624372]

Yeaman GR, et al. Unique CD8+ T cell-rich lymphoid aggregates in human uterine endometrium. J. Leukoc. Biol. 1997; 61:427–435. [PubMed: 9103229]

Yeaman GR, et al. CD8+ T cells in human uterine endometrial lymphoid aggregates: evidence for accumulation of cells by trafficking. Immunology. 2001; 102:434–440. [PubMed: 11328377]

Yeaman GR, et al. Human immunodeficiency virus receptor and coreceptor expression on human uterine epithelial cells: regulation of expression during the menstrual cycle and implications for human immunodeficiency virus infection. Immunology. 2003; 109:137–146. [PubMed: 12709027]

Yeaman GR, et al. Chemokine receptor expression in the human ectocervix: implications for infection by the human immunodeficiency virus-type I. Immunology. 2004; 113:524–533. [PubMed: 15554931]

Zhang Z, et al. Sexual transmission and propagation of SIV and HIV in resting and activated CD4+ T cells. Science. 1999; 286:1353–1357. [PubMed: 10558989]

## Table 1

Effect of menstrual cycle stage on receptor expression in freshly isolated human endometrial and ectocervical epithelial cells

| | Endometrial | | Ectocervical | |
|---|---|---|---|---|
| | **Proliferative** | **Secretory** | **Proliferative** | **Secretory** |
| CD4 | Increase | Decrease | Increase | Decrease |
| CCR5 | Increase | Decrease | No Change | No Change |
| CXCR4 | Decrease | Increase | Absent | Absent |
| GalC | No Change | No Change | No Change | No Change |