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A new strategy to understand how HIV infects women: identification of a window of vulnerability during the menstrual cycle

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Introduction

Although 85% of new HIV cases are due to sexual transmission from men to women, little attention is being paid to the immune system in the female reproductive tract (FRT), and to how it meets the conflicting challenges of protecting from pathogens and permitting procreation. As a new approach we have tried to envision how HIV evades FRT mucosal immune protection and have been led to the unexpected conclusion that in a normal menstrual cycle, there is a window of vulnerability (7–10 days following ovulation) in which the potential for viral infectivity in the FRT is enhanced. During that period, aspects of the innate, humoral, and cell-mediated immune systems are suppressed by sex hormones to optimize conditions for procreation. Suppression occurs in the upper (Fallopian tubes, uterus, endocervix) and lower (ectocervix and vagina) FRT, and coincides with the recruitment of potentially infectable cells and upregulation of coreceptors essential for viral uptake. Implications of these findings are that the entire FRT is a potential target for HIV infection, immune cells and antibodies in blood are not surrogate markers for immune protection in the FRT, and immune protection against HIV will require an understanding of the hormone-induced regulation of humoral, cell-mediated, and innate immune systems throughout the FRT.

The need to understand the interplay between the immune and endocrine systems in the human female reproductive tract

Despite unprecedented efforts by scientists worldwide, the solution to the ever-growing HIV/AIDS crisis remains elusive. HIV/AIDS is unique in modern human history in its rapid spread, its extent, and the depth of its impact. Since the first AIDS case was diagnosed in 1981, the world has struggled to come to grips with its extraordinary toll. Approaching 25 million deaths worldwide with an additional 33.2 million (of which 15.4 million are women) estimated to be infected worldwide, HIV/AIDS will soon be the world's worst pandemic [1].

With the recent failures of the Diaphragm trial, the Merck vaccine trial, and the Microbicide gel trial [2–6] along with recognition that for each person treated with antiretrovirals, six are newly infected with HIV [7], it remains unclear when safe and effective protection will become available. The failure of apparently promising approaches highlights the urgency to better understand how to prevent HIV transmission in women. Women are approximately twice as likely to contract HIV infection from men as men are from women during vaginal intercourse [8]. Each year brings an increase in the percentage of women infected with HIV. In particular, women and girls make up about 57% of all people infected with HIV in sub-Saharan Africa,

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where a striking 76% of those with HIV in the 15–24 year age group are female [9]. In the United States, the proportion of AIDS cases reported among women increased from 7% in 1985 to 28% in 2005 [1,8].

Our interest in the reproductive tract immune system over the past 25 years has been refocused by the human tragedy of AIDS. As 80% of new HIV infections are due to heterosexual transmission, our efforts are concentrated on mucosal protection [10]. What is difficult to reconcile is that whereas the number of women infected has reached 20 million, the estimated rate of HIV transmission per coital act is low, 1 : 122 to 1 : 1000 [11,12]. These findings suggest that, while transmission is related to the viral load [13], exposure time following seroconversion [14] and pre-existence of other sexually transmitted infections (STIs), there exists within the FRT a window of vulnerability through which HIV, and probably other STI, can gain access to the body.

Lacking in many studies is an appreciation of the reproductive process and the complexity of the FRT. To understand the immunological response of the FRT to pathogenic challenge, one must first appreciate that, unlike other mucosal immune sites, the FRT has evolved to complement the reproductive events occurring each month. The FRT consists of five distinct anatomical sites (Fallopian tubes, uterus, endocervix, ectocervix, and vagina), which function separately yet in coordinated fashion. Each site is controlled by estradiol and progesterone. Extensive studies [15] have defined how these functions are synchronized to optimize the chances for successful fertilization, implantation, and pregnancy.

Our working hypothesis

Critical to the reproductive process is the ability of the immune system to distinguish between semiallogeneic sperm, an allogeneic fetal placental unit, and potential pathogens. Herein lies the problem of understanding the realities of heterosexual transmission of HIV from men to women at FRT mucosal surfaces. As have others, we have designed our studies to define the components of the immune system present in the FRT, how these protect against pathogens, and how they are controlled by sex hormones [16–18]. Recently, we challenged ourselves to understand how the physiology of the FRT can lead to increased vulnerability to viral infection. This led us to the following question: from a viral perspective, what times during the menstrual cycle come closest to being optimal for infection?

By examining multiple immunological parameters, as described in detail below, we reached the unexpected conclusion that within the FRT during a normal menstrual cycle, there is a period lasting 7–10 days when important components of innate, humoral, and cell-mediated immunity are suppressed by estradiol and/or progesterone, enhancing the potential for viral infection. Our working hypothesis is that immunological suppression occurs in both the upper and the lower FRT as an integral part of the physiological processes that underlie successful reproduction, and that this suppression coincides with recruitment of potentially infectable cells and upregulation of coreceptors on target cells that are essential for viral uptake.

Common misconceptions about the human female reproductive tract

High on the list of misconceptions about the FRT are several items that have compromised research relevant to HIV. First, the lower FRT (ectocervix and vagina) is the only significant primary site of HIV infection. Recent observations [19–21] suggest that the upper FRT (endocervix and uterus) might also be a portal of entry for HIV following sexual intercourse. Second, the upper FRT is sterile. In reality, the upper FRT is continuously exposed to commensals and pathogens present in the lower FRT. Labeled-albumin microspheres and dyes as well as sperm enter the uterus and Fallopian tubes within minutes of placement in the vagina [22–25]. Because HIV in the FRT can be cell-free, cell-associated, and attached to sperm

[26,27], HIV is likely disseminated throughout the entire FRTwithin minutes of deposition in the vagina. Third, because the FRT lacks organized lymphoid follicles, it is not an inductive site for eliciting immune responses. In fact, lymphoid follicles are found in the uterus, and antigen-presenting cells (APCs) throughout the FRT can present antigen to naive and memory T cells [28–31]. Moreover, when added to the vagina, Toll-like receptor (TLR) agonists enhance protection against immune challenge beyond that seen with vaccine alone [32]. Fourth, hormonal balance does not matter in immune protection in the FRT. As discussed in detail elsewhere, most aspects of the immune system in the FRT are hormonally regulated [18]. Finally, immune cells in the FRT are identical to those in peripheral blood. Both in terms of phenotype and immune function, subsets of blood leukocytes [including neutrophils, natural killer (NK) cells, monocytes, and T cells] mature and/or differentiate shortly after entering the FRT, becoming distinct from their blood counterparts [18,33,34].

A series of observations from the 1960s to the present clearly defined the changing pattern of sex hormones in blood over the menstrual cycle and the consequences of these hormonal effects throughout the FRT. Under the influence of the hypothalamic–pituitary axis (Fig. 1), estradiol levels, which are low during the first half of the menstrual cycle (proliferative stage) rise and peak 2–3 days before ovulation. After ovulation estradiol levels transiently decline and then increase along with progesterone for 7–10 days (secretory stage), after which both decline to initiate menstruation. These hormonal changes prepare the vagina and cervix to optimize sperm survival and migration to the Fallopian tube where fertilization occurs. Hormones regulate the movement of the ovum into the Fallopian tube, the provision of nutrients for cell division, and retention of the fertilized egg at this site for 3–4 days. In the uterus, estradiol and progesterone prepare this site for implantation by providing an adequate vascular supply and nutrients both prior to and following implantation and successful pregnancy. Along with the reproductive functions, the sex hormones regulate the immune system in the FRT to protect against pathogens (including HIV) without rejecting sperm, zygote, and blastocyst.

Immune protection in the upper reproductive tract

Figure 2 schematically illustrates how sex hormones regulate immune function in the upper FRT. Figure 2a depicts key immunological mechanisms. Each of these is essential for successful reproduction and directly or indirectly impacts pathogens that enter the upper FRT and threaten reproductive health. The ovals represent immune cell migration, cytotoxic T cell activity, coreceptor expression, antibodies and antimicrobials in secretions, and innate immune cells. Each plays a role in normal physiological defense functions [35,36].

Figure 2b shows these immune mechanisms under hormonal influence. The expanding concentric rings represent estradiol released at mid-cycle along with progesterone released during the secretory stage of the cycle, which not only enhance immune cell migration into the uterus but alter the architectural relationship of these cells so that they form lymphoid aggregates throughout the endometrium [29]. These aggregates consist of a B-cell core surrounded by CD8+ T cells with a halo of macrophages. Occasionally, some aggregates consist of CD4+ T cells. Under hormonal control, aggregates increase in size from 300 to 3000–4000 cells, and in many cases make physical contact with the basolateral surfaces of epithelial cells lining the uterine lumen [29,37]. White *et al.* [38] found that coincident with aggregate formation, CD8+ cytotoxic T lymphocyte (CTL) activity, measured in a redirected lysis assay, is suppressed in the uterus and Fallopian tubes during the secretory stage of the cycle. This suppression is confined to the upper FRT and occurs without any concomitant drop in CD8+ T cell numbers. We also found that uterine CTL from HIV-infected women displayed anti-HIV CTL activity that did not parallel that seen in blood [39]. In other studies, we found that epithelial cells in the uterus express CD4, CXCR4, and CCR5 and that expression on the apical surfaces of these cells varies with the stage of the menstrual cycle [19]. Of particular interest

was our finding that all three coreceptors are under hormonal control: they are low during the proliferative stage of the cycle, peak at the time of ovulation and then either plateau (CXCR4, CD4) or decline (CCR5) during the secretory stage of the cycle [19]. Equally important are findings of others showing that immature and mature dendritic cells, when cultured with transforming growth factor-β (TGF-β), upregulate coreceptor expression (CXCR4, CCR5) [40]. Given that estradiol stimulates FRT secretion of TGF-β [41], these studies suggest that estradiol may be acting indirectly to alter coreceptor expression on immune cells in the FRT. In Fig. 2b we suggest that estradiol/progesterone regulates antibody [immunoglobulin A (IgA) or immunoglobulin G (IgG)] movement from tissue to lumen in the upper FRT. This conclusion is based on our findings that the level of IgA receptor responsible for transporting IgA is elevated in uterine secretions during the secretory stage of the menstrual cycle [42]. Under normal conditions, however, IgA and IgG levels are low in uterine secretions and are therefore indicated with a single thin arrow.

An unexpected recent finding from our laboratory is that estradiol has direct effects on epithelial cell synthesis and secretion of β-defensins and secretory leukocyte protease inhibitor (SLPI), which have potent antimicrobial (bacterial and viral) activity [43,44]. Using primary polarized epithelial cells, we demonstrated that estradiol enhances the secretion of these antimicrobials while simultaneously suppressing the secretion of proinflammatory chemokines and selected cytokines of TLR agonists [44]. In other studies [45], we found that antimicrobial products secreted by epithelial cells are biologically active in that they inhibit the growth of gram-positive and gram-negative bacteria (*Staphylococcus aureus, Escherichia coli*), as well as *Neisseria gonorrheae, Candida albicans* and HIV (X4 and R5) (Fahey *et al.*, unpublished results).

The upper left oval in Fig. 2b refers to three types of innate immune cells and indicates that through the direct and/or indirect effects of estradiol on TGF-β, other cytokines and growth factors [16,41], innate immune protection is damped. Sentman and colleagues demonstrated that uterine NK cells (CD56 bright) express relatively low levels of intracellular interferon-γ (IFN-γ) when cultured in the presence of uterine secretions. Under conditions of antibody neutralization of TGF-β, intracellular IFN-γ production by uterine NK cells increased in cells stimulated with the TLR3 agonist, poly I:C [46]. The complexity of this system is further evidenced by our findings that estradiol attenuates lipopolysaccharide (LPS)-induced expression of IL-8 in monocytes. Treatment of monocytes with estradiol prior to LPS reduced IL-8 message and protein production [47]. These results suggest that estradiol acts through monocytes to suppress the migration of neutrophils in the FRT, which decreases innate immune protection. In contrast, when macrophages are challenged with LPS in the presence of estradiol, IL-1β secretion is enhanced, which leads to increased apical secretion of human β-defensin-2 (HBD2) by uterine epithelial cells and enhanced antimicrobial activity [48]. In other studies, we found that TGF-β acts on neutrophils to inhibit inflammatory degranulation and reduce the secretion of lactoferrin that could protect against pathogens but potentially damage the oocyte or fetus [49]. Sato *et al.* [40] demonstrated that TGF-β enhanced the chemotactic migratory ability of immature dendritic cells in response to CC and CXC chemokines while suppressing major histocompatibility complex (MHC) class II expression and presumed antigen recognition and presentation. Using the mouse FRT as a model system, we have found that estradiol suppresses antigen presentation by epithelial cells as well as APC in the uterine and vaginal stroma by downregulating MHC class II and CD80/86 expression ([50], Wira, unpublished observation). Taken together, these studies indicate several immunological parameters in the upper FRT are altered in response to increased hormone levels during the menstrual cycle.

Figure 2c illustrates our hypothesis that sex hormones in the upper FRT generally suppress the immune system to optimize chances for fertilization and implantation. A consequence is to

open several windows of vulnerability for HIV infection. On one hand, macrophages, CD4+ T cells and possibly epithelial cells are placed in juxtaposition with the lumen so that coreceptors essential for infectivity are upregulated. On the other hand, innate (NK cells, neutrophils, and dendritic cells) and adaptive (CD8+ T cells) immune cells are suppressed. From a viral standpoint, only the presence of antimicrobials in Fallopian tube, uterine, and endocervical secretions stands as an obstacle to successful infection. Whether sex hormone-induced increases in antimicrobials are sustained during the secretory stage of the cycle remains to be determined. According to our hypothesis, the high levels of estradiol at midcycle followed by the continued presence of estradiol and/or progesterone during the secretory stage of the cycle, prepare the FRT for conception at the risk of susceptibility to HIV and other infections.

Immune protection in the lower reproductive tract

We have noted that in the lower reproductive tract sex hormones introduce a window of vulnerability separate from that of the upper FRT. Figure 3 illustrates some major immunological parameters in the ectocervix and vagina. Over the course of the menstrual cycle, subtle changes occur in migration of macrophages, B cells and neutrophils into the lower tract [28,33], and in dendritic cells entering the squamous epithelium [51]. In the ectocervix and vagina, in contrast to the upper FRT, we found that CTL activity was measurable in tissues from women at the proliferative or secretory stages of the menstrual cycle [52]. As in the upper FRT, we postulate that NK, neutrophils, and dendritic cell function is suppressed by TGF-β and HIV-coreceptor expression is enhanced on macrophage/dendritic cells. Of particular interest is the finding that coreceptors are expressed on epithelial cells in the ectocervix. Yeaman *et al.* [20] showed that basal and parabasal epithelial cells of the ectocervix express CD4, CCR5 and GalCer, unlike the midzone and superficial cells lining the lumen. Although changes in protein expression were not as pronounced as those seen in the uterus, histological evidence supported the conclusion that CD4 and CCR5 expression was greater during the proliferative stage than during the secretory stage of the cycle.

Other differences from the upper tract are the effects of sex hormones on secretions from the ectocervix and vagina. Schumacher [53] demonstrated that IgA, IgG, and lactoferrin levels in secretions declined 10–100-fold at midcycle, only to rise toward the end of the menstrual cycle. When women were placed on oral contraceptives, immunoglobulins and lactoferrin levels were suppressed for the duration of hormone exposure. In other studies, in which cervical mucus was evaluated from 5 days before to 3 days after ovulation, IgA and IgG had a biphasic pattern with a peak before ovulation followed by a small increase after ovulation [54]. Nardelli-Haefliger *et al.* [55] demonstrated that titers of antihuman papillomavirus 16 virus-like particle (VLP) IgG in cervical secretions dropped approximately nine-fold at midcycle during ovulatory cycles. These changes would not be expected to enhance viral infectivity in an immunologically naive individual, but might decrease resistance in individuals in which humoral anti-HIV responses were induced. Of importance is the midcycle decline in lactoferrin, produced by neutrophils, which has been shown to have anti-HIV activity [56]. Recently, we found that midcycle suppression by estradiol extends to endogenous antimicrobials in cervical–vaginal lavages (CVLs) [56]. Analysis of the concentrations of cytokines, chemokines, and antimicrobials in CVL indicated that SLPI, HBD2, human neutrophil peptide (HNP)-1–3, and lactoferrin dropped significantly at midcycle (day 13) and remained depressed for 7–10 days, returning to proliferative stage levels just before menstruation. In contrast, total protein and TGF-β levels remained unchanged throughout the menstrual cycle. In other studies, human intestinal defensin-5 was highest in CVL during the secretory stage of the menstrual cycle [57]. More recently, Cole and colleagues demonstrated an anti-HIV (X4 and R5) function of cationic polypeptides within human vaginal fluid and suggested a synergism between polypeptides and proteins in vaginal fluid [58,59]. These findings support the proposal of

Lehrer that the innate immune system contains a repository for future antimicrobial agents [60].

It remains unclear whether sex hormones act directly on immune cells and their secretions in the lower FRT, or whether changes are due to alterations in mucus content or volume. What is clear is that the ectocervical and vaginal secretions exhibit a pattern of innate immune protection that is physiologically suppressed at midcycle. Given that regulatory T cells are located throughout the FRTand are responsive to estradiol [61], we postulate that the immune suppression of NK cells and dendritic cells in the ectocervix and vagina is similar to that seen in the uterus. Figure 3c depicts what we postulate to be the window of vulnerability for HIV infection in the lower FRT at midcycle and during the secretory stage of the menstrual cycle. Starting with a broad spectrum of physiological immunosuppression of antimicrobials in CVL secretions it appears that protection depends on CTLs in the ectocervix and vagina. Although immune cell migration might be expected to enhance protection, further suppression of immune cell function (NK cells, dendritic cells), when coupled with enhanced coreceptor expression, increases the potential for successful HIV infection at this time.

Influence of oral contraceptives, menopausal status, and endocrine manipulation to HIV infection

Of potential importance in the spread of HIV is the role of hormonal contraceptives. As reviewed by Baeten and Overbaugh, biological and epidemiological studies suggest that use of hormonal contraceptives could influence susceptibility (infectivity) to HIV and disease progression [62–64]. Not all studies have shown a relationship, and questions remain about contraceptive pills used (combination, depot, etc.), dosage, and length of use. Consistent with our hypothesis is evidence that oral contraceptives upregulate CCR5 expression on CD4+ T cells in the cervix, and that progesterone prevents the induction of mucosal responses in the FRT following intravaginal immunization with herpes simplex virus type 2 [65,66]. Other studies [53,55] show that oral contraceptives reverse the cyclic changes in total IgA and IgG levels as well as specific anti-HPV antibody levels in cervical secretions of women on oral contraceptives. Less clear is the extent to which postmenopausal status alters HIV susceptibility in women. The spread of STI in the elderly suggests that critical components of immune protection may be compromised. As discussed previously, we have found that epithelial cells from the uteri of postmenopausal women lack the capacity to secrete antimicrobials, relative to that seen with cells from premenopausal women [45]. In contrast, using a redirected lysis assay, we found that CTL activity in uterine tissues (CD8+) from postmenopausal women is three to four fold higher [38].

Implications

Over the past 35–40 years significant progress has been made in our understanding of how the immune system in the FRT is regulated for successful procreation. The studies we have presented have important implications for HIV research. First, attention must be paid to the entire FRT to understand the effects on immune protection. Sites for HIV infection exist throughout the FRT. Studies confined exclusively to the lower FRT may miss the pathophysiological processes involved in HIV infection. Second, subtle changes in hormone levels have profound effects on innate and adaptive immune protection. Failure to consider these changes may have dire consequences for interpretation of findings, particularly when hormones are administered to render animal models susceptible to infection. Although effects may be observed, continuous treatment or pharmacological doses of sex hormones may lead to erroneous conclusions. Third, immune cells throughout the FRT (NK cells, macrophages, T cells, and neutrophils) differ from their counterparts in blood, so blood immune cells cannot be used as surrogate markers for FRT immune function. Finally, recognizing that HIV infection is rapid, an effective vaccine to protect against heterosexual transmission must elicit humoral,

cell-mediated and innate immune protection, as under physiological conditions all three function in complementary fashion throughout the FRT to confer protection.

In conclusion, our identification of a window for viral infectivity of 7–10 days during which components of innate, humoral, and cell-mediated immunity are suppressed by sex hormones, provides an opportunity for the development of experimental approaches to restore the needed protection without compromising procreation. Numerous vaccine/microbicide trials have been carried out or will be undertaken in future. Building on a strategy to determine how HIVevades FRT mucosal immune protection, trials need to include an awareness of how the innate and adaptive immune systems in the FRT fluctuate during the menstrual cycle. Similarly, attention needs to be paid to other factors such as commensals and pH in the FRT and the roles of semen in HIV infection. Overall, these studies suggest the consideration of approaches that would break endocrine immunological tolerance throughout the FRT that normally occurs during the menstrual cycle.

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References

1. UNAIDS 2007. AIDS epidemic update JUNPoHAUaWHOW. Geneva, Switzerland: UNAIDS; 2007.
2. Padian N, van der Straten A, Ramjee G, Chipato T, de Bruyn G, Blanchard K, et al. Diaphragm and lubricant gel for prevention of HIV acquisition in southern African women: a randomised controlled trial. Lancet 2007;370:251–261. [PubMed: 17631387]
3. Cohen J. Promising prevention interventions perform poorly in trials. Science 2007;317:440. [PubMed: 17656693]
4. Cohen J. AIDS research. Promising AIDS vaccine's failure leaves field reeling. Science 2007;318:28–29. [PubMed: 17916696]
5. Editorial team. Phase III anti-HIV microbicide trial in Africa and India stopped as preliminary results show gel may increase risk of infection. Euro Surveill 2007;12:1–2.E070208.070206 [Epub ahead of print]
6. Honey K. Microbicide trial screeches to a halt. J Clin Invest 2007;117:1116. [PubMed: 17476340]
7. Piot P. San Francisco Chronicle. May 9;2007
8. CDC. HIV/AIDS Surveillance Report 2003 AUDoHaHS. CDC; 2003.
9. UNAIDS. AIDS epidemic update [global summary]. UNAIDS; 2004.
10. European Study Group on Heterosexual Transmission of HIV. Comparison of female to male and male to female transmission of HIV in 563 stable couples. BMJ 1992;304:809–813. [PubMed: 1392708]
11. Wawer MJ, Gray RH, Sewankambo NK, Serwadda D, Laeyendecker XLO, Kiwanuka N, et al. Rates of HIV-1 transmission per coital act, by stage of HIV-1 infection, in Rakai, Uganda. J Infect Dis 2005;191:1403–1409. [PubMed: 15809897]
12. Mastro TD, de Vincenzi I. Probabilities of sexual HIV-1 transmission. AIDS 1996;10(Suppl A):S75–S82. [PubMed: 8883613]
13. Quinn TC. Epidemiologic trends in HIV infection. Curr Opin Infect Dis 1992;5:189–200.
14. Colfax GN, Buchbinder SP, Cornelisse PG, Vittinghoff E, Mayer K, Celum C. Sexual risk behaviors and implications for secondary HIV transmission during and after HIV seroconversion. AIDS 2002;16:1529–1535. [PubMed: 12131191]

15. Wira, CR.; Fahey, JV.; White, HD.; Yeaman, GR.; Given, AL.; Howell, AL. The mucosal immune system in the human female reproductive tract: influence of stage of the menstrual cycle and menopause on mucosal immunity in the uterus. In: Glasser, S.; Aplin, J.; Guidice, L.; Tabibzadeh, S., editors. The endometrium. New York: Taylor and Francis; 2002. p. 371-404.

17. Wira, CR.; Crane-Godreau, MA.; Grant-Tschudy, KS. Role of sex hormones and cytokines in regulating the mucosal immune system in the female reproductive tract. In: Mestecky, J.; Bienenstock, J.; Lamm, ME.; Mayer, L.; McGhee, JR.; Strober, W., editors. Mucosal immunology. New York: Academic Press; 2005. p. 1661-1678.

18. Wira, CR.; Fahey, JV.; Sentman, CL.; Pioli, PA.; Shen, L. Innate and adaptive immunity in the female genital tract: cellular responses and interactions. In: Mayer, L., editor. Immunological reviews. Blackwell Munskgaard; 2005. p. 306-335.

19. Yeaman GR, Howell AL, Weldon S, Damien DJ, Collins JE, O'Connell DM, et al. HIV receptor and coreceptor expression on human uterine epithelial cells during the menstrual cycle. Immunology 2003;109:137–146. [PubMed: 12709027]

20. Yeaman GR, Asin S, Weldon S, Demian DJ, Collins JE, Wira CR, et al. Chemokine receptor expression in the human cervix: implications for infection by the human immunodeficiency virus-type I (HIV-1). Immunology 2004;113:524–533. [PubMed: 15554931]

21. Haase A. Perils at mucosal front lines for HIV and SIV and their hosts. Nat Rev Immunol 2005;5:783–792. [PubMed: 16200081]

22. Kunz G, Beil D, Deininger H, Wildt L, Leyendecker G. The dynamics of rapid sperm transport through the female genital tract: evidence from vaginal sonography of uterine peristalsis and hysterosalpingoscintigraphy. Hum Reprod 1996;11:627–632. [PubMed: 8671281]

23. Kunz GD, Beil H, Deiniger A, Einspanier G, Mall G, Leyendecke G. The uterine peristaltic pump. Normal and impeded sperm transport within the female genital tract. Adv Exp Med Biol 1997;424:267–277. [PubMed: 9361805]

24. Parsons, AK.; Cone, RA.; Moench, TR. Uterine uptake of vaginal fluids: implications for microbicides. Presented at Microbicides 2002; Antwerp, Belgium. 2002. p. 136(abstract B-175)

25. Settlage D, Motoshima M, Tredway D. Sperm transport from the external cervical os to the fallopian tubes in women: a time and quantitation study. Fertil Steril 1973;24:655–661. [PubMed: 4737661]

26. Bagasra O, Freund M, Weidmann J, Harley G. Interaction of human immunodeficiency virus with human sperm in vitro. J Acquir Immune Defic Syndr 1988;1:431–435. [PubMed: 3065475]

27. Brogi A, Presentini R, Solazzo D, Piomboni P, Costantino-Ceccarini E. Interaction of human immunodeficiency virus type 1 envelope glycoprotein gp120 with a galactoglycerolipid associated with human sperm. AIDS Res Hum Retroviruses 1996;12:483–489. [PubMed: 8679303]

28. 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]

29. Yeaman GR, Guyre PM, Fanger MW, Collins JE, White HD, Rathbun W, et al. Unique CD8+ T cell-rich lymphoid aggregates in human uterine endometrium. J Leuk Biol 1997;61:427–435.

30. Wallace PK, Yeaman GR, Johnson K, Collins JE, Guyre PM, Wira CR. MHC class II expression and antigen presentation by endometrium cells. J Steroid Biochem Mol Biol 2001;76:203–211. [PubMed: 11384879]

31. Wira CR, Rossoll RM, Young RC. Polarized uterine epithelial cells preferentially present antigen at the basolateral surface: role of stromal cells in regulating class II mediated epithelial cell antigen presentation. J Immunol 2005;175:1795–1804. [PubMed: 16034121]

32. McCluskie M, Cartier J, Patrick A, Sajic D, Weeratna R, Rosenthal K, Davis H. Treatment of intravaginal HSV-2 infection in mice: a comparison of CpG oligodeoxynucleotides and resiquimod (R-848). Antiviral Res 2006;69:77–85. [PubMed: 16377001]

33. Smith JM, Wira CR, Fanger MW, Shen L. Human fallopian tube neutrophils: a distinct phenotype from blood neutrophils. AJRI 2006;56:218–229. [PubMed: 16938110]

34. Sentman CL, Meadows SK, Wira CR, Eriksson M. Recruitment of uterine NK cells: induction of CXC chemokine ligands 10 and 11 in human endometrium by estradiol and progesterone. J Immunol 2004;173:6760–6766. [PubMed: 15557169]

35. Wira CR, Fahey J, Wallace P, Yeaman GR. Effect of the menstrual cycle on immune parameters in the human female reproductive tract. J Acquir Immune Defic Syndr 2005;38:S34–S36. [PubMed: 15867615]
36. Wira, C.; Fahey, J.; Schaefer, T.; Paoli, P.; Sentman, C.; Shen, L. Innate and adaptive immunity in the human female reproductive tract: influence of the menstrual cycle and menopause on the mucosal immune system in the uterus. In: Glasser, S.; Aplin, J.; Giudice, L.; Tabibzadeh, S., editors. The endometrium: molecular, cellular and clinical perspectives. Vol. 2nd ed. Vol. Chapter 33. London: Informa Healthcare; 2007. p. 491-521.
37. Yeaman GR, Fazleabas AT, Wira CR. Endometrial lymphoid aggregates. Mucosal Immunol Update 2004;12:6–8.
38. White HD, Crassi KM, Givan AL, Stern JE, Gonzalez JL, Memoli VA, et al. CD3+ CD8+ CTL activity within the human female reproductive tract: influence of stage of the menstrual cycle and menopause. J Immunol 1997;158:3017–3027. [PubMed: 9058841]
39. White HD, Musey L, Andrews MM, Yeaman GR, DeMars LR, Manganiello PD, et al. HIV-specific and CD3-redirected cytotoxic T lymphocyte activity in human female reproductive tract tissues: lack of correlation between mucosa and peripheral blood. J Infect Diseases 2001;183:977–983. [PubMed: 11237817]
40. Sato K, Kawasaki H, Nagayama H, Enomoto M, Morimoto C, Tadokoro K, et al. TGF-beta 1 reciprocally controls chemotaxis of human peripheral blood monocyte-derived dendritic cells via chemokine receptors. J Immunol 2000;164:2285–2295. [PubMed: 10679062]
41. Casslén B, Sandberg T, Gustavsson B, Willén R, Nilbert M. Transforming growth factor beta1 in the human endometrium. Cyclic variation, increased expression by estradiol and progesterone, and regulation of plasminogen activators and plasminogen activator inhibitor-1. Biol Reprod 1998;58:1343–1350. [PubMed: 9623591]
42. Sullivan DA, Richardson GS, MacLaughlin DT, Wira CR. Variations in the levels of secretory component in human uterine fluid during the menstrual cycle. J Steroid Biochem 1984;20:509–513. [PubMed: 6708533]
43. Schaefer TM, Fahey JV, Wright JA, Wira CR. Innate immunity in the human female reproductive tract: antiviral response of uterine epithelial cells to the TLR3 agonist poly (I:C). J Immunol 2005;174:992–1002. [PubMed: 15634923]
44. Fahey JV, Wright JA, Shen L, Smith JA, Ghosh M, Rossoll RM, Wira CR. Estradiol modulation of innate immune function by polarized human uterine epithelial cells in culture. Nature Mucosal Immunol. in press
45. Fahey JV, Wira CR. Effect of menstrual status on antibacterial activity and secretory leukocyte protease inhibitor production by human uterine epithelial cells in culture. J Infect Dis 2002;185:1606–1613. [PubMed: 12023766]
46. Eriksson M, Meadows S, Wira C, Sentman C. Endogenous transforming growth factor-beta inhibits toll-like receptor mediated activation of human uterine natural killer cells. Am J Reprod Immunol 2006;56:321–328. [PubMed: 17076676]
47. Pioli P, Jensen A, Weaver L, Amiel E, Shen Z, Shen L, et al. Estradiol attenuates lipopolysaccharide-induced CXC chemokine ligand 8 production by human peripheral blood monocytes. J Immunol 2007;179:6284–6290. [PubMed: 17947704]
48. Pioli PA, Weaver LK, Schaefer TM, Wright JA, Wira CR, Guyre PM. LPS-induced IL-1β production by human uterine macrophages upregulates uterine epithelial cell expression of human β-defensin 2. J Immunol 2006;176:6647–6655. [PubMed: 16709823]
49. Shen L, Smith JM, Shen Z, Eriksson M, Sentman C, Wira CR. Inhibition of human neutrophil degranulation by transforming growth factor-beta1. Clin Exp Immunol 2007;149:155–161. [PubMed: 17403059]
50. Wira CR, Rossoll RM, Ochiel DO, Haddad SN, Schaefer TM. Effect of estradiol and PAMPs on class II mediated antigen presentation and immunomodulatory molecule expression in the mouse female reproductive tract [abstract]. Am J Reprod Immunol 2006;55:412.
51. Piguet V, Steinman R. The interaction of HIV with dendritic cells: outcomes and pathways. Trends Immunol 2007;28:503–510. [PubMed: 17950666]

52. White, HD.; Crassi, K.; Wira, CR. Cytolytic functional activities of NK cells and cytotoxic T lymphocytes (CTL) are coordinately regulated in the human female reproductive tract. In: Husband, AJ.; Beagley, KW.; Clancey, RL.; Collins, AM.; Cripps, AW.; Emery, DL., editors. Mucosal solutions: advances in mucosal immunology. Sydney, Australia: The University of Sydney; 1997. p. 385-391.

53. Schumacher, GFB. Soluble proteins in cervical mucus. In: Blandau, RJ.; Moghissi, K., editors. The biology of the cervix. Chicago: The University of Chicago Press; 1973. p. 201-233.

54. Kutteh KW, Moldoveanu Z, Mestecky J. Mucosal immunity in the female reproductive tract: correlation of immunoglobulins, cytokines, and reproductive hormones in human cervical mucus around the time of ovulation. AIDS Res Hum Retroviruses 1998;14:S51–S55. [PubMed: 9581884]

55. Nardelli-Haefliger DJ, Wirthner D, Schiller J, Lowy D, Hildesheim A, Ponci F, Grandi P. Specific antibody levels at the cervix during the menstrual cycle of women vaccinated with human papillomavirus 16 virus-like particles. J Nat Cancer Inst 2003;95:1128–1137. [PubMed: 12902442]

56. Keller M, Guzman E, Hazrati E, Kasowitz A, Cheshenko N, Wallenstein S, et al. PRO 2000 elicits a decline in genital tract immune mediators without compromising intrinsic antimicrobial activity. AIDS 2007;21:467–476. [PubMed: 17301565]

57. Quayle AJ, Martin Porter E, Nussbaum AA, Wang YM, Brabec C, Yip K-P, Mok SC. Gene expression, immunolocalization and secretion of human defensin-5 in human female reproductive tract. Am J Pathol 1998;152:1247–1258. [PubMed: 9588893]

58. Venkataraman N, Cole A, Svoboda P, Pohl J, Cole AL. Cationic polypeptides are required for anti-HIV-1 activity of human vaginal fluid. J Immunol 2005;175:7560–7567. [PubMed: 16301665]

59. Cole A, Cole A. Antimicrobial polypeptides are key anti-HIV-1 effector molecules of cervicovaginal host defense. AJRI 2008;59:27–34. [PubMed: 18154593]

60. Sambhara S, Lehrer RI. The inate immune system: a repository for future drugs? Expert Rev Anti Infect Ther 2007;5:1–5. [PubMed: 17266447]

61. Tai P, Wang J, Jin H, Song X, Yan J, Kang Y, et al. Induction of regulatory T cells by physiological level estrogen. J Cell Physiol 2008;214:456–464. [PubMed: 17654501]

62. Cohen M. HIV and sexually transmitted diseases: lethal synergy. Top HIV Med 2004;12:104–107. [PubMed: 15516707]

63. Baeten J, Ludo Lavreys L, Overbaugh J. The influence of hormonal contraceptive use on HIV-1 transmission and disease progression. HIV/AIDS 2007;45:360–369.

64. Bulterysa M, Smith D, Chao A, Jaffee H. Hormonal contraception and incident HIV-1 infection: new insight and continuing challenges. AIDS 2007;21:97–99. [PubMed: 17148973]

65. Prakash M, Kapembwa M, 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]

66. Gillgrass AE, Ashkar AA, Rosenthal KL, Kaushic C. Prolonged exposure to progesterone prevents induction of protective mucosal responses following intravaginal immunization with attenuated herpes simplex virus type 2. J Virol 2003;77:9845–9851. [PubMed: 12941893]

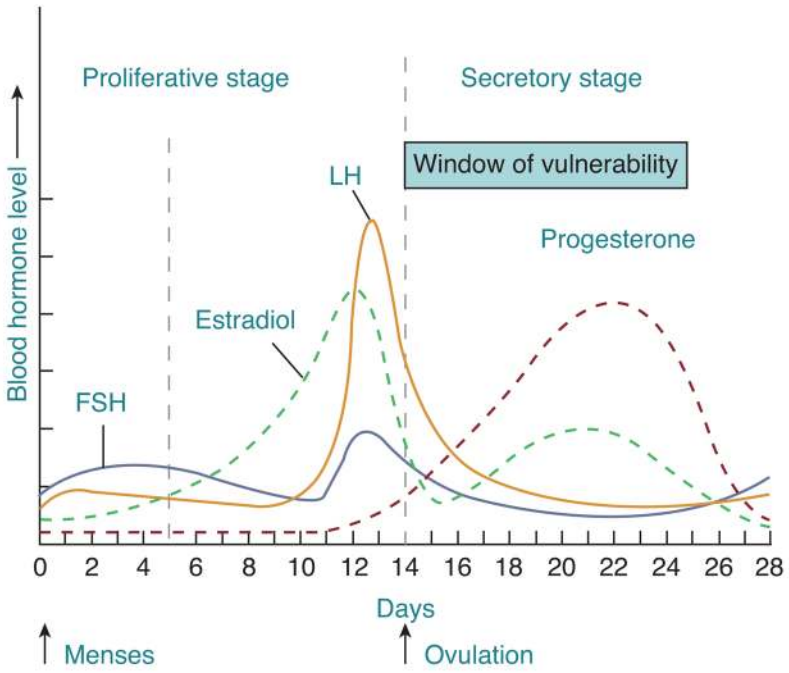

Fig. 1. Relative changes in levels of estradiol and progesterone during the proliferative and secretory stages of the menstrual cycle

Indicated on days 14–23 is the suggested window of vulnerability to HIV infection. FSH, follicle-stimulating hormone; LH, luteinizing hormone.

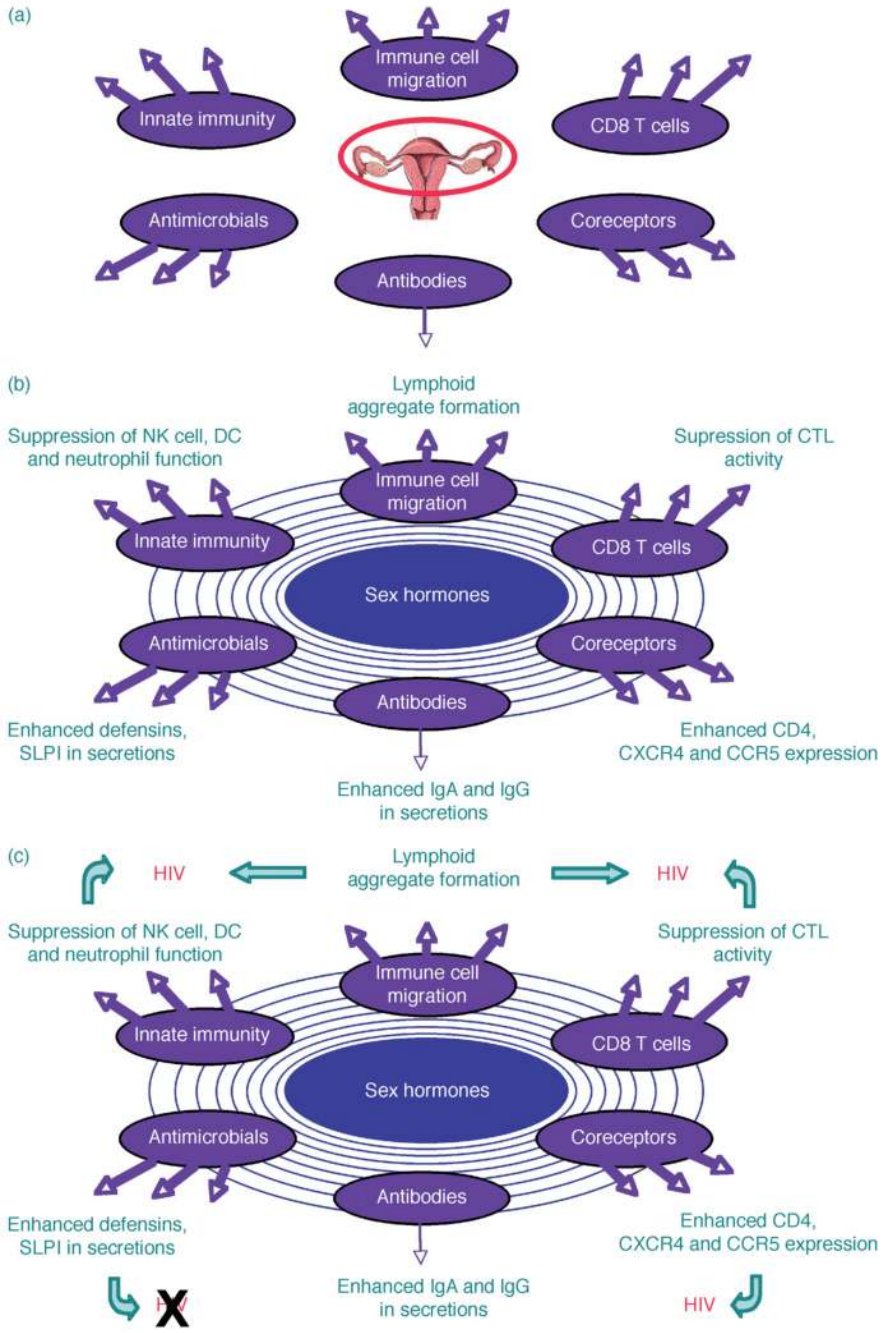

Fig. 2. The role of sex hormones in regulating immune function in the upper human female reproductive tract

(a, top) Depicts key immunological mechanisms present in the Fallopian tubes, uterus and endocervix that are essential for successful reproduction. These directly or indirectly impact pathogens that enter the upper female reproductive tract (FRT) and threaten reproductive health. (b, middle) Indicates that these immune mechanisms are under hormonal control. The expanding concentric rings represent estradiol released at mid-cycle along with progesterone released during the secretory stage of the menstrual cycle. (c, bottom) Depicts our hypothesis that estradiol and/or progesterone generally suppress immune protection, resulting in a window

of potential HIV infectivity. CTL, cytotoxic T lymphocyte; SLPI, secretory leukocyte protease inhibitor.

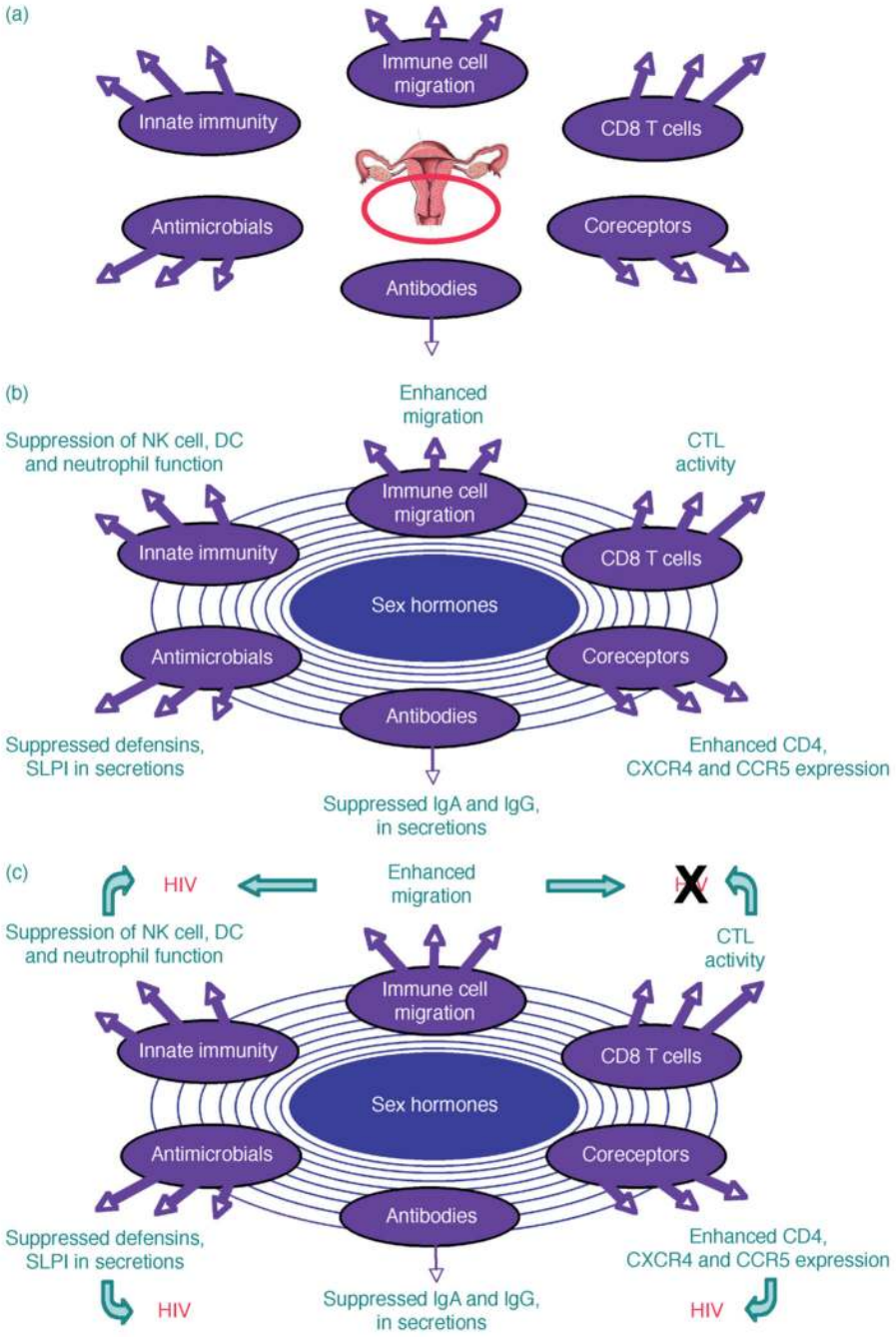

Fig. 3. The role of sex hormones in regulating immune function in the lower human female reproductive tract (ectocervix and vagina)

Panels a, b and c are described in detail in Fig. 2 and the text.