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Innate Immunity in the Human Female Reproductive Tract: Endocrine Regulation of Endogenous Antimicrobial Protection Against HIV and Other Sexually Transmitted Infections

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Abstract

Mucosal surfaces of the female reproductive tract (FRT) contain a spectrum of antimicrobials that provide the first line of defense against viruses, bacteria, and fungi that enter the lower FRT. Once thought to be a sterile compartment, the upper FRT is periodically exposed to pathogens throughout the menstrual cycle. More recently, secretions from the upper FRT have been shown to contribute to downstream protection in the lower FRT. In this review, we examine the antimicrobials in FRT secretions made by immune cells and epithelial cells in the upper and lower FRT that contribute to innate protection. Because each site is hormonally regulated to maintain fertility, this review focuses on the contributions of hormone balance during the menstrual cycle to innate immune protection. As presented in this review, studies from our laboratory and others demonstrate that sex hormones regulate antimicrobials produced by innate immune cells throughout the FRT. The goal of this review is to examine the spectrum of antimicrobials in the FRT and the ways in which they are regulated to provide protection against pathogens that compromise reproductive health and threaten the lives of women.

Keywords

Antimicrobials; female reproductive tract; innate immunity

Introduction

Sexually transmitted infections (STI) are a major worldwide health problem.¹ Despite extensive efforts, only limited success has been achieved in dealing with a growing list of STI that include bacteria (group B streptococcus, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Treponema pallidum*), parasites (*Trichomonas vaginalis*), and viruses [herpes simplex (HSV), human papilloma (HPV) and human immunodeficiency (HIV) virus]. Taken together, more than 20 pathogens, all of which are transmissible through sexual intercourse, account for approximately 340 million new STI cases annually.²

Since 1975, HIV has accounted for approximately 25 million deaths with an additional 33.4 million people (of which approximately 50% are female) currently infected worldwide.³ In sub-Saharan Africa, the area hardest hit by the pandemic, women living with HIV/AIDS make up approximately 60% of the number of HIV-infected people.³ Depending on the African country analyzed, infection rates vary from 5 to 25% of the population. Not widely

recognized are recent findings that major cities in the United States such as Washington DC have infection rates (approximately 3%) that are comparable to those seen in Africa.⁴

The mucosal surfaces of the human FRT are protected against pathogens by both the adaptive and the innate immune systems. Important components of innate defense are the antimicrobials in fluids that bathe the luminal surfaces of the upper (Fallopian tubes, uterus and endocervix) and lower (ectocervix and vagina) FRT. This thin layer of fluid covers all luminal surfaces and contains multiple antimicrobial factors secreted by epithelial cells and immune cells strategically distributed throughout the FRT. These secretions contribute in a number of ways to the defense of the FRT against bacterial, viral and fungal pathogens. In addition to physically protecting columnar and squamous epithelial cells that line the FRT, secretions promote ciliary clearance and contain mucus that serves both as a physical barrier and as a trap for bacteria and viruses. FRT secretions contain surfactant proteins that enhance phagocytosis of bacteria, immunoglobulins that bind bacteria, and lactoferrin that deprives bacteria of iron. Also present are antimicrobials that contribute to the protective shield against potential pathogens.

As presented in this review, FRT secretions contain a spectrum of antimicrobials totaling more than 20 molecules that are able to kill or inhibit bacteria, viruses, and fungi without inducing inflammation. Because survival depends on protection against pathogens, antimicrobial redundancy and synergism have evolved to provide a spectrum of protection greater than that present with a single factor.⁵ Less well recognized is the realization that antimicrobials in FRT tissues and secretions are under hormonal control. As a result, some antimicrobials are enhanced while others are suppressed in response to estradiol and/or progesterone. These changes lead to protection against STI at times during the menstrual cycle when aspects of the adaptive immune system are suppressed.⁶ Our goal in this review is to identify the innate immune cells at different sites in the FRT that provide antimicrobial protection, characterize the antimicrobials present in FRT secretions of the upper and lower FRT, and examine the changes in antimicrobials expression that occurs during the menstrual cycle, pregnancy, and following menopause. Important biologic factors are presented under the broad headings of epithelial cells and immune cells in the FRT; antimicrobials in the upper and lower FRT; endocrine regulation of antimicrobial protection; and relationship of antimicrobials to STI.

Epithelial cells and immune cells in the FRT

Leukocytes in the FRT play a central role in providing cellular, humoral, and innate immune protection against bacterial and viral invasion. Using human reproductive tract tissues dispersed by enzymatic digestion prior to quantitative flow cytometry, Givan et al.⁷ demonstrated that CD3+ T lymphocytes were present in substantial numbers, not only in the uterine endometrium, but throughout the FRT, including the ovary, Fallopian tube, uterine endometrium, endocervix, ectocervix, and vagina. Using three-color FACS analysis, leukocytes were found to be 6–20% of the total number of cells within the FRT; the uterine endometrium and Fallopian tube contained a higher proportion of leukocytes (mean of $14 \pm 2\%$ in the uterine endometrium) relative to other sites within the FRT. T lymphocytes were a major constituent of reproductive tract leukocytes from all tissues. Fallopian tubes contained granulocytes as a second major constituent. Granulocytes were significantly less numerous in the other tissues. All tissues contained B-lymphocytes and monocytes as clearly detectable but minor components. The proportions of leukocyte subsets in tissues from pre-menopausal women showed only small differences related to stage of the menstrual cycle. Numbers of leukocytes were decreased in post-menopausal endometrial samples relative to pre-menopausal samples, when analyzed on a percentage of total cells or per gram basis,

possibly reflecting, in part, a decreased population of immune cells in post-menopausal endometrium.

Antimicrobials in the upper and lower FRT

The complete antimicrobial repertoire in FRT secretions is unknown. Furthermore, there is considerable variability in reports of antimicrobial concentrations within the FRT. While the best-studied antimicrobials present in the FRT are shown in Table I, this list is incomplete in that other molecules exist in the FRT whose functional capacity is understudied (Table II).

Endogenous antimicrobials are small peptides mainly produced by epithelial and immune cells (leukocytes) that possess antibacterial, antifungal, and antiviral activity against a broad range of pathogens.⁸ They have distinct immunomodulatory functions including chemotaxis, cell proliferation, cytokine induction, and regulation of antigen uptake, which can be independent of or complementary to their direct protective effects.⁹ Importantly, while each antimicrobial is addressed individually below, *in vivo* they function as part of an intricate interconnected system. Several antimicrobials, for example, human beta defensin (HBD)2 and cathelicidin antimicrobial peptide LL-37,¹⁰ secretory leukocyte protease inhibitor (SLPI) and lysozyme,¹¹ lactoferrin and lysozyme,¹¹ display synergistic effects that potentially increase innate immune protection in the FRT.⁵

Despite their structural and functional differences, antimicrobials possess some common elements. They are generally cationic amphipathic molecules that can directly interact with cell membranes with high acidic phospholipid content, subsequently forming pores that destabilize cells through the abolition of pH and ionic concentration gradients.^{5,9,12,13} The varying composition of cell membranes has been postulated as a reason for the differential activity of antimicrobials toward a range of pathogens.¹² In addition, they are susceptible to the effects of pH, ion concentration (e.g. Na⁺, Mg²⁺), serum proteins, and protease inhibitor levels in the FRT, many of which, especially at higher physiological concentrations, are antagonistic toward antimicrobial activity.^{9,12,14–19}

Human defensins cluster on chromosome 8 and are composed of two main functional families: alpha and beta defensins.^{12,13} They have a common α -sheet structure and unique disulfide linkages between six specific conserved cysteine residues.^{8,12,13} There are six alpha defensins: human neutrophil peptide (HNP)1–4 and human defensin (HD) 5 and 6. HNPs 1–3 share a high degree of homology with only the amino terminal amino acid differing between them. Alpha defensins are synthesized as pre-prodefensins that are cleaved by proteases to create an active peptide which displays antibacterial activity against Gram-positive and Gram-negative bacteria, fungi, and yeast; and antiviral effects against HIV-1, HSV-1, and HSV-2.¹² Intriguingly, however, HD5 and HD6 enhance HIV replication by themselves as well as in the presence of gonorrheal infection.²⁰ However, the exact mechanism of infection remains to be determined.

Beta defensins HBD1–6 are structurally similar to alpha defensins and have broad inhibitory activity against a range of pathogens including HIV-1.¹² Genome scans have revealed at least 28 putative human beta defensins; though, only six have been discovered, of which four are present in the FRT.^{8,12,13} HBD1–3 have direct and indirect anti-HIV-1 activity.^{21,22} Similar to other antimicrobials, they interact directly with the viral envelope.^{12,21} Furthermore, they act upon target cell populations to decrease levels of the HIV-1 CXCR4 co-receptor as well as inhibit the early steps of viral replication.^{21–23}

Cathelicidins are a family of cationic antimicrobial peptides of which only one is found in humans, cathelicidin (hCAP-18/LL-37).²⁴ LL-37 is present in the FRT and is composed of three domains: a signal peptide region, an N-terminal cathelin-like domain, and a C-terminal

antimicrobial domain.^{9,24} The mature peptide LL-37 is generated from hCAP-18 by protease cleavage, is broadly antibacterial, and inhibits HIV-1 replication *in vitro* independently of changes in HIV-1 co-receptor expression. Intriguingly, the cathelin-domain also has antibacterial activity but no disclosed anti-HIV-1 activity.^{5,25} Uniquely, hCAP-18 is cleaved to form ALL-38 by gastricsin, a protease present in seminal fluid that is reaction dependent on low pH found in the vagina.²⁶ ALL-38 has a similar antibacterial profile to LL-37, but its anti-HIV activity is unknown. This remarkable mechanism for antimicrobial activation highlights the importance of male sexual fluids in modulating the protective response in the FRT.^{9,13}

Secretory leukocyte protease inhibitor and Elafin, located together on chromosome 20, are members of whey acidic protein (WAP) family that possess a conserved whey four disulfide core domain (WFDC).^{27,28} The pair are endogenous protease inhibitors involved in the control of inflammatory responses and tissue remodeling.^{27,28} Unlike SLPI, Elafin is relatively restricted in its target population acting mainly on neutrophil and pancreatic elastase and neutrophil proteinase 3. Both proteins also demonstrate anti-HIV-1 activity that is independent of their protease inhibitor function.^{27–30} SLPI, the better studied of the pair, inhibits HIV-1 infection by competing for annexin-binding sites on the cell membrane and thus decreasing viral entry into a cell.^{27,28,31,32} The cationic nature of SLPI may also allow it to directly destabilize viral envelop. The mechanism for Elafin inhibition of HIV-1 is unknown, but may be similar to SLPI given their homology (approximately 40%).^{29,30}

Lysozyme, another component of FRT secretions, derives its antibacterial activity from the ability to cleave peptidoglycan present on bacterial cell walls.¹³ Like other antimicrobials, it can directly interact with cell membranes via its positively charged amino acids.^{13,33} It inhibits HIV-1 infection of target cells, most likely via its HL9 and HL18 peptide regions, by blocking viral entry and replication.^{8,34,35} Lactoferrin, a homolog of the iron-carrier protein transferrin, inhibits bacterial growth by sequestering iron under acidic conditions similar to those in the lower FRT.¹³ It blocks HIV-1 infection of target cells by interfering with viral fusion and entry through interactions with the V3 loop of HIV-1 gp120.^{8,36} Furthermore, it inhibits HIV-1 adsorption to target cells. MIP3 /CCL20 is a neutrophil chemoattractant, and similar to other chemokines and cytokines, also functions as an antimicrobial agent.³⁷ Recently, it was shown to inhibit HIV-1 infection of target cells through an unknown mechanism.³⁸

Endocrine regulation of antimicrobial protection

Antimicrobial Changes During the Menstrual cycle

The pioneering studies of Schumacher in the 1960s and 1970s demonstrated that components of the reproductive tract milieu vary with specific stages of the menstrual cycle. For example, IgG and IgA in cervical mucus both decrease at ovulation but remain elevated during the proliferative and secretory phases of the cycle. Reflecting this initial work, other investigators have shown that, in addition to antibodies, specific cytokines, chemokines and antimicrobials also change with the menstrual cycle.

As seen in Table III, HNPs 1–3, HBD2, lactoferrin, and SLPI in cervico-vaginal lavages (CVL) transiently and dramatically decrease at mid-cycle/ovulation, before increasing during the latter portion of the secretory phase.^{39,40} A similar trend for lysozyme has also been reported elsewhere.⁴¹ The greatest decreases were observed in HNPs 1–3 (80%) and HBD2 (70%).³⁹ Multiple cytokines, which potentially have antimicrobial activity,³⁷ also demonstrated this trend.³⁹ Some of the highest concentrations of antimicrobials (HNPs 1–3, HBD2 and SLPI) were detected during the menstrual phase. However, this is likely due to blood contamination of CVL during endometrial breakdown and may not reflect endogenous

FRT production. In contrast to CVL findings, studies using tampons for collection of vaginal fluid reported increased levels of HNPs 1–3, HBD2, and lysozyme while lactoferrin, HBD1, and SLPI decreased from proliferative to secretory stages of the menstrual cycle with no apparent mid-cycle drop.⁴² Another study used Dacron swabs and found that SLPI peaked at mid-cycle compared to proliferative and secretory stages.⁴³ It remains to be determined which recovery technique (CVL, tampon, or swab) most accurately reflects antimicrobial levels in the lower FRT. Whether upper FRT secretions, which contain elevated levels of antimicrobials at mid-cycle, mix with vaginal fluid to mask cycle-dependent differences remains to be determined. Furthermore, it is important to accurately identify the cycle stage from which samples are recovered. Thus, self-reporting based upon the idealized 28-day cycle, while useful in some cases, can be replaced by direct measurement of serum estradiol and progesterone.

Within the upper FRT, HBD1–4 mRNA levels peak in endometrial tissue at different times during the menstrual cycle with HBD4 highest during the proliferative phase and HBD2 peaking at menstruation. Similar to HBD2, Elafin increases late in the cycle,⁴⁴ while HBD1 is highest during the mid-secretory stage. In contrast, HBD3 is maximal at early and late secretory, with a transient decline at midsecretory. SLPI mRNA and protein also peak during the secretory phase.⁴⁵ In the Fallopian tube, SLPI and Elafin mRNA expression remain constant across the cycle.⁴⁶ The reason behind this exquisite regulation of upper FRT antimicrobial expression may reside either in their unique antimicrobial activities or in non-antimicrobial functions related to fertility that remain to be determined.

Over 90% of sexually active women in the United States have used some form of contraception at least once.⁴⁷ Given its widespread use, the effect of hormonal contraceptives on antimicrobial levels is understudied. In a seminal study, Schumacher⁴⁸ demonstrated that sequential oral contraceptives suppress the cyclic changes of a spectrum of proteins including IgG, IgA, and lysozyme. In other studies with a combination oral contraceptive, no effect on antimicrobial expression was observed except for a significant decrease in HBD3 when compared to the secretory phase.⁴⁹ In the upper FRT, women taking the combined oral contraceptive had decreased SLPI in luminal epithelial secretions compared to women in the proliferative phase.⁵⁰ Future studies need to separate the different classes of oral contraceptives to determine their effects on the innate immune system throughout the FRT.

Antimicrobial Activity in FRT of Pregnant Women

Traditionally, pregnancy has been defined as a general state of immune suppression. However, this notion has been challenged recently with an evolution of our understanding; pregnancy seems to be both a pro-inflammatory and an anti-inflammatory state depending on the stage of gestation (reviewed by Ref. 51). The trophoblast, which is the cellular unit of the placenta, acts as an immune-regulatory interface between the maternal and fetal units. The placenta can recognize microorganisms and initiate response by producing cytokines, chemokines, and antimicrobials. Specifically, trophoblastic cells have been shown to produce HBDs, SLPI, and IFN in response to pathogenic stimuli.

Protection of the uterus and prevention of infection is of critical importance during pregnancy. A host of endogenous antimicrobials play an active role in protecting the pregnant uterus. Both alpha (HNPs) and beta (HBDs) defensins have been detected in amniotic fluid, chorion, and placenta (reviewed by Ref. 52). Defensins have also been detected in the cervical mucus plug that, during pregnancy, forms a physical barrier between the vagina and the uterus and prevents the upward movement of harmful pathogens. In addition, HNPs have been detected in the vernix caseosa (substance covering the skin of fetus and newborn), which has antimicrobial properties and protects the fetus during

delivery and immediately after birth. Increases in the levels of alpha and beta defensins in amniotic fluid are strongly indicative of uterine inflammation or infection which can result in preterm labor and delivery.⁵² Both alpha and beta defensins have been detected in vaginal fluids of healthy pregnant women.⁵³ However, changes in vaginal microflora during pregnancy correlate with the presence of alpha defensins in vaginal fluid.⁵⁴ Asymptomatic trichomoniasis in pregnancy has also been associated with higher HNP in vaginal fluids.⁵⁵

Both SLPI and Elafin are present in the healthy pregnant uterus.⁵⁶ SLPI has been detected in the decidua, amnion epithelium, vernix caseosa, and at very high concentrations (750 mg/g) in cervical mucus plugs.⁵² Elafin, in contrast, is confined to fetal membranes and placenta at term pregnancy. Both SLPI and Elafin possess anti-protease/anti-inflammatory activities beyond their antimicrobial capabilities and are believed to regulate inflammation during pregnancy and labor. Both SLPI and Elafin have been reported to decrease significantly in women with premature rupture of membrane (PROM). This correlates with increases in protease activity [matrix metalloproteases (MMPs) and neutrophil elastase] that contribute to rupture and/or infection. Interestingly, although levels of Elafin in amnion epithelium have been reported to rise in chorioamnionitis, SLPI concentrations did not appear to change. It has been suggested that this might occur as SLPI is degraded by certain pathogens (*Trichomonas*,⁵⁷ *Pseudomonas*,⁵⁸ *Staphylococcus aureus*²⁸ and *Chlamydia*⁴⁶). In studies using CVL, SLPI was found to be increased in pregnant women,⁵⁶ but decreased in the presence of bacterial vaginosis (BV).⁵⁹ Sachdeva et al.⁶⁰ confirmed these findings and further demonstrated that SLPI is down-regulated in HIV-infected pregnant women. Elafin has also been detected in pregnant CVLs and reported to be diminished by BV.⁶¹

In addition to SLPI, Elafin and the defensins, several other natural antimicrobials are also present in the pregnant uterus although most have not been studied in great detail. Lactoferrin is present during pregnancy and has been detected in amniotic fluid, cervical mucus, and vernix caseosa.⁵² Surfactant protein A (SP-A) is a lung antimicrobial that can facilitate phagocytosis of pathogens; it is expressed by amniotic epithelium, trophoblast, and amniotic macrophages although the main source is fetal lung.

Antimicrobial Activity in FRT of Post-Menopausal Women

It is well established that the innate immune system changes with aging or immune senescence.^{62–65} In elderly patients, NK cells, macrophages, dendritic cells, and neutrophils show impaired function as well as decreased toll-like receptor (TLR)-mediated cytokine responses. Aging has been shown to impair responses to viral infections including HIV, HSV, CMV, and Influenza; one mechanism is thought to be the functional impairment of plasmacytoid dendritic cells, the major producer of type I interferons, which are essential for combating viral infections.⁶⁶

Several studies have demonstrated that innate immune factors are compromised in the FRT of postmenopausal women. A general decline in several immunomodulatory factors has been reported that appear to be age related as well as attributed to the loss of endocrine responsiveness.⁶⁷ As multiple immune factors of the FRT are estrogen responsive, the loss of estrogen with aging results in loss of TLR function, secretory antimicrobial components, commensal lactobacilli, and acidity of vaginal microenvironment.⁶⁸ Vaginal epithelium thins significantly in the non-estrogenic post-menopausal state. There is also lack of production of cervical mucus, which itself is a protective barrier against pathogens.⁶⁹ Gender-specific decline of immune responses in the elderly have been described (reviewed by Refs ^{62,70}). Post-menopausal women show higher chronic levels of proinflammatory cytokines IL-6, MCP1, and TNF as well as a reduced ability to respond to pathogens or stimuli (Reviewed by Refs ^{62,70}). Mselle et al.⁷¹ have shown that inactive endometrium has lower numbers of NK cells compared to endometrium of cycling women.

A few studies have addressed the loss of specific antimicrobials in the FRT of post-menopausal women. Production of defensins has been shown to change under the influence of sex hormones.⁷² Han et al.,⁷³ demonstrated that estradiol can enhance the production of HBD2 whereas progesterone can decrease it. Fahey et al.⁷⁴ reported a loss of antibacterial activity against both Gram-positive and Gram-negative bacteria in the uterine secretions of post-menopausal women and correlated this with a loss of SLPI secretion, a molecule well known for bactericidal and viricidal activity.^{74,75} Shimoya et al.⁷⁶ confirmed lower SLPI levels in cervical vaginal secretions from post-menopausal women and further showed that hormone replacement therapy in elderly women increased SLPI levels.

In our studies (M. Ghosh, J. V. Fahey, S. Cu-Uvin, C. R. Wira, unpublished observations), we observed a reduction in anti-HIV activity in CVL from postmenopausal compared to pre-menopausal women. Using Luminex analyses we found that post-menopausal CVL contained higher levels of proinflammatory IL1 and lower levels of Elafin (Ghosh, unpublished observation) when compared to premenopausal controls. This suggests that changes in innate immune components in FRT secretions might increase the risk of infection by HIV and other STIs in post-menopausal women.

Direct and Indirect Effects of Sex Hormones on Upper Versus Lower FRT Antimicrobials

Immune cells in the pre-menopausal FRT exist in an environment that is continuously exposed to changing levels of sex hormones. As previously described, several antimicrobials in CVL or CVM vary with stage of the menstrual cycle. However, the contribution of individual cell types within the FRT toward total antimicrobial production remains relatively understudied with the bulk of research being performed on FRT epithelial cells. As seen in Table IV, we and others have isolated purified uterine epithelial and stromal cells from hysterectomy patients. Under estradiol stimulation, uterine epithelial cells upregulate the production of SLPI, HBD2 and Elafin.^{72,77} However, the antimicrobial profile of human uterine stromal cells and their response to hormonal stimulation is unknown. In the lower FRT, we observed a very different response, with vaginal epithelial cells decreasing the secretion of HBD2 and Elafin after 48 hrs of estradiol treatment (Patel et al. unpublished observation). Inhibition progressively increases from 10^{-8} to 10^{-10} M. In our system, uterine epithelial cells were strong constitutive producers of MIP3³⁸ – an antimicrobial absent from vaginal epithelial cell cultures (Patel et al. unpublished observation). Thus, the vaginal compartment possesses markedly dissimilar responses compared to the uterus – possibly the result of their different embryonic origins, or the differential expression of co-activator molecules in epithelial cells. Estradiol can also modulate innate immune responses to pathogenic stimuli. For example, estradiol inhibits the LPS-mediated upregulation of IL-6 in uterine epithelial cells.^{72,77} Whether estradiol influences antimicrobial production in a similar manner remains unknown.

The effects of progesterone upon epithelial cells are less well studied (Table IV). We found that progesterone has no effect on HBD2 and Elafin production by fresh primary human vaginal epithelial cells (Patel et al. unpublished observation). Endometrial explants from the proliferative or secretory phase show a differential response to progesterone. Proliferative phase tissue decreased the mRNA production of HBD1 and HBD2 but increased SLPI in response to progesterone (10^{-6} M).⁷⁸ In contrast, no progesterone effect was observed with secretory tissue. As neither estradiol nor progesterone exists alone in the FRT, further studies are needed to investigate the combined effects of these hormones to more accurately represent the *in vivo* environment.

Studies on immune cells recovered from the FRT are limited. It is essential to understand the effects of hormonal stimulation on these cells, as they are a rich source of antimicrobials. For example, neutrophils contain high concentrations of alpha defensins in their granules

and are present in greater numbers in the upper FRT during ovulation.⁷⁷ It is not unreasonable to ask whether their antimicrobial production, like that of epithelial cells, also changes with hormonal balance. Studying hormonal effects on systemic immune cells may not be an appropriate system for defining the responses of FRT mucosal immune cells. Immune cells in the FRT have a different phenotype from those in systemic circulation.⁷⁹ For example, uterine NK cells express higher levels of specific markers and have greater anti-HIV activity than blood NK cells.⁸⁰ Neutrophils and macrophages also possess distinct characteristics from their counterparts in the blood. FRT neutrophils have lower levels of lactoferrin and matrix metaloproteinase- 9, but appear to be primed for a more rapid induction of innate immune defense.⁸¹

Biologic Activity of Antimicrobials

Typically, levels of antimicrobials in mucosal fluids are measured by ELISA. In some cases, antimicrobial levels correlate with biologic activity while others do not.⁸² As discussed elsewhere, molecules in CVL may be quantitatively detected in an ELISA, but might not be biologically active, depending on the local environment in FRT secretions.⁸³ Several factors determine biologic activity of antimicrobials in the FRT.

Proteases—Female reproductive tract secretions contain both proteases and protease inhibitors, many of which are hormonally regulated.⁶⁹ For example, several proteases with trypsin-like activity in cervical vaginal secretions are regulated throughout the menstrual cycle with levels highest at ovulation and during the secretory phase. Families of proteases include cathepsins, kallikreins, MMPs, CD26, and others, all of which are responsible for activating and/or deactivating a variety of antimicrobial peptides.⁸⁴ In addition, antimicrobials such as SLPI and Elafin are themselves protease inhibitors and can therefore regulate the endogenous proteases.

Effects of microenvironment—Factors such as pH, salt, serum, and presence of sperm can affect biologic activity of antimicrobials. For example, the activity of the antimicrobial LL-37 is altered in the presence of sperm. LL-37 is processed and activated by prostate-derived protease gastricsin in a pH-dependent manner.²⁶ Many antimicrobials are sensitive to salt as well as the presence of serum. The activity and efficacy of defensins have been shown to change with pH and salt concentration.⁸⁵ Daher et al.¹⁶ showed that the addition of serum inhibited neutralization of HSV by HNPs. More recently, Mackewicz et al.⁸⁶ demonstrated that HIV inhibition by alpha defensins was almost completely abrogated by the presence of 10% fetal calf serum.

Synergy between antimicrobials—Many antimicrobials present in mucosal fluids can act in synergy. Lactoferrin and lysozyme have been shown to be synergistic against Gram-negative bacteria.⁸⁷ HBD2 and LL-37 also show synergistic effects.¹⁰ Singh et al.¹¹ has shown that SLPI, lactoferrin, and lysozyme, in combination, have significantly higher antimicrobial activity than each of the molecules individually. Van Wetering et al.⁸⁸ has also demonstrated that alpha defensins can enhance production of SLPI and Elafin by bronchial epithelial cells. Overall, endogenous antimicrobials interact in a complex pattern with biologic activity dependent on a host of factors. This finding most likely explains why a single mucosal immune factor is unlikely to be utilized as a therapeutic intervention against a given pathogen.

Relationship of antimicrobials to STI

The secretions of the FRT mucosa contain a spectrum of immune factors, many of which have direct or indirect antimicrobial functions. Antimicrobials present in the FRT are shown

in Tables I and II. However, Shaw et al.⁸⁹ have characterized the protein repertoire of CVL and identified 685 distinct proteins, many of which may have antimicrobial activity. The classical broad-spectrum antimicrobials like defensins are small cationic peptides that can form pores in bacterial cell walls or destabilize charges in viral envelopes, thereby neutralizing them.^{90,91} Chemokines are traditionally defined based on their ability to attract immune cells to sites of infections thereby connecting the innate to the adaptive immune systems. However, a majority of chemokines are also antimicrobials with activity against bacteria, viruses, and fungi.³⁷

As stated in the introduction of this review, there are an estimated 340 million new cases each year of STI from bacteria (*Neisseria gonorrhoeae*, *Chlamydia trachomatis*), parasites (*Trichomonas vaginalis*), and viruses (HSV, HPV, HIV). In addition, the yeast *C. albicans*, which can exist as a commensal but become pathogenic under certain conditions, is responsible for 85–90% of cases of vulvovaginal candidiasis.⁹² Many of these organisms are inhibited by antimicrobials through a variety of mechanisms.

Human Immunodeficiency Virus (HIV)

Our studies have shown that secretions from primary uterine, Fallopian tube, endocervix, and ectocervix cells are capable of inhibiting both CXCR4 and CCR5 strains of HIV-1.⁹² Anti-HIV activity was also detected in CVL of both HIV(+) and HIV(–) women⁸² with considerable decline with disease progression (M. Ghosh, J. V. Fahey, C. R. Wira, in preparation). We and others have demonstrated the presence of numerous antimicrobials in FRT secretions,^{39,82,84,92,93} many of which have anti-HIV activity. Some of the known anti-HIV molecules include SLPI, Elafin, MIP3, HNP1–3, and HBD2. Chemokines MIP1, MIP1, RANTES, and SDF1, also found in secretions and CVL, can act by blocking the co-receptors CXCR4 and CCR5 that HIV needs to bind to infect. In addition, these molecules can also inhibit HIV through post-infection mechanisms.⁹⁴

Herpes Simplex Virus

HSV-2 is the predominant sexually transmitted strain of Herpes. More than 20% of women of child-bearing age in the United States are HSV-2 seropositive, and in developing countries up to 80% of the population can be infected.⁹⁵ Studies have shown intrinsic anti-HSV activity in CVL.^{39,96} Several factors with specific anti-HSV activity have been identified. Lactoferrin and lysozyme have both been shown to inhibit cell-to-cell spread of HSV.⁹⁷ HNP1–4, HD5, 6, and HBD3 can all inhibit HSV through multiple mechanisms although HBD1 and HBD2 cannot.⁹⁸ Fakioglu et al.⁹⁵ reported that HSV-2 down-regulates SLPI suggesting that this is a mechanism for immune evasion.

Human Papilloma Virus

Human Papilloma Virus can be separated into high and low-risk HPVs depending on their oncogenic potential. Persistent high-risk HPV16 and HPV18 infection are the major causes of cervical cancer. Low-risk HPV types are associated with benign ano-genital warts. Human α -defensins 1–3 and human α -defensin 5 inhibit sexually transmitted HPV infection.⁹⁹ This may explain why a majority of women infected with HPV clear the infection with time. Another antimicrobial peptide, MIP3/CCL20, is decreased in squamous intraepithelial lesions caused by HPV16.¹⁰⁰ Whether high levels of MIP3 have a direct protective effect against HPV remains to be determined. In addition, Duffy et al.¹⁰¹ have observed that the HPV E6 oncoprotein is able to down-regulate Elafin, perhaps as an immune escape mechanism.

Neisseria gonorrhea—*Neisseria gonorrhea* is responsible for 700,000 infections in women in the USA each year.¹⁰² In women, untreated *Neisseria* infection can result in

pelvic inflammatory disease (PID), which can lead to ectopic pregnancy and an increased risk of infertility. We recently demonstrated that epithelial cell secretions from upper and lower FRT significantly inhibit *Neisseria*.⁹² In other studies, *Neisseria* has been described to be highly sensitive to LL-37.¹⁰³ *Neisseria* has also been shown to induce HD5 and 6 which in turn enhances HIV replication, underlining the significance of *Neisseria* as a co-factor in increased HIV transmission.²⁰

Chlamydia trachomatis—*Chlamydia* infection is a known cause of PID, infertility, and ectopic pregnancy because of scarring of the Fallopian tubes.¹⁰⁴ *Chlamydia* is also a co-factor for increased risk of HIV acquisition.¹⁰⁵ Several antimicrobials play a role in *Chlamydia* infection. A decrease in SLPI levels in vaginal secretions is related to infection of the lower reproductive tract by *C. trachomatis*.¹⁰⁶ This implies that reduced levels of SLPI in the lower FRT may increase susceptibility to *C. trachomatis* infection. Elafin expression is upregulated in oviduct epithelial cells infected with *C. trachomatis*, suggesting that Elafin plays a role in innate immunity response to chlamydial infection.⁴⁶ High levels of HBD1 and 2 have been observed in CVL of women infected with *Chlamydia*.¹⁰⁷ Specifically, HNP2 has been shown to inhibit *C. trachomatis*.¹⁰⁸

Candida albicans—*Candida* is described as a commensal microbe in the vagina because of its presence in up to 20% of healthy asymptomatic women. However, perturbations of the normal vaginal ecosystem can cause overgrowth of *Candida* and result in vulvovaginal candidiasis or yeast infection; it affects 75% of all women at least once during their lifetime, and also causes recurrent infections. We tested the effects of upper and lower FRT epithelial cell secretions on both the non-pathogenic yeast and the pathogenic hyphal forms of *Candida*. These studies indicated that FRT secretions inhibit both forms of *Candida*. *Candida albicans* is affected by alpha defensins, LL-37, calprotectin, and HBD1.^{107,109} In addition, *C. albicans* is inhibited by both SLPI and Elafin.²⁸

Bacterial Vaginosis

Bacterial vaginosis has been described as a co-factor for HIV acquisition. Cu-Uvin et al.¹¹⁰ have shown BV to be significantly associated with genital tract shedding of HIV. BV is characterized by loss of the normal protective Lactobacilli and overgrowth of diverse anaerobes.¹¹¹ The microorganisms involved in BV are many, but include *Gardnerella vaginalis*, *Mobiluncus*, *Bacteroides*, and *Mycoplasma*. Low levels of SLPI and an increase in lactoferrin in cervicovaginal fluid have been associated with BV.^{59,112} The increase in lactoferrin could be attributed to higher levels of neutrophil activation and degranulation, but was not sufficient to protect against HIV infection.⁵⁹ Elafin decreases in CVL from women with BV.⁶¹

Trichomonas vaginalis—*Trichomonas* is an extracellular protozoa that adheres to and damages vaginal epithelial cells.¹¹³ *T. vaginalis* infection predisposes women to HIV infection and increases HIV shedding in the FRT.^{114,115} *Trichomonas vaginalis* lipophosphoglycans induce a dose-dependent upregulation of IL-8 and MIP3 in vaginal, ectocervical, and endocervical epithelial cells.¹¹⁶ TV Infection by *T. vaginalis* results in significantly higher concentrations of vaginal fluid neutrophil defensins and cervical IL-8 in women with asymptomatic trichomoniasis compared to uninfected counterparts.⁵⁵

Lactobacillus—Multiple distinct species of Lactobacilli colonize the lower genital tract of women. In healthy women of reproductive age, major phylotypes of *Lactobacillus* includes *L. crispatus*, *L. iners*, *L. gasseri*, *L. jensenii*, *L. gallinarum*, and *L. vaginalis*.¹¹⁷ These commensals play a very important role in maintaining a healthy vaginal ecosystem that protects women against sexually transmitted pathogens. The presence of Lactobacilli creates

an acidic environment that is detrimental to pathogens. In addition, they secrete bacteriocins that directly kill pathogens. Loss of Lactobacilli through illness or antibiotics intake increases a woman's chance of getting infected by a sexually transmitted pathogen.¹¹⁷ However, in one study, lactobacilli were reported to enhance HIV infection.¹¹⁸ We and others have shown that FRT secretions contain antimicrobials that act either alone or in synergy to inhibit a number of sexually transmitted pathogens (J. V. Fahey, R. M. Rossoll, C. R. Wira, unpublished observation).^{40,82,84,92,119} Recently, we tested FRT secretions against *L. crispatus* and found no effects.⁹² This suggests an intricate balance in which constitutive secretions containing endogenous antimicrobials can affect pathogens but not commensals, which maintain a healthy vaginal ecosystem.

Given the number of proteins with antimicrobial properties found in the FRT, it is likely there are many others yet to be discovered. Several promising candidates are shown in Table II. These include SP-A and D, thrombospondin (TSP-1), eppin, HE4, lipophilin, lipocalin, and histones. All are mucosal peptides with antimicrobial functions but have not been studied in great detail. Some of these were discussed in an earlier review.¹²⁰

Summary

The complexities of the innate immune system in the human FRT are profound, both between the upper and lower FRT, and in the ways each site is regulated during the menstrual cycle, pregnancy, and menopause. These differences have evolved to meet multiple challenges of viral, bacterial, and fungal pathogens. The purpose of this review is to emphasize the complexity of innate immune protection in the FRT by including the spectrum of antimicrobials present, the recognition that many work in synergy, and the realization that antimicrobial activity is influenced by the complex milieu of proteases, protease inhibitors, pH, and hormonal balance. Understanding how reproductive demands for fertility interact with the immune system in the FRT are crucial to developing novel approaches to prevent the spread of HIV and other STI.

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Antimicrobials in the FRT

Table I

Antimicrobials	Location	Source	Detection				References
			RNA	IH	WB	ELISA	
-defensins (HNP1-3)	U/L FRT, VF, CM	Neutrophils	ND	Yes	Yes	ND	12,39,42,49,121-125
-defensins (HBD1-4)	U/L FRT, VF, CM	Macrophages	ND	Yes	ND	ND	12,39,42,49,72,78,124-127
		Monocytes	ND	Yes	ND	ND	
		VEC	Yes	ND	ND	Yes	
		VEC	Yes	ND	ND	Yes	
MIP3 /CCL20	U/L FRT, VF	Macrophages	Yes	Yes	ND	ND	38,128 (M. Ghosh, J. V. Fahey, C. R. Wira, in preparation)
		Monocytes	Yes	Yes	ND	ND	
		Dendritic Cells	Yes	ND	ND	ND	
		VEC	Yes	ND	ND	Yes	
Elafin	U/L FRT, CM, VF	VEC	Yes	ND	ND	Yes	29,44,46,129 (M. V. Patel, C. R. Wira, in preparation)
		VEC	Yes	Yes	ND	Yes	
		VEC	Yes	Yes	ND	Yes	
		VEC	ND	Yes	ND	Yes	
SLPI	U/L FRT, CM, VF	Neutrophils	Yes	Yes	ND	ND	39,40,42,43,45,46,49,59,76,124,125,130-132
		VEC	Yes	Yes	Yes	Yes	
Cathelicidin LL37	U/L FRT, VF, CM,	Superficial VEC	Yes	Yes	ND	ND	42,124,133,134
		Neutrophils	ND	ND	Yes	ND	
Calprotectin	L FRT, VF, CM,	Basal VEC	Yes	Yes	Yes	Yes	13,42,125,135,136
		Neutrophils	ND	ND	ND	ND	
		Monocytes	Yes	ND	ND	Yes	
		Superficial VEC	ND	Yes	ND	ND	
Lysozyme	L FRT, VF, CM,	Neutrophils	ND	ND	ND	Yes	41,42,49,125,137-139
Lactoferrin	L FRT, VF, CM	Neutrophils	ND	ND	ND	Yes	41,42,49,59,81,125,139,140

U, upper; L, lower; FRT, female reproductive tract; VF, vaginal fluid; CM, cervical mucus; UEC, upper FRT epithelial cells; VEC, lower FRT epithelial cells; STC, stromal fibroblasts; NK, natural killer; SLPI, secretory leukocyte protease inhibitor.

Table II

Potential Antimicrobial Compounds in the FRT

Name	Found in FRT?	Epithelial origin?	Bacteriocidal?	Other sites	Anti-HIV?	Comments	References
SP-D	FRT and MRT	Yes	Yes (Chlamydia)	Respiratory	?	Lectin-binding domains	141-143
SP-A	FRT, MRT	Yes (prostate, vagina)	Yes	Respiratory	?	Lectin-binding domains	144-146
TSP-1	FRT (cervical cancer)	Yes	Yes	Oral	Yes	Angiogenic inhibitor; anti-HIV in saliva	147-149
Eppin and HE4	MRT	Yes (epididymis)	Yes	Seminal fluid	?	Androgen regulated; SLPI related	150,151
Lipophilin	MRT (prostate, testis); FRT (uterus, ovary)	?	Yes	Tears, mammary glands, nasal fluids	?	Steroid hormone regulated	152,153
Lipocalin (glycodefin)	MRT, FRT	Yes	Yes (also fungicide)	Tears, nasal fluids	?		153-155
Histones	Yes	Yes	Yes	Colon, nasal fluids, skin	Yes		13,156-158

FRT, female reproductive tract; MRT, male reproductive tract; SLPI, secretory leukocyte protease inhibitor; SP, surfactant protein; TSP-1, thrombospondin.

Antimicrobial Changes in Cervical Vaginal Secretions from the Female Reproductive Tract Attributed to Menstrual Status, Pregnancy, and Contraceptive Use

Table III

Antimicrobial	Proliferative	Mid-cycle	Secretory	Pregnancy	Post-menopausal	Oral contraceptive	References
-defensins (HNP1-3)	+++	+	+++	Present	ND	NC	39,49,53
SLPI	++++	++	+++	Present	Decrease	ND	39,49,50,52,76,159
Lysozyme	+++	+	+++	ND	ND	NC	39,41,49
Lactoferrin	++++	++	+++	ND	ND	NC	39,41,49,140
-defensin 1	ND			Present	ND	ND	50,52
-defensin 2	++++	++	+++	Present	ND	NC	39,49,50,52
-defensin 3	ND			Present	ND	Decrease	49,50,52,78
-defensin 4	ND			Present	ND	ND	78
MIP3 /CCL20	ND			Present	ND	ND	M. Ghosh, J. V. Fahey, C. R. Wira (in preparation)
Elafin	ND			Present	ND	ND	52

+ symbols indicate level of each antimicrobial determined in CVL relative to the proliferative phase with increasing numbers of + symbols indicating higher levels of antimicrobial.

NC, no change; ND, not determined; SLPI, secretory leukocyte protease inhibitor.

Direct Hormonal Effects on Antimicrobial Production by Upper and Lower Female Reproductive Tract (FRT) Epithelial Cells

Table IV

Cells	Antimicrobials	Constitutive	Hormone		References
			Estrogen	Progesterone	
Upper FRT epithelial	HBD2	Present	Increase	ND	72
	HBD3	Present	ND	Increase	78
	HBD4	Present	ND	Increase	78
	SLPI	Present	Increase	NC	72
	Elafin	Present	Increase	ND	J. V. Fahey, C.R. Wira (in preparation)
Lower FRT epithelial	HBD2	Present	Decrease	NC	M.V. Patel, C.R. Wira (in preparation)
	HBD3	ND	ND	ND	
	HBD4	ND	ND	ND	
	SLPI	Present	ND	ND	
	Elafin	Present	Decrease	NC	M.V. Patel, C.R. Wira (in preparation)

NC, no change; ND, not determined; SLPI, secretory leukocyte protease inhibitor; HBD, human beta defensin.