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Current Opinion in Virology

Review

Mechanisms of mucosal immunity at the female reproductive tract involved in defense against HIV infection

Margaret WY Choi, Carmina A Isidoro and Amy Gillgrass

Human immunodeficiency virus-1 remains a major global health threat. Since the virus is often transmitted through sexual intercourse and women account for the majority of new infections within the most endemic regions, research on mucosal immunity at the female reproductive tract (FRT) is of paramount importance. At the FRT, there are intrinsic barriers to HIV-1 infection, such as epithelial cells and the microbiome, and immune cells of both the innate and adaptive arms are prepared to respond in case the virus overcomes the first line of defense. In this review, we discuss recent findings on FRT mucosal mechanisms of HIV-1 defense and highlight research gaps. While defense from HIV-1 infection at the FRT has been understudied, current and future research is essential to develop new therapeutics and vaccines that can protect this unique mucosal site from HIV-1.

Address
McMaster Immunology Research Centre, Michael G. DeGroote Institute for Infectious Disease Research, Department of Medicine, McMaster University, Hamilton, ON L8S 4K1, Canada

Corresponding author: Gillgrass, Amy (gillgra@mcmaster.ca)

Current Opinion in Virology 2024, **66**:101398

This review comes from a themed issue on **Mucosal Immunology**

Edited by **Zhou Xing** and **Jean-Michel Sallenave**

For complete overview about the section, refer "Mucosal Immunology (2024)"

Available online 13 March 2024

https://doi.org/10.1016/j.coviro.2024.101398

Background

In 2022, 39 million people were living with human immunodeficiency virus-1 worldwide, and 53% were women [1,2]. Sub-Saharan Africa (SSA) is disproportionately burdened by the HIV-1 epidemic, and in SSA, women are disparately impacted as they account for 63% of all new infections, contrasting with other regions where men represent most new infections [2]. The discrepancy can be attributed to complex societal factors, including the dominant patriarchal culture in the region, which perpetuates risky sexual behaviors and stigma around women's health, increasing their HIV-1 risk [3]. Social drivers are compounded with immunobiological factors. Heterosexual vaginal–penile intercourse is a common route of HIV-1 transmission, and the risk of HIV-1 acquisition for women is estimated to be twice as high as that for their male counterparts [4]. Here, we summarize recent research on mucosal immunity at the female reproductive tract (FRT) and how it contributes to defense against HIV-1.

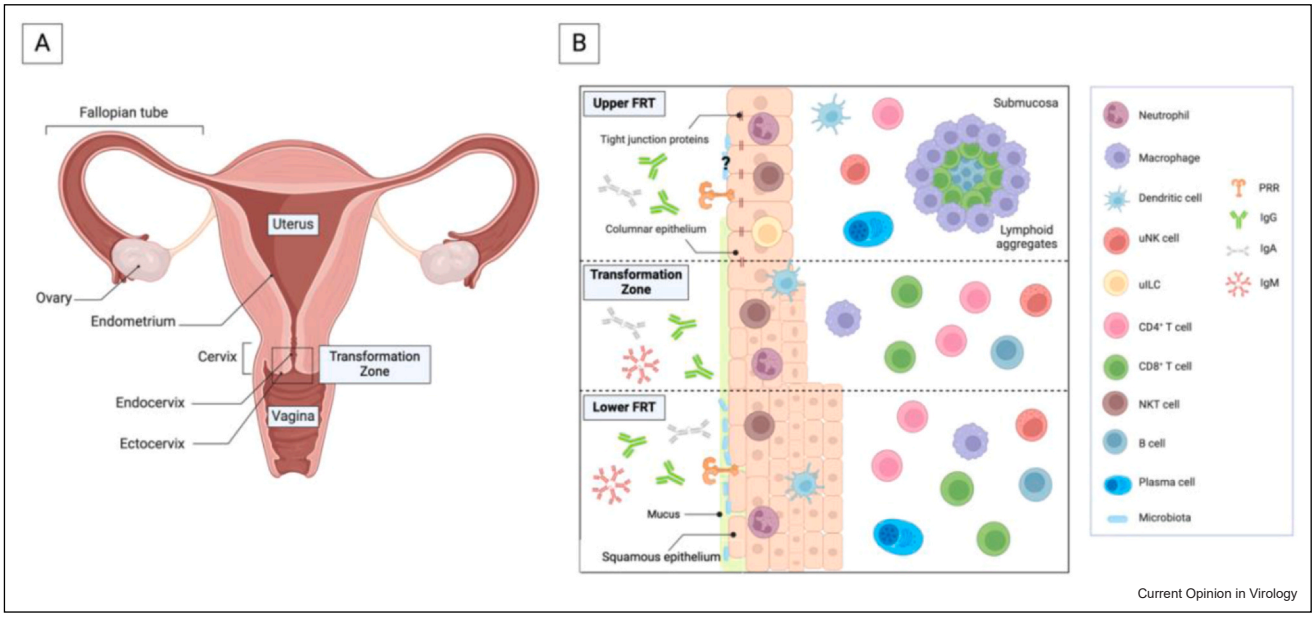
HIV-1 pathogenesis

HIV-1 pathogenesis has been extensively studied and was detailed recently by Bekker et al. [5]. In brief, HIV-1 enters cells by binding to the CD4 receptors and the CCR5 (R5 strain) or CXCR4 co-receptors (X4 strain) [5]. Mucosal transmission in the FRT is almost exclusively established by the R5 or dual-tropic R5X4 strain capable of using both co-receptors [6]. HIV-1 preferentially targets CD4+ T cells but can also infect other immune cells, such as monocytes, macrophages, and dendritic cells (DCs) [7]. The majority of CD4+ T cells die rapidly upon infection due to the virus's cytopathic effects or host immune responses, but a small portion can persist, in which HIV-1 establishes latency and can reactivate [8,9]. While monocytes and macrophages resist productive infection with antiviral restriction factor expression and escape cytopathic effects, they can become viral reservoirs as well [10,11].

Female reproductive tract

The FRT has a wide range of functional demands, and its mucosal immune system faces the unique challenge of creating an environment to support the growth of a fetal allograft during pregnancy and simultaneously provide protection against sexually transmitted infections, all amidst cyclic hormonal changes and contraceptive use [12]. The immune mechanisms in place at the FRT are remarkably effective as the risk of HIV-1 infection is 0.08% per exposure act, whereas the risk is 17-fold higher (1.38%) at the rectal mucosa [13]. The lower FRT comprises the vagina and ectocervix, while the upper FRT comprises the endocervix, uterus, and fallopian tubes [12]

Figure 1



FRT structure and immune cell distribution. **(a)** The FRT is divided into upper and lower FRT. The lower FRT includes the vagina and cervix, while the upper FRT includes the endocervix, uterus and fallopian tubes. (**b**) The lower FRT is covered by several layers of stratified squamous epithelium, and the upper FRT is lined by a single layer of columnar epithelial cells joined by tight junction proteins. The area near the opening of the cervix is referred to as the transformation zone, which represents the area where squamous epithelial cells of the ectocervix transition to single columnar epithelial cells of the endocervix. Lymphoid aggregates are unique to the endometrium. These aggregates comprise an outer layer of macrophages, with CD8+ T cells underneath and wrapping an inner core of B cells. Note that the immune cell populations and expression of antibodies or receptors depicted in this illustration do not accurately reflect their frequencies in the FRT. Microbiota presence has been confirmed in the lower FRT, and its presence and composition remain to be confirmed in the upper FRT. Figure created using BioRender. uNK, uterine natural killer; NKT, natural killer T. Created with BioRender.com.

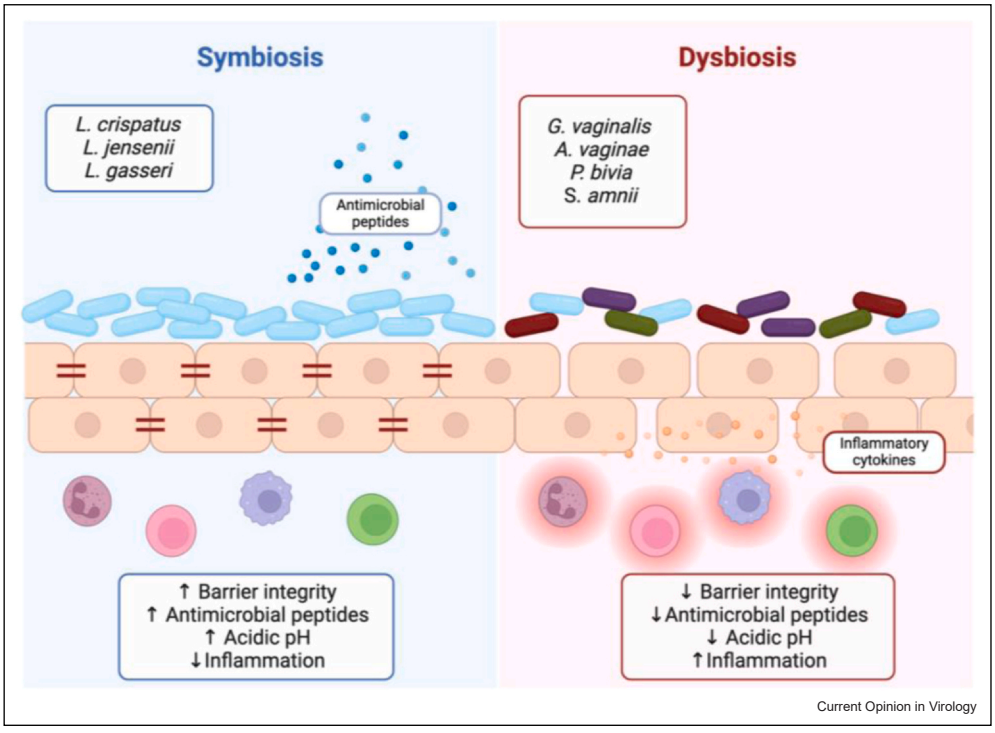
(Figure 1). The lower tract is covered with stratified squamous epithelium, while the upper tract is lined by a single layer of columnar epithelial cells joined by tight junction proteins [7]. The multilayered aspect of the lower tract does provide some protection against HIV-1, but its surface area is 15 times that of the upper tract's, which significantly heightens the risk of virus contact [14]. Although the upper tract is single layered, its tight junction proteins offer a more rigid barrier against HIV-1 infection [14,15]. Nevertheless, research in rhesus macaques has shown that simian immunodeficiency virus (SIV) can infect target cells in both the upper and lower FRT [16]. Hormones can affect the FRT mucosa, and recent papers have linked the use of depot medroxyprogesterone acetate contraceptive with increased HIV target cells in the FRT and disruption of cervical epithelial integrity [17–19].

Intrinsic immunity

The mucosal epithelium

As mentioned, the epithelial cells lining the FRT form a physical barrier that serve as an obstacle for HIV-1 infection of target cells in the submucosa, but they are also critical sensors of pathogens and sources of antivirals [20,21]. Alongside the immune cells in the FRT, epithelial cells express an array of pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), which recognize pathogens and elicit defense by triggering signaling cascades to produce antiviral interferons (IFNs) [13,20]. Of the many PRRs, the most well-studied ones are the TLRs [13]. The cells in the lower tract express TLRs 1–3, 5, and 6; and the cells in the upper tract express TLRs 1–9 [13]. A study by Nazli et al. found that exposure to HIV-1 Env gp120 activates TLR-2 on endometrial and endocervical cells to produce IFN-β, inhibiting HIV infection of T cells and reducing barrier impairment that often occurs when the virus stimulates upregulation of proinflammatory cytokines [20]. In addition to TLRs, other PRRs such as NOD-like receptors are essential pathogen sensors in the FRT as well [13]. NOD-1 and NOD-2 receptors can similarly be found throughout the FRT, with a more noted presence in the endometrium of the upper tract [22]. It has been reported that NOD-2 is selectively upregulated during HIV-1 infection in the FRT, which is suggested to reduce viral replication [23,24]. Furthermore, akin to other mucosal areas in the body, the FRT is coated by mucus composed of mucins, epithelial cell secretions, antimicrobial peptides, and immunoglobulins [13]. The complex role of antimicrobials in HIV infection was

Figure 2



Effects of a symbiotic and dysbiotic FRT microbiota. When the FRT microbiota is dominated by *Lactobacillus* species (left), the epithelial barrier integrity is enhanced as they promote the expression of tight junction proteins, reducing chances of penetration by invading pathogens through the epithelial layers. The also maintain an acidic pH, which creates an environment that would not favor the survival of pathogens, such as HIV-1. On the other hand, a diverse microbiota that is not dominated by the *Lactobacillus* species is often characterized by compromised barrier integrity, less antimicrobial peptide production, reduced acidity, and increased inflammation, which would diminish protection against pathogens. Created with BioRender.com.

reviewed by Ghosh [25]. Collectively, these mechanisms constitute a stringent barrier to pathogen penetration.

Microbiota

The vaginal microbiota plays a pivotal role in immunity against pathogens. The influence of FRT microbiota on disease pathology and outcome is highly dependent on the dominant species. A eubiotic FRT microbiome dominated by *Lactobacillus* species — particularly *L. crispatus*, *L. jensenii*, and *L. gasseri* — is associated with lower susceptibility to HIV infection [26] (Figure 2). *Lactobacillus* species ferment carbohydrates in the FRT and produce lactic acid (LA) as a metabolite [26]. LA helps maintain an acidic pH (3.5) in the FRT and its secretions, which can trap and inactivate HIV virions [26]. A recent study by Delgado-Diaz et al. revealed that LA increases tight junction protein expression (claudin-1) on ectocervical epithelial cells and fortifies the epithelial barrier [27••]. A follow-up study by Schwecht et al. found that treatment with LA and the short-chain fatty acids specifically associated with a eubiotic microbiota can alleviate HIV-1-induced epithelial barrier disruption *in vitro* [28••]. *L. crispatus* can also confer protection through the secretion of extracellular vesicles, which exert steric hindrance to diminish the exposure of HIV-1 envelope glycoproteins (gp) to the receptors on host cells [29]. Colonization of the FRT with *Lactobacillus*-deficient and highly diverse bacterial communities, referred to as dysbiosis, has been linked to local inflammation and elevated HIV acquisition risk [26,30••]. Bacterial vaginosis (BV) is a dysbiotic vaginal condition that is defined by an increase of anaerobic bacteria, such as *Gardnerella vaginalis*, *Atopobium vaginae*, *Prevotella bivia*, and *Sneathia amnii* [26,31]. These bacteria alter the immune landscape in the FRT by diminishing the expression of mucins MUC1 and MUC16, which compromises the epithelial barrier integrity and induces higher levels of proinflammatory cytokines such as interleukin (IL)-6, IL-8, and tumor necrosis factor α, which may in turn promote recruitment of permissive target cells to the area [26,31]. In vaginal fluids from women with dysbiosis, researchers found that a bacterial metabolite called 2-hydroxyisovalerate (2-HV) was linked with increased HIV replication in resting T cells [32•]. In a study of young South African women, researchers observed that the FRT microbiome of those undergoing maturational changes was dominated by non-*Lactobacillus*-dominant species, which has been found to

correlate with higher HIV acquisition risk [30]. Together, these findings highlight the critical role of the FRT microbiota in defense against HIV-1. Recent work has focused in this area, but the complexities indicate more research is needed to determine the full influence of microbial communities on risk of HIV-1 acquisition and how to harness this to reduce risk.

HIV and immunity

If pathogens maneuver past the intrinsic barriers of the FRT, they will meet immune cells (Figure 1) [13]. In the case of HIV-1, immune cells can be a double-edged sword. On the one hand, activated innate and adaptive immune cell populations can aid in the clearance of HIV-1, contributing to the less probable penile–vaginal transmission estimates [13,33]. On the other hand, activated macrophages and DCs — alongside adaptive CD4+ T cells — are targets for HIV-1 infection, which is why inflammation and the resulting surge of immune cells to the FRT may be undesirable [33].

Innate immune cells

Neutrophils

Neutrophils represent 10–20% of all the immune cells in the FRT [34]. Blood neutrophils can inactivate HIV *in vitro*, but we lacked information about their role at the mucosa until recently [35]. Barr et al. found that when human ectocervix, endocervix, and endometrium neutrophils were incubated with HIV-1 virus-like particles, TLRs –7 and –8 recognized HIV-1 and released neutrophil extracellular traps (NETs) [34]. NETs are the release of intracellular DNA fragments tangled with antimicrobial proteins that can ensnare and immobilize HIV-1 virions upon contact and prevent them from infecting $CD4^+$ T cells [34]. A number of factors can influence the efficacy of FRT neutrophils' response. Recent studies have found that aging could impair FRT neutrophils' signaling pathways and delay their NET release in response to HIV [36••]. In addition, a non-*Lactobacillus* dominant microbiome is associated with increased neutrophil influx and activation, which contributes to damaging of the epithelium [37••].

Macrophages and dendritic cells

Macrophages and DCs are found in all FRT compartments [38]. Derived from the same lineage, they are involved in surveillance as well as antigen-presenting to bridge the innate and adaptive immune responses [12]. In the context of HIV-1 infection, they may inhibit infection or act as HIV-1 targets. Macrophages are armed with restriction factors, such as SAMHD1, that make them less susceptible to productive infection by HIV-1 compared with CD4+ T cells [39]. But because HIV-1 can evade such factors, macrophages are not exempt from infection and often serve as viral reservoirs [39]. FRT DCs constitutively express elafin and secretory leukocyte peptidase inhibitor that are anti-inflammatory and inhibitory to HIV-1 [40]. Conversely, FRT DCs expressing CD14 are efficient in capturing HIV-1 virions, and their subsequent transport of virions to the lymph nodes could facilitate dissemination of the virus to a large pool of $CD4^+$ T cells, thus circumventing their normal purpose in defense [40]. Recently, Teijlingen et al. examined human vaginal Langerhans cells (LCs) — a subset of DCs [41••]. These LCs express TLRs 1–8, implying that they can recognize other invading pathogens, which would in turn contribute to defense against HIV-1 because co-infections with other bacteria and viruses such as *Chlamydia trachomatis* and human papillomavirus could increase the risk of HIV-1 acquisition [41,42]. An earlier study found that immature skin LCs' characteristic C-type lectin receptor langerin can effectively capture HIV-1 virions and subsequently internalize them into Birbeck granules, which are rod-shaped cytoplasmic vesicles unique to LCs [43]. This structure restricts HIV's further dissemination, although whether the virions become degraded within the Birbeck granules is still under investigation [43]. In agreement with this finding about skin LCs, Teijlingen et al. observed that the immature vaginal LCs could effectively capture HIV-1 virions through binding to their gp120 using langerin and restrict their replication, whereas mature vaginal LCs that express low levels of langerin could be efficiently infected by HIV-1 and subsequently transmit the virus to $CD4^+$ T cells [41]. Intriguingly, the authors found that activation of immature vaginal LCs with bacterial ligands would abrogate their ability to restrict HIV-1, which coincides with the fact that certain bacterial co-infections in the FRT could sabotage the immune system's defense against HIV-1 [41].

Innate lymphoid cells

Natural killer (NK) cells are present from the uterus to the vagina [44]. The NK cells from different FRT compartments have distinct phenotypes and display varying levels of cytotoxicity, which are summarized in a review by Ivanova et al. [44]. In the last decade, there is a growing interest in the innate lymphoid cells (ILCs), which belong to the same family as NK cells and are called ILC1s, ILC2s and ILC3s [45]. ILCs are enriched at mucosal barriers, and the FRT is no exception. All three subsets are present in the human uterus, and they are referred to as uterine ILCs (uILC), with uILC1 and uILC3 at higher frequency than uILC2s [46]. Certain ILC subsets have been found in the mouse vagina tissues, but their frequency in the other compartments of human FRT remains to be determined [47]. As ILCs 1–3 mirror the $CD4^+$ helper T cell subsets in terms of cytokine production, their potential involvement in pathogen defense at the mucosal sites is igniting increasing interest [45]. It is known that HIV-1 infection irreversibly depletes ILCs from the blood, but these cells'

dynamics in infected mucosal tissues are yet to be elucidated [48]. Xu et al. demonstrated that uILC3s help promote endometrial immunity against *Chlamydia trachomatis* infection via IFN-γ-dependent mechanisms; in the gut, they have also been reported to aid in maintaining epithelial barrier integrity through secretion of IL-22 [48,49]. Given these observations, it is likely that they hold critical roles in the FRT during HIV-1 infection, whether it be bolstering protection or inadvertently contributing to the disease in varying contexts.

Adaptive immune responses

If the virus breaches both the intrinsic and innate immune barriers, the adaptive immune response will be initiated to aid in infection clearance.

CD4+ T cells

$CD4^+$ T cells play a central role in orchestrating the immune responses in various diseases. However, in the context of HIV infection, they are the preferential permissive targets of the virus, although various subsets have different susceptibility. For example, memory and activated $CD4^+$ T cells are more prone to infection compared to naïve or resting $CD4^+$ T cells [50]. Rodriguez-Garcia et al. found that Th17 cells make up a significant portion of $CD4^+$ T cells in the FRT and are also the main $CD4^+$ T cells that express CCR5 [51]. Th17 cells are particularly abundant in the endocervix and ectocervix and are at significantly lower frequency in the endometrium [51]. In line with this, McKinnon et al. demonstrated that Th17 cells are preferentially targeted and depleted from the ectocervix, whereas another study by Ma et al. observed that they are not the preferential targets by HIV in the endometrium [52,53]. Studies suggest that ectocervix Th17 cells' heightened susceptibility is due to their high expression of HIV coreceptors and the α4β7 integrin, which can make HIV virion attachment more efficient [54,55]. While they act as HIV-1 targets, Th17 cells participate in mucosal defense as they help maintain a stringent epithelial barrier through their production of IL-17 and IL-22 [56,57]. Wang et al. found that gp140 on HIV-1 downregulates the expression of tight junction-associated genes, which compromises integrity of intestinal epithelial cells *in vitro*, but that IL-17 reverses this HIV-induced damage [57]. Another study observed that cervical epithelial cells treated with IL-22 had increased expression of the tight junction proteins and antiviral factors against HSV-2 [56]. While more work needs to be done on the role of Th17 cells in HIV defense, evidence suggests they play a role in epithelial barrier maintenance.

$CD4^+$ T cells also provide critical help to potentiate other immune cells' functionality, and certain $CD4^+$ T cells exert cytotoxicity in a similar manner to $CD8^+$ T cells [58•]. In SIV-infected rhesus macaques, Olwenyi et al. identified high expression of CD29 as a reliable marker for cytotoxic $CD4^+$ T cells and concluded that a loss of these cells is associated with higher viral loads and more severe loss of functionality in NK cells during the infection [58]. Studies have explored cytotoxic $CD4^+$ T cells in the peripheral blood, but these cells could be recruited to the FRT during infection and play a role in defense, although this remains to be confirmed in humans.

CD8+ T cells

In the FRT, $CD8^+$ T cells make up 50% or more of the total T cell population interspersed in the vagina and ectocervix or within lymphoid aggregates in the endometrial tissues [7,12,19,38]. As with other infections, a high-magnitude HIV-specific $CD8^+$ T cell response would be mounted soon after an individual gets infected with HIV-1 [59]. Lately, tissue-resident effector memory (T_{RM}) $CD8^+$ T cells in the FRT have become of interest because upon antigen re-encounter, they respond with greater efficiency and alert other local cells to prompt pathogen defense [60,61]. Rosato et al. evaluated the ability of $CD8^+$ T_{RM} cells to induce relocalization of circulating protective antiviral antibodies to the FRT [60]. They established a mouse model with FRT $CD8^+$ T_{RM} cells specific for an epitope of the lymphocytic choriomeningitis virus (LCMV) and then performed a passive transfer of broadly neutralizing anti-HIV-antibodies intravenously [60]. The FRT $CD8^+$ T_{RM} cells were subsequently reactivated through a secondary exposure to the LCMV antigen, and they observed rapid exudation of the antibodies into the FRT tissues as local $CD8^+$ T_{RM} cells enhanced vascular permeability [60]. This provides insight into how these $CD8^+$ T_{RM} cells may control disease severity in the FRT and could be leveraged in potential HIV immunization regimens to heighten protection in mucosal sites where vaccine-induced antibodies may not be available locally [60].

B cells

B cells are scarce throughout the FRT and make up only 5% of all immune cells there, with antibody-producing plasma cells mostly in the cervix and in the vagina at a lower frequency [7]. The endocervix contains the most IgG and IgA antibody-producing plasma cells compared with the other compartments, and unlike other mucosal areas, IgG is the most abundant in the FRT [12]. Antibodies are thought to contribute to HIV defense as high levels of broadly neutralizing antibodies have been found in highly HIV-exposed seronegative individuals [62]. According to a study by Fourcade et al., highly exposed seronegative women showed increased frequencies of total B cells with B cell receptors that could bind HIV-1 gp120 in their cervicovaginal secretions [63]. Recently, Schaefer et al. provided novel insight on mechanisms through modeling the interactions between the virus and antibodies in FRT mucus [64••]. The

authors demonstrated that IgG antibodies are capable of cross-linking HIV-1 virus-like particles to the mucus, thereby trapping the particles and hindering their penetration of the mucus to reach target cells [64]. Interestingly, the effectiveness of these antibodies at trapping the particles was also correlated with a eubiotic microbiota [64].

Conclusion

Altogether, the complex array of intrinsic barriers and immune cells from both the innate and adaptive arms work together to provide a formidable barrier to prevent transmission of HIV-1 in the FRT. However, the ability of HIV to target immune cells and exploit inflammation induced by the body as it mounts an antiviral response often complicates virus eradication. As women continue to face significantly elevated the risk of HIV-1 acquisition, it is imperative to gain a better understanding of these mucosal immune mechanisms in the FRT, which represents a major portal for viral entry and therefore signifies a critical anatomical site to study for potential localized immunization strategies. With ongoing research continuing to uncover the intricacies of mucosal immunity in the FRT, it is hopeful that there will be novel therapeutics and vaccines to mitigate women's disproportional risk of HIV-1 acquisition in the future.

Funding

MC was supported by Institute for Infectious Disease Research (IIDR) Summer Fellowship, Canadian Institutes of Health Research (CIHR) Canadian Graduate Scholarship Master's Award, CI was supported by a BHSc Program Summer Research Scholarship. Research is supported by CIHR Project Grant Priority Announcement: HIV/AIDS and STBBI.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

None.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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This study collected CVL samples from women of different age groups and characterized their vaginal microbiome composition. The study found that younger women are more likely to have a polymicrobial microbiome with lower abundance of *Lactobacillus* species, which could be associated with increased inflammation and hence higher risk of vaginal epithelial barrier disruption, coinciding with previous observations of higher HIV acquisition risk among women in this age group.

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This study analyzed the differences in the metabolites present in the CVL fluids from women with *Lactobacillus*-dominant and highly diverse *Lactobacillus*-deficient microbiomes. It was found that 2-HV, a metabolite consistently identified in dysbiotic microbiome, is present at significantly higher levels in those with a diverse *Lactobacillus*-deficient microbiome compared with *Lactobacillus*-dominant microbiome. The authors also confirmed that presence of 2-HV could potentiate HIV-1 infection in $CD4^+$ T cells *ex vivo*.

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