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REVIEW

Blocking HIV-1 transmission in the female reproductive tract: from microbicide development to exploring local antiviral responses

Sahar G Eid[1,2], Niamh E Mangan[3], Paul J Hertzog[3] and Johnson Mak[1,2]

The majority of new HIV-1 infections are transmitted sexually by penetrating the mucosal barrier to infect target cells. The development of microbicides to restrain heterosexual HIV-1 transmission in the past two decades has proven to be a challenging endeavor. Therefore, better understanding of the tissue environment in the female reproductive tract may assist in the development of the next generation of microbicides to prevent HIV-1 transmission. In this review, we highlight the important factors involved in the heterosexual transmission of HIV-1, provide an update on microbicides' clinical trials, and discuss how different delivery platforms and local immunity may empower the development of next generation of microbicide to block HIV-1 transmission in the female reproductive tract.
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HIV-1/AIDS has been a public health priority for the past three decades. In 2013, an estimated of 35 million people were living with HIV/AIDS worldwide while women accounted for 16 million people, which represent 46% of the HIV infected population.[1] HIV-1 sexual transmission remains the main mechanism for its spread worldwide. The infection rates of HIV are higher in women than in men, and that could be because of less selection bias and a more permissive environment in the female reproductive tract.[2] Because of gender inequality, women (particularly in developing countries) have limited power to implement HIV-1 prevention options. The reliance on the male sexual partner in HIV prevention practices often make women more susceptible to acquire the virus infection 5–7 years earlier and eightfold higher than men.[3] Finding an effective method to reduce these high infection rates among women is vital to control HIV-1 epidemic.

To curb the new HIV-1 infections, extensive efforts have been made to develop HIV prevention methods involving anti-retroviral drugs (ARVs) and microbicides with varying mechanisms.[4] Since 1987 there has been substantial development of anti-retroviral therapies, whereas a combination of anti-retrovirals are used to lower HIV-1 viremia to undetectable levels ($\leqslant$50 copies/ml).[5] Achievement of persistent low-level viremia is dependent on drug adherence and compliance with the medication dosing. Individuals failing to adhere to drug regimen increased the probability of generation of drug-resistant mutants and viral shedding in the genital compartment thus raising the risk of new HIV-1 infection.[6] Currently, the US Food and Drug Administration has approved 25 anti-retroviral agents in the treatment against HIV-1. These anti-retroviral agents can be categorized based on their mechanism of action into (i) nucleoside analog reverse transcriptase inhibitors (NRTIs), (ii) non-nucleoside reverse transcriptase inhibitors (NNRTIs), (iii) protease inhibitors (PI), (iv) entry inhibitors (such as CCR5 co-receptor antagonists and fusion inhibitors) and (v) integrase strand transfer inhibitors. Timing the start of an anti-retroviral therapy is vital in reducing HIV-1 transmission. It has been suggested that early treatment against HIV-1 may expose the patients to the cytotoxic effect of the drugs (for example, rash, renal and hepatic abnormalities),[7,8] although some of these concerns have subsided with improved understanding of the pharmacokinetics and pharmacodynamics of these anti-retroviral agents. The potential development of drug resistance has also been a concern when monotherapy was used in treatment. Amongst all limitations, poor adherence is likely to be a major factor in the development of drug resistance HIV.[9]

The updated guidelines (April 2015) for first-line of anti-HIV regimen often consist of two NRTIs in combination with either an integrase strand transfer inhibitors, or a PI, or a NNRTI. Patients failing with the first-line anti-retrovirals regimen will often be given an alternative of either a NNRTI-based regimen, or a PI-based regimen, or an integrase strand transfer inhibitors-based regimen, or all combined. The choice of drugs to be included in the second-line of anti-retroviral regimen is based on the patient's treatment history, and drug-resistance testing.[10] The burden of the HIV/AIDS related disease has been greatest in the poorest countries where the switch to the second-line ARV regimen is not always an option because of: high cost, inaccessibility to the drugs, and inadequate health care infrastructures.[11] The implementation of the aforementioned ARVs

[1]Deakin University, Greelong, Victoria, Australia; [2]CSIRO Australian Animal Health Laboratory, Newcomb, Victoria, Australia and [3]Hudson Institute of Medical Research, Clayton, Victoria, Australia
Correspondence: Professor J Mak, School of Medicine, Deakin University, 75 Pigdons Road, Geelong, Victoria 3216, Australia.
E-mail: j.mak@deakin.edu.au
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Table 1 First- and second-generation microbicides clinical trials summary (modified from Cottrell and Kashuba[12] and D'cruz and Uckun[108])

Candidate microbicide	*Category*	*Formulation*	*Clinical trial phase*	*Outcome*
Nonoxynol-9	Surfactant	1000 mg Sponge gel	II	No success and vaginal epithelial disruption
		70 mg Film	II	No success and 30% increase of vaginal irritation
		3.5% Gel	II/III	Failed; higher rate of HIV acquisition associated with frequency of use plus epithelial disruption
C31G (SAVVY)	Surfactant	1% Gel	III	Failed (early termination) in preventing HIV and was associated with genital irritations
Carraguard (sulfate polysaccharide)	Blocker	3% Gel	II	Failed to provide protection against HIV
Celllose sulfate	Blocker	6% Gel	III	Failed to provide protection against HIV and possible harm
PRO2000	Blocker	0.5–2% Gel	IIb/III	No statistical difference and uselessness in preventing HIV
BufferGel (polyacrylic acid)	Buffer	Gel	IIb	No statistical difference with trend toward increase in HIV incidence
Tenofovir (TFV)	HIV replication inhibitor (NtRTI)	1% Gel	II (CAPRISA 004)	39% reduction of HIV acquisition to 54% in high adherer's women
TDF	HIV replication inhibitor (NtRTI)	1% Gel	III (VOICE)	Failed to show efficacy in preventing HIV
Stampidine	HIV replication inhibitor (NRTI)	5–25 mg kg^{-1} Capsule	I	Clinically safe; no toxicity
Dapivirine	HIV replication inhibitor (NNRTI)	Intravaginal ring (IVR 25 mg or IVR 200 mg)	I	Considered safe and tolerated by prticipants
Dapivirine+Maraviroc	NNRTI+CCR5 antagonist	IVR (25 +100 mg)	I	Completed but results have not been published yet
GSK'744	Integrase strand-inhibitor	Monthly injection	I	Tolerated by the participants

Abbreviations: NtRTI, nucleotide reverse transcriptase inhibitor; TDF, TFV-disoproxil fumarate.

regimen often provides an undetectable viral load and lower transmission rate. Although combinational ARV treatment is unable to remove the last trace of HIV-1 virus from the infected patient, loweing the cost of ARVs and making them universally accessible would significantly impair the spread of HIV in a global scale. In the last two decades, development of microbicides has emerged to be an alternative strategy to reduce the incidence of new HIV infection.

In this review, we will discuss the unique relationship between HIV-1, type-I interferons (IFNs) and mucosal immunity. In addition, we will discuss the recent advances in microbicides development, and the lessons learnt from these experiences for future design and generation of effective microbicides against HIV.

MICROBICIDES PAST AND CURRENT CHALLENGES

Microbicides, by definition, act as a barrier and prevent acquisition of HIV-1 via sexual transmission. Microbicides can roughly be divided into two groups: (1) the non-anti-retroviral candidate microbicides; and (2) the anti-retroviral based candidate microbicides. These two groups of microbicides are sometimes grouped as 'first generation' and 'second generation' of microbicide, respectively.[12] One of the latest microbicide developments consists of combination of anti-retroviral agents with distinct mechanisms of action against HIV-1 in an attempt to achieve greater level of protection. There is also strong inclination to combine non-hormonal contraceptives with the candidate microbicides as a preventive measure against HIV-1 transmission and unwanted pregnancy.

A number of advances have been made to deliver microbicide candidates into the female reproductive tract. These delivery systems can be divided into coitally dependent (gels, films, tablets and diaphragms) and coitally independent (rings and fibers) means. Ideally, delivery of microbicides should not cause disruption to the vaginal epithelium and to the innate microflora (complex vaginal microbiome), as this might induce adverse effects. For example, alteration of microflora profiles was reported in clinical trials upon usage of 1% Tenofovir gel, whereas 3% of participants developed asymptomatic vaginal candidiasis and sometimes bacterial vaginosis.[13] As the association of bacterial vaginosis and high risk of HIV-1 transmission/susceptibility has been confirmed,[14,15] Pyles *et al.*[16] highlighted the importance of screening vaginally applied compounds (including microbicides), for impact on vaginal microbiome, using colonized epithelial multilayer cultures method to detect unwanted alterations in bacterial community profiles.[16] Other considerations include the timing of the product application and menses, as they are imperative to maintain the effective dosage of microbicide needed to block the transmission of HIV-1 and success in protection. In addition, sustaining vaginal pH ($pH \leqslant 4.5$) level, mainly via the presence of L-lactic acid, is a critical determinant to having a broad-spectrum HIV virucidal activity.[17] Ultimately, an effective microbicide against HIV-1 should offer protection against HIV-1 without compromising the existing delicate balance in the female reproductive tract to prevent pathogen invasions.

NON-ANTI-RETROVIRAL BASED MICROBICIDE CANDIDATES

Only a small number of candidate microbicides have successfully gone through the preclinical pipeline (high efficacy and minimal toxicity, both *in vitro* and in animal models) to clinical trial testing in humans. First-generation microbicides include surfactants, blockers and buffers (Table 1). Among the surfactants group, two products (nonoxynol-9 and C31G) were clinically investigated. They act by disrupting the viral envelopes (solubilizing lipids or denaturing proteins). Nonoxynol-9 (N-9) is a known spermicide that went through clinical trials between 1992–2002 based on different formulations (gel, vaginal film and

suppositories). The clinical trials showed an inverse association between HIV-1 incidence and frequency of suppository use; whereas an increase of the administered dose to twice and four times daily has led to epithelial disruption.[18] The N-9 vaginal film formula showed no benefit but possible harm, and 30% increase of vaginal irritation.[19] Two studies using the gel formulation (COL-1492) demonstrated a higher rate of HIV-1 acquisition and positively associated with frequency of use, plus epithelial disruption.[20] Like N-9, C31G (SAVVY) showed disappointing results (studied in four trials for safety evaluation and two trials for effectiveness between 2004 and 2008) in preventing HIV-1 transmission, and was associated with genital irritations.[21,22] The next class of candidate microbicides assessed was the 'blockers' (cellulose sulfate gel, Carraguard and PRO 2000). They act by interfering with HIV-1 attachment to the host cells. The first product assessed was Carraguard (sulfate polysaccharide derived from seaweed). Perotti *et al.*[23] showed effectiveness of carrageenan in blocking HIV-1 infection of cervical epithelial cells and preventing macrophages trafficking from the vagina to lymph nodes. Although Carraguard (gel) was safe, it failed to provide protection against HIV-1 in the efficacy trial[24] (Table 1). The second 'blocker' investigated was cellulose sulfate (vaginal gel). Two phase III clinical trials of 6% cellulose sulfate gel showed ineffectiveness in preventing HIV-1 infection.[25,26] Later on, Mesquita *et al.*[27] showed that disruption of epithelial cells tight junctions by cellulose sulfate reduced epithelial barrier and activated proinflammatory signaling pathway, thus facilitating HIV-1 infection. The last product in this category is PRO 2000 vaginal gel (naphthalene sulfonate). PRO 2000 vaginal gel was evaluated in two trials and proven ineffective in preventing HIV infection in women.[28,29]

In the category of 'Buffers', the last in the first-generation microbicides, BufferGel (polyacrylic acid) was checked in efficacy trial and demonstrated no benefit in protection against HIV-1[28] (Table 1). The underlined principle for this category of microbicide is to preserve the acidic/low pH of the vaginal milieu that can be elevated by the presence of semen or reduction of lactobacilli (vaginal flora bacteria).

As an alternative to the abovementioned non-anti-retroviral candidates, LACTIN-V (*Lactobacuillus crispatus*) and a live recombinant *Lactobacillus jensenii* (producing cyanovirin-N lectin which has high affinity recognition of gp120 carbohydrate moieties) was shown to be safe, tolerated among women, reduced SIV (63%) in animal trials and may proceed in the microbicides development pipeline.[30,31]

ANTI-RETROVIRAL BASED MICOBICIDE CANDIDATE

Anti-retroviral based microbicide candidates comprise HIV replication inhibitors (Tenofovir, Dapivirine, and Stampidine), HIV fusion inhibitors (Maraviroc) and HIV integrase inhibitor (GSK'744), which were evaluated in clinical trials, in addition to promising candidates in preclinical studies (MIV-150, HI-443, Rilpivirine and PSC-RANTES). Tenofovir (TFV) is a Food and Drug Administration-approved drug for HIV-1 treatment. TFV is a nucleotide reverse transcriptase inhibitor (NtRTI), and it blocks the synthesis of viral cDNA during infection. TFV was formulated as a vaginal gel by containing the TFV or TFV- disoproxil fumarate (TDF) (Table 1). Application of TFV gel (1%) before and after sex was included in a phase II clinical trial (CAPRISA 004). The CAPRISA 004 trial showed 39% reduction of HIV-1 acquisition but two seroconverters were super-infected with HIV subtype C.[32] However, HIV-1 incidence was reduced to 54% in high adherer' women.[32]

The VOICE trial (phase IIb) dealt with evaluation of daily dosage of 1% TDF gel, oral TDF and TFV+FTC (entricitabine) or Truvada. The VOICE trial failed to show efficacy in preventing HIV-1 transmission in women.[33] It is thought that the lack of protection against HIV-1 was attributed to low adherence of the participants. In addition, a number of ongoing trials will provide invaluable insight for the future development of this microbicide. For example, FACTS 001 (phase III) is a trial that has been completed in April 2015. This study will reveal the effectiveness of TDF gel in preventing the transmission of HIV-1 and HSV-2,[34] whereas CAPRISA 009 trial was recently completed in April 2015, and will determine whether exposure to TFV gel alters the therapeutic responses to future TFV containing ARVs regimen.[35] Other ongoing trial, such as CAPRISA 008 (phase III), is the continuation of CAPRISA 004 trial. It is aimed to assess the effectiveness and safety of TFV gel provision through family planning services.[36] Recently, two safety studies have been completed by investigating the effect of tenofovir gel (1%) application on pregnancy and lactation (MTN-008), plus the effect of contraception and menstrual cycle on the gel use and effectiveness in protection (A10–114).[37,38]

Volk *et al.*[39] provided analyses on data collected between July 2013 and February 2015 from pre-exposure prophylaxis—PrEP (tenofovir/enticitabine) initiators and non-initiators among members of the Kaiser Permanente Medical Center in San Francisco. The authors observed high rates of sexually transmitted infection (STIs) (50%) after 12 months use of PrEP. However, there was no HIV diagnosis among PrEP initiators and was associated with 41% decrease in the reported use of condoms. Despite the limitation of this study, the authors' recommendations were to increase understanding of 'risk compensation' and how sexual risk behavior may impact PrEP users,[39] In addition, the risks from prolonged exposure to ARV-based microbicide candidates, such as kidney toxicity, mitochondrial toxicity, osteoporosis and emergence of drug resistance HIV-1 mutants because of poor adherence, still require more attention and careful evaluation. However, a number of barriers and challenges remain including behavioral disinhibition/risk compensation, societal stigmatization against HIV and healthy individuals' reluctance of using anti-HIV-1 drug as preventive strategy. Recently, Biswas *et al.*[40] assessed the effect of TFV on epithelial cells, fibroblasts, $CD4^+$ T-cells and $CD14^+$ cells isolated from the female reproductive tract (endometrium, endocervix and ectocervix). They found that TFV induces proinflammatory responses in the female reproductive tract (site-specific) by upregulating Interleukin (IL)-8, tumor necrosis factor alpha (TNF-α) and macrophage inflammatory protein 3α (MIP-3α), which may potentially increase the risk of HIV-1 acquisition. Adding up, TFV modulation of proinflammatory cytokines (TNF-α, IL-2, IL-7, and IL-12p70) and activation of natural killer cells were associated with HIV-1 acquisition in women showing high systemic innate immune activation before infection.[41]

Aside from TFV, a distinct new HIV anti-retroviral, stampidine, was evaluated recently in phase I clinical trial, and showed no toxicity at dosage ranging from 5 to 25 mg kg^{-1} when taken daily as capsules,[42] which offers an alternative option of using anti-retrovirals as microbicide strategy. To overcome barriers to adherence, intravaginal ring (IVR 25 mg or IVR 200 mg) formulated with a non-nucleoside RT inhibitor (Dapivirine) has been developed. Intravaginal ring was demonstrated to be safe and tolerated in phase I trials (Table 1),[43,44] which is likely to help maintain adherence for future clinical application.

Several anti-retroviral agents (for example, NNRTIs: MIV-150, Rilpivirine and HI-443; CCR5 antagonist: PSC-RANTES) are being considered as potential candidate microbicides. These compounds have good records in preclinical studies and are being considered for different formulations for delivery. One of the current focuses is on

the development of combination microbicides targeting different steps in the HIV-1 life cycle in parallel with improving the efficacy of the delivery systems (for example, gels, rings). Lehman *et al.*[45] have showed that selected drug resistance mutations can be detected in patients who acquired HIV-1 after participating in microbicide clinical trials. Drug resistance remains a major concern when ARV is being used as part of the microbicide strategy. Furthermore, the rise of the drug-resistant virus transmission (5–15%) in areas where ARVs have been used for long period of time is an important public health concern,[46] which can negatively impact on the effectiveness of ARV-based microbicides. In addition, the lack of comprehensive knowledge of the long-term effects of these ARV based microbicides on the female reproductive tract (such as vaginal cells and tissues, epithelial integrity, inflammatory responses and vaginal microbiota) is also a concern. It is therefore important to consider an alternative strategy to develop a non-ARV-based microbicide to empower the natural host defenses within the female reproductive tract to prevent HIV-1 infection.

FEMALE REPRODUCTIVE TRACT IMMUNOBIOLOGY AND HIV-1 HETEROSEXUAL TRANSMISSION

Understanding the female genital tract immunobiology is important in designing any preventive methods to HIV-1 spread. The female reproductive tract represents a unique site where a balance must be maintained to ensure successful pregnancy and at the same time to offer protection against sexually transmitted diseases. The female reproductive tract consists of two different types of mucosal surface: type I mucosa (endocervix, endometrium, and the fallopian tubes) and type II mucosa (vagina and ectocervix). Type I mucosal surface includes a monolayer of columnar epithelial cells with tight junction contrary to type II mucosal surface, which is covered with multilayer of squamous epithelial cells[47] (Figure 1). It is well known that the multilayer of squamous epithelial cells and the tight junctions between columnar epithelial cells present good barrier protection in upper and lower female reproductive tract. The transition between the squamous and columnar epithelial cells occurs at the cervical transformation zone (Figure 1) and it is believed to provide a more susceptibility site for HIV-1 penetration and transmission. However, the actual anatomical site(s) of initial HIV-1 infection establishment is still highly debated. It was demonstrated that hysterectomized women (lacking a cervix) and hysterectomized macaques (lacking a cervix) can be infected with HIV-I and SIV (simian immunodeficiency virus), respectively, by crossing the vaginal mucosa.[48,49] This could be explained either by infection of mucosa cells (epithelial cells) or that the virus can cross an intact mucosal barrier. Micsenyi *et al.*[50] reported infection of epithelial cells with HIV-1 leading to *de novo* infection of underlying $CD4^+$ T-cells in a contact-dependent manner. On the other hand, SIV infection of macaques showed that the virus can cross intact mucosal barrier, and infect intraepithelial Langerhans cells, that can extend through epithelial cells into the vaginal lumen.[51] In addition, resident immature dendritic cells (DC) can trap HIV-1 through C-type lectin DC-SIGN, and migrate to secondary lymphoid tissue and undergo maturation where they can subsequently infect neighboring $CD4^+$ T-cells via *trans*-infection.[52] Recently, it was shown that *trans*-infection is strongly enhanced via sialic acid binding Immunoglobulin-like lectin 1 (Siglec-1), which is a type-I interferon (IFN) inducible gene product, and highly expressed on mature DC.[53,54] This data shows that, while many IFN-stimulated genes (ISGs) are expressed in the early stages of HIV infection to suppress viral infection,[55,56] HIV-1 has evolved ways to recruit host IFN-induced genes to help establish infection *In vivo*.

It is well known that the vaginal submucosa area is heavily populated with a spectrum of HIV-1 target immune cells including mainly $CD4^+$ T-lymphocytes cells, antigen-presenting cells (DC and Langerhans cells), natural killer cells, neutrophils and macrophages.[57,58] Therefore, understanding the biological impacts of candidate microbicides on this target population of immune cells is crucial for the development of an effective microbicide formulation.

Whether cell-free or cell-associated HIV-1 virus particles are responsible of initiating infection; HIV-1 heterosexual transmission is initiated by a presumably fitter genetic variant (founder virus) in the vaginal mucosal compartment.[2] Founder virus requires cell surface receptors (CD4) and co-receptors (CCR5 rather than CXCR4) for attachment and entry. Infection will eventually expand locally then spread via lymphatic drainage to establish in the draining lymph nodes. Shortly thereafter, HIV-1 viral RNA is detected in the circulation around 21–28 days after sexual mucosal exposure at which time mucosal (for example, genitorectal and gastrointestinal tracts) $CD4^+$ T-cells undergo severe depletion related to viral cytopathogenicity and failing of CD4+ memory T-cell homeostasis.[59] This depletion of T-cells can be restored slowly or incompletely under combined anti-retroviral therapy.[60,61] In addition, increased risk of HIV-1 transmission has been found to be associated with sexually transmitted infections including herpes simplex type-2,[62] bacterial vaginosis,[15] pregnancy,[63] as well as hormonal contraception options.[64–66]

HOW SEX HORMONES AND HIV-1 WINDOW OF VULNERABILITY ARE RELATED

The host cell responses in the female reproductive tract are affected by sex hormones and the continuous variation of the ratio between the levels of progesterone and those of estrogen. It has been suggested that a 'window of vulnerability' of HIV-1 infection coincides with the high levels of progesterone in the secretory phase.[67] This vulnerability may be due to thinning of the vaginal epithelium during the secretory phase resulting in increased susceptibility to HIV-1 by reaching the underlying target immune cells as showed in macaque experiments.[68–70] Others have demonstrated increased frequency of $CCR5^+CD4^+$ T-cells (HIV-I target cells) and $\alpha4\beta7$ expression on DCs (its expression influences preferential trafficking to gut lamina propria and associated lymphoid tissues) in the vaginal tissue and endocervix tissue respectively in progesterone treated macaques.[71]

On the other hand, the female reproductive tract epithelial cells act as a barrier and contribute to protection by secreting factors that display potent antimicrobial activity (that is, HD5, HBD1–4, Elafin, and SLP1), chemokines/cytokines (that is, IL-8, CCL20, RANTES).[72] More recently, a novel cytokine IFN-epsilon (IFN-ε) with potent antimicrobial activity has been shown to be expressed in the luminal and glandular epithelial cells of the endometrium.[73] However, these factors are also regulated by sex hormones and dependent on the anatomic site within the female reproductive tract. Therefore, it is important to study the anti-HIV-1 activity of candidate microbicides throughout the menstrual cycle.

HIV-1 TRANSMISSION—CYTOKINES AND ROLES OF TYPE-I IFNS

Exposure to HIV-1 and activation of innate immunity can result in rapid upregulation and release of cytokines, chemokines and chemokine receptors leading to recruitment of additional DCs and T-cells. In addition, epithelial cells, fibroblasts and immune cells in the female reproductive tract contribute to this pool of cytokines and chemokines through an autocrine or paracrine loop. For instance, epithelial cells secrete proinflammatory cytokines (IL-6, IL-8 and MIF), IFN-β,

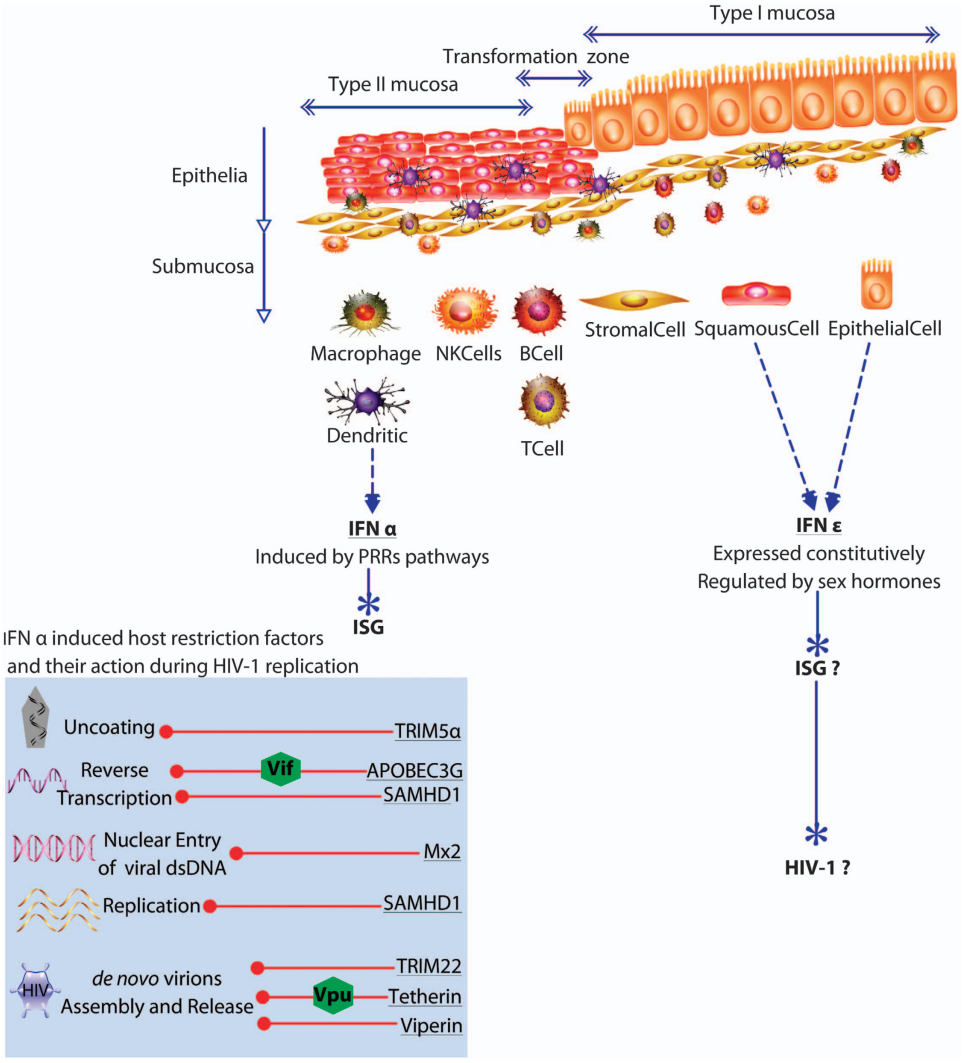

Figure 1 Schematic representation of the human female reproductive tract mucosa surveyed by innate and adaptive immune cells and antiviral responses (generated using ePath3D: http://www.epath3d.com). First part: type I mucosa, type II mucosa, transformation zone and spectrum of resident immune cells. Second part: secretion of IFNα by dendritic cells occurs upon infection while IFNε by epithelial cells is constitutively expressed and hormonally regulated. Target stages of IFNα-Stimulated genes within HIV-1 life cycle: Red connectors with round head represent inhibition of particular stage of HIV-1 life cycle by the designated cellular restriction factor(s) and the green hexagons represent the viral counterpartners. Question marks (?) represent unresolved questions.

defensins (antimicrobial peptides) and MIP3-α/CCL20 (recruits CCR6$^+$ plasmacytoid DCs), whereas resident macrophages and DCs secrete antiviral factors such as type-I IFNs and cytokines.[74] Furthermore, migrating cells were shown to secrete IFN-α, IFN-β, MIP-1α, MIP-1β, and CCL5 or RANTES (CCR5$^+$ chemoattractant chemokines), which recruit additional HIV-1 target cells and participate in expanding the infection.[75] HIV-1 founder viruses have been shown to be less sensitive to IFN-α[76,77] thus avoiding this cytokine storm, despite a high level production of IFN-α by plasmacytoid DCs, by counteracting IRF-3 and modulating NF-κB, thus disrupting the innate signaling pathways in infected cells.[78,79]

IFN-α THROUGHOUT THE COURSE OF HIV-I DISEASE

IFN-α is produced and secreted following cellular detection of pathogen-associated molecular patterns by pattern-recognition receptors to pilot the upregulation of ISGs.[80] ISGs that exhibit restriction to HIV-1 infection will be briefly introduced here by order of their obstruction with HIV-1 life cycle from entry to *de novo* virions production and the correspondent antagonist HIV-1 proteins (Figure 1).

Shortly after HIV-1 entry, the virus encounters the tripartite motif (TRIM) protein TRIM5α that binds to the virus capsid and presumably accelerates the disassembly of the virus capsid structure before reverse transcription can occur. Therefore, it is thought that TRIM5α block HIV infection via dismantling the virus, although the exact mechanism is still unclear.[81] Another protein of the same family TRIM22 was found to inhibit HIV-1 Gag protein trafficking to host cell membrane. TRIM22 interferes in the very last stage of the virus life cycle and decreases virus particle production.[82] After virus entry and uncoating, apoliprotein B-editing catalytic polypeptide-3G protein, APOBEC3G, exerts its antiviral activity during reverse transcription by driving hypermutation in the newly synthesized viral cDNA, or reducing priming and initiation of viral cDNA synthesis. As a result, this hypermutation fate leads to degradation by a cellular DNA repair mechanism yielding proviruses that are non-functional.[83,84] HIV-1 counteracts APOBEC3G restriction by the viral infectivity factor (Vif).

Vif induces proteasomal degradation of APOBEC3G and blocks its catalytic activity. In addition, Vif reduces APOBEC3G translation and packaging into the viral particle, which ultimately lessen the APOBEC3G mediated antiviral effect.[85,86] The next step in the HIV-1 life cycle is the transportation of the synthesized double-stranded viral cDNA into the cell nucleus. It has been suggested that the murine myxovirus resistance 2 (Mx2) can act at the level of nuclear entry and ultimately obstruct chromosomal integration of the viral cDNA.[87] Other ISG products that may impair retrovirus infection include SAMHD1. The cytoplasm and nucleus localized sterile alpha motif and histidine-aspartate domain containing protein 1 (SAMHD1) reduces the intracellular nucleotide pools and consequently affects retrovirus reverse transcription and replication. SAMHD1 is expressed in monocytes, macrophages, DCs, and resting $CD4^+$ T-cells.[88–90] SAMHD1 has a greater antiviral activity against both HIV-2 and SIV over HIV-1.[91] No anti-SAMHD1 counteracting protein has yet discovered in HIV-1 infected cells and it seems that HIV-1 can tolerate lower levels of nucleotides available in macrophage during cDNA synthesis owing to the enzyme kinetics of HIV-1 RT to support viral replication in low concentrations of substrates.[92] At the late stage of HIV-1 replication cycle, the release of virus particles is barred by the type 2 transmembrane protein 'Tetherin' through physical binding and trapping of HIV-1 particles on the plasma membrane.[93] Tetherin is constitutively expressed in B cells and plasmacytoid DCs.[94] Antagonizing Tetherin function by HIV-1 Viral protein unique (Vpu) includes ubiquitination followed by Tetherin degradation.[95] Another ISG protein that have been shown to have anti-HIV-1 activity is Viperin. It is thought that Viperin block HIV infection via inhibiting farnesyl diphosphate synthase, which alters the plasma membrane fluidity and thus particles' release.[96]

Despite the available of large number of ISGs to suppress HIV-1 infection, once HIV-1 transmission has occurred and infection is established in the host, type-I IFN sensitivity itself is unable to stop the spread of HIV-1 amongst different compartments of the infected host.[97,98] *In vivo* studies have been conducted to assess the impacts of interferon administration on the spread of retrovirus infection and progress of the disease. Treatment of macaques with pegylated IFN-α2a during SIV challenge increased host resistance to systemic infection, despite the evidence suggesting that inflammation intensifies virus acquisition and disease progression.[97]Although once SIV infection is established in the host, continued pegylated IFN-α2a treatment resulted in a decrease of antiviral gene expression, which increases the susceptibility to the spread of infection and greater $CD4^+$ T-cell depletion compared to placebo,[97] and a similar conclusion was depicted by Asmuth *et al.*[98] when chronically SIV-infected macaques were injected with pegylated IFN-α2a. Sandler *et al.*[97] highlighted the importance of timing and duration of type-I IFN administration in shaping the course of the disease development. More specifically, early type-I IFN signaling was critical for early and long-term control of SIV replication and virus reservoir in macaques. At the same time, a delay of as few as 3 days in antiviral gene expression after SIV infection is established can result in accelerated disease progression.[97] Accordingly, type-I IFN treatment requires different therapeutic strategies at each stage of the course of HIV-1 disease.

IFN-ε ROLE IN FEMALE REPRODUCTIVE TRACT

IFN-ε, a novel type-I IFN, was identified in humans (chromosome 9p21) and mice (chromosome 4).[99] IFN-ε was found to be located within type-I IFN locus as a single copy. It comprises conserved progesterone receptor binding site in the proximal promoter but interestingly the response elements for pattern-recognition receptors are limited as opposed to other type-I IFN.[73,99] IFN-ε shares only 30% amino acid homology to consensus IFN-α and to IFN-β. Based on coding sequence, IFN-ε is most closely related to IFN-β. Human IFN-ε is 15 residues longer than mouse IFN-ε and they share 54% amino acid identity and 15% amino acid similarity.[99] The predicted structures of IFN-ε have overall similarity to the type-I IFN and it was confirmed later on by the ability to bind to the type-I IFN receptor complex.[73,99] Contrary to other classical type-I IFN, IFN-ε is not induced by pattern-recognition receptors pathways, IFN-ε is expressed constitutively by epithelial cells of the female reproductive tract and its expression level is regulated by sex hormones. The detectable levels of IFN-ε expression are highest in the proliferative stage (estrogen dominant) and lower in secretory phase (progesterone dominant) of the menstrual cycle.[73] The expression level of IFN-ε (with potential antimicrobial activity) concurs with the 'window of vulnerability for HIV-1' when the conditions are optimized for fertilization and pregnancy. Fung *et al.*[73] revealed the protective role of IFN-ε in the female reproductive tract by maintaining basal levels of ISGs (*2'5'oas, Irf-7, Isg15*) in the murine model. *IFN-ε*-deficient mice, challenged with genital HSV-2 or *Chlamydia muridarum* infection, showed increased susceptibility to infection compared to wild-type mice. There was no change in the expression of *Ifn-ε* in wild-type mice, as it is not pathogen induced, and protection was granted by activating recruitment of natural killer cells.[73] The quite distinct role and importance of IFN-ε in mucosal immunity was the focal point in different studies but still the mechanism is not fully understood. It is intriguing to evaluate IFN-ε in HIV-1 heterosexual transmission (Figure 1), which raises the question of how IFN-ε may protect the female reproductive tract from HIV-1 infection. Which HIV-1-related restriction factors are favorably induced by IFN-ε? What immune cell types are activated? Does IFN-ε support trans-infection mechanism from DCs to target cells? Could it be considered as a candidate microbicide?

CONSIDERATIONS FOR PROSPECTIVE CANDIDATE MICROBICIDE

Finding safe and effective non-ARV-based anti-HIV-1 microbicides, delivered efficiently and accepted by users, would provide new venues for the control of the HIV/AIDS epidemic. Candidate microbicide that progress in the production pipeline will be tested *in vitro, ex vivo* (vaginal mucosal explants, female reproductive tract immune cells, microflora) and *in vivo* on animal models (bone/liver/thymus mice, and non-human primates), despite the fact that impressive preclinical data are not always predictive of the success of such microbicides formulation to prevent HIV infection in humans. However, studying the effects of candidate microbicides throughout the menstrual cycle on vaginal cells and tissues (first cells to encounter HIV-1 in heterosexual transmission), epithelial integrity, inflammatory responses and vaginal microbiota is becoming a prerequisite. In addition, it is important to test the efficacy of candidate microbicides in the presence of semen as shown by Zirafi *et al.*[100] where there was an enhancement of HIV-1 infectivity in presence of semen resulting in the impaired antiviral activity of most candidate microbicides except maraviroc (CCR5 antagonist).[100] It is thought that the amyloid fibrils in semen enhance HIV infectivity by promoting viral attachment to the target cells.[101,102] A mutually non-exclusive explanation would be the induction of proinflammatory cytokines (IL-6 and IL-1β), chemokines (IL-8 and MCP-1), and IL-7 by seminal plasma that facilitate HIV infection *In vivo*.[103–105]

IFN-ε has a unique role at the mucosae immunity and the probability of considering it as future candidate microbicide is

dependent on many factors. IFN-ε (i) is not induced by PPR as seen with other type-I IFNs, (ii) endogenous IFN-ε is well tolerated as it is expressed constitutively by epithelial cells in the female reproductive tract where levels are hormonally regulated, (iii) is known to provide protection against common sexual transmitted infections in mice (HSV-2 and *Chlamydia*), (iv) is not suppressed by seminal fluids for its expression, (v) is induced by human ectocervical epithelial cells following exposure to seminal fluid, (vi) is required to maintain basal levels of ISGs in the female reproductive tract, (vii) is activated to facilitate recruitment of natural killer cells, (viii) is exclusively expressed by epithelial cells of mucosal tissues in *Indian* rhesus macaques (can be used for preclinical studies) and (ix) is important to enhance lymphocytes recruitment (cytotoxic $CD8^+CD4^+$ T-cells subset in lymph nodes but not memory $CD8^+$ T-cells responses), and to promote migration of antigen-specific $CD8^+$ T-cells to the gut mucosae, and reduced inflammation.[73,103,106,107] Together, these features highlight the antiviral potential of this naturally produced cytokine IFN-ε, which may offer unique protection against HIV-1 in the female reproductive tract.

The anti-HIV mechanisms of type-I IFNs have most impact at the acute stage of infection, but they are 'too late' and 'less efficient' to suppress HIV-1 replication once infection is firmly established within the host. IFN-ε provides a new modality of immune protection of the female reproductive tract in comparison with other type-I IFNs. These initial results of IFN-ε contribution to mucosal immunity fit with the principal mechanisms of HIV-1 protection. It would be very important to study the effect of exogenous application of IFN-ε throughout the menstrual cycle on the epithelial cells (tight junctions), the immune cells population in the female reproductive tract, fertilization, pregnancy and fetus.

The key question is whether IFN-ε is able to elicit protective immune responses without fueling immune activation to support HIV-1 replication *in vivo*; whereas detection of a proinflammatory signature will be a major criterion. Investigating the effect of IFN-ε on the residing immune cells will give an insight on its relevance in antiviral protection. Some of the focal points will be around its effect on DCs as they are the most potent antigen-presenting cells and the connection between the innate and adaptive immune systems. In addition, scrutinizing the induced ISGs and their antiviral activity will be central to understand IFN-ε protective mechanism. Despite the fact that IFN-ε is an endogenous protein, side effects may occur and this demands caution in timing and dosages. IFN-ε could be considered for different formulations for delivery as vaginal gel or intravaginal ring, plus the ability to combine it with other candidate microbicides may add a boost protection. The big challenge would be identifying the exact set of effector molecules induced by IFN-ε at the vaginal mucosa and their roles in protection against heterosexual transmission of HIV-1. The answers to these questions may hold the key for the development of the next generation of microbicide to offer greater protection against HIV-1 and/or other sexual transmitted diseases via the female reproductive tract.

CONCLUDING REMARKS

Although none of the candidate microbicides has progressed for clinical application currently, the innovation in microbicide's formulations and advancement in preclinical and clinical trials have already paved the way for more rapid development of future microbicides. Understanding the female reproductive tract immunobiology, and the transmission of HIV-1, is a prerequisite in the quest of finding a safe and effective candidate microbicide to prevent HIV-1 acquisition. Candidate microbicides offering novel and promising prevention modality should be investigated in depth. Better knowledge will lead to improved approaches to prevent HIV-1 transmission and address important issues in women's sexual and reproductive health needs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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