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Review

Preventing mucosal HIV transmission with topical microbicides: Challenges and opportunities

Florian Hladik[a,b], Gustavo F. Doncel[c,*]

[a] Departments of Obstetrics and Gynecology and Medicine, University of Washington, 1959 NE Pacific Street, Box 356460, Seattle, WA 98195, United States
[b] Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, United States
[c] CONRAD, Department of Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, VA 23507, United States

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ABSTRACT

A combination of prevention and treatment modalities will be needed to successfully control the global spread of HIV. Microbicides, drug products topically applied to mucosal surfaces to prevent HIV infection, are one of these biomedical interventions that hold great promise. In order to be efficacious, microbicides must overcome several challenges imposed by the mucosal microenvironment they intend to protect and the mischievous human immunodeficiency virus with its enormous capacity to adapt. Recent data, however, supports the establishment of the primo-infection by only a small number of founder viruses, which are highly vulnerable to microbicidal intervention at the initial stages of mucosal invasion. The biological foundation of these challenges and opportunities in microbicide development is explored in this review. This article forms part of a special supplement on presentations covering HIV transmission and microbicides, based on the symposium "Trends in Microbicide Formulations", held on 25 and 26 January 2010, Arlington, VA.

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1. Introduction

While global prevalence of HIV infection appears to have stabilized in recent years, the total number of people living with HIV is increasing because of ongoing accumulation of new infections and longer survival times due to the beneficial impact of antiretroviral therapy, measured over a continuously growing general population (UNAIDS, 2009). As of December 2008, approximately 4 million people in low- and middle-income countries were receiving antiretroviral therapy. However, we are not going to "treat our way out" of the epidemic. For every two people put on treatment, five more become infected. A combination of prevention and treatment modalities is necessary to successfully control the global spread of HIV. Microbicides, drug products topically applied to mucosal surfaces to prevent HIV infection, are one of these biomedical interventions that hold great promise.

Approximately 50 candidates are currently at different stages of development in the microbicide pipeline (for a full list see www.microbicide.org). So far mostly non-specific microbicides, such as surfactants and polyanions, have completed phase III clinical testing and none has demonstrated clear statistical evidence of protection (Feldblum et al., 2008; Halpern et al., 2008; Karim, 2010; Peterson et al., 2007; Van Damme et al., 2002, 2008). There is hope, however, that better results will be achieved with agents that specifically inhibit HIV, some of which have shown quite remarkable efficacy in explant tissue and animal infection models (Cranage et al., 2008; Cummins et al., 2007; Denton et al., 2008; Lederman

* Corresponding author. Tel.: +1 757 446 5020; fax: +1 757 446 8998.
E-mail addresses: fhladik@fhcrc.org (F. Hladik), DoncelGF@evms.edu (G.F. Doncel).

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et al., 2004; Parikh et al., 2009; Rohan et al., 2010; Veazey et al., 2005). Tenofovir, a nucleotide analogue reverse transcriptase inhibitor (NtRTI) used in HIV/AIDS therapy, represents the most advanced candidate within this category. A 1% vaginal gel formulation of tenofovir reduced HIV acquisition by nearly 40% overall in the recently completed CAPRISA 004 Phase IIb HIV prevention trial in South African women (Karim et al., 2010).

Tenofovir now represents the first vaginal microbicide proven to be safe and efficacious in the primary prevention of HIV in women. This proof-of-concept has invigorated the field with renewed optimism. Several other clinical trials are underway investigating non-nucleoside reverse transcriptase inhibitors (NNRTI) anti-HIV microbicides such as dapivirine and UC781. Other classes of microbicide candidates involve specific entry inhibitors, including gp120 blockers, gp41 blockers and CCR5 antagonists, integrase inhibitors, and protease inhibitors (Buckheit et al., 2010). For the most part these compounds are small molecules, which have been developed by the pharmaceutical industry and, in combination with the above-mentioned reverse transcriptase inhibitors, represent the next generation of microbicide products. Another category of microbicide candidates consists of peptides and proteins with potent anti-HIV activity, including cyanovirin-N, griffithsin, and actinohivin (Alexandre et al., 2010; Matoba et al., 2010; Veazey et al., 2009). Due to the high cost of synthetic protein manufacture, these candidates are currently being explored in combination with live delivery systems such as genetically engineered lactobacilli (Liu et al., 2006), or are being produced by alternative manufacturing strategies such as in plants or yeast (Colgan et al., 2010).

In order to be effective, microbicides must overcome several challenges imposed by the mucosal microenvironment they intend to protect and the enormous capacity of HIV to adapt. A deep understanding of how HIV establishes initial infection in the mucosa and how and where a particular topical microbicide interacts with the virus is important in the development of a safe and efficacious microbicide and will support incremental improvements in product development.

This paper forms part of a group of seven reviews covering presentations from the Trends in Microbicide Formulations Workshop that was held on 25–26 January, 2010 in Arlington, VA, USA. The other articles discuss gel, film, and tablet formulations (Garg et al., 2010), intravaginal rings (Malcolm et al., 2010), preclinical evaluation of microbicides (Doncel and Clark, 2010), clinical evaluation of microbicides (Morrow and Hendrix, 2010), dual protection (Friend and Doncel, 2010) and novel approaches to microbicide delivery and safety assessment (Whaley et al., 2010).

2. Progress in understanding HIV mucosal infection

Impressive progress has been made in recent years in elucidating the events that occur between exposure to HIV at the mucosal surface and systemic spread of infection. Macaque models of SIV infection and human explant models of HIV infection are in concordance in singling out mucosal $CD4^+$ T lymphocytes as the prime targets for initial infection (Greenhead et al., 2000; Gupta et al., 2002; Hladik et al., 2007; Hu et al., 2004; Kader et al., 2009; Li et al., 2005; Saba et al., 2010; Zhang et al., 1999). There is no doubt that CD4 and CCR5 are the main receptors for HIV-1 fusion and cytoplasmic entry on these cells, yet the discovery that fusion primarily occurs after endocytosis of HIV into endosomes rather than directly on the cell surface is a relatively novel insight (Miyauchi et al., 2009).

In male-to-female transmission HIV can infect the vaginal, ectocervical, endocervical, and endometrial mucosa, although the relative contribution of each site to the establishment of the initial infection is not known. When intact, the multilayered squamous epithelium that covers the vagina and ectocervix provides better mechanical protection against HIV invasion than the single layer columnar epithelium that lines the endocervix and endometrium; however, HIV penetration and infection have been demonstrated in all four sites (Hladik and Hope, 2009; Hladik and McElrath, 2008).

HIV infection *in vivo* can be established by cell-free and cell-associated viruses. Infection by both cell-free and cell-associated virus has been observed in female macaques infected with simian immunodeficiency and chimeric viruses (SIV/SHIV) (Gupta et al., 2002; Kaizu et al., 2006; Khanna et al., 2002; Salle et al., 2010; Zhu et al., 1996), mice infected with HIV (Khanna et al., 2002), and indirectly in humans through genetic matching of HIV viruses sequenced from acutely infected women and from seminal cells and plasma from their infected partners (Zhu et al., 1996). Human cervical explant studies have also confirmed transmission of cell-free and cell-associated HIV (Gupta et al., 2002). Both forms of HIV are carried by semen and deposited in the vagina during intercourse. Interestingly, semen is more than just a carrier—it neutralizes the harmful acidic pH of the vagina (Tevi-Benissan et al., 1997), enhances virion attachment to target cells (Kim et al., 2010), and stimulates epithelial chemokines that attract new HIV-target cells to the mucosa (Berlier et al., 2006; Thompson et al., 1992).

The surface of the cervicovaginal mucosa provides a large portal of entry for HIV. The virus has been shown to penetrate several layers from the luminal surface into the thin gaps between squamous epithelial cells (Hladik and Hope, 2009). This penetration may bring the virus in direct contact with two key cell types presumably involved in the initial stages of mucosal infection: intraepithelial Langerhans cells (LCs) and $CD4^+$ T lymphocytes (Fig. 1). In addition, the virus may reach basal epithelial cells that are susceptible to viral binding, endocytosis, or transcytosis, or may penetrate even further, reaching subepithelial targets, such as T cells and dendritic cells, through breaches in the epithelium caused by microabrasions (Shattock and Moore, 2003). Pre-existing inflammation, caused by lower genital tract infections such as bacterial vaginosis and trichomoniasis, also facilitates infection by thinning and disrupting the multilayered lining, recruiting a pool of target cells for local HIV expansion, and interfering with innate antimicrobial activity (Thurman and Doncel, 2010).

Utilizing single-genome amplification (SGA) and mathematical modeling, it has been reported in several patient cohorts and non-human primates that most (60–90%) mucosal infections originate from single-variant transmissions (Salazar-Gonzalez et al., 2009; Stone et al., 2009). The remaining 10–40% of infections are initiated by a limited number of transmitted/founder HIV variants. Therefore, for each individual infected, the potential viral diversity in the period of acute infection is limited to a single or a few HIV lineages. This genetic bottleneck is less pronounced in individuals engaged in high-risk behaviors (Keele et al., 2008) and in patients with sexually transmitted infections (Haaland et al., 2009).

The small, focally infected population is initially composed mainly of resting $CD4^+$ T cells lacking conventional markers of activation (Haase, 2010). HIV expands locally in these “resting” and in activated $CD4^+$ T cells, and then disseminates, initially to the draining lymph node, and subsequently to secondary lymphoid organs, to generate a systemic infection. Exposure of reproductive tract epithelium to virus increases expression of chemokines which recruit plasmacytoid dendritic cells (pDCs) (Li et al., 2009). They in turn recruit, through secretion of additional chemokines, more $CD4^+$ T cells that fuel local expansion. Interferons and chemokines from the pDCs also suppress viral replication, but the balance is tipped in favor of the virus by the cells that fuel the local expansion

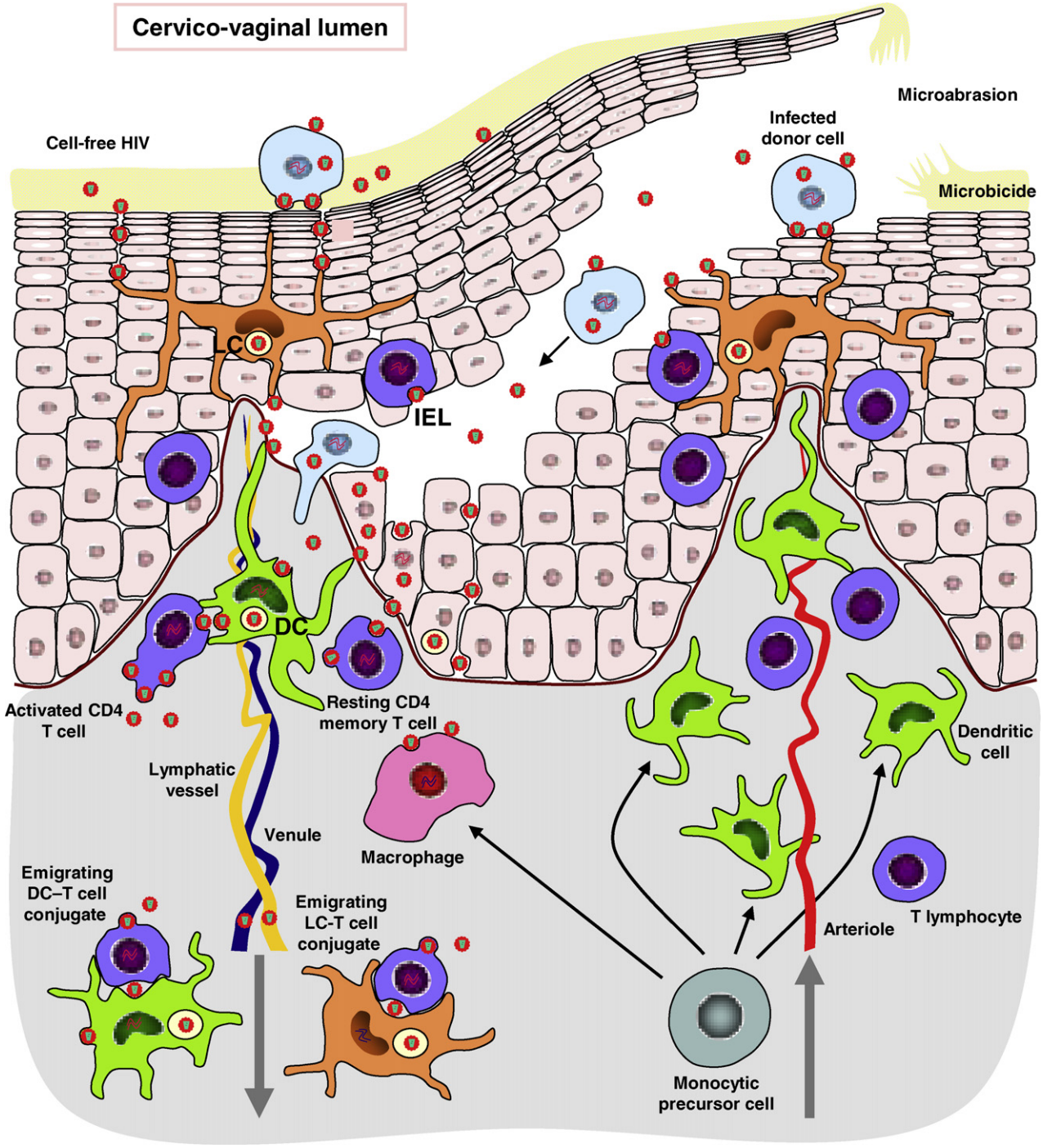

Fig. 1. Sexual transmission of HIV-1 and topical microbicide targets. Cell-free and cell-associated HIV-1 penetrate the cervicovaginal epithelium through microabrasions and/or intact tissue. They quickly reach Langerhans cells (LC) and intraepithelial $CD4^+$ T lymphocytes (IEL) within the epithelium or dendritic cells (DC) and resting $CD4^+$ T cells in the lamina propria. $CD4^+$ T cells are activated by direct contact with antigen-presenting (AP) LC or DC, or indirectly through cytokine secretion by epithelial and other immune cells. This happens focally at the port(s) of entry. Pre-existing inflammation and chemokine-mediated recruitment of new cells expand the number of activated $CD4^+$ T cells, which fuel the initial infection by a small number of founder viruses. Dissemination of infected T cells, DC, LC and APC/T cell complexes from the initial cervicovaginal infection foci to the draining lymph nodes or directly into systemic circulation leads to an established infection. Microbicide formulations must deliver their active ingredient to all these cells and places if they want to prevent the irrevocable step of systemic dissemination.
Modified from Hladik and Hope (2009), and reproduced with permission.

necessary for dissemination and establishment of systemic infection.

The initial steps of HIV mucosal infection described above represent vulnerabilities for the virus as it enters the host organism and establishes a systemic infection. These vulnerabilities create opportunities for the development of prevention strategies such as vaccines and microbicides.

3. Potential hurdles for microbicides in preventing mucosal HIV transmission

Although a microbicide might reliably block HIV infection of $CD4^+$ T cells *in vitro*, the complex *in vivo* tissue environment presents a number of challenges that the drug must overcome to provide successful protection.

First, HIV might manage to quickly disseminate beyond the reach of the microbicide. This possibility emphasizes both the enormous importance of formulating a microbicide with adequate mucosal penetration properties and the necessity to thoroughly understand the mucosal niches where HIV could potentially hide from a microbicide. Furthermore, mucosal HIV target cells are in constant turnover, and the influx of new immune cells that have not been in contact with the microbicide makes the task of inhibiting viral entry and replication much more challenging.

The need to adapt microbicidal compounds for mucosal application is nicely exemplified by the pronounced difference in the 50% inhibitory concentration (IC_{50}) between two versions of the fusion inhibitor T-20 for blocking HIV-1 infection in vaginal explant mucosa. Topical application to the explants of the N-acetylated T-20 peptide, which is available for systemic treatment under the label Fuzeon, is nearly 3 log less efficacious in inhibiting infection than the less-hydrophilic T-20 peptide with free N- and C-terminal amino acids (McElrath et al., 2010). Clearly, mucosal distribution, concentration and retention of a topical microbicide are critical parameters and should be determined for each candidate compound. For example, assays measuring the intracellular concentration of di-phosphorylated active tenofovir in the mucosa, in particular within the potential target cell populations for HIV infection, have become an integral, albeit still experimental, part of current clinical trials testing tenofovir as a microbicide (Hawkins et al., 2005; Hendrix et al., 2009; King et al., 2006; Kiser et al., 2008).

Achieving a sufficiently high concentration of an active compound in HIV target cells depends on numerous factors related to drug properties (e.g., molecular weight, hydrophobicity, ionization), formulation characteristics (e.g., compatibility, release rate, coverage of the mucosal surface), anatomical site (e.g., vagina, cervix, endometrium), and concurrent physiopathological conditions (e.g., menstrual cycle and hormonal contraceptive use, genital infections, presence of semen). All these factors, in addition to others not mentioned above such as the ability of target cells to activate compounds that require activation or actively transport them in and out of the cells, will influence the concentration gradient between the delivery system/formulation and the target cells. Determining that gradient is key to calculating a safe and effective dose of a microbicide, and will dictate its efficacy *in vivo*.

Several niches and routes in the mucosa may be exploited by HIV to evade the inhibitory action of a microbicide. It has long been known that dendritic cells (DCs), professional antigen-presenting cells with migratory capacity, can be invaded by HIV and in some cases productively infected (Cameron et al., 1992; Wu and KewalRamani, 2006). In the human vagina, $CD1a^+$ Langerhans cells (LCs), a subpopulation of intraepithelial DCs in squamous epithelia, efficiently and rapidly internalize HIV virions via endocytosis (Hladik et al., 2007). The particular receptors mediating HIV endocytosis in vaginal LCs are not known, however some evidence indicates that it is not langerin. Langerin has been identified in skin LCs as a receptor for HIV-1 that subsequent to endocytosis channels the virions into lysosomes, where they are degraded (de Witte et al., 2007). This dead-end pathway does not appear to be recapitulated in the vaginal mucosa. Unpublished data by Robinson et al. show that vaginal LCs, despite not being productively infected, are capable of passing infectious virions on to $CD4^+$ T cells. Pre-treatment with mannan, a polysaccharide that blocks HIV-1 binding to C-type lectin receptors, including langerin, only weakly inhibits the endocytosis of HIV-1 into vaginal LCs (Hladik et al., 2007). Moreover, a subpopulation of vaginal LCs lacks langerin expression (Hervouet et al., 2010; Robinson et al., 2009). Thus, HIV-1 likely uses other receptors instead of, or in addition to, langerin to enter vaginal LCs.

Clarifying how HIV-1 invades LCs in the vagina and the foreskin, or DCs at other mucosal sites, is an important question for microbicide research. In their migratory capacity, LCs and DCs can potentially transport endocytosed infectious HIV virions away from the mucosa and into the local lymphatics (Hladik et al., 2007; Hu et al., 2004). If they merely act as storage unit and carrier for HIV-1 but are not productively infected, drugs like tenofovir that interrupt the intracellular reproductive cycle of HIV are unlikely to have an effect in these cells. If virus-bearing LCs or DCs travel to the local lymphatics whereas a sufficient inhibitory concentration of the topically applied microbicide does not, HIV virions released from the carrier cells would gain an opportunity to infect and then spread unabated to lymphatic $CD4^+$ T cells. To prevent this from happening a microbicide would either have to distribute efficiently from the mucosa to the local lymphatics or it would have to inhibit viral endocytosis into mucosal LCs and DCs. No clinical drugs are currently available or under investigation that specifically inhibit HIV endocytosis. Clear understanding of viral entry into mucosal LCs will help determine the relevance of this pathway in HIV mucosal infection as well as aid in the development of potential inhibitors.

Alternatively to hitching a ride with local LCs or DCs, HIV may also take advantage of breaches in the mucosal epithelium, as caused by microtraumata or genital ulcers. Genital ulcer disease may be associated with higher HIV transmission rates for several reasons (Coombs et al., 2003; Weiler et al., 2008), including presenting an opportunity for hematogenous systemic spread that could disseminate HIV beyond the reach of a topical microbicide immediately following exposure.

A second potential challenge is that HIV may evade a microbicide by persisting in a protected mucosal compartment until the microbicide concentration has fallen below the threshold of antiviral efficacy, if the product is not being regularly re-applied. For instance, DCs not only provide a means for HIV virions to travel but also to potentially survive dormant in the cytoplasm for several days until they are passed on to $CD4^+$ T cells, which they can then productively infect (Hladik et al., 2007). However, this may require a low level productive infection of DCs (Burleigh et al., 2006; Nobile et al., 2005; Popov et al., 2005). Beside LCs and DCs, some anecdotal evidence exists that the cytoplasm of mucosal epithelial cells may also provide safe harbor to intact HIV virions (Berlier et al., 2005; Bobardt et al., 2007; Bomsel, 1997; Dezzutti et al., 2001; Hladik and McElrath, 2008; Wu et al., 2003). Whether HIV can be released from these epithelial cells at a later time point and still infect $CD4^+$ T cells remains unclear.

Third, an exposure to HIV that by itself does not lead to persistent infection may still provide stimuli to the mucosa that facilitate infection when re-exposure to the virus occurs. Early HIV infection in the mucosa is accompanied by broad innate immune stimulation (Abel et al., 2005; Haase, 2010), which is designed to protect against persistent infection. Much more needs to be learned about this early mucosal immune response to HIV, but many of these innate defenses are likely to be triggered by abortive as well as persistent infection. For example, human genital mucosal epithelial cells produce thymic stromal lymphopoetin (TSLP) not only in response to live HIV-1 but also following exposure to inactivated virions (Fontenot et al., 2009). TSLP potently activates myeloid DCs, which induce homeostatic expansion of naïve $CD4^+$ T cells, and this in turn may deliver a trigger for DC-mediated amplification of HIV infection in $CD4^+$ T cells (Fontenot et al., 2009; Ziegler and Artis, 2010). Thus, paradoxically, by creating an inflammatory state with increased numbers of susceptible target cells and possibly also higher HIV receptor densities, an infection that was successfully aborted by a microbicide may pave the way for a subsequently successful transmission event. This concept is illustrated by the failure of nonoxynol-9 (N-9) as an anti-HIV-1 vaginal microbicide (Van Damme et al., 2002). Although N-9 was virucidal to HIV-1 *in vitro*, the sub-clinical vaginal inflammation caused by N-9 *in vivo* resulted in an increased incidence of HIV-1, particularly among frequent users (Cummins and Doncel, 2009; Van Damme et al., 2002).

Pathogens causing lower genital tract infections (LGTIs), highly prevalent in HIV-endemic populations, activate Toll-like receptors (TLRs), which in turn trigger secretion of pro-inflammatory cytokines and chemokines. Epithelial and mucosal immune cells use TLRs to recognize pathogens and elicit an immunoinflammatory defensive response (Lavelle et al., 2010). Recruitment and activation of HIV target cells to mucosal sites is one of the mechanism by which LGTIs facilitate HIV transmission (Thurman and Doncel, 2010). Immortalized THP-1 cells, latently infected human monocytes expressing multiple specific TLRs, treated with the cervico-vaginal fluid lavage (CVL) of women with bacterial vaginosis showed higher levels of NF-κB and HIV-1 long terminal repeats (LTRs) than those treated with CVLs of women with normal vaginal flora (Hladik and Hope, 2009; Mares et al., 2008). Microbicides may interfere with TLR signaling, changing the immunoinflammatory status of the cervicovaginal mucosa and making it more prone to HIV infection (Trifonova et al., 2009). In the case of herpes simplex virus 2 (HSV-2) infection, enriched target cell populations for HIV persist beyond ulcer healing and even despite acyclovir therapy (Gebhardt et al., 2009; Zhu et al., 2009), providing a potential explanation for the failure of HSV-2 treatment to reduce HIV-1 acquisition (Celum et al., 2008). Thus, in the face of cervicovaginal co-infections, the task of a protective microbicide gets appreciably harder. Innate immunity modulators such as glycerol monolaureate, which has been shown to protect macaques from SIV infection (Li et al., 2009), have been postulated as potential adjuvants to more specific microbicides. However, the acute and chronic effects of these immune modulators on the epithelium and immune system of the reproductive tract mucosa and their use in combination with other microbicides should be further evaluated.

Fourth, HIV is remarkably effective in developing resistance to antiretroviral drugs (Johnson et al., 2009). Breakthrough HIV infections that occur while using systemic pre-exposure prophylaxis with a single antiretroviral carry all the theoretical ingredients for spreading drug resistance in a population: monotherapy, intermittent usage of the drug and ignorance of the newly acquired infection. Much has been written about HIV drug resistance, but the potential epidemiological effect and the impact on therapeutic options of breakthrough infections acquired while using single-agent antiretroviral microbicides is worth devoting attention to. It has been argued, however, that the estimated number of averted infections would outweigh the potential risk of inducing drug resistant virus (Ramjee et al., 2008). Only clinical trials and post-marketing surveillance will provide answers for these concerns.

4. Conclusions

The above discussion of ways in which HIV can potentially evade a protective microbicide is not meant to be comprehensive. However, it serves to remind us of the complexity of mucosal HIV transmission, the opportunities for HIV to exploit these complexities, and the challenges to be considered while developing a microbicide formulation. Improved knowledge of initial HIV infection at the major sites of sexual HIV transmission, and the mucosal responses to it, will provide the foundation to successfully navigate the path towards a safe and efficacious microbicide product.

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