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Preventing mucosal HIV transmission with topical microbicides – challenges and opportunities

Florian Hladik, MD, PhD^a and Gustavo F. Doncel, MD, PhD^b

^aDepartments of Obstetrics and Gynecology and Medicine, University of Washington, 1959 NE Pacific Street, Box 356460, Seattle, WA 98195; and Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109. fhladik@fhcrc.org

^bCONRAD, Department of Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, Virginia 23507. DoncelGF@evms.edu

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.

Keywords

Microbicides; HIV transmission; HIV prevention; HIV mucosal infection; sexually transmitted infections

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

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Corresponding Author: Gustavo F. Doncel, MD, PhD, CONRAD-Dept. of Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, VA 23507, Tel: (757) 446-5020, FAX: (757) 446-8998, DoncelGF@evms.edu.

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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., 2008; Van Damme et al., 2002). 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 et al., 2004; Parikh et al., 2009; Rohan et al.; 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, Virginia, 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.; 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 (Miyachi 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.; 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% to 90%) mucosal infections originate from single-variant transmissions (Salazar-Gonzalez et al., 2009; Stone et al., 2009). The remaining 10% to 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 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 (IC₅₀) 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 lymphopoietin (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 immuno-inflammatory 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- κ B 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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References

- Abel K, Rocke DM, Chohan B, Fritts L, Miller CJ. Temporal and Anatomic Relationship between Virus Replication and Cytokine Gene Expression after Vaginal Simian Immunodeficiency Virus Infection. *J Virol* 2005;79:12164–12172. [PubMed: 16160143]
- Alexandre KB, Gray ES, Lambson BE, Moore PL, Choge IA, Mlisana K, Karim SS, McMahon J, O'Keefe B, Chikwamba R, Morris L. Mannose-rich glycosylation patterns on HIV-1 subtype C gp120 and sensitivity to the lectins, Griffithsin, Cyanovirin-N and Scytovirin. *Virology* 2010;402:187–196. [PubMed: 20392471]
- Berlier W, Bourlet T, Lawrence P, Hamzeh H, Lambert C, Genin C, Verrier B, Dieu-Nosjean MC, Pozzetto B, Delezay O. Selective sequestration of X4 isolates by human genital epithelial cells: Implication for virus tropism selection process during sexual transmission of HIV. *J Med Virol* 2005;77:465–474. [PubMed: 16254974]
- Berlier W, Cremel M, Hamzeh H, Levy R, Lucht F, Bourlet T, Pozzetto B, Delezay O. Seminal plasma promotes the attraction of Langerhans cells via the secretion of CCL20 by vaginal epithelial cells: involvement in the sexual transmission of HIV. *Hum Reprod* 2006;21:1135–1142. [PubMed: 16531471]
- Bobardt MD, Chatterji U, Selvarajah S, Van der Schueren B, David G, Kahn B, Gallay PA. Cell-free human immunodeficiency virus type 1 transcytosis through primary genital epithelial cells. *J Virol* 2007;81:395–405. [PubMed: 17050597]
- Bomsel M. Transcytosis of infectious human immunodeficiency virus across a tight human epithelial cell line barrier. *Nat Med* 1997;3:42–47. [PubMed: 8986739]
- Buckheit RW Jr, Watson KM, Morrow KM, Ham AS. Development of topical microbicides to prevent the sexual transmission of HIV. *Antiviral Res* 2010;85:142–158. [PubMed: 19874851]
- Burleigh L, Lozach PY, Schiffer C, Staropoli I, Pezo V, Porrot F, Canque B, Virelizier JL, Arenzana-Seisdedos F, Amara A. Infection of dendritic cells (DCs), not DC-SIGN-mediated internalization of

- human immunodeficiency virus, is required for long-term transfer of virus to T cells. *J Virol* 2006;80:2949–2957. [PubMed: 16501104]
- Cameron PU, Freudenthal PS, Barker JM, Gezelter S, Inaba K, Steinman RM. Dendritic cells exposed to human immunodeficiency virus type-1 transmit a vigorous cytopathic infection to CD4+ T cells. *Science* 1992;257:383–387. [PubMed: 1352913]
- Celum C, Wald A, Hughes J, Sanchez J, Reid S, Delany-Moretlwe S, Cowan F, Casapia M, Ortiz A, Fuchs J, Buchbinder S, Koblin B, Zwierski S, Rose S, Wang J, Corey L. Effect of aciclovir on HIV-1 acquisition in herpes simplex virus 2 seropositive women and men who have sex with men: a randomised, double-blind, placebo-controlled trial. *Lancet* 2008;371:2109–2119. [PubMed: 18572080]
- Colgan R, Atkinson CJ, Paul M, Hassan S, Drake PM, Sexton AL, Santa-Cruz S, James D, Hamp K, Gutteridge C, Ma JK. Optimisation of contained *Nicotiana tabacum* cultivation for the production of recombinant protein pharmaceuticals. *Transgenic Res* 2010;19:241–256. [PubMed: 19588264]
- Coombs RW, Reichelderfer PS, Landay AL. Recent observations on HIV type-1 infection in the genital tract of men and women. *Aids* 2003;17:455–480. [PubMed: 12598766]
- Cranage M, Sharpe S, Herrera C, Cope A, Dennis M, Berry N, Ham C, Heeney J, Rezk N, Kashuba A, Anton P, McGowan I, Shattock R. Prevention of SIV rectal transmission and priming of T cell responses in macaques after local pre-exposure application of tenofovir gel. *PLoS Med* 2008;5:e157. discussion e157. [PubMed: 18684007]
- Cummins JE Jr, Doncel GF. Biomarkers of cervicovaginal inflammation for the assessment of microbicide safety. *Sex Transm Dis* 2009;36:S84–S91. [PubMed: 19218890]
- Cummins JE Jr, Guarner J, Flowers L, Guenther PC, Bartlett J, Morken T, Grohskopf LA, Paxton L, Dezzutti CS. Preclinical testing of candidate topical microbicides for anti-human immunodeficiency virus type 1 activity and tissue toxicity in a human cervical explant culture. *Antimicrob Agents Chemother* 2007;51:1770–1779. [PubMed: 17353237]
- de Witte L, Nabatov A, Pion M, Fluittsma D, de Jong MA, de Gruijl T, Piguet V, van Kooyk Y, Geijtenbeek TB. Langerin is a natural barrier to HIV-1 transmission by Langerhans cells. *Nat Med* 2007;13:367–371. [PubMed: 17334373]
- Denton PW, Estes JD, Sun Z, Othieno FA, Wei BL, Wege AK, Powell DA, Payne D, Haase AT, Garcia JV. Antiretroviral pre-exposure prophylaxis prevents vaginal transmission of HIV-1 in humanized BLT mice. *PLoS Med* 2008;5:e16. [PubMed: 18198941]
- Dezzutti CS, Guenther PC, Cummins JE Jr, Cabrera T, Marshall JH, Dillberger A, Lal RB. Cervical and prostate primary epithelial cells are not productively infected but sequester human immunodeficiency virus type 1. *J Infect Dis* 2001;183:1204–1213. [PubMed: 11262202]
- Doncel GF, Clark MR. Preclinical evaluation of anti-HIV microbicide products: New models and biomarkers. *Antiviral Res.* 2010 in press.
- Feldblum PJ, Adeiga A, Bakare R, Wevill S, Lendvay A, Obadaki F, Olayemi MO, Wang L, Nanda K, Rountree W. SAVVY vaginal gel (C31G) for prevention of HIV infection: a randomized controlled trial in Nigeria. *PLoS One* 2008;3:e1474. [PubMed: 18213382]
- Fontenot D, He H, Hanabuchi S, Nehete PN, Zhang M, Chang M, Nehete B, Wang YH, Wang YH, Ma ZM, Lee HC, Ziegler SF, Courtney AN, Miller CJ, Sun SC, Liu YJ, Sastry KJ. TSLP production by epithelial cells exposed to immunodeficiency virus triggers DC-mediated mucosal infection of CD4+ T cells. *Proc Natl Acad Sci U S A* 2009;106:16776–16781. [PubMed: 19805372]
- Friend DR, Doncel GF. Combining prevention of HIV-1, other sexually transmitted infections and unintended pregnancies: Development of dual-protection technologies. *Antiviral Res.* 2010 in press.
- Garg S, Goldman D, Krumme M, Rohan LC, Smoot S, Friend D. Advances in development, scale-up and manufacturing of microbicide gels, films, and tablets. *Antiviral Res.* 2010 in press.
- Gebhardt T, Wakim LM, Eidsmo L, Reading PC, Heath WR, Carbone FR. Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. *Nat Immunol* 2009;10:524–530. [PubMed: 19305395]

- Greenhead P, Hayes P, Watts PS, Laing KG, Griffin GE, Shattock RJ. Parameters of human immunodeficiency virus infection of human cervical tissue and inhibition by vaginal virucides. *J Virol* 2000;74:5577–5586. [PubMed: 10823865]
- Gupta P, Collins KB, Ratner D, Watkins S, Naus GJ, Landers DV, Patterson BK. Memory CD4(+) T cells are the earliest detectable human immunodeficiency virus type 1 (HIV-1)-infected cells in the female genital mucosal tissue during HIV-1 transmission in an organ culture system. *J Virol* 2002;76:9868–9876. [PubMed: 12208964]
- Haaland RE, Hawkins PA, Salazar-Gonzalez J, Johnson A, Tichacek A, Karita E, Manigart O, Mulenga J, Keele BF, Shaw GM, Hahn BH, Allen SA, Derdeyn CA, Hunter E. Inflammatory genital infections mitigate a severe genetic bottleneck in heterosexual transmission of subtype A and C HIV-1. *PLoS Pathog* 2009;5:e1000274.
- Haase AT. Targeting early infection to prevent HIV-1 mucosal transmission. *Nature* 2010;464:217–223. [PubMed: 20220840]
- Halpern V, Ogunsola F, Obunge O, Wang CH, Onyejebu N, Oduyebo O, Taylor D, McNeil L, Mehta N, Umo-Otong J, Otusanya S, Crucitti T, Abdellati S. Effectiveness of cellulose sulfate vaginal gel for the prevention of HIV infection: results of a Phase III trial in Nigeria. *PLoS One* 2008;3:e3784. [PubMed: 19023429]
- Hawkins T, Veikley W, St Claire RL 3rd, Guyer B, Clark N, Kearney BP. Intracellular pharmacokinetics of tenofovir diphosphate, carbovir triphosphate, and lamivudine triphosphate in patients receiving triple-nucleoside regimens. *J Acquir Immune Defic Syndr* 2005;39:406–411. [PubMed: 16010161]
- Hendrix CW, Cao YJ, Fuchs EJ. Topical microbicides to prevent HIV: clinical drug development challenges. *Annu Rev Pharmacol Toxicol* 2009;49:349–375. [PubMed: 19006450]
- Hervouet C, Luci C, Rol N, Rousseau D, Kissenpfennig A, Malissen B, Czerkinsky C, Anjuere F. Langerhans cells prime IL-17-producing T cells and dampen genital cytotoxic responses following mucosal immunization. *J Immunol* 2010;184:4842–4851. [PubMed: 20351191]
- Hladik F, Hope TJ. HIV infection of the genital mucosa in women. *Curr HIV/AIDS Rep* 2009;6:20–28. [PubMed: 19149993]
- Hladik F, McElrath MJ. Setting the stage: host invasion by HIV. *Nat Rev Immunol* 2008;8:447–457. [PubMed: 18469831]
- Hladik F, Sakchalathorn P, Ballweber L, Lentz G, Fialkow M, Eschenbach D, McElrath MJ. Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. *Immunity* 2007;26:257–270. [PubMed: 17306567]
- Hu Q, Frank I, Williams V, Santos JJ, Watts P, Griffin GE, Moore JP, Pope M, Shattock RJ. Blockade of attachment and fusion receptors inhibits HIV-1 infection of human cervical tissue. *J Exp Med* 2004;199:1065–1075. [PubMed: 15078900]
- Johnson VA, Brun-Vezinet F, Clotet B, Gunthard HF, Kuritzkes DR, Pillay D, Schapiro JM, Richman DD. Update of the drug resistance mutations in HIV-1: December 2009. *Top HIV Med* 2009;17:138–145. [PubMed: 20068260]
- Kader M, Wang X, Piatak M, Lifson J, Roederer M, Veazey R, Mattapallil JJ. Alpha4(+)beta7(hi)CD4(+) memory T cells harbor most Th-17 cells and are preferentially infected during acute SIV infection. *Mucosal Immunol* 2009;2:439–449. [PubMed: 19571800]
- Kaizu M, Weiler AM, Weisgrau KL, Vielhuber KA, May G, Piaskowski SM, Furlott J, Maness NJ, Friedrich TC, Loffredo JT, Osborne A, Rakasz EG. Repeated intravaginal inoculation with cell-associated simian immunodeficiency virus results in persistent infection of nonhuman primates. *J Infect Dis* 2006;194:912–916. [PubMed: 16960778]
- Karim ASS. Results of effectiveness trials of PRO 2000 gel: lessons for future microbicide trials. *Future Microbiol* 2010;5:527–529. [PubMed: 20353292]
- Karim Q, Karim SS, Frohlich JA, Grobler AC, Baxter C, Mansoor LE, Kharsany AB, Sibeko S, Mlisana KP, Omar Z, Gengiah TN, Maarschalk S, Arulappan N, Mlotshwa M, Morris L, Taylor D. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science* 2010;329:1168–1174. [PubMed: 20643915]
- Keele BF, Giorgi EE, Salazar-Gonzalez JF, Decker JM, Pham KT, Salazar MG, Sun C, Grayson T, Wang S, Li H, Wei X, Jiang C, Kirchherr JL, Gao F, Anderson JA, Ping LH, Swanstrom R,

- Tomaras GD, Blattner WA, Goepfert PA, Kilby JM, Saag MS, Delwart EL, Busch MP, Cohen MS, Montefiori DC, Haynes BF, Gaschen B, Athreya GS, Lee HY, Wood N, Seoighe C, Perelson AS, Bhattacharya T, Korber BT, Hahn BH, Shaw GM. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proc Natl Acad Sci U S A* 2008;105:7552–7557. [PubMed: 18490657]
- Khanna KV, Whaley KJ, Zeitlin L, Moench R, Mehrazar K, Cone RA, Liao Z, Hildreth JE, Hoen TE, Shultz L, Markham RB. Vaginal transmission of cell-associated HIV-1 in the mouse is blocked by a topical, membrane-modifying agent. *J Clin Invest* 2002;109:205–211. [PubMed: 11805132]
- Kim KA, Yolamanova M, Zirafi O, Roan NR, Staendker L, Forssmann WG, Burgener A, Dejuq-Rainsford N, Hahn BH, Shaw GM, Kirchhoff F, Munch J. Semen-mediated enhancement of HIV infection is donor-dependent and correlates with the levels of SEVI. *Retrovirology* 2010;7:55. [PubMed: 20573198]
- King T, Bushman L, Kiser J, Anderson PL, Ray M, Delahunty T, Fletcher CV. Liquid chromatography-tandem mass spectrometric determination of tenofovir-diphosphate in human peripheral blood mononuclear cells. *J Chromatogr B Analyt Technol Biomed Life Sci* 2006;843:147–156.
- Kiser JJ, Aquilante CL, Anderson PL, King TM, Carten ML, Fletcher CV. Clinical and genetic determinants of intracellular tenofovir diphosphate concentrations in HIV-infected patients. *J Acquir Immune Defic Syndr* 2008;47:298–303. [PubMed: 18398970]
- Lavelle EC, Murphy C, O'Neill LA, Creagh EM. The role of TLRs, NLRs, and RLRs in mucosal innate immunity and homeostasis. *Mucosal Immunol* 2010;3:17–28. [PubMed: 19890268]
- Lederman MM, Veazey RS, Offord R, Mosier DE, Dufour J, Mefford M, Piatak M Jr, Lifson JD, Salkowitz JR, Rodriguez B, Blauvelt A, Hartley O. Prevention of vaginal SHIV transmission in rhesus macaques through inhibition of CCR5. *Science* 2004;306:485–487. [PubMed: 15486300]
- Li N, Chen Y, He W, Yi T, Zhao D, Zhang C, Lin CL, Todorov I, Kandeel F, Forman S, Zeng D. Anti-CD3 preconditioning separates GVL from GVHD via modulating host dendritic cell and donor T-cell migration in recipients conditioned with TBI. *Blood* 2009;113:953–962. [PubMed: 18922852]
- Li Q, Duan L, Estes JD, Ma ZM, Rourke T, Wang Y, Reilly C, Carlis J, Miller CJ, Haase AT. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. *Nature* 2005;434:1148–1152. [PubMed: 15793562]
- Liu X, Lagenaur LA, Simpson DA, Essenmacher KP, Frazier-Parker CL, Liu Y, Tsai D, Rao SS, Hamer DH, Parks TP, Lee PP, Xu Q. Engineered vaginal lactobacillus strain for mucosal delivery of the human immunodeficiency virus inhibitor cyanovirin-N. *Antimicrob Agents Chemother* 2006;50:3250–3259. [PubMed: 17005802]
- Malcolm RK, Edwards KL, Kiser P, Romano J, Smith T. Advances in microbicide vaginal rings. *Antiviral Res.* 2010 in press.
- Mares D, Simoes JA, Novak RM, Spear GT. TLR2-mediated cell stimulation in bacterial vaginosis. *J Reprod Immunol* 2008;77:91–99. [PubMed: 17532476]
- Matoba N, Husk AS, Barnett BW, Pickel MM, Arntzen CJ, Montefiori DC, Takahashi A, Tanno K, Omura S, Cao H, Mooney JP, Hanson CV, Tanaka H. HIV-1 neutralization profile and plant-based recombinant expression of actinohivin, an Env glycan-specific lectin devoid of T-cell mitogenic activity. *PLoS One* 2010;5:e11143. [PubMed: 20559567]
- McElrath MJ, Ballweber L, Terker A, Kreger A, Sakchalathorn P, Robinson B, Fialkow M, Lentz G, Hladik F. Ex vivo comparison of microbicide efficacies for preventing HIV-1 genomic integration in intraepithelial vaginal cells. *Antimicrob Agents Chemother* 2010;54:763–772. [PubMed: 19949052]
- Miyauchi K, Kim Y, Latinovic O, Morozov V, Melikyan GB. HIV enters cells via endocytosis and dynamin-dependent fusion with endosomes. *Cell* 2009;137:433–444. [PubMed: 19410541]
- Morrow K, Hendrix C. Clinical evaluation of microbicide formulations. *Antiviral Res.* 2010 in press.
- Nobile C, Petit C, Moris A, Skrabal K, Abastado JP, Mammano F, Schwartz O. Covert human immunodeficiency virus replication in dendritic cells and in DC-SIGN-expressing cells promotes long-term transmission to lymphocytes. *J Virol* 2005;79:5386–5399. [PubMed: 15827153]
- Parikh UM, Dobard C, Sharma S, Cong ME, Jia H, Martin A, Pau CP, Hanson DL, Guenther P, Smith J, Kersh E, Garcia-Lerma JG, Novembre FJ, Otten R, Folks T, Heneine W. Complete

protection from repeated vaginal simian-human immunodeficiency virus exposures in macaques by a topical gel containing tenofovir alone or with emtricitabine. *J Virol* 2009;83:10358–10365. [PubMed: 19656878]

- Peterson L, Nanda K, Opoku BK, Ampofo WK, Owusu-Amoako M, Boakye AY, Rountree W, Troxler A, Dominik R, Roddy R, Dorfänger L. SAVVY (C31G) gel for prevention of HIV infection in women: a Phase 3, double-blind, randomized, placebo-controlled trial in Ghana. *PLoS One* 2007;2:e1312. [PubMed: 18091987]
- Popov S, Chenine AL, Gruber A, Li PL, Ruprecht RM. Long-term productive human immunodeficiency virus infection of CD1a-sorted myeloid dendritic cells. *J Virol* 2005;79:602–608. [PubMed: 15596853]
- Ramjee G, Doncel GF, Mehendale S, Tolley EE, Dickson K. Microbicides 2008 Conference: From Discovery to Advocacy. *AIDS Res Ther* 2008;5:19. [PubMed: 18702834]
- Robinson B, Ballweber L, Kreger A, Lentz G, Fialkow M, McElrath MJ, Hladik F. Vaginal Langerhans cells resist genomic integration of HIV-1 but transmit endocytosed virions to susceptible target cells. *J. Immunol* 2009;182 39.27.
- Rohan LC, Moncla BJ, Kunjara Na Ayudhya RP, Cost M, Huang Y, Gai F, Billitto N, Lynam JD, Pryke K, Graebing P, Hopkins N, Rooney JF, Friend D, Dezzutti CS. In vitro and ex vivo testing of tenofovir shows it is effective as an HIV-1 microbicide. *PLoS One* 2010;5:e9310. [PubMed: 20174579]
- Saba E, Grivel JC, Vanpouille C, Brichacek B, Fitzgerald W, Margolis L, Lisco A. HIV-1 sexual transmission: early events of HIV-1 infection of human cervico-vaginal tissue in an optimized ex vivo model. *Mucosal Immunol* 2010;3:280–290. [PubMed: 20147895]
- Salazar-Gonzalez JF, Salazar MG, Keele BF, Learn GH, Giorgi EE, Li H, Decker JM, Wang S, Baalwa J, Kraus MH, Parrish NF, Shaw KS, Guffey MB, Bar KJ, Davis KL, Ochsenbauer-Jambor C, Kappes JC, Saag MS, Cohen MS, Mulenga J, Derdeyn CA, Allen S, Hunter E, Markowitz M, Hraber P, Perelson AS, Bhattacharya T, Haynes BF, Korber BT, Hahn BH, Shaw GM. Genetic identity, biological phenotype, and evolutionary pathways of transmitted/founder viruses in acute and early HIV-1 infection. *J Exp Med* 2009;206:1273–1289. [PubMed: 19487424]
- Salle B, Brochard P, Bourry O, Mannioui A, Andrieu T, Prevot S, Dejucq-Rainsford N, Dereuddre-Bosquet N, Le Grand R. Infection of macaques after vaginal exposure to cell-associated simian immunodeficiency virus. *J Infect Dis* 202;337–344. [PubMed: 20569157]
- Shattock RJ, Moore JP. Inhibiting sexual transmission of HIV-1 infection. *Nat Rev Microbiol* 2003;1:25–34. [PubMed: 15040177]
- Stone M, Ma ZM, Genesca M, Fritts L, Blozis S, McChesney MB, Miller CJ. Limited dissemination of pathogenic SIV after vaginal challenge of rhesus monkeys immunized with a live, attenuated lentivirus. *Virology* 2009;392:260–270. [PubMed: 19647847]
- Tevi-Benissan C, Belec L, Levy M, Schneider-Fauveau V, Si MA, Hallouin MC, Matta M, Gresenguet G. In vivo semen-associated pH neutralization of cervicovaginal secretions. *Clin.Diagn.Lab Immunol* 1997;4:367–374. [PubMed: 9144379]
- Thompson WJ, Fitzgerald PM, Holloway MK, Emini EA, Darke PL, McKeever BM, Schleif WA, Quintero JC, Zugay JA, Tucker TJ, et al. Synthesis and antiviral activity of a series of HIV-1 protease inhibitors with functionality tethered to the P1 or P1' phenyl substituents: X-494 ray crystal structure assisted design. *J Med Chem* 1992;35:1685–1701. [PubMed: 1588551]
- Thurman AR, Doncel GF. Innate Immunity and Inflammatory Response to *Trichomonas vaginalis* and Bacterial Vaginosis: Relationship to HIV Acquisition. *Am J Reprod Immunol*. 2010
- Trifonova RT, Doncel GF, Fichorova RN. Polyanionic microbicides modify Toll-like receptor-mediated cervicovaginal immune responses. *Antimicrob Agents Chemother* 2009;53:1490–1500. [PubMed: 19139286]
- Van Damme L, Govinden R, Mirembe FM, Guedou F, Solomon S, Becker ML, Pradeep BS, Krishnan AK, Alary M, Pande B, Ramjee G, Deese J, Crucitti T, Taylor D. Lack of effectiveness of cellulose sulfate gel for the prevention of vaginal HIV transmission. *N Engl J Med* 2008;359:463–472. [PubMed: 18669425]
- Van Damme L, Ramjee G, Alary M, Vuylsteke B, Chandey V, Rees H, Sirivongrangsorn P, Mukenge-Tshibaka L, Ettiegn-Traore V, Uaheowitchai C, Karim SS, Masse B, Perriens J, Laga

- M. Effectiveness of COL-1492, a nonoxynol-9 vaginal gel, on HIV-1 transmission in female sex workers: a randomised controlled trial. *Lancet* 2002;360:971–977. [PubMed: 12383665]
- Veazey RS, Klasse PJ, Schader SM, Hu Q, Ketas TJ, Lu M, Marx PA, Dufour J, Colonna RJ, Shattock RJ, Springer MS, Moore JP. Protection of macaques from vaginal SHIV challenge by vaginally delivered inhibitors of virus-cell fusion. *Nature* 2005;438:99–102. [PubMed: 16258536]
- Veazey RS, Ling B, Green LC, Ribka EP, Lifson JD, Piatak M Jr, Lederman MM, Mosier D, Offord R, Hartley O. Topically Applied Recombinant Chemokine Analogues Fully Protect Macaques from Vaginal Simian-Human Immunodeficiency Virus Challenge. *J Infect Dis* 2009;199:1525–1527. [PubMed: 19331577]
- Weiler AM, Li Q, Duan L, Kaizu M, Weisgrau KL, Friedrich TC, Reynolds MR, Haase AT, Rakasz EG. Genital ulcers facilitate rapid viral entry and dissemination following intravaginal inoculation with cell-associated SIVmac239. *J Virol.* 2008
- Whaley KJ, Hanes J, Shattock R, Friend DR. Novel approaches to vaginal delivery and safety of microbicides: Biopharmaceuticals, nanoparticles and vaccines. *Antiviral Res.* 2010 in press.
- Wu L, KewalRamani VN. Dendritic-cell interactions with HIV: infection and viral dissemination. *Nat Rev Immunol* 2006;6:859–868. [PubMed: 17063186]
- Wu Z, Chen Z, Phillips DM. Human genital epithelial cells capture cell-free human immunodeficiency virus type 1 and transmit the virus to CD4+ Cells: implications for mechanisms of sexual transmission. *J Infect Dis* 2003;188:1473–1482. [PubMed: 14624372]
- Zhang Z, Schuler T, Zupancic M, Wietgreffe S, Staskus KA, Reimann KA, Reinhart TA, Rogan M, Cavert W, Miller CJ, Veazey RS, Notermans D, Little S, Danner SA, Richman DD, Havlir D, Wong J, Jordan HL, Schacker TW, Racz P, Tenner-Racz K, Letvin NL, Wolinsky S, Haase AT. Sexual transmission and propagation of SIV and HIV in resting and activated CD4+ T cells. *Science* 1999;286:1353–1357. [PubMed: 10558989]
- Zhu J, Hladik F, Woodward A, Klock A, Peng T, Johnston C, Remington M, Magaret A, Koelle DM, Wald A, Corey L. Persistence of HIV-1 receptor-positive cells after HSV-2 reactivation is a potential mechanism for increased HIV-1 acquisition. *Nat Med* 2009;15:886–892. [PubMed: 19648930]
- Zhu T, Wang N, Carr A, Nam DS, Moor-Jankowski R, Cooper DA, Ho DD. Genetic characterization of human immunodeficiency virus type 1 in blood and genital secretions: evidence for viral compartmentalization and selection during sexual transmission. *J Virol* 1996;70:3098–3107. [PubMed: 8627789]
- Ziegler SF, Artis D. Sensing the outside world: TSLP regulates barrier immunity. *Nat Immunol* 2010;11:289–293. [PubMed: 20300138]

Figure 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.