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HIV-1 VAGINAL TRANSMISSION: CELL-FREE OR CELL-ASSOCIATED VIRUS?

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Abstract

The vast majority of new HIV infections in male-to-female transmission occurs through semen, where HIV-1 is present in two different forms: as free and as cell-associated virus. In the female lower genital tract, semen mixes with female genital secretions that contain various factors, some of which facilitate or inhibit HIV-1 transmission. Next, HIV-1 crosses the genital epithelia, reaches the regional lymph nodes, and disseminates through the female host. Cervico-vaginal mucosa contains multiple barriers, resulting in a low probability of vaginal transmission. However, in some cases HIV-1 is able to break these barriers. Although the exact mechanisms of how these barriers function remain unclear, their levels of efficiency against cell-free and cell-associated HIV-1 are different, and both cell-free and cell-associated virions seem to use different strategies to overcome these barriers. Understanding the basic mechanisms of HIV-1 vaginal transmission is required for the development of new antiviral strategies to contain HIV-1 epidemics.

Keywords

Cell; HIV-1; mucosa; semen; transmission

INTRODUCTION

The vast majority of new HIV infections occurs in women through male-to-female sexual transmission[1, 2]. In such intercourse, HIV is transferred from semen of an infected male to an uninfected female partner and overcomes some host's defensive barriers. The presence of mucus, epithelial layers, secreted neutralizing antibodies, defensive proteins (eg. α-defensins, immunoglobulins, complement, antimicrobial peptides, lysozyme and lactoferrin) in genital tracts, are examples of the host barriers against these microbes[3-5]

Although the mechanisms of vaginal HIV-1 transmission are widely studied (see[6-8]), important aspects of this process remain to be elucidated. In particular, it is not known what the source of transmitted HIV is: does it come from infected cells present in semen, or from a cell-free HIV-1? What are the roles of seminal cytokines and other seminal compounds in

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HIV-1 transmission? What are the protective barriers against HIV transmission in the female lower genital tract? These questions and their possible answers are discussed in the present review.

In particular, we discuss below the biological mechanisms of the male-to-female vaginal HIV-1 transmission, the role of seminal components in this process, the barriers to HIV-1 transmission present in the female genital tract and the strategies HIV uses to overcome them. In our review, we specifically address data regarding the roles of cell-free and cell-associated viruses in HIV-1 vaginal transmission.

SEMEN AS A VEHICLE FOR HIV-1 TRANSMISSION

Since the discovery of HIV-1 in genital secretions, several groups have evaluated the relation between viral concentrations in these fluids and the risk of transmission and found that higher concentration in genital secretions augments the probability of HIV-1 sexual transmission[1, 9-11]. The level of seminal HIV-1 depends on the stage of the infection: for example more viral particles are detected in semen of HIV-1-infected men in the acute phase of the infection[11, 12] and the risk of HIV transmission varies from 8 cases per 1000 vaginal coital acts in the acute to 1-2 per 1,000 in chronic phase[10, 13-18]. Administration of antiviral therapy decreases seminal viral load, consequently diminishing the risk of transmission[19-22].

The origin of HIV-1 in semen has been debated for years. Blood does not seem to be the only source of seminal HIV-1, since genetic discordances were observed between viruses isolated from blood and from semen[22-25]. In particular, some genetic signatures are present only in seminal HIV-1 (e.g., specific glycosylation patterns in the viral envelope gp120)[25]. Other genetic analysis of HIV-1 demonstrated that seminal HIV-1 originates predominantly from male genital tract tissues[22-26]. This was confirmed in macaques: male genital organs, such as testis, epididymis, prostate, and seminal vesicles were infected by SIV in both acute and chronic phases of the infection and thus may be the source of HIV-1 found in semen[27, 28]. Moreover, cultures of human testis and prostate contained cells that can be productively infected by HIV-1, i.e., $CD4^+$ $CCR5^+$ T cells and macrophages[29, 30]. On the basis of the phylogenetic analysis of viral sequences in the blood and semen, Anderson *et al*. proposed that viral populations in semen derive from multiple sources including direct import of virus from blood and an oligoclonal amplification within the male genital tract[31]. However, vasectomy does not preclude the presence of virus in ejaculate, indicating that most cell-free HIV in seminal plasma arises distally to the vas deferens[32, 33]. Thus, seminal HIV-1 is composed of an heterogeneous population of viruses produced by semen-producing organs and blood.

Whichever the source of the virus in semen is, HIV-1 exists in two different forms: free and cell-associated[12, 34-39]. Monocytes/macrophages and $CD4^+$ T cells are the major populations infectable by HIV-1 and can both produce free virus and carry it in semen. Both forms of viruses were proved to be infectious (reviewed in[12]). Anderson and colleagues estimated that roughly 0.2% of macrophages and $CD4^+$ T cells are infected in human semen of HIV-1 therapy naïve infected patients[12]. These results are in general agreement with the enumeration of infected cells in semen of SIV infected macaques[40].

SEMEN AFFECTS HIV ACQUISITION

In the course of heterosexual intercourse, HIV-1 carried by semen is deposited in the vaginal mucosa. Semen is more than a mere carrier of HIV-1, since it contains many biological factors that may facilitate or inhibit HIV-1 transmission[41]. For instance, semen neutralizes the acidic cervical mucus increasing HIV-1 diffusion[42] otherwise trapped in mucus[43]. Furthermore, semen can significantly modify the chemical and physical properties of the female genital mucus. For example, semen contains amyloid fibrils derived from the prostatic alkaline phosphatase (SEVI)[44] and semenogelins (SEM1 & SEM2)[45] that promote viral attachment to target cells enhancing HIV-1 transmission. These fibrils increase the infectivity of cell free HIV-1 but should have no effect on cell-associated HIV-1 infectivity. Also, semen contains inhibitors of the complement system, such as CD59, which could facilitate viral escape from complement-mediated virucidal activity[46].

Other seminal factors affecting HIV-1 transmission are cytokines. The modulation of the cytokine network in semen affects viral transmission. Indeed, it was showed that seminal plasma induces chemokine (C-C motif) ligand (CCL)-2 secretion by ectocervical epithelial cells. CCL-2, also referred as MCP-1, may in turn recruit immune cells to the female genital tract following ejaculation, providing new targets for HIV infection[47]. More recently, we[48], and others[49], reported that interleukin (IL)-7, one of the most prominent seminal cytokine is upregulated in the seminal plasma of HIV-1-infected individuals. We used an *ex vivo* system of human cervical tissues to investigate the role of IL-7 in HIV-1 transmission. We found that, *ex vivo*, IL-7 facilitates HIV-1 transmission predominantly by preventing apoptosis of HIV-1 infected cells. IL-7 is not the only cytokine that modulates HIV transmission, since Olivier *et al*. reported that concentrations of G-CSF in semen significantly predicted both HIV shedding and T-cell activation[49].

Upregulation of different cytokines in semen of HIV-1 infected individuals may be related to a local inflammation or immunoactivation in the male genital tract caused by HIV-1 itself of by HIV-associated infections. By residing and replicating in the genital tract of HIV-1 infected individuals, copathogens such as Chlamydia trachomatis, Trichomonas vaginalis, Neisseria gonorrhoeae, Human papillomavirus, cytomegalovirus (CMV) and herpes simplex viruses (HSV)[48, 50-54] may promote subclinical inflammation, therefore modulating HIV-1 replication via cytokine network alteration or/and the recruitment of new target cells[55]. As examples, CMV, HSV, and *Neisseria gonorrhoeae* reactivation in the seminal compartment augment HIV shedding in semen[53, 56-59]. In contrast, GBV-C has recently been showed to decerease T cell activation and inflammation and therefore may decrease HIV-1 transmission[60].

Furthermore, some genital pathogens, such as *Trichomonas* or HSV-2, can facilitate HIV acquisition directly by disrupting the mucosal epithelia or by inducing the infiltration of susceptible cells[50, 52, 61]. In summary, male-to-female transmission of HIV-1 is a multiregulated process affected by various seminal factors and other pathogens.

SEMINAL FREE AND CELL-ASSOCIATED VIRUS IN THE ESTABLISHMENT OF HIV-1 INFECTION

Most experiments on HIV-1 or SIV transmission were performed with cell-free viruses. However, the literature regarding the role of cell-associated virus in HIV-1 transmission is now growing. Operatively, it is often difficult to distinguish between virus deposited on the vaginal mucosa in cell-free or cell-associated form, since HIV-1 can be temporarily adsorbed on the cell surface and subsequently released as free virus. We believe that virus adsorbed to seminal cells should be considered as cell-associated only if it remains on the cell surface (e.g., spermatozoa[62, 63]) during its contact with female genital epithelia.

The idea of infection transmitted by cells containing pathogens ("Trojan Horses") predates the discovery of HIV as the agent of AIDS[64, 65]. Later, two independent groups found *in vivo* evidence that mouse spleen mononuclear cells are able to cross mouse vaginal epithelium after atraumatic inoculation in the vaginal lumen[66, 67]. Furthermore, it was demonstrated in hu-SCID mice that HIV-infected human cells migrate transepithelially and transmit infection[68, 69]. Similarly, in a non-human primate model, intravaginal inoculation of SIV-infected cells resulted in persistent infection of exposed animals[70-72]. In humans, a longitudinal study reported that the HIV-1 genotype found in women in acute infection matched the viruses integrated in the seminal cells of their infected male partners, suggesting that HIV originated from infected cells present in semen[23], mainly lymphocytes and macrophages. This is in agreement with experiments showing that intravaginal inoculation of semen simulant containing ^{111}In-radiolabeled autologous leukocytes together with ^{99m}Tc-radiolabeled nanoparticles result in migration of both labeled components in the human cervical tract[73]. Thus it seems that not only free virus but also infected cells are able to interact with cervico-vaginal tissue, transmitting infection in heterosexual intercourse.

Whether free or cell-associated HIV-1 is more prone to overcome the multiple barriers that defend the female tract from HIV-1 transmission remains to be elucidated. Free virus seems to diffuse where water diffuses[74]. Thomas Hope's group found that different cell-free HIV-1 clones penetrated on average approximately 7 to 9 μm and in some cases up to 50 μm in ecto- or endocervixes[74]. Unlike cell-free HIV-1 particles, which move passively, cells are capable of active locomotion through barriers such as epithelia.

Let us consider how these barriers ("gatekeepers") insure a low probability of HIV-1 transmission through vaginal sex[1]. The notion of biological barriers for HIV sexual transmission evolved when it was noticed that the only HIV strain detected at the early stages of HIV-1 sexual transmission was of the R5 (CCR5 coreceptor-using) phenotype, while in semen both R5 and X4 (CXCR4 coreceptor-using) HIV-1 variants were present. While these viruses use different co-receptors often expressed by the same cells, their physiological features are dramatically different. At least in B-clade HIV-1 R5 dominates early stages of transmission/infection while X4 HIV-1 often evolves at the later stage. Since both R5 and X4 HIV-1 are present in semen, female host's barriers seem to block X4 viruses as R5 HIV-1 are found ubiquitously in almost all reported HIV-1 sexual transmission events[75]. Moreover, it seems that there are barriers that not only select R5 over X4 but also may operate among R5 HIV-1 variants. Genetic analysis of HIV-1 diversity at the earliest

stages of HIV infection indicates that, in majority of cases, infection is transmitted by a single R5 viral particle[76, 77]. HIV-1 transmission by a single virion can be explained by stochastic mechanisms[78]. However, several characteristics of the transmitted virus that distinguish them from the bulk have been reported (i.e., glycosylation pattern[79]). If, only these selected HIV-1 virions are transmitted, the gatekeeping mechanism is even more selective than previously anticipated.

Where do these gatekeepers reside? The genital mucus is the first barrier against HIV on its way to dissemination. Mucus can protect underlying epithelia by decreasing HIV infectivity via various soluble factors and by temporarily trapping virions or infected cells in the protein mesh, slowing their movement by several orders of magnitude compared with water[42, 43, 80, 81]. Since free HIV, due to its fragility, cannot remain outside of cells for a long time, its infectivity may be significantly decreased if mucus slows viral penetration[81]. However, if transmission is mediated by direct contact of an infected cell with a target cell, the slowing of its movement by mucus may not be critical for infection. While virus can be protected inside the cell, HIV-1 may be protected from antiviral compounds as well in the mucus because virions may be covered by seminal proteins and other seminal colloid constituents. This aspect of HIV-semen interactions has not been exhaustively studied yet.

Viral particles or virus-infected cells that go through the cervical mucus reach the epithelial layer, which constitutes another major barrier to efficient transmission of HIV and other pathogens. According to some data[82, 83], HIV crosses the epithelial barrier predominantly through lesions that commonly occur as a result of various infections or coital/sexual abrasion[84]. However, the efficiency of epithelial protection doesn't seem to be uniform through the entire surface of the female lower genital tract. It is believed that the main site for HIV transmission in the female genital tract is the cervix, especially the endocervix and the transitional zone, which are covered by a single-layer columnar epithelium. Such a layer is less protective against HIV-1 than the stratified epithelia of the vagina[85, 86]; reviewed in[87]). Also, the ectocervix, together with the transition zone, contains a high number of potential cell targets for HIV[82]. However, it is known that HIV-1 transmission through the vaginal mucosa does happen as well, as HIV genital transmission to women with a congenital absence of cervix has been reported[88]. Similarly, SIV has been transmitted intravaginally to hysterectomized rhesus macaques[12, 34].

HIV-1 transmission through epithelia has been simulated in various models *ex vivo*. Conventional cultures of cell lines or peripheral blood mononuclear cells have been sucessfully used in many areas of HIV research. However. these cultures have an important limitation: they neither reproduce the morphology nor mimic the functions of living tissues, and therefore they lack the potential to predict tissue responses to viral challenges. In contrast, cervico-vaginal tissue explants have several advantages: first, these explants preserve tissue architecture for 2–3 weeks; second, they retain the majority of cell types, which express key cell surface molecules relevant for HIV infection[89-91]; third, unlike single-cell cultures they do not require exogenous activation or stimulation to support productive HIV infection. Cervico-vaginal tissue *ex vivo* as a model to study early events in HIV-1 infection has been extensively reviewed by Merbah et al.,2011[92].

In particular, several studies using polarized epithelial monolayers exposed to HIV-1-infected cells showed that HIV-1-infected cells were able to transcytose and infect underlying susceptible target cells[93-95]. Also, it was reported that *in vitro* cells are able to migrate through ecto-/endocervical tissues when placed on the luminal side[12, 96-98]. Collins'group used cervical tissue polarized in 3% agarose and reported that HIV-1-infected cells cross cervical explants and transmit infection to susceptible cells underneath[96]. However, the methodology of this study was criticized because of the problems with reliable tissue polarization in their experiments: HIV-1 could potentially penetrate tissue explant from the (wounded) lateral sides[99]. Later, Maher and colleagues observed that seminal cells penetrate beneath the most external layer of ectocervix[97]. More recently, Anderson et al. observed macrophages protruding their membranes into interepithelial spaces of endocervical tissues, thus potentially carrying and delivering HIV to sub-epithelial cells[12]. Finally, Soto-Rivera *et al*. (2013) exposed cervical explants to HIV-1-infected cells and reported that these cells transmit HIV-1 to isolated tonsillar cells located in the lower chamber in a transwell system[98]. Although it has been shown in *ex vivo* models that HIV-1-infected cells can penetrate and cross the cervical epithelium, it remains to be demonstrated whether such processes account for HIV-1 infection *in vivo*, especially in vaginal and cervical multilayered epithelium.

Adhesion proteins may play an important role in the transmission of virus through epithelia. Immune cells use adhesion proteins to migrate from the apical to the basal part of epithelia[100]. The expression of these proteins is a process coordinated by chemokines and inflammatory mediators, which are present in high concentration in the vaginal mucosal and submucosal tissues[9, 83]. For example, LFA-1 adhesion molecules on seminal macrophages and on T cells from SIV-infected macaques could interact with intercellular adhesion molecules (ICAMs) on mucosal epithelia, triggering the penetration of infected cells into the epithelia[40, 49, 101, 102]. Acordingly, semen from HIV-1-infected patients is enriched in the chemokine CXCL-12[48] (SDF-1), which enables the activation of LFA-1[102]. These reports showed the possible role of adhesion proteins in cell-associated virus penetration through epithelia but the role of these proteins in cell-free HIV-1 penetration remains to be elucidated. LFA-1 was demonstrated on the surface of HIV-1[103] (reviewed in[104]) and recently Arakelyan et al.[105] reported that LFA-1 is present on selected virions. Thus, it is conceivable that these particular cell-free viruses cross epithelia utilizing adhesion molecules as conduits, similar to cell-associated virions do.

Moreover, free HIV-1 may interact with molecules on the surface of host epithelial cells, such as cell surface heparan sulfate proteoglycans and glycosphingolipids[106, 107]. It has been shown that virions attachment to glycosphingolipids promotes their endocytosis in cervico-vaginal tissue cells[74, 108-112]. On the other hand, the trapping of HIV-1 by epithelium surface-molecules may constitute another barrier for HIV transmission.

Once the epithelial barrier has been overcome, migrant free HIV-1 or HIV-1-infected cells reach the submucosal tissue, wherein they can interact with HIV-1 target cells, such as activated T cells and macrophages[113]. The first (founding) infected cells seem to be CD4 lymphocytes[114, 115] rather than macrophages. In submucosa, infected cells transmit HIV-1

to uninfected cells through a cell-to-cell specific structure called a virological synapse and through nanotubes and filopodia[116-119].

Cell-to-cell transfer seems to be efficient[120, 121] because it facilitates contacts between virus and its receptor(s) on the target cells, as this contact occurs in the intercellular space of a synapse that also may protect HIV-1 from extracellular soluble antiviral compounds[122-127]. Another mechanism that may protect virus from extracellular soluble antiviral compounds is HIV uptake by host cervical dendritic cells (DCs). DCs are specialized cells that take up antigens, and transfer them to local lymph nodes[128]. This way they transfer HIV-1 to these lymph nodes, where they transmit viruses to T cells, contributing to viral disseminatation[8, 112, 129]. It is still under debate whether DCs are infected by HIV-1 or just carry virions without being productively infected[130-134]. Although free HIV virions are capable to be transmitted by this mechanism via binding to DC's C-type lectin DC-SIGN, or langerin in the case of Langerhans cells[135-137], recently it was suggested that DCs can capture and transmit cell-associated virus[63, 138].

CONCLUSION

In semen of infected men, HIV-1 is present as free virions and as cell-associated ones. In heterosexual vaginal intercourse, semen carrying HIV-1 is deposited in the female lower genital tract. In case of efficient transmission, HIV-1 penetrates the genital epithelia, reaches the draining lymph nodes, and disseminates through the female host. Cervico-vaginal tissues provide efficient multiple barriers against the vast majority of cell-free and cell-associated HIV virions, resulting in a low probability of vaginal transmission. Although the exact mechanisms by which these barriers function are unclear, their levels of efficiency against cell-free and cell-associated HIV-1 are different, and both free and cell-associated virions seem to use different strategies to overcome them. Seminal components play an important role in HIV transmission, both facilitating and inhibiting transmission.

Data from *in vivo* and *ex vivo* studies convincingly show that both free and cell-associated HIV virions are able to penetrate the female genital mucosa, although by different pathways, and reach the regional lymph nodes. However, the relative contribution of free and cell-associated HIV-1 in transmission of HIV-1 infection from an infected man to his uninfected female partner remains to be understood. Towards this goal, it is necessary (i) to evaluate and characterize cells that carry HIV in semen as well as to characterize free virions that are preferentially transmitted; (ii) to investigate the effects of female genital mucus mixed with semen on free or cell-associated HIV-1; (iii) to identify seminal factors and evaluate their effect on free or cell-associated HIV in vaginal transmission; and (iv) to investigate the distinct strategies used by free HIV and HIV-carrying seminal cells to penetrate vaginal epithelial layers and reach regional lymph nodes.

As these strategies seem to be different, different counter-measures, should be developed to prevent transmission of free and cell-associated HIV-1. A better knowledge of these strategies will lead to the development of new approaches to prevent HIV-1 transmission.

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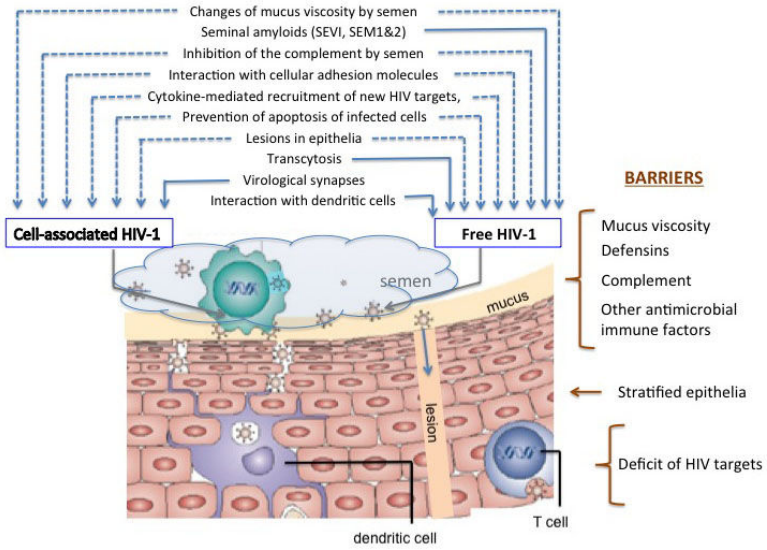

Fig. Overcoming barriers to vaginal transmission by free or cell-associated HIV-1

Female host's natural barriers prevent HIV-1 vaginal transmission, (text in brown) HIV-1 is overcoming these barriers via various mechanisms. Some of these mechanisms seem to be common for free and cell associated viruses (dashed blue arrows) whereas others predominantly facilitate either free or cell-associated HIV-1 transmission (solid blue arrows).

Table

The role of cell-free and cell-associated in HIV-1 vaginal transmission: Questions to answer

Free and cell-associated HIV-1 transmission	Question to answer
Cell-associated HIV-1 is present in semen	Which types of cells carry HIV-1 in semen? What is the relative proportions of these type of these cells?
Free and cell-associated HIV-1 are present in semen	What are the sources of free HIV-1 and cell-associated HIV-1 in semen
Both free and cell-associated HIV-1 transmit infection	What are the relative contributions of free and cell-associated virus to HIV-1 transmission?
Different cytokines differentially affect HIV-1 transmission/infection	What are the effects of various cytokine spectra on HIV-1 transmission
Sexually transmitted pathogens facilitate HIV-1 transmission	What are the mechanisms of this facilitation?
DCs transmit HIV-1 infection	Are these DCs productively infected or do they passively carry HIV-1?
Female genital barriers decrease the probability of HIV-1 transmission	Which is more apt to overcome barriers that defend the female genital tract: free or cell-associated HIV-1?
Mucus slows penetration of free HIV, probably diminishing its infectivity.	Is the same true for cell-associated virus?
Free HIV particles cross epithelia through lesions.	Can HIV-infected cells penetrate mucosal epithelia via a similar pathway?
Cell-associated HIV transmitted through virological synapsis is protected from many soluble factors	Are there antiviral factors (e.g., neutralizing antibodies, antiretrovirals) that penetrate virological synapses?
Most experiments on protection from HIV-1 transmission were performed with free virus	Would the results be the same for cell-associated virus?