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Setting the stage – HIV host invasion

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Abstract

For more than two decades, HIV has infected millions of people worldwide each year through mucosal transmission. Our knowledge of how HIV secures a foothold at both the molecular and cellular levels has expanded by recent investigations that have applied new technologies and used improved techniques to isolate *ex vivo* human tissue and generate *in vitro* cellular models, as well as more relevant *in vivo* animal challenge systems. Here, we review the current concepts of the immediate events that follow viral exposure at genital mucosal sites where most documented transmissions occur. Furthermore, we discuss the gaps in our knowledge that are relevant to future studies, which will shape strategies for effective HIV prevention.

Introduction

HIV vaccines and microbicides hold promise of preventing the acquisition of HIV-1 and HIV-2, the two viruses that cause AIDS, but the success of designing such agents necessitates a clear understanding of where HIV first encounters its target cells — primarily T cells, macrophages and dendritic cells (DCs) — and how it gains entry at various sites to eventually establish infection. HIV infection has rapidly spread since the early 1980's to become an epidemic that is maintained by sexual transmission through the lower genital and rectal mucosa, and it is these routes of infection that account for the vast majority of current and new infections (Figure 1, Table 1)[1]. Here, we have endeavored to synthesize the current knowledge on the acquisition of HIV at these mucosal sites, confining our discussion to the lower genital mucosa. We 1 clearly recognize that other sites of entry, such as blood, placenta and gastrointestinal mucosa, are important considerations but these are beyond the scope of this Review.

Many elegant studies have provided insights into selective aspects of how HIV and simian immunodeficiency virus (SIV) enter the mucosa through detailed examination of the relevant tissues and target cells following *in vivo*, *ex vivo* and *in vitro* virus exposure. We have organized the discussion in this Review based on the experimental system used from the same mucosal source to alleviate confusion and inconsistencies that often emerge when findings from one system are extrapolated to those of another. When appropriate, we have emphasized the

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benefits and limitations of the experimental approaches, important considerations in the interpretation of findings and their relevance for future studies.

HIV invasion in the female genital tract

Anatomical sites

An estimated 30–40% of all new HIV-1 infections occur in women through vaginal intercourse, which carries a lower HIV transmission probability per exposure event than anal intercourse or parenteral inoculation (Table 1). Although HIV-1 can infect the vaginal, ectocervical and endocervical mucosa (Figure 1), the relative contribution of each site to the establishment of the initial infection is not known. The multilayered squamous epithelium that covers the vagina and ectocervix, when intact, provides better mechanical protection against HIV invasion than the single-layer columnar epithelium that lines the endocervix. However, the greater surface area of the vaginal wall and ectocervix, which often exceeds 15 times that of the endocervix, provides more potential access for HIV entry, particularly when breaches occur in the epithelial-cell layer. HIV or SIV can establish an initial infection solely in the vagina, as shown in women who lack a uterus at birth[2] and in female macaques after surgical removal of the uterus[3]. In fact, selective transmission of HIV through the vaginal mucosa rather than the cervix may commonly occur, as suggested by a recent large randomized, controlled prevention clinical trial in African women. In this study, no significant reduction in HIV-1 acquisition occurred in women using a diaphragm compared with the control group[4]. However, the observed potential benefit of blocking HIV-1 exposure to the cervix may have been undermined because the sexual partners reported lower condom usage in the diaphragm intervention group than the control group.

The region where the ectocervix transforms into the endocervix (Figure 1) can be enriched with $CD4^+$ T cells and may be a particularly susceptible site of HIV entry. Whether HIV can cross the endocervical mucus plug, reach the uterine cavity and invade through the mucosa of the upper genital tract has not been well examined. In principle, uterine tissue is susceptible to infection if directly inoculated with HIV[5]. Uterine simian–HIV (SHIV) infection has been shown in one monkey following vaginal inoculation two days earlier, an interval that is probably too short for stromal or lymphatic spread from the lower genital tract[6]. This indicated that ascent of virus through the endocervical mucus plug may be possible, but confirmation in humans is lacking. Conceivably, the upper mucosal tract may become more vulnerable to HIV-1 penetration during ovulation, a period when rising estrogen levels alter the endocervical mucus to a less viscous and more alkaline consistency.

HIV entry through the female genital epithelium

Both free HIV and SIV virions, and HIV and SIV virions from seminal leukocytes (cell-associated virus) can establish mucosal infection (Figure 2)[7–11]. This has been shown directly *in vivo* in female macaques[12] and in mice[13], and indirectly in humans through genetic sequence comparisons of viral isolates from acutely infected women and from seminal cells and plasma from their infected source partners[14]. *Ex vivo* studies using human cervical explants have also confirmed transmission of cell-free and cell-associated HIV-1[15,16]. Initially, cervical mucus can trap seminal cells or free virions[17,18]. Conceivably, this could facilitate transmission by prolonging mucosal contact time. However, while immobilized, the virions may also become more susceptible to innate antiviral substances.

HIV virions that are initially free or following their release from infected donor cells interact with epithelial cells and traverse the epithelium through several pathways, including transcytosis, endocytosis, or productive infection, or they merely penetrate through gaps between epithelial cells (Figure 2). Understanding these events has been hindered by

inconsistent findings, largely because experimental systems have used epithelial cell types derived from different anatomical sites, and primary and immortalized cell lines. Several reports demonstrate that HIV-1 binds to and enters epithelial cells from the lower female genital tract[19–21]. Transcytosis has been shown to occur in both cell lines and primary cells, but not definitively within intact tissue. Upon release, the virions readily infect susceptible leukocytes[22,23]. Interestingly, cell-associated virions secreted from infected leukocytes appear markedly more efficient in transcytosis than cell-free virions[8,11,22]. Productive infection may also occur within cervical epithelial cells themselves[7], although this remains controversial[19,24]. Conceivably, HIV-1 can also be transported through the cervicovaginal epithelium to the draining lymphatics by donor lymphocytes and macrophages, as has been suggested in mouse studies[7,25].

Our *ex vivo* experiments using sheets of isolated vaginal epithelium, devoid of mucosal stroma, confirmed that the sequestration of HIV-1 virions in endocytic compartments and in the cytosol of epithelial cells occurs (Figure 3a–c). However, although the experimental conditions permitted HIV-1 access to both the luminal and basal sides of the epithelium, the virions were detected exclusively in the basal and suprabasal epithelial cells (F. Hladik, P. Sakchalathorn, M. J. McElrath, unpublished observations). Therefore, rather than entering and traversing superficial epithelial cells in the vagina and ectocervix, HIV-1 probably disperses through the narrow gaps between them[17], as depicted in Figure 2. This route might permit HIV-1 to directly contact and then infect intraepithelial Langerhans cells and $CD4^+$ T cells[26] (see later), or it might allow HIV-1 to reach suprabasal or basal epithelial cells that are susceptible to viral sequestration and transcytosis. Importantly, factors in human semen, most notably amyloid fibrils forming from naturally occurring fragments of seminal prostatic acidic phosphatase, can capture virions and promote attachment to epithelial cells and leukocytes, thus increasing infectivity[27].

Several proteins expressed on the surface of epithelial cells may mediate attachment of HIV-1. Two cell surface glycosphingolipids, sulfated lactosylceramide expressed by vaginal epithelial cells[28] and galactosylceramide expressed by ectocervical epithelial cells[19,29], bind HIV-1 gp120 and foster transcytosis[22]. Interactions of HIV-1 gp120 with transmembrane heparan sulfate proteoglycans (syndecans) expressed by genital epithelial cells can also contribute to HIV-1 attachment and entry[20,23]. Recently, glycoprotein 340, a splice variant of salivary agglutinin expressed by cervical and vaginal epithelial cells, was shown to specifically bind to the HIV envelope protein and to enhance the passage of HIV through the epithelium to susceptible leukocytes[30]. One group found that the β_1 subunit of integrins expressed by explant cervical epithelial cells bound virions that were presumably coated with fibronectin, which is abundant in human semen, although this was not observed across all explants[17]. Detection of HIV-1 chemokine co-receptor expression has been inconsistent: one study did not detect the expression of either CC-chemokine receptor 5 (CCR5) or CXC-chemokine receptor 4 (CXCR4) by cervical epithelial cells[19], another reported the expression of CXCR4 by these cells[21], and another reported the exclusive expression of CCR5[29].

Regardless of the mode, the penetration of virus through the cervicovaginal epithelium *in vivo* occurs rapidly within 30–60 minutes of exposure, as shown in SIV infected macaques[31]. Once within the epithelium, HIV encounters $CD4^+$ T cells as well as Langerhans cells (LCs). LCs have dendrites that extend and retract through the intercellular spaces[32], and even reach up to the epithelium surface[33] where HIV can bind directly to these cells (T. Hope, personal communication). Based on observations of gut dendritic cells[34,35], this could be particularly true for DCs that are located just beneath the endocervical columnar epithelium. However, direct sampling of luminal pathogens by endocervical DCs or vaginal LCs, which could be exploited by HIV to bypass the epithelial-cell barrier, has not yet been formally demonstrated.

Finally, mechanical microabrasions of the mucosal surface induced by intercourse may allow HIV to directly access target cells, such as DCs, T cells and macrophages, at the basal epithelium and the underlying stroma[36]. Areas above the stromal papillae, where the epithelium is relatively thin and where LCs on the epithelial-cell side (F. Hladik, L. Ballweber, M. J. McElrath, unpublished observations) and T cells and macrophages on the stromal side[29] congregate, appear particularly vulnerable to viral invasion. Consistent with this notion, *in vivo* SIV infection of the genital mucosa of macaques is initially established in a highly focal manner, and continuous seeding from this nidus of infection is crucial for establishing systemic infection[18]. Similarly, chemical microabrasions with the use of certain topical microbicides and microabrasions due to genital ulcers caused by sexually transmitted diseases (for example, syphilis, chancroid and those caused by infection by *Herpes simplex virus*) are also likely to expose vulnerable target cells in the basal epithelium and stroma[37].

Importance of cervicovaginal LCs in HIV invasion

LCs are a DC subtype residing within the outer squamous epithelium of the skin or mucosae. For many years, HIV-1 acquisition in the lower genital tract has been assumed to occur through internalization of HIV-1 by LCs. This view was supported by evidence that skin LCs are susceptible to infection by HIV-1[38–41] and that genital mucosal LCs harbour SIV virions within 24 hours of intravaginal inoculation of macaques[31]. However, soon after *ex vivo* organ culture, LCs migrate out of the epithelium[15,24,42–44]; therefore, examination of LC infection specifically within the human vaginal epithelium has been technically difficult. For example, one landmark study demonstrated that after exposure of human complete cervical mucosa to HIV-1, emigrating DCs had efficiently captured HIV and were capable of transmitting the virus *in trans*[43]. However, determining whether the cells originated from the epithelium as LCs or the underlying stroma as DCs was impossible. More recently, we resolved this issue by preparing sheets of vaginal epithelium separated from the underlying stroma, and observed that vaginal LCs efficiently internalized HIV-1 into their cytoplasmic compartments[26]. As LCs exit the epithelium at the basal side, they transport intact virions, thereby enabling infection to spread beyond the site of viral entry (Figure 2).

The ability of LCs in the cervicovaginal epithelium to produce and release new HIV-1 virions is uncertain. HIV-specific receptors are expressed by these LCs, including CD4, CCR5 and the C-type lectin langerin (CD207), but not CXCR4 and dendritic-cell-specific ICAM3-grabbing non-integrin (DC-SIGN, CD209)[26,45–48]. Antibodies that bind CD4 and CCR5 partially block the uptake of R5-tropic HIV-1 by LCs, but blocking the binding of C-type lectin to mannan on HIV-1 had little effect on uptake[26]. Although low-level CD4- and CCR5-mediated productive infection of LCs in human skin explants has been shown[40,41,49], we were unable to confirm this finding in our imaging studies of vaginal LCs[26]. Therefore, if *de novo* production of virions occurs, it appears relatively inefficient in contrast to the high capacity of vaginal LCs to endocytose HIV-1. Nevertheless, even low levels of productive HIV-1 infection of cutaneous DCs and LCs lead to profound viral replication in co-cultured T cells[41,49,50]. Therefore, future investigations must conclusively determine if LCs in cervicovaginal epithelium can support productive HIV infection *in vivo* and if this property is required for the passage of the virus to T cells, as has been reported for other types of DCs[51–54].

The relative inefficiency of mannan, a mannose polymer, to block binding and endocytosis of HIV-1 by vaginal LCs was surprising[26], because C-type lectins, which recognize mannose containing carbohydrate structures, mediate viral entry in other types of DCs[47]. However, HIV-1 can bind DC subsets independent of C-type lectins and CCR5[55–57]. So, although HIV is efficiently captured by langerin expressed by epidermal LCs[58], HIV appears to largely bypass langerin expressed by vaginal LCs in favour of alternative endocytic routes. This

distinction may be highly relevant for mucosal HIV transmission. Langerin expressed by epidermal LCs can direct HIV-1 to Birbeck granules for degradation[58]. By contrast, by gaining entry to the vaginal LCs independently of langerin, HIV may survive by reaching endocytic compartments, such as early phagosomes, where antigens are preserved for cross-presentation[59]. This is consistent with our observations that intact virions were still present in LCs that had migrated out of the vaginal epithelium at 60 hours after viral challenge[26]. Thus, it appears that HIV-1 enters vaginal LCs through a different route than skin LCs, resulting in a distinct fate of the endocytosed virions. More detailed studies are now warranted to uncover which endocytic pathways HIV uses in vaginal LCs, and how this can be harnessed therapeutically.

Infection of DCs in the cervicovaginal stroma

Unlike genital LCs, stromal DCs express both DC-SIGN[47,48] and CCR5[60,61] and have been implicated in SIV and HIV infection, but their exact role in mucosal transmission is not clear. *In situ* studies in the human explant model have failed to identify DCs in the cervicovaginal stroma as foci for productive HIV infection[15,24,42,44]. By contrast, SIV-infected DCs were present in the lamina propria of the cervicovaginal mucosa of macaques shortly after intravaginal SIV challenge[31,62], as well as in chronically infected animals[63]. Likewise, HIV-infected DCs were identified in tissue biopsies of the vaginal stroma of asymptomatic HIV-1 infected women[64]. The failure to reveal infection of stromal DCs in the human explant models may have been due to the relatively low sensitivity of the detection methods employed and the migration of stromal DCs from the tissue, which may drastically decrease the number of infected cells *in situ* over time. Indeed, when DCs were harvested from the culture supernatants of human cervical explants that were challenged with HIV-1, significant *in trans* infectivity was detected[43] and massive budding of virions was observed among emigrant DCs five days after virus exposure[61] (Figure 4b–d). However, it could not be determined if the original source of DCs was from the epithelium proper or from the stromal tissue. In addition, inferring the initial susceptibility of DCs while confined to the mucosal stroma, based on findings from emigrated DCs that undergo phenotypic changes as they exit the mucosa, may be less reliable. Thus, stromal DCs exhibit different HIV-1 receptor expression patterns than LCs, potentially permitting different HIV-1 entry pathways than in the epithelial LCs. Much still remains to be learned about stromal DCs in the human genital mucosa in general, about whether different subsets exist similar to those found in skin dermis[65], and about the interaction of these DC subsets with HIV in particular. More sensitive *in situ* detection methods of HIV infection, as well as assays distinguishing *de novo* virus production from endocytically engulfed virions, as recently reported[66], will be helpful in sorting out the contribution of stromal DCs to HIV-1 propagation.

HIV infection of cervicovaginal CD4$^+$ T cells

CD4$^+$ T cells are dispersed throughout the lamina propria of the human vagina, ectocervix and endocervix, often clustering just beneath the basal membrane[67,68]. They also reside at variable numbers within the vaginal and ectocervical squamous epithelium[67,68]. The majority are memory T cells that express higher levels of CCR5 than T cells that circulate in peripheral blood[26,69–71]. One day post HIV-1 inoculation of vaginal, ectocervical and endocervical tissue cultures, infected CD4$^+$ T cells were shown to be confined to the mucosal stroma[15,17,24,43]. This result was surprising, given the presence of CCR5$^+$ CD4$^+$ T cells within the squamous epithelium. However, by analyzing the fate of fluorescence-tagged virions as early as two hours after viral exposure, we observed that R5-tropic HIV-1 bound to intraepithelial vaginal CD4$^+$ T cells very efficiently, followed by fusion and productive infection[26]. Therefore, infected T cells must rapidly leave the epithelium, and those found in the stroma may be the same or early progeny of intraepithelial T cells.

Findings in the human explant studies show that HIV-1 very effectively targets $CD4^+$ T cells in the genital mucosa for productive infection[15,26,43], and that the initial infection of intraepithelial $CD4^+$ T cells is probably independent of LCs[26]. The central role for genital $CD4^+$ T cells in early infection and propagation is also evident from SIV challenge experiments in macaques[63,72,73]. Interestingly, not only does SIV productively infect activated T cells, characterized by HLA-DR and Ki67 expression, but also T cells that are in the HLA-DR- and Ki67-negative resting state[72]. Consistent with this finding, we observed binding of HIV-1 to both HLA-DR^+ and HLA-DR^- intraepithelial T cells in our human vaginal explant model (M. J. McElrath, P. Sakchalathorn, L. Ballweber, F. Hladik, unpublished observations). In addition, the contribution of resting $CD4^+$ T cells to viral production is substantial during the very earliest stages of infection[74]. The fact that vaginal $CD4^+$ T cells are rapidly depleted following intravenous SIV inoculation of macaques[6,73], similar to that observed in $CD4^+$ T cells of the gut during acute SIV infection[75], further illustrates their high susceptibility to infection *in vivo*.

Other leukocyte targets for HIV in the female genital tract

Macrophages in the female genital mucosa are also susceptible targets for early HIV-1 infection, as demonstrated in studies using human explant models[24,42,44], and in two reports were the major cell type infected by R5-tropic HIV-1[24,44]. Whether or not resident macrophages in the female genital tract constitutively express CCR5 *in situ* is not known, but most macrophages do so when harvested from supernatants of vaginal organ cultures[61], suggesting that the expression of CCR5 by macrophages may occur during the period of activation and emigration from the mucosa[76]. By contrast, SIV-infected macrophages in genital tissues were either rare[72], or undetectable[31,62]. Likewise, macrophages in the human intestinal mucosa were reported to lack CCR5 expression and to possess low permissibility for HIV-1 infection[77]. These discrepancies illustrate a potential limitation of organ cultures. If indeed explantation activates stromal macrophages and as a consequence increases surface CCR5 expression, this would lead to an overestimation of their susceptibility to infection *in vivo*. Of note, in addition to chemokine receptor-mediated fusion, monocyte-derived macrophages can also trap intact virions through syndecans[78], or even without specific envelope-receptor interactions through a process known as macropinocytosis[79]. Once captured, HIV-1 can be archived for several days and then transmitted to T cells *in trans*[80,81]. If genital macrophages similarly archive infectious virions, their role in viral propagation once HIV-1 invades the stroma may be significant.

Other leukocyte subpopulations also interact with HIV. For example, productive infection of natural killer (NK) cells has been reported[82], as well as transmission of virus through DC-SIGN expressed by B cells to T cells *in trans*[83]. The significance of B cells for HIV invasion in the genital mucosa remains unknown. Lastly, monocyte precursor cells enter the mouse dermis in large numbers following intracutaneous infection with *Leishmania major* and differentiate into stromal DCs[84]. This finding raises the possibility that the influx of inflammatory cells into the genital tract following HIV-1 exposure may create new potential target cells that normally do not reside in the mucosa, and further heighten the initial local infection.

The role of DCs in enhancement of infection

A growing body of literature suggests that HIV exploits DCs to enhance its infectivity of T cells[85]. First reported in 1992, DCs, even when seemingly uninfected themselves, invoked vigorous cytopathic infection to $CD4^+$ T cells[86]. The potential relevance of these findings for the transmission of HIV at mucosal sites was subsequently highlighted by reports of increased HIV-1 replication in DC–T-cell conjugates derived from human skin[87] and cervicovaginal

mucosa[61]. The enhancement of HIV transmission by DCs probably occurred by facilitating T-cell activation[72,88] as well as *de novo* T-cell infection.

Four mechanisms for how DCs can augment *de novo* infection of T cells have been proposed. In classic HIV *trans* infection[47], the DCs are not productively infected but trap and preserve the virus, which is subsequently transferred to T cells across an "infectious synapse", which is a zone of DC–T-cell contact where HIV itself and the HIV receptors are concentrated[89,90]. Alternatively, *trans* infection may occur by HIV association with DC-derived exosomes, which, intriguingly, appear to markedly increase the infectivity of virions that are coupled to them[91]. In a third pathway, productively infected DCs transmit new viral progeny across the infectious synapse to T cells[49,52–54]. In this case, the contact zone has also been termed a "virological synapse", to signify that the donor cell, in analogy to cell-associated HIV transmission between $CD4^+$ T cells[92], is productively infected[93]. Furthermore, efficient retroviral transfer between cells has recently been described, in which retroviruses, including HIV-1, "surf" along the outer surface of filopodia or cytonemes that extend from an uninfected cell and interact through their tips with an infected cell[94]. These narrow filopodial contact zones may be special cases of virological synapses[95], or may be analogous to nanotubules that are formed between immune cells[96]. In T cells, migration of HIV-1 also occurs within nanotubules[130]. Nanotubules have been shown to functionally connect DCs with other cells[96], but their significance for viral transfer from DCs remains to be determined.

Clear evidence for any of these described modes of viral transmission from DCs to $CD4^+$ T cells in the genital mucosa is still lacking. We have shown that HIV concentrates along the cell–cell junction between emigrant LC–T-cell conjugates from human vaginal epithelium, supporting the formation of an infectious synapse[26]. No consensus has been reached over whether *in trans* infection occurs primarily from surface bound virions[47,97], internalized virions[98] or both[57]. At any rate, once HIV makes its way into the genital DC–T-cell conjugate, a profound productive infection ensues[61]. Of note, in these conjugates viral budding was not only observed from the surface of T cells but also of the DCs. Visualization of budding by electron microscopy signifies massive infection. So, in DC–T-cell conjugates not only T cells but also DCs, which by themselves are generally weak producers of virus[85], acquire the ability to produce large amounts of viral progeny. This is consistent with findings obtained in co-cultures of monocyte-derived DCs and T cells[99]. Thus, DC–T-cell cross-talk in the genital tract appears to drive productive HIV infection in both cell types. Moreover, transmission from DCs to T cells may provide a means for HIV to avoid antibody-mediated neutralization[100–102]. Taken together, unravelling the precise mechanisms by which the interaction between genital DCs and $CD4^+$ T cells enhance HIV transmission will be important in developing effective strategies to counteract this process.

HIV invasion in the male genital tract

Of the nearly 15 million infected men, an estimated 70–75% acquired HIV-1 through vaginal intercourse (Table 1), making the male genital tract the second leading site of HIV invasion following the cervicovaginal mucosa. HIV-1 target cells are abundant in the foreskin, and include $CD1a^+$ LCs and $CD4^+$ T cells in the squamous lining, as well as T cells, macrophages and DCs in the underlying stroma[46,103–105]. Variable fractions of these cells express CD4, CCR5 and CXCR4[46,103,104]. As in the vagina, LCs do not express DC-SIGN, whereas at least some of the stromal DCs do[103]. Therefore, similar mechanisms that have been described for viral invasion into the female genital tract above are likely to occur in the male genital tract as well.

The highly protective effect of circumcision[106,107], which has been reviewed comprehensively elsewhere[108], suggests that the penile foreskin is particularly vulnerable to

HIV infection. The foreskin is lined by stratified squamous epithelium, and the external surface is more heavily keratinized than the internal surface[103,104]. Consequently, the inner foreskin may be more susceptible to infection, a view that was supported by investigations of penile autopsy tissues[103], and corroborated in an *in vitro* foreskin explant model that showed infectious foci, predominantly containing LCs and $CD4^+$ T cells, at the base of the epithelial-cell layer, exclusively in the inner foreskin[104]. Alternatively, circumcision may reduce the risk for genital tears, abrasions and ulcer disease, resulting in decreased HIV infection risk[108,109].

Although circumcision appears to provide a protective effect against HIV infection, entry sites other than the foreskin must exist for circumcised males. The glans penis has a heavily keratinized squamous epithelium similar to the outer foreskin, and effective viral penetration seems relatively improbable here. By contrast, the penile urethra is a more likely candidate as it is lined by a narrowly stratified, non-keratinized columnar epithelium and contains high numbers of $CD4^+$ and $CD8^+$ T cells, and macrophages, within the epithelium and the lamina propria[110]. Interestingly, DCs are not observed in the urethral mucosa[46,110]. The presence of CCR5 and CXCR4 mRNA in urethral swabs indicates that these HIV-1 co-receptors may be expressed by urethral cells[111], particularly the predominant $CD4^+CD45RO^+$ memory T cells[110]. Of note, intraurethral infusion of SIV resulted in infection of all six inoculated male macaques[112]. In HIV-1 infected men, antibiotic treatment of urethral *Neisseria gonorrhoeae* infection reduces HIV-1 shedding in semen but not the viral load in blood[113]. This finding, as well as HIV-1 shedding in the ejaculate of vasectomized HIV-positive men[114] and in the pre-ejaculatory fluid[115,116] suggest a distal source for virus, pointing to the urethra as a site harboring significant numbers of cells susceptible to HIV-1 infection. Taken together, these data suggest that $CD4^+$ T cells and macrophages in the male urethra provide a suitable portal of entry for HIV, although the specific events of urethral invasion are still unknown.

Conclusions

Although studies that focus on mucosal HIV infection continue to be painstaking, many investigators have overcome some of the technical difficulties that have previously precluded active research in this area. Recent investigations summarized here provide invaluable insights into the distinct cellular and molecular interactions of mucosal HIV infection. Clearly, the challenge emerging from these findings is to counteract the rapid acceleration of infection in local reservoirs of the lower genital and gastrointestinal tracts. Two key questions to address in future studies lie in determining whether DC–T-cell interactions that markedly amplify HIV-1 production, which are consistently observed *in vitro*, are relevant in the mucosal epithelium *in vivo*, and whether common mechanisms of HIV-1 entry apply to both the lower genital and gastrointestinal tract. This information can guide the development of innovative strategies to protect susceptible target cells from HIV-1 infection in both women and men, such as with barrier protection methods, topical microbicides and mucosal immunization.

Although there are an increasing number of large-scale HIV prevention clinical trials that have recently reported a lack of efficacy, one favourable approach has been male circumcision. The opportunity to gain a more thorough understanding of how HIV-1 invades its target cells in the human foreskin should be exploited. The recent emphasis of $CD4^+$ T-cell depletion by HIV-1 in the gut and vagina during acute infection has sparked a renewed interest in abating infection at the sites where massive replication occurs[117]. Although important in counteracting HIV disease, $CD4^+$ T-cell depletion appears to be a secondary event that commonly occurs through various routes of transmission. Therefore, the best opportunity to prevent HIV disease clearly lies at the sites of mucosal entry, and investigations to directly counterattack HIV infection must continue to focus on these portals.

Box 1. HIV invasion in the lower gastrointestinal tract

HIV-1 and SIV infection commonly targets the lower gastrointestinal tract as an initial site following receptive anal intercourse in humans and direct inoculation in macaques, and as a secondary infection site following rapid dissemination from mucosal foci[18] or acute systemic infection[118–120]. The rectal mucosa contains simple columnar epithelial cells, and the lamina propria is a rich source of lymphoid cells and lymphoid nodules. Numerous reports have documented the pathogenic effect of the virus in the gastrointestinal tract, and have shown a severe depletion of $CD4^+$ T cells that express CCR5 in the gut, regardless of the route of infection[117]. The relevant target cells for infection in the lower gastrointestinal tract are thus likely to be primarily $CD4^+$ memory T cells[121]. At present, detailed features of HIV-1 entry into the lower gastrointestinal tract that may be distinct from the genital tract have not been elucidated, but this remains an important question to address for prevention strategies.

References

1. 2007 AIDS epidemic update. UNAIDS/WHO; Geneva:
2. Kell PD, Barton SE, Edmonds DK, Boag FC. HIV infection in a patient with Meyer-Rokitansky-Kuster-Hauser syndrome. J R Soc Med 1992;85:706–707. [PubMed: 1474562]
3. Miller CJ, Alexander NJ, Vogel P, Anderson J, Marx PA. Mechanism of genital transmission of SIV: a hypothesis based on transmission studies and the location of SIV in the genital tract of chronically infected female rhesus macaques. J Med Primatol 1992;21:64–68. [PubMed: 1433268]
4. Padian NS, et al. Diaphragm and lubricant gel for prevention of HIV acquisition in southern African women: a randomised controlled trial. Lancet. 2007
5. Howell AL, et al. Human immunodeficiency virus type 1 infection of cells and tissues from the upper and lower human female reproductive tract. J Virol 1997;71:3498–3506. [PubMed: 9094621]
6. Joag SV, et al. Animal model of mucosally transmitted human immunodeficiency virus type 1 disease: intravaginal and oral deposition of simian/human immunodeficiency virus in macaques results in systemic infection, elimination of CD4+ T cells, and AIDS. J Virol 1997;71:4016–4023. [PubMed: 9094679]
7. Phillips DM, Tan X, Perotti ME, Zacharopoulos VR. Mechanism of monocyte-macrophage-mediated transmission of HIV. AIDS Res Hum Retroviruses 1998;14(Suppl 1):S67–70. [PubMed: 9581887]
8. Alfsen A, Yu H, Magerus-Chatinet A, Schmitt A, Bomsel M. HIV-1-infected blood mononuclear cells form an integrin- and agrin-dependent viral synapse to induce efficient HIV-1 transcytosis across epithelial cell monolayer. Mol Biol Cell 2005;16:4267–4279. [PubMed: 15975901]
9. Sourisseau M, Sol-Foulon N, Porrot F, Blanchet F, Schwartz O. Inefficient human immunodeficiency virus replication in mobile lymphocytes. J Virol 2007;81:1000–1012. [PubMed: 17079292]
10. Muratori C, et al. Macrophages Transmit Human Immunodeficiency Virus Type 1 Products to CD4-Negative Cells: Involvement of Matrix Metalloproteinase 9. J Virol 2007;81:9078–9087. [PubMed: 17581988]
11. Van Herrewege Y, et al. A dual chamber model of female cervical mucosa for the study of HIV transmission and for the evaluation of candidate HIV microbicides. Antiviral Res 2007;74:111–124. [PubMed: 17097156]
12. Kaizu M, et al. 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]
13. Khanna KV, et al. 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]
14. Zhu T, et al. 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]

15. Gupta P, et al. 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]
16. Zussman A, Lara L, Lara HH, Bentwich Z, Borkow G. Blocking of cell-free and cell-associated HIV-1 transmission through human cervix organ culture with UC781. AIDS 2003;17:653–661. [PubMed: 12646787]
17. Maher D, Wu X, Schacker T, Horbul J, Southern P. HIV binding, penetration, and primary infection in human cervicovaginal tissue. Proc Natl Acad Sci U S A 2005;102:11504–11509. [PubMed: 16061810]
18. Miller CJ, et al. Propagation and dissemination of infection after vaginal transmission of simian immunodeficiency virus. J Virol 2005;79:9217–9227. [PubMed: 15994816]
19. Dezzutti CS, et al. 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]
20. 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]
21. Berlier W, et al. 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]
22. Bomsel M. Transcytosis of infectious human immunodeficiency virus across a tight human epithelial cell line barrier. Nature Med 1997;3:42–47. [PubMed: 8986739]This study introduced the concept of HIV-1 transcytosis, a process by which virions are transported intact through the interior of epithelial cells, retaining their infectivity
23. Bobardt MD, et al. Cell-free human immunodeficiency virus type 1 transcytosis through primary genital epithelial cells. J Virol 2007;81:395–405. [PubMed: 17050597]
24. Greenhead P, et al. Parameters of human immunodeficiency virus infection of human cervical tissue and inhibition by vaginal virucides. J Virol 2000;74:5577–5586. [PubMed: 10823865]
25. Ibata B, Parr EL, King NJ, Parr MB. Migration of foreign lymphocytes from the mouse vagina into the cervicovaginal mucosa and to the iliac lymph nodes. Biol Reprod 1997;56:537–543. [PubMed: 9116158]
26. Hladik F, et al. Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. Immunity 2007;26:257–270. [PubMed: 17306567]This investigation found that HIV-1 rapidly penetrates intraepithelial vaginal $CD1a^{+}$ Langerhans cells and $CD4^{+}$ T cells, and that viral fusion predominates in T cells and viral endocytosis in LCs
27. Munch J, et al. Semen-Derived Amyloid Fibrils Drastically Enhance HIV Infection. Cell 2007;131:1059–1071. [PubMed: 18083097]The authors discovered that amyloidogenic fragments of prostatic acidic phosphatase in semen can serve as strong enhancing factors for HIV infection
28. Furuta Y, et al. Infection of vaginal and colonic epithelial cells by the human immunodeficiency virus type 1 is neutralized by antibodies raised against conserved epitopes in the envelope glycoprotein gp120. Proc Natl Acad Sci U S A 1994;91:12559–12563. [PubMed: 7809077]
29. Yeaman GR, et al. Chemokine receptor expression in the human ectocervix: implications for infection by the human immunodeficiency virus-type I. Immunology 2004;113:524–533. [PubMed: 15554931]
30. Stoddard E, et al. gp340 Expressed on Human Genital Epithelia Binds HIV-1 Envelope Protein and Facilitates Viral Transmission. J Immunol 2007;179:3126–3132. [PubMed: 17709527]
31. Hu J, Gardner MB, Miller CJ. Simian immunodeficiency virus rapidly penetrates the cervicovaginal mucosa after intravaginal inoculation and infects intraepithelial dendritic cells. J Virol 2000;74:6087–6095. [PubMed: 10846092]
32. Nishibu A, et al. Behavioral responses of epidermal Langerhans cells in situ to local pathological stimuli. J Invest Dermatol 2006;126:787–796. [PubMed: 16439974]
33. Miller CJ, McChesney M, Moore PF. Langerhans cells, macrophages and lymphocyte subsets in the cervix and vagina of rhesus macaques. Lab Invest 1992;67:628–634. [PubMed: 1434540]

34. Rescigno M, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nature Immunol 2001;2:361–367. [PubMed: 11276208]
35. Niess JH, et al. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. Science 2005;307:254–258. [PubMed: 15653504]
36. Norvell MK, Benrubi GI, Thompson RJ. Investigation of microtrauma after sexual intercourse. J Reprod Med 1984;29:269–271. [PubMed: 6716372]
37. Weiler AM, et al. Genital ulcers facilitate rapid viral entry and dissemination following intravaginal inoculation with cell-associated SIVmac239. J Virol. 2008
38. Tschachler E, et al. Epidermal Langerhans cells--a target for HTLV-III/LAV infection. J Invest Dermatol 1987;88:233–237. [PubMed: 3100656]
39. Zaitseva M, et al. Expression and function of CCR5 and CXCR4 on human Langerhans cells and macrophages: implications for HIV primary infection. Nature Med 1997;3:1369–1375. [PubMed: 9396607]
40. Dittmar MT, et al. Langerhans cell tropism of human immunodeficiency virus type 1 subtype A through F isolates derived from different transmission groups. J Virol 1997;71:8008–8013. [PubMed: 9311896]
41. Kawamura T, et al. Candidate microbicides block HIV-1 infection of human immature Langerhans cells within epithelial tissue explants. J Exp Med 2000;192:1491–1500. [PubMed: 11085750]
42. Collins KB, Patterson BK, Naus GJ, Landers DV, Gupta P. Development of an in vitro organ culture model to study transmission of HIV-1 in the female genital tract. Nature Med 2000;6:475–479. [PubMed: 10742159]
43. Hu Q, et al. Blockade of attachment and fusion receptors inhibits HIV-1 infection of human cervical tissue. J Exp Med 2004;199:1065–1075. [PubMed: 15078900]This investigation clearly demonstrates that DCs migrating from HIV-1 exposed cervical tissue can efficiently disseminate virus. Inhibition of this pathway can occur only by simultaneous blockade of CD4 and mannose-binding C type lectin receptors
44. Cummins JE Jr, et al. 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]
45. Turville SG, et al. Diversity of receptors binding HIV on dendritic cell subsets. Nature Immunol 2002;3:975–983. [PubMed: 12352970]
46. Hussain LA, Lehner T. Comparative investigation of Langerhans' cells and potential receptors for HIV in oral, genitourinary and rectal epithelia. Immunology 1995;85:475–484. [PubMed: 7558138]
47. Geijtenbeek TB, et al. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. Cell 2000;100:587–597. [PubMed: 10721995]In this report, DC-SIGN (CD209) is identified as an HIV-1 receptor on DCs and promotes efficient infection in trans of $CD4^+$ T cells
48. Jameson B, et al. Expression of DC-SIGN by dendritic cells of intestinal and genital mucosae in humans and rhesus macaques. J Virol 2002;76:1866–1875. [PubMed: 11799181]
49. Kawamura T, et al. Significant Virus Replication in Langerhans Cells following Application of HIV to Abraded Skin: Relevance to Occupational Transmission of HIV. J Immunol 2008;180:3297–3304. [PubMed: 18292554]
50. Pope M, Gezelter S, Gallo N, Hoffman L, Steinman RM. Low levels of HIV-1 infection in cutaneous dendritic cells promote extensive viral replication upon binding to memory CD4+ T cells. J Exp Med 1995;182:2045–2056. [PubMed: 7500050]
51. Cameron PU, et al. Preferential infection of dendritic cells during human immunodeficiency virus type 1 infection of blood leukocytes. J Virol 2007;81:2297–2306. [PubMed: 17166903]
52. Nobile C, et al. 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]
53. Burleigh L, et al. 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]
54. Turville SG, et al. Immunodeficiency virus uptake, turnover, and 2-phase transfer in human dendritic cells. Blood 2004;103:2170–2179. [PubMed: 14630806]

55. Gummuluru S, Rogel M, Stamatatos L, Emerman M. Binding of human immunodeficiency virus type 1 to immature dendritic cells can occur independently of DC-SIGN and mannose binding C-type lectin receptors via a cholesterol-dependent pathway. J Virol 2003;77:12865–12874. [PubMed: 14610207]
56. Boggiano C, Manel N, Littman DR. Dendritic cell-mediated trans-enhancement of human immunodeficiency virus type 1 infectivity is independent of DC-SIGN. J Virol 2007;81:2519–2523. [PubMed: 17182696]
57. Wang JH, Janas AM, Olson WJ, Wu L. Functionally distinct transmission of human immunodeficiency virus type 1 mediated by immature and mature dendritic cells. J Virol 2007;81:8933–8943. [PubMed: 17567699]
58. de Witte L, et al. Langerin is a natural barrier to HIV-1 transmission by Langerhans cells. Nature Med 2007;13:367–371. [PubMed: 17334373]This study showed that HIV-1 captured by the C type lectin receptor langerin (CD207) was internalized into Birbeck granules and degraded, thus preventing HIV-1 transmission by skin LCs
59. Savina A, et al. NOX2 controls phagosomal pH to regulate antigen processing during crosspresentation by dendritic cells. Cell 2006;126:205–218. [PubMed: 16839887]
60. Prakash M, Kapembwa MS, Gotch F, Patterson S. Chemokine receptor expression on mucosal dendritic cells from the endocervix of healthy women. J Infect Dis 2004;190:246–250. [PubMed: 15216457]
61. Hladik F, et al. Dendritic cell-T-cell interactions support coreceptor-independent human immunodeficiency virus type 1 transmission in the human genital tract. J Virol 1999;73:5833–5842. [PubMed: 10364335]This is the first reported observation that DCs isolated from the vaginal mucosa internalize HIV-1 into cytoplasmic endosomes and produce new virions that bud from the cell membrane
62. Spira AI, et al. Cellular targets of infection and route of viral dissemination after an intravaginal inoculation of simian immunodeficiency virus into rhesus macaques. J Exp Med 1996;183:215–225. [PubMed: 8551225]Intravaginal inoculation of macaques with simian immunodeficiency virus (SIV) led to infection of stromal dendritic cells and cells in the draining lymph nodes within two days
63. Hu J, Pope M, Brown C, O'Doherty U, Miller CJ. Immunophenotypic characterization of simian immunodeficiency virus- infected dendritic cells in cervix, vagina, and draining lymph nodes of rhesus monkeys. Lab Invest 1998;78:435–451. [PubMed: 9564888]
64. Bhoopat L, et al. In vivo identification of Langerhans and related dendritic cells infected with HIV-1 subtype E in vaginal mucosa of asymptomatic patients. Mod Pathol 2001;14:1263–1269. [PubMed: 11743049]
65. Nestle FO, Nickoloff BJ. Deepening our understanding of immune sentinels in the skin. J Clin Invest 2007;117:2382–2385. [PubMed: 17786233]
66. Turville SG, Aravantinou M, Stossel H, Romani N, Robbiani M. Resolution of de novo HIV production and trafficking in immature dendritic cells. Nature Methods 2008;5:75–85. [PubMed: 18059278]
67. Johansson EL, Rudin A, Wassen L, Holmgren J. Distribution of lymphocytes and adhesion molecules in human cervix and vagina. Immunology 1999;96:272–277. [PubMed: 10233705]
68. Edwards JN, Morris HB. Langerhans' cells and lymphocyte subsets in the female genital tract. Br J Obstet Gynaecol 1985;92:974–982. [PubMed: 2931100]
69. Hladik F, Lentz G, Delpit E, McElroy A, McElrath MJ. Coexpression of CCR5 and IL-2 in human genital but not blood T cells: implications for the ontogeny of the CCR5+ Th1 phenotype. J Immunol 1999;163:2306–2313. [PubMed: 10438976]
70. Zhang L, et al. In vivo distribution of the human immunodeficiency virus/simian immunodeficiency virus coreceptors: CXCR4, CCR3, and CCR5. J Virol 1998;72:5035–5045. [PubMed: 9573273]
71. Prakash M, Kapembwa MS, Gotch F, Patterson S. Higher levels of activation markers and chemokine receptors on T lymphocytes in the cervix than peripheral blood of normal healthy women. J Reprod Immunol 2001;52:101–111. [PubMed: 11600181]
72. Zhang Z, et al. Sexual transmission and propagation of SIV and HIV in resting and activated CD4+ T cells. Science 1999;286:1353–1357. [PubMed: 10558989]The authors identified $CD4^+$ T cells in

the rhesus macaque genital mucosa as the predominant targets for SIV infection, and they noted that both activated and resting T cells propagate virus

73. Veazey RS, Marx PA, Lackner AA. Vaginal CD4+ T cells express high levels of CCR5 and are rapidly depleted in simian immunodeficiency virus infection. J Infect Dis 2003;187:769–776. [PubMed: 12599050]
74. Zhang ZQ, et al. Roles of substrate availability and infection of resting and activated CD4+ T cells in transmission and acute simian immunodeficiency virus infection. Proc Natl Acad Sci U S A 2004;101:5640–5645. [PubMed: 15064398]
75. Picker LJ, Watkins DI. HIV pathogenesis: the first cut is the deepest. Nature Immunol 2005;6:430–432. [PubMed: 15843796]
76. Tuttle DL, Harrison JK, Anders C, Sleasman JW, Goodenow MM. Expression of CCR5 increases during monocyte differentiation and directly mediates macrophage susceptibility to infection by human immunodeficiency virus type 1. J Virol 1998;72:4962–4969. [PubMed: 9573265]
77. Meng G, et al. Lamina propria lymphocytes, not macrophages, express CCR5 and CXCR4 and are the likely target cell for human immunodeficiency virus type 1 in the intestinal mucosa. J Infect Dis 2000;182:785–791. [PubMed: 10950772]
78. Saphire AC, Bobardt MD, Zhang Z, David G, Gallay PA. Syndecans serve as attachment receptors for human immunodeficiency virus type 1 on macrophages. J Virol 2001;75:9187–9200. [PubMed: 11533182]
79. Marechal V, et al. Human immunodeficiency virus type 1 entry into macrophages mediated by macropinocytosis. J Virol 2001;75:11166–11177. [PubMed: 11602756]
80. Sharova N, Swingler C, Sharkey M, Stevenson M. Macrophages archive HIV-1 virions for dissemination in trans. EMBO J 2005;24:2481–2489. [PubMed: 15920469]
81. Groot F, Welsch S, Sattentau QJ. Efficient HIV-1 transmission from macrophages to T cells across transient virological synapses. Blood. 2008Epub ahead of print
82. Harada H, Goto Y, Ohno T, Suzu S, Okada S. Proliferative activation up-regulates expression of CD4 and HIV-1 co-receptors on NK cells and induces their infection with HIV-1. Eur J Immunol 2007;37:2148–2155. [PubMed: 17628860]
83. Rappocciolo G, et al. DC-SIGN on B lymphocytes is required for transmission of HIV-1 to T lymphocytes. PLoS Pathog 2006;2:e70. [PubMed: 16839201]
84. Leon B, Lopez-Bravo M, Ardavin C. Monocyte-derived dendritic cells formed at the infection site control the induction of protective T helper 1 responses against Leishmania. Immunity 2007;26:519–531. [PubMed: 17412618]
85. Wu L, KewalRamani VN. Dendritic-cell interactions with HIV: infection and viral dissemination. Nature Rev Immunol 2006;6:859–868. [PubMed: 17063186]
86. Cameron PU, et al. 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]This is the first study demonstrating HIV-1 transmission from DCs to $CD4^+$ T cells
87. Pope M, et al. Conjugates of dendritic cells and memory T lymphocytes from skin facilitate productive infection with HIV-1. Cell 1994;78:389–398. [PubMed: 7914836]This investigation showed that DCs and T cells derived from human epithelium form stable conjugates which can enhance HIV replication
88. Geijtenbeek TB, et al. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. Cell 2000;100:575–585. [PubMed: 10721994]
89. McDonald D, et al. Recruitment of HIV and its receptors to dendritic cell-T cell junctions. Science 2003;300:1295–1297. [PubMed: 12730499]The investigators introduce the concept of an infectious synapse between DCs and T cells to which HIV is recruited on the DC side and CD4 and CCR5 on the T cell side, thus promoting efficient in trans infection
90. Arrighi JF, et al. DC-SIGN-mediated infectious synapse formation enhances X4 HIV-1 transmission from dendritic cells to T cells. J Exp Med 2004;200:1279–1288. [PubMed: 15545354]
91. Wiley RD, Gummuluru S. Immature dendritic cell-derived exosomes can mediate HIV-1 trans infection. Proc Natl Acad Sci U S A 2006;103:738–743. [PubMed: 16407131]

92. Jolly C, Sattentau QJ. Human immunodeficiency virus type 1 assembly, budding, and cell-cell spread in T cells take place in tetraspanin-enriched plasma membrane domains. J Virol 2007;81:7873–7884. [PubMed: 17522207]

93. Jolly C, Sattentau QJ. Retroviral spread by induction of virological synapses. Traffic 2004;5:643–650. [PubMed: 15296489]

94. Sherer NM, et al. Retroviruses can establish filopodial bridges for efficient cell-to-cell transmission. Nature Cell Biol 2007;9:310–315. [PubMed: 17293854]Retroviruses are transmitted between cells not only across large-surface interfaces (infectious synapses) but also through movement along the outer surface of thin filopodial bridges that are extended from the non-infected to the infected cells

95. Hope TJ. Bridging efficient viral infection. Nature Cell Biol 2007;9:243–244. [PubMed: 17330113]

96. Watkins SC, Salter RD. Functional connectivity between immune cells mediated by tunneling nanotubules. Immunity 2005;23:309–318. [PubMed: 16169503]

97. Cavrois M, Neidleman J, Kreisberg JF, Greene WC. In Vitro Derived Dendritic Cells trans-Infect CD4 T Cells Primarily with Surface-Bound HIV-1 Virions. PLoS Pathog 2007;3:e4. [PubMed: 17238285]

98. Kwon DS, Gregorio G, Bitton N, Hendrickson WA, Littman DR. DC-SIGN-mediated internalization of HIV is required for trans- enhancement of T cell infection. Immunity 2002;16:135–144. [PubMed: 11825572]

99. Granelli-Piperno A, Finkel V, Delgado E, Steinman RM. Virus replication begins in dendritic cells during the transmission of HIV-1 from mature dendritic cells to T cells. Curr Biol 1999;9:21–29. [PubMed: 9889121]

100. van Montfort T, Nabatov AA, Geijtenbeek TB, Pollakis G, Paxton WA. Efficient capture of antibody neutralized HIV-1 by cells expressing DC-SIGN and transfer to CD4+ T lymphocytes. J Immunol 2007;178:3177–3185. [PubMed: 17312166]

101. Ganesh L, et al. Infection of specific dendritic cells by CCR5-tropic human immunodeficiency virus type 1 promotes cell-mediated transmission of virus resistant to broadly neutralizing antibodies. J Virol 2004;78:11980–11987. [PubMed: 15479838]

102. Chen P, Hubner W, Spinelli MA, Chen BK. Predominant mode of HIV transfer between T cells is mediated by sustained Env-dependent neutralization-resistant virological synapses. J Virol. 2007

103. McCoombe SG, Short RV. Potential HIV-1 target cells in the human penis. AIDS 2006;20:1491–1495. [PubMed: 16847403]

104. Patterson BK, et al. Susceptibility to human immunodeficiency virus-1 infection of human foreskin and cervical tissue grown in explant culture. Am J Pathol 2002;161:867–873. [PubMed: 12213715]

105. Donoval BA, et al. HIV-1 target cells in foreskins of African men with varying histories of sexually transmitted infections. Am J Clin Pathol 2006;125:386–391. [PubMed: 16613341]

106. Meier AS, Bukusi EA, Cohen CR, Holmes KK. Independent association of hygiene, socioeconomic status, and circumcision with reduced risk of HIV infection among Kenyan men. J Acquir Immune Defic Syndr 2006;43:117–118. [PubMed: 16885771]

107. Gray RH, et al. Male circumcision for HIV prevention in men in Rakai, Uganda: a randomised trial. Lancet 2007;369:657–666. [PubMed: 17321311]

108. Quinn TC. Circumcision and HIV transmission. Curr Opin Infect Dis 2007;20:33–38. [PubMed: 17197879]

109. Wawer MJ, et al. Rates of HIV-1 Transmission per Coital Act, by Stage of HIV-1 Infection, in Rakai, Uganda. J Infect Dis 2005;191:1403–1409. [PubMed: 15809897]

110. Pudney J, Anderson DJ. Immunobiology of the human penile urethra. Am J Pathol 1995;147:155–165. [PubMed: 7604877]

111. McClure CP, et al. HIV coreceptor and chemokine ligand gene expression in the male urethra and female cervix. AIDS 2005;19:1257–1265. [PubMed: 16052080]

112. Miller CJ, et al. Genital mucosal transmission of simian immunodeficiency virus: animal model for heterosexual transmission of human immunodeficiency virus. J Virol 1989;63:4277–4284. [PubMed: 2778875]

113. Cohen MS, et al. Reduction of concentration of HIV-1 in semen after treatment of urethritis: implications for prevention of sexual transmission of HIV-1. AIDSCAP Malawi Research Group. Lancet 1997;349:1868–1873. [PubMed: 9217758]

114. Krieger JN, et al. Vasectomy and human immunodeficiency virus type 1 in semen. J Urol 1998;159:820–825. [PubMed: 9474159]discussion 825–826
115. Pudney J, Oneta M, Mayer K, Seage G 3rd, Anderson D. Pre-ejaculatory fluid as potential vector for sexual transmission of HIV-1. Lancet 1992;340:1470. [PubMed: 1360584]
116. Ilaria G, et al. Detection of HIV-1 DNA sequences in pre-ejaculatory fluid. Lancet 1992;340:1469. [PubMed: 1360583]
117. Brenchley JM, Price DA, Douek DC. HIV disease: fallout from a mucosal catastrophe? Nature Immunol 2006;7:235–239. [PubMed: 16482171]
118. Wang X, et al. Massive infection and loss of CD4+ T cells occurs in the intestinal tract of neonatal rhesus macaques in acute SIV infection. Blood 2007;109:1174–1181. [PubMed: 17047153]
119. Mattapallil JJ, et al. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature 2005;434:1093–1097. [PubMed: 15793563]
120. Mehandru S, et al. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. J Exp Med 2004;200:761–770. [PubMed: 15365095]
121. Haase AT. Perils at mucosal front lines for HIV and SIV and their hosts. Nature Rev Immunol 2005;5:783–792. [PubMed: 16200081]
122. CDC HIV/AIDS Fact Sheet. Centers for Diseases Control and Prevention; Atlanta: 2007. HIV/AIDS among men who have sex with men.
123. The Global HIV/AIDS pandemic. Morb Mort Wkly Rep 2006;55:841–844.
124. Page-Shafer K, et al. Risk of HIV infection attributable to oral sex among men who have sex with men and in the population of men who have sex with men. AIDS 2002;16:2350–2352. [PubMed: 12441814]
125. Aceijas C, Stimson GV, Hickman M, Rhodes T. Global overview of injecting drug use and HIV infection among injecting drug users. AIDS 2004;18:2295–2303. [PubMed: 15577542]
126. Lehman DA, Farquhar C. Biological mechanisms of vertical human immunodeficiency virus (HIV-1) transmission. Rev Med Virol 2007:381–403. [PubMed: 17542053]
127. Galvin SR, Cohen MS. The role of sexually transmitted diseases in HIV transmission. Nature Rev Microbiol 2004;2:33–42. [PubMed: 15035007]
128. Royce RA, Sena A, Cates W Jr, Cohen MS. Sexual transmission of HIV. N Engl J Med 1997;336:1072–1078. [PubMed: 9091805]
129. Halperin DT. Heterosexual anal intercourse: prevalence, cultural factors, and HIV infection and other health risks, Part I. Aids Patient Care STDS 1999;13:717–730. [PubMed: 10743535]
130. Sowinski S, et al. Membrane nanotubes physically connect T cells over long distances presenting a novel route for HIV-1 transmission. Nature Cell Biol 2008;10:211–219. [PubMed: 18193035]

Glossary terms

Simian–HIV (SHIV)

SHIVs are chimeric viruses that are created by inserting the envelope protein (Env), the transcriptional transactivator (Tat) and the regulator of virion gene expression (Rev) of HIV into the SIV_{MAC239} clone. Depending on the particular HIV Env protein, these SHIVs have different *in vivo* characteristics. The SHIV chimeric viruses are best used for testing antibodies specific for HIV in non-human primate models.

Transcytosis

A process by which various macromolecules, including HIV-1 virions, are transported across the interior of a cell.

Syndecans

Single transmembrane domain proteins that carry three to five heparan sulfate and chondroitin sulfate chains which allow for interaction with a large variety of ligands including residues on the HIV-1 gp120 protein.

Langerhans cell

A type of dendritic cell that is localized in the squamous epithelial layer of the skin and certain mucosae.

Stromal papillae

Superficial areas of the mucosal stroma that interdigitate with the epithelium.

C-type lectin receptors

A large family of receptors that bind glycosylated ligands and have multiple roles, such as in cell adhesion, endocytosis, natural-killer-cell target recognition and dendritic-cell activation.

R5-tropic HIV-1

An HIV strain that uses CC-chemokine receptor 5 (CCR5) as the co-receptor to gain entry to target cells.

Birbeck granules

Membrane-bound rod- or tennis racket-shaped structures with a central linear density, found in the cytoplasm of Langerhans cells. The formation of Birbeck granules is induced by langerin, an endocytic C-type lectin specific to Langerhans cells.

Phagosomes

Vacuolar compartments that confine bacteria after enforced endocytosis or after phagocytosis. Unless counteracted by a bacterial survival strategy, the phagosome matures into a hostile environment that is designed to kill and digest microorganisms.

Cross-presentation

The initiation of a $CD8^+$ T-cell response to an antigen that is not present within antigen-presenting cells (APCs). This exogenous antigen must be taken up by APCs and then re-routed to the MHC-class-I pathway of antigen presentation.

Lamina propria

Connective tissue that underlies the epithelium of the mucosa and contains various myeloid and lymphoid cells, including macrophages, dendritic cells, T cells and B cells.

Exosomes

Small lipid-bilayer vesicles that are released from activated cells. They comprise either plasma membrane or membrane derived from intracellular vesicles.

Filopodia

Slender cytoplasmic projections, which extend from the leading edge of migrating cells.

Cytonemes

Actin-based filopodial cell extensions.

Nanotubules

Cytonemes that connect blood cells over a distance of several cell diameters and transport membrane proteins, lipids and ions from one of the connected cells to another, thus executing long range intercellular communications.

Macropinocytosis

A mechanism of endocytosis in which large droplets of fluid are trapped underneath extensions (ruffles) of the cell surface. Can be exploited by some pathogens as a route for entry into cells.

Glans penis

Sensitive tip of the penis. When the penis is flaccid it is wholly or partially covered by the foreskin, except in men who have been circumcised.

Biographies

Florian Hladik

Florian Hladik obtained his M.D., Ph.D. and dermatology training at the University of Vienna in Austria. He carried out postdoctoral research at the Johannes Gutenberg University in Mainz, Germany, and the University of Washington, USA. He is currently Research Assistant Professor in the Departments of Gynecology and Medicine, University of Washington, and Affiliate Investigator at the Fred Hutchinson Cancer Research Center in Seattle. His research focuses on mucosal HIV transmission pathogenesis, microbicide development and infectious causes of fetal prematurity.

M. Juliana McElrath

After receiving her M.D., Ph.D. and internal medicine residency training at the Medical University of South Carolina, Dr. McElrath undertook a clinical fellowship in infectious diseases at Columbia University and a faculty position at the Rockefeller University. For nearly two decades she has focused her research efforts on HIV pathogenesis, mucosal immunity and vaccine development in Seattle. She currently is a Member and Co-Director of the Vaccine and Infectious Disease Institute at Fred Hutchinson Cancer Research Center and a Professor of Medicine at the University of Washington.

Online Summary

- **HIV invasion through the mucosae of the female lower genital tract contributes the largest number of new HIV-1 infections worldwide. The second leading site of viral invasion is the lower male genital tract, followed by invasion via the rectal mucosa in both women and men.**
- **Explant models of human genital tissues have provided new insights into the mechanisms of sexual HIV transmission.**
- **Initial attachment of HIV-1 to the mucosa may be aided by cervical mucus and a variety of gp120-binding surface receptors on epithelial cells. HIV-1 penetration into the genital mucosa occurs rapidly after exposure and is possibly enhanced by microabrasions or genital ulcer disease.**
- **In the human vagina, intraepithelial $CD4^+$ T cells and $CD1a^+$ Langerhans cells are the first cells infected by HIV-1.**
- **Vaginal Langerhans cells exhibit a high capacity to endocytose HIV-1 virions. C-type lectins such as DC-SIGN (CD209) or langerin (CD207) appear to play little to no role in mediating this infection pathway.**
- **Genital $CD4^+$ T cells express high levels of CCR5, are rapidly infected by HIV-1 and produce large quantities of viral progeny.**
- **Dendritic cells utilize several pathways to enhance viral propagation to $CD4^+$ T cells for productive infection. Presumably these occur as well in the genital mucosa, but direct evidence is lacking.**
- **The highly protective effect of circumcision indicates that viral invasion in men occurs predominantly through the inner foreskin, where both $CD1a^+$ Langerhans cells and $CD4^+$ cells are abundant. The**

second leading site of viral invasion in the male genital tract is likely the penile urethra.

Glossary terms

Simian–HIV (SHIV)

SHIVs are chimeric viruses that are created by inserting the envelope protein (Env), the transcriptional transactivator (Tat) and the regulator of virion gene expression (Rev) of HIV into the SIV_{MAC239} clone. Depending on the particular HIV Env protein, these SHIVs have different *in vivo* characteristics. The SHIV chimeric viruses are best used for testing antibodies specific for HIV in non-human primate models.

Transcytosis

A process by which various macromolecules, including HIV-1 virions, are transported across the interior of a cell.

Syndecans

Single transmembrane domain proteins that carry three to five heparan sulfate and chondroitin sulfate chains which allow for interaction with a large variety of ligands including residues on the HIV-1 gp120 protein.

Langerhans cell

A type of dendritic cell that is localized in the squamous epithelial layer of the skin and certain mucosae.

Stromal papillae

Superficial areas of the mucosal stroma that interdigitate with the epithelium.

C-type lectin receptors

A large family of receptors that bind glycosylated ligands and have multiple roles, such as in cell adhesion, endocytosis, natural-killer-cell target recognition and dendritic-cell activation.

R5-tropic HIV-1

An HIV strain that uses CC-chemokine receptor 5 (CCR5) as the co-receptor to gain entry to target cells.

Birbeck granules

Membrane-bound rod- or tennis racket-shaped structures with a central linear density, found in the cytoplasm of Langerhans cells. The formation of Birbeck granules is induced by langerin, an endocytic C-type lectin specific to Langerhans cells.

Phagosomes

Vacuolar compartments that confine bacteria after enforced endocytosis or after phagocytosis. Unless counteracted by a bacterial survival strategy, the phagosome matures into a hostile environment that is designed to kill and digest microorganisms.

Cross-presentation

The initiation of a $CD8^+$ T-cell response to an antigen that is not present within antigen-presenting cells (APCs). This exogenous antigen must be taken up by APCs and then re-routed to the MHC-class-I pathway of antigen presentation.

Lamina propria

Connective tissue that underlies the epithelium of the mucosa and contains various myeloid and lymphoid cells, including macrophages, dendritic cells, T cells and B cells.

Exosomes

Small lipid-bilayer vesicles that are released from activated cells. They comprise either plasma membrane or membrane derived from intracellular vesicles.

Filopodia

Slender cytoplasmic projections, which extend from the leading edge of migrating cells.

Cytonemes

Actin-based filopodial cell extensions.

Nanotubules

Cytonemes that connect blood cells over a distance of several cell diameters and transport membrane proteins, lipids and ions from one of the connected cells to another, thus executing long range intercellular communications.

Macropinocytosis

A mechanism of endocytosis in which large droplets of fluid are trapped underneath extensions (ruffles) of the cell surface. Can be exploited by some pathogens as a route for entry into cells.

Glans penis

Sensitive tip of the penis. When the penis is flaccid it is wholly or partially covered by the foreskin, except in men who have been circumcised.

Biographies

Florian Hladik

Florian Hladik obtained his M.D., Ph.D. and dermatology training at the University of Vienna in Austria. He carried out postdoctoral research at the Johannes Gutenberg University in Mainz, Germany, and the University of Washington, USA. He is currently Research Assistant Professor in the Departments of Gynecology and Medicine, University of Washington, and Affiliate Investigator at the Fred Hutchinson Cancer Research Center in Seattle. His research focuses on mucosal HIV transmission pathogenesis, microbicide development and infectious causes of fetal prematurity.

M. Juliana McElrath

After receiving her M.D., Ph.D. and internal medicine residency training at the Medical University of South Carolina, Dr. McElrath undertook a clinical fellowship in infectious diseases at Columbia University and a faculty position at the Rockefeller University. For nearly two decades she has focused her research efforts on HIV pathogenesis, mucosal immunity and vaccine development in Seattle. She currently is a Member and Co-Director of the Vaccine

and Infectious Disease Institute at Fred Hutchinson Cancer Research Center and a Professor of Medicine at the University of Washington.

Online Summary

- HIV invasion through the mucosae of the female lower genital tract contributes the largest number of new HIV-1 infections worldwide. The second leading site of viral invasion is the lower male genital tract, followed by invasion via the rectal mucosa in both women and men.
- Explant models of human genital tissues have provided new insights into the mechanisms of sexual HIV transmission.
- Initial attachment of HIV-1 to the mucosa may be aided by cervical mucus and a variety of gp120-binding surface receptors on epithelial cells. HIV-1 penetration into the genital mucosa occurs rapidly after exposure and is possibly enhanced by microabrasions or genital ulcer disease.
- In the human vagina, intraepithelial $CD4^+$ T cells and $CD1a^+$ Langerhans cells are the first cells infected by HIV-1.
- Vaginal Langerhans cells exhibit a high capacity to endocytose HIV-1 virions. C-type lectins such as DC-SIGN (CD209) or langerin (CD207) appear to play little to no role in mediating this infection pathway.
- Genital $CD4^+$ T cells express high levels of CCR5, are rapidly infected by HIV-1 and produce large quantities of viral progeny.
- Dendritic cells utilize several pathways to enhance viral propagation to $CD4^+$ T cells for productive infection. Presumably these occur as well in the genital mucosa, but direct evidence is lacking.
- The highly protective effect of circumcision indicates that viral invasion in men occurs predominantly through the inner foreskin, where both $CD1a^+$ Langerhans cells and $CD4^+$ cells are abundant. The second leading site of viral invasion in the male genital tract is likely the penile urethra.

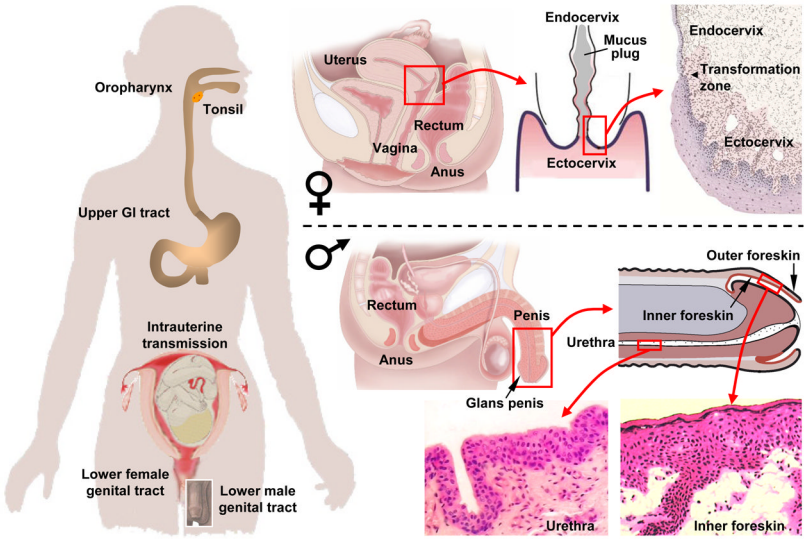

Figure 1. HIV invasion sites

HIV mucosal invasion sites of the lower genital tract, the rectum, and the upper intestinal tract. In women, viral invasion occurs mostly through the non-keratinized squamous epithelium of the vagina and ectocervix, as well as through the single-layer columnar epithelium of the endocervix. The endocervical canal is filled with mucus, providing a barrier against ascent of pathogens. However, ovulation is accompanied by hydration and alkalinization of the mucus plug, possibly decreasing its barrier function. Infection in women can also ensue when HIV-1 invades the single-layer columnar epithelium of the rectum following receptive anal intercourse. In men, viral invasion occurs most frequently through the inner foreskin and the penile urethra as a consequence of penile–vaginal or penile–anal intercourse. Thinly stratified columnar epithelial cells line most of the urethra except for the fossa navicularis near the external meatus (exit hole), which is covered by non-keratinized squamous epithelium. The glans penis and the outer foreskin are protected by keratinized squamous epithelium, which provides a strong mechanical barrier against HIV invasion. By contrast, a thin and poorly keratinized squamous epithelium covers the inner foreskin, rendering this site vulnerable to HIV invasion. Men are also infected by viral invasion through the rectum. In fact, receptive anal intercourse carries the highest per exposure probability of infection among all mucosal transmission sites. The upper gastrointestinal (tract, lined by non-keratinized squamous epithelium in the oropharynx and the esophagus, and by single layer columnar epithelium in the stomach and the small intestine, is another site of mucosal HIV invasion. In adults, transmission in the upper gastrointestinal tract occurs following contact with HIV-containing semen during fellatio, but the efficiency of this route is low. In infants, HIV invasion in the upper gastrointestinal tract occurs after exposure to or ingestion of infected maternal blood and genital secretions during birth as well as infected milk during breast feeding.

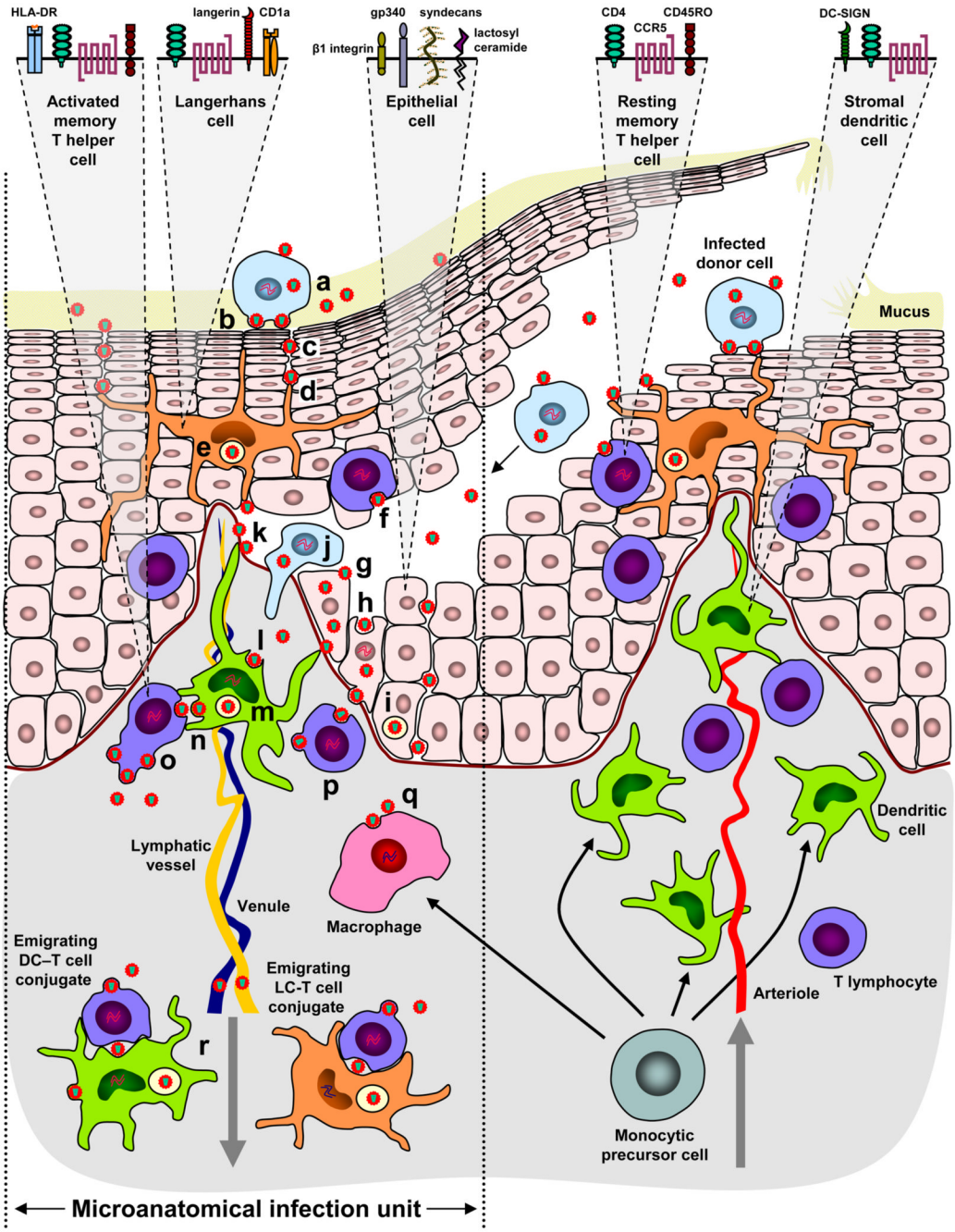

Figure 2. Pathways of HIV invasion in the mucosa of the vagina and uterine ectocervix, part A

The human vagina and ectocervix are covered by non-keratinized squamous epithelium. Shearing during sexual intercourse can lead to physical abrasions of the epithelium, in particular in microanatomical regions where the stromal papillae, enriched with stromal DCs, reach close to the luminal surface of the mucosa. Depicted are two stromal papillae containing arterioles, venules and lymphatic vessels. The stromal papilla on the right signifies the afferent arm shuttling blood cells to the mucosa. Monocytic precursor cells differentiate upon arrival either into macrophages or dendritic cells (DCs), and DCs may differentiate further into subsets. Three stromal DC subsets have been identified in human skin, distinguished by BDCA-1, CD1 and CD14 expression patterns[65], but their presence and susceptibility to HIV

have not been determined in the mucosa. An abrasion of the outer epithelium exposes the stromal papilla tip (left), as well as several epithelial cells located close to or within the basal layer. Infected donor cells and free virions may migrate along such an abrasion, as shown here, and directly contact various target cells in the mucosal epithelium and stroma. Resident mucosal leukocytes such as DCs and T cells tend to cluster in these regions (see Figure 3e), creating susceptible foci for infection. Possible pathways for HIV penetration are depicted on the left side of the illustration and are indicated by letters. Characteristic phenotypic cell receptors and receptors relevant for HIV binding and infection are shown on the top of the figure. **a.** Trapping of free HIV virions or HIV-infected donor cells in mucus covering the mucosa. **b.** Attachment of HIV-infected donor cells to the luminal surface of the mucosa and secretion of virions upon contact. **c.** Penetration of virions into gaps between epithelial cells. **d.** Capture of penetrating virions by Langerhans cells (LCs) residing within the epithelium, which extend processes toward the vaginal lumen. **e.** Internalization of virions into endocytic compartments of LCs. **f.** Fusion of HIV with the surface of intraepithelial $CD4^+$ T cells, followed by productive infection. **g.** Transcytosis of virions through epithelial cells located close to or within the basal layer of the squamous epithelium (Figure 3a–c). **h.** Productive infection of basal epithelial cells. **i.** Internalization of virions into endocytic compartments of basal epithelial cells. **j.** Immigration of infected donor cells along physical abrasions of the epithelium into the mucosal stroma, where they are taken up by lymphatic or venous microvessels and transported to local lymph nodes or the blood circulation. **k.** Immigration of free virions along microabrasions into the stroma, where they can make direct contact with stromal DCs. **l.** Productive infection of stromal DCs by HIV. **m.** Internalization of virions into endocytic compartments of stromal DCs. **n.** Passage of virus from stromal DCs to $CD4^+$ T cells across an infectious synapse (see also Figure 4). **o.** Massive productive infection of mucosal $CD4^+$ T cells activated by contact with antigen-presenting DCs. **p.** Productive infection of resting mucosal $CD4^+$ memory T cells. **q.** Binding of HIV and possibly productive infection of stromal macrophages. **r.** Emigration of productively infected $CD4^+$ T cells and DCs into the submucosa and the draining lymphatic and venous microvessels. T cells may derive from the epithelium or the stroma. Likewise, emigrating DCs may originate from intraepithelial LCs or stromal DCs. DCs and T cells often form conjugates, and HIV may accumulate between the two cells along an infectious synapse. DCs carry virions in endocytic compartments and some are also productively infected, but it remains unclear at which differentiation stage this occurs.

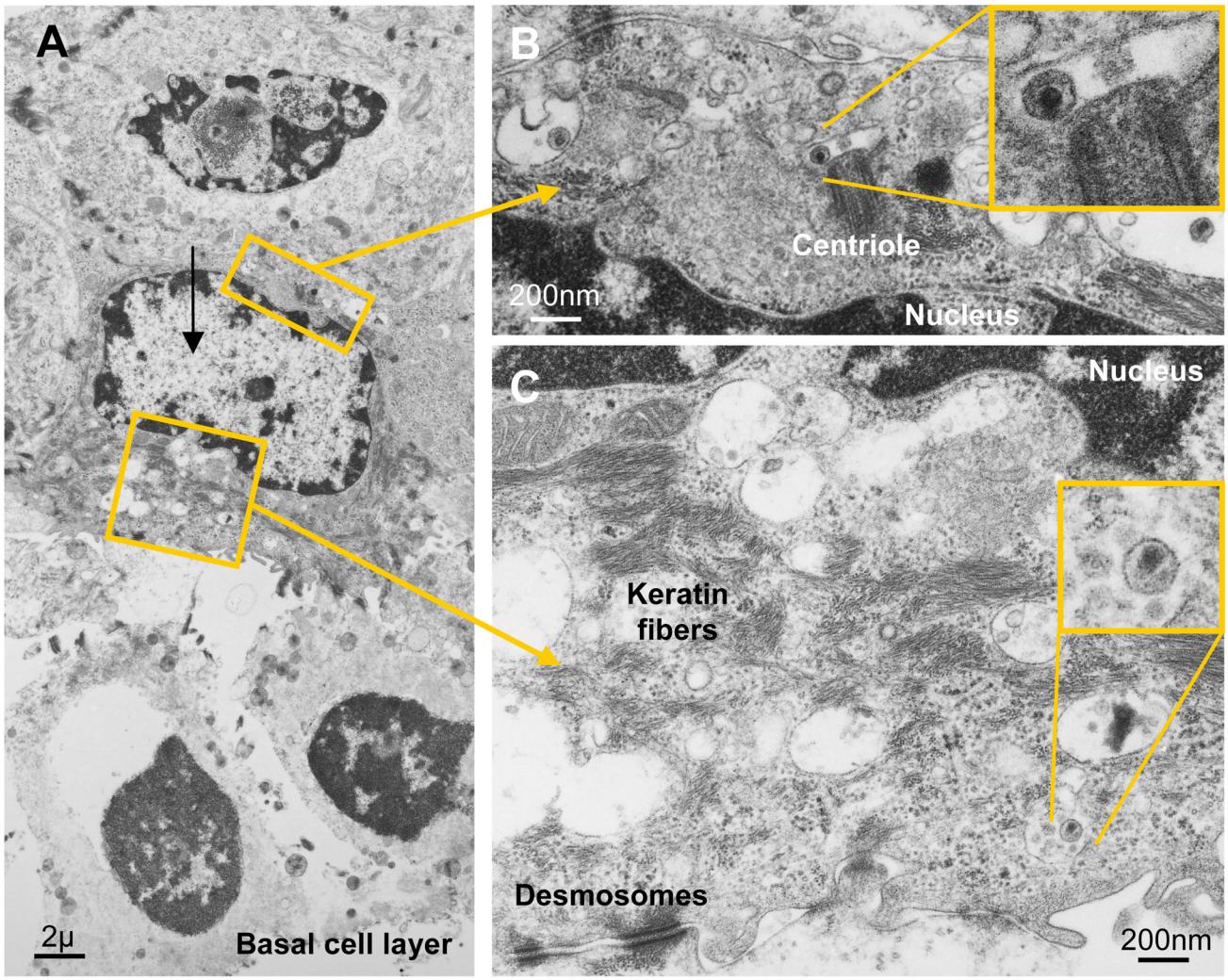

Figure 3. Pathways of HIV invasion in the mucosa of the vagina and uterine ectocervix, part B

A–C. Likely HIV transcytosis in a vaginal epithelial cell *in situ* located one layer above the basal cell layer. Virions can be seen in the cytoplasm on both sides of the nucleus. Desmosomes and keratin fibers identify the cell as epithelial.

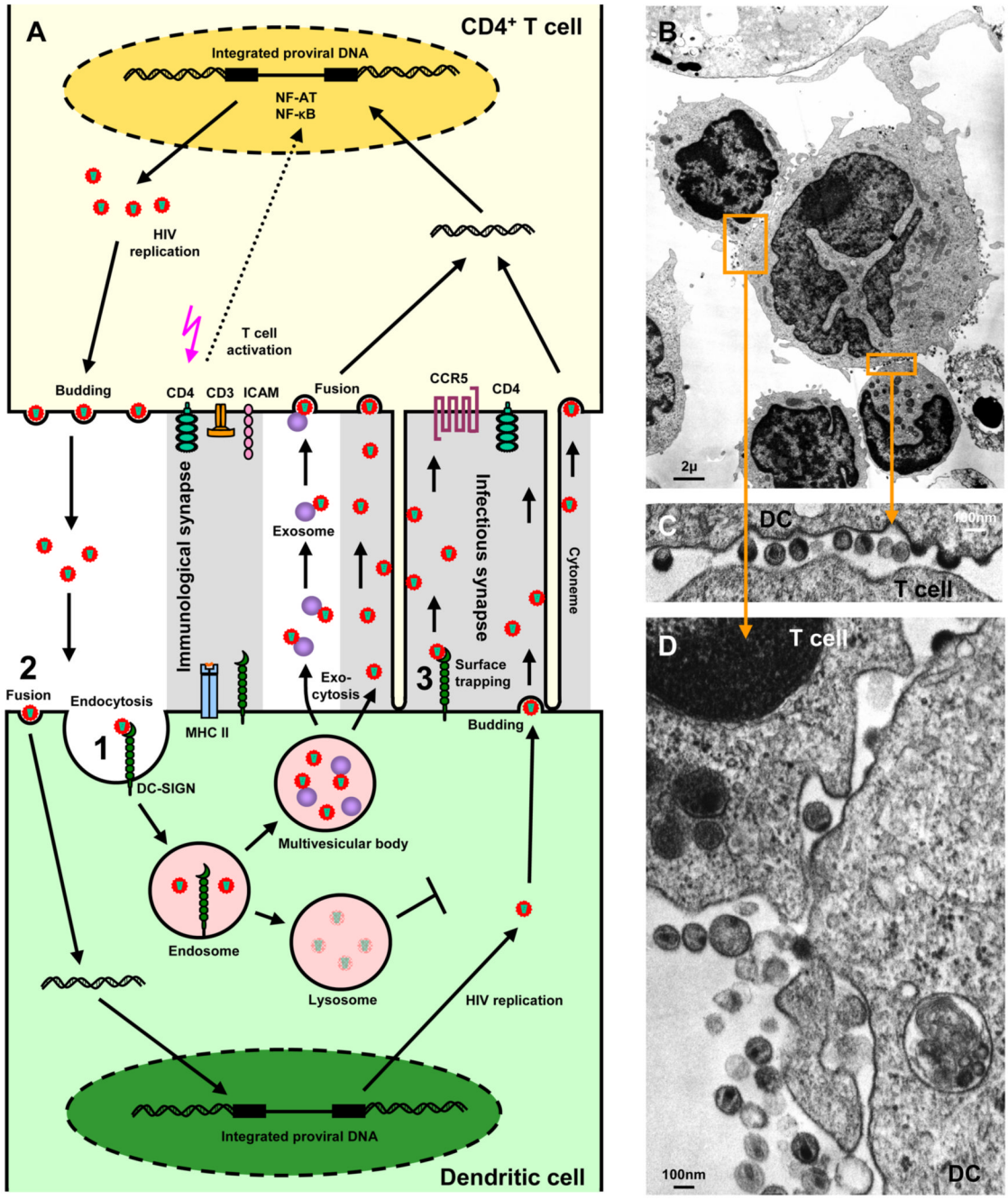

Figure 4. Significance of DC–T-cell interactions for HIV-1 transmission

A. Pathways of HIV-1 passage between dendritic cells (DCs) and T cells. DCs can store HIV-1 in three forms for eventual infection of $CD4^+$ T cells. (1) Endocytosed intact virions. Endocytic entry via C-type lectins such as DC-specific ICAM3-grabbing non-integrin (DC-SIGN, CD209) directs HIV to early and late endosomes. From there it enters multivesicular bodies and remains intact, or traffics to lysosomes where it is degraded. (2) Integrated provirus. Entry of HIV-1 by CD4- and co-receptor-mediated fusion leads to productive infection of DCs. (3) Surface-bound intact virions. Binding and trapping of intact virions on the cell surface can also occur by C-type lectins such as DC-SIGN.

Passage of HIV-1 from DCs to $CD4^+$ T cell occurs most effectively across an infectious synapse, formed by concentration of HIV-1 on the DC side and of HIV receptors such as CD4 and CC-chemokine receptor 5 (CCR5) on the T cell side. HIV is released into the infectious synapse either by exocytosis of stored virions from multivesicular bodies (MVBs) or by budding of newly formed virions following active viral replication. Surface bound virions may also accumulate at the infectious synapse. Migration of HIV toward the T cell may be further enhanced by "surfing" of virions along the outer surface of filopodia or cytonemes that are extended from the T cell toward the DC. Coupling of virions with exosomes as they are being released from MVBs may also increase their infectivity. Exosome-associated virions are likely to be transmitted to $CD4^+$ T cells through membrane binding and fusion, either within the infectious synapse or over longer distances. In parallel to transmission of virus from the DC to the $CD4^+$ T cell, the DC also presents antigenic peptides through MHC class II molecules to the T-cell receptor CD3. During peptide recognition, additional receptor–ligand pairs that are important for T-cell stimulation accumulate in this region and form an immunological synapse. Signals delivered through the immunological synapse lead to T-cell activation, which ultimately causes transcription factors such as nuclear factor-κB (NF-κB) and nuclear factor of activated T cells (NFAT) to translocate into the nucleus of the T cell. There, they bind to the enhancer region of the viral long terminal repeat (LTR) and activate viral gene transcription, driving HIV-1 replication. **B–D.** Example of infectious synapse formation between DCs and T cells in the human vagina (taken from Hladik F. et al.[69]). HIV-1 buds from the surface of the productively infected DC toward the contact zone between the DC and the two T cells. Viral budding is also seen along other areas of the DC surface. The DC contains the typical veiled nucleus as well as multiple large mitochondria, and one large cytoplasmic process is formed at the top right. **C–D.** The two contact zones with the lymphocytes are further magnified, displaying virus budding from the DC into the infectious synapse.

Table 1

Contribution of HIV invasion sites to global HIV infections (adapted from[1,123–130])

HIV invasion site	Anatomical sub-location	Type of epithelium	Transmission medium	Transmission probability per exposure event	Estimated contribution to HIV cases worldwide
Female genital tract	Vagina	Squamous, non-keratinized			
	Ectocervix	Squamous, non-keratinized			
	Endocervix	Columnar, single layer	Semen	1 in 200 - 1 in 2,000	12.6 million
	Other	Various epithelia			
Male genital tract	Inner foreskin	Squamous, poorly keratinized			
	Penile urethra	Columnar, stratified	Cervicovaginal and rectal secretions and desquamations	1 in 700 - 1 in 3,000	10.2 million*
	Other	Various epithelia			
	Rectum	Columnar, single layer	Semen	1 in 20 - 1 in 300	3.9 million**
Intestinal tract			Semen	1 in 2,500	1.5 million
	Upper GI tract	Various epithelia	Maternal blood, genital secretions¶	1 in 5 - 1 in 10	960,000***
			Breast milk¶¶	1 in 5 - 1 in 10	960,000***
Placenta	Chorionic villi	Two layer epithelium(cyto- and syncytiotrophoblast)	Maternal blood¶¶¶	1 in 10 - 1 in 20	480,000***
Blood stream			Blood products, sharps	95 in 100 - 1 in 150	2.6 million****

* Includes men having sex with men (MSM), bisexual men and heterosexual men

** Includes MSM, bisexual men and women infected via anal receptive intercourse

*** Mother-to-child transmission:

¶ intrapartum

¶¶ breastfeeding

¶¶¶ intrauterin

**** Mostly intravenous drug use (IDU), but includes infections by transfusions and health care related accidents