Setting the stage: host invasion by HIV

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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 been 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.

HIV vaccines and microbicides hold promise for preventing the acquisition of HIV-1 and HIV-2, the two viruses that cause AIDS, but the success of designing such agents needs 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 1980s to become an epidemic disease (see the UNAIDS/WHO AIDS epidemic update) that is largely maintained by sexual transmission through the lower genital and rectal mucosa (FIG. 1, TABLE 1). Here, we have endeavoured to assemble the current knowledge on the acquisition of HIV at mucosal sites, confining our discussion to the lower genital mucosa. We clearly recognize that other sites of entry, such as the blood, placenta and gastrointestinal mucosa (BOX 1), are also important for HIV acquisition but these are beyond the scope of this Review.

Many studies have provided insights into certain aspects of HIV and simian immunodeficiency virus (SIV) mucosal entry by carrying out detailed examinations of the relevant tissues and target cells following their *in vivo*, *ex vivo* or *in vitro* exposure to the virus. This Review discusses experimental systems that use the same mucosal source to avoid confusion and the inconsistencies that often emerge when findings from one system are extrapolated to those of another. Where appropriate, we have emphasized the 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 in women occur 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 (FIG. 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 sites for HIV entry, particularly when breaches occur in the epithelial-cell layer. HIV or SIV can establish an initial infection solely through invasion of the vaginal mucosa, as shown in women who lack a uterus at birth[1] and in female macaques after surgical removal of the uterus[2]. 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[3]. However, the observed potential benefit of blocking HIV-1 exposure to the cervix may have been undermined because the sexual partners of the women in the group using a diaphragm reported lower condom use than those in the control group.

The region where the ectocervix transforms into the endocervix (FIG. 1) can have enriched CD4+ T-cell populations and therefore may be a particularly susceptible site for HIV entry. Whether HIV can cross the endocervical mucus plug, reach the uterine cavity and

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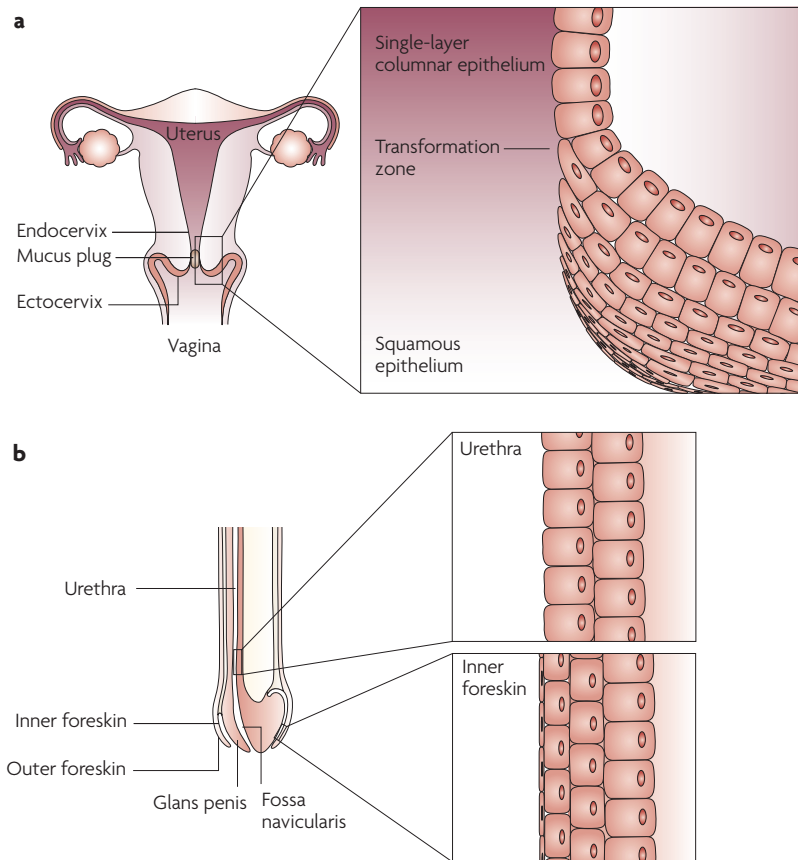
a

b

Figure 1 | **HIV invasion sites. a** | 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 the 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. **b** | 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.

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.

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[4]. 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[5]. This indicated that ascent of the virus through the endocervical mucus plug may be possible, but this observation has not been confirmed in humans. Conceivably, the upper mucosal tract may become more vulnerable to HIV-1 penetration during ovulation, a period when rising oestrogen levels alter the endocervical mucus making its consistency less viscous and more alkaline.

HIV entry through the female genital epithelium. Both free and cell-associated HIV and SIV virions can establish mucosal infection (FIG. 2)[6–10]. This has been shown directly *in vivo* in female macaques[11] and in mice[12], and indirectly in humans through genetic sequence comparisons of viral isolates from acutely infected women with those from seminal leukocytes (cell-associated virions) and plasma from their infected source partners[13]. *Ex vivo* studies using human cervical explants have also confirmed transmission of cell-free and cell-associated HIV-1 (REFS 14,15). Initially, cervical mucus can trap infected seminal cells or free virions[16,17]. Conceivably, this could facilitate viral transmission by prolonging contact time of the virions with the mucosa. However, while immobilized, the virions may also become more susceptible to innate antiviral substances.

HIV virions that are initially free, or those that are released from infected donor seminal cells, interact with epithelial cells and traverse the epithelium through several pathways, including transcytosis, endocytosis and subsequent exocytosis, by causing productive infection, or merely by penetrating through the gaps between epithelial cells (FIG. 2). The understanding of these events has been hindered by inconsistent findings, and these are largely a result of the use in experimental systems of epithelial-cell types derived from different anatomical sites, as well as primary and immortalized cell lines. Several reports have shown that HIV-1 binds to and enters epithelial cells from the lower female genital tract[18–20]. Transcytosis has been shown to occur in cell lines and also in primary cells, but this has not been definitively shown in intact tissue. On release from the epithelial cells, the virions readily infect susceptible leukocytes[21,22]. Interestingly, cell-associated virions secreted from infected seminal leukocytes appear markedly more efficient at transcytosis than cell-free virions[7,10,21]. It has been reported that virions can also productively infect the cervical epithelial cells themselves[6], although this remains controversial[18,23]. 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[6,24].

Our *ex vivo* experiments using sheets of isolated vaginal epithelium, devoid of mucosal stroma, confirmed that HIV-1 virions are sequestered in endocytic compartments and in the cytosol of epithelial cells (FIG. 3). Interestingly, 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.H., P. Sakchalathorn and M.J.M., unpublished observations). This suggests that initially, rather than entering and traversing superficial epithelial cells in the vagina and ectocervix, HIV-1 probably disperses through the narrow gaps between them[16], as depicted in FIG. 2. This route might then permit HIV-1 to directly contact and infect intraepithelial Langerhans cells (LCs) and CD4+ T cells[25] (see later), or it might allow HIV-1 to reach suprabasal or basal epithelial cells that are more susceptible to viral sequestration and transcytosis. Importantly, components of human semen, most notably amyloid fibrils that form

Table 1 | **Contribution of HIV invasion sites to global HIV infections***

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	Semen	1 in 200 – 1 in 2,000	12.6 million
	Ectocervix	Squamous, non-keratinized			
	Endocervix	Columnar, single layer			
	Other	Various			
Male genital tract	Inner foreskin	Squamous, poorly keratinized	Cervicovaginal and rectal secretions and desquamations	1 in 700 – 1 in 3,000	10.2 million[‡]
	Penile urethra	Columnar, stratified			
	Other	Various			
Intestinal tract	Rectum	Columnar, single layer	Semen	1 in 20 – 1 in 300	3.9 million[§]
	Upper GI tract	Various	Semen	1 in 2,500	1.5 million
			Maternal blood, genital secretions (intrapartum)	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 (intrauterine)	1 in 10 – 1 in 20	480,000[‖]
Blood stream			Blood products, sharps	95 in 100 – 1 in 150	2.6 million[¶]

*Table adapted from the <u>UNAIDS/WHO AIDS epidemic update</u> and REFS 121–128. [‡]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. [¶]Mostly intravenous drug use, but includes infections by transfusions and health-care-related accidents. GI, gastrointestinal.

from naturally occurring fragments of seminal prostatic acidic phosphatase, can capture virions and promote their attachment to epithelial cells and leukocytes, thus increasing infectivity[26].

Several proteins expressed on the surface of epithelial cells may mediate the attachment of HIV-1. Two cell-surface glycosphingolipids, sulphated lactosylceramide expressed by vaginal epithelial cells[27] and galactosyl-ceramide expressed by ectocervical epithelial cells[18,28], bind HIV-1 gp120 and foster transcytosis[21]. Interactions of HIV-1 gp120 with transmembrane heparan sulphate proteoglycans (syndecans) expressed by genital epithelial cells can also contribute to HIV-1 attachment and entry[19,22]. Recently, gp340, a splice variant of salivary agglutinin that is 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 giving it access to susceptible leukocytes[29]. One research group found that the β_1 subunit of integrins expressed by cervical epithelial cells from some explants, but not all, bound virions that were presumably coated with fibronectin, which is abundant in human semen[16]. 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[18], another reported the expression of CXCR4 by these cells[20], whereas another reported the exclusive expression of CCR5 (REF. 28).

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[30]. Once within the epithelium,

Transcytosis
The process of transport of material, including HIV virions, across a cell layer by uptake on one side of the cell into a coated vesicle. The vesicle might then be sorted through the *trans*-Golgi network and transported to the opposite side of the cell, where its contents are released into the extracelluar space.

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

Syndecans
Single transmembrane domain proteins that carry three to five heparan sulphate and chondroitin sulphate chains that allow for interaction with various ligands including residues on the HIV-1 gp120 protein.

Box 1 | **HIV invasion in the gastrointestinal tract**

HIV-1 and simian immunodeficiency virus (SIV) infection commonly targets the lower gastrointestinal tract as an initial infection site following receptive anal intercourse in humans and direct inoculation in macaques, and as a secondary infection site following rapid dissemination from mucosal foci[17] or acute systemic infection[117–119]. 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 CC-chemokine receptor 5 (CCR5; an HIV co-receptor) in the gut, regardless of the route of infection[116]. The relevant target cells for infection in the lower gastrointestinal tract are thus likely to be primarily CD4+ memory T cells[120]. 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.

The upper gastrointestinal tract, lined by non-keratinized squamous epithelium in the oropharynx and the oesophagus, 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.
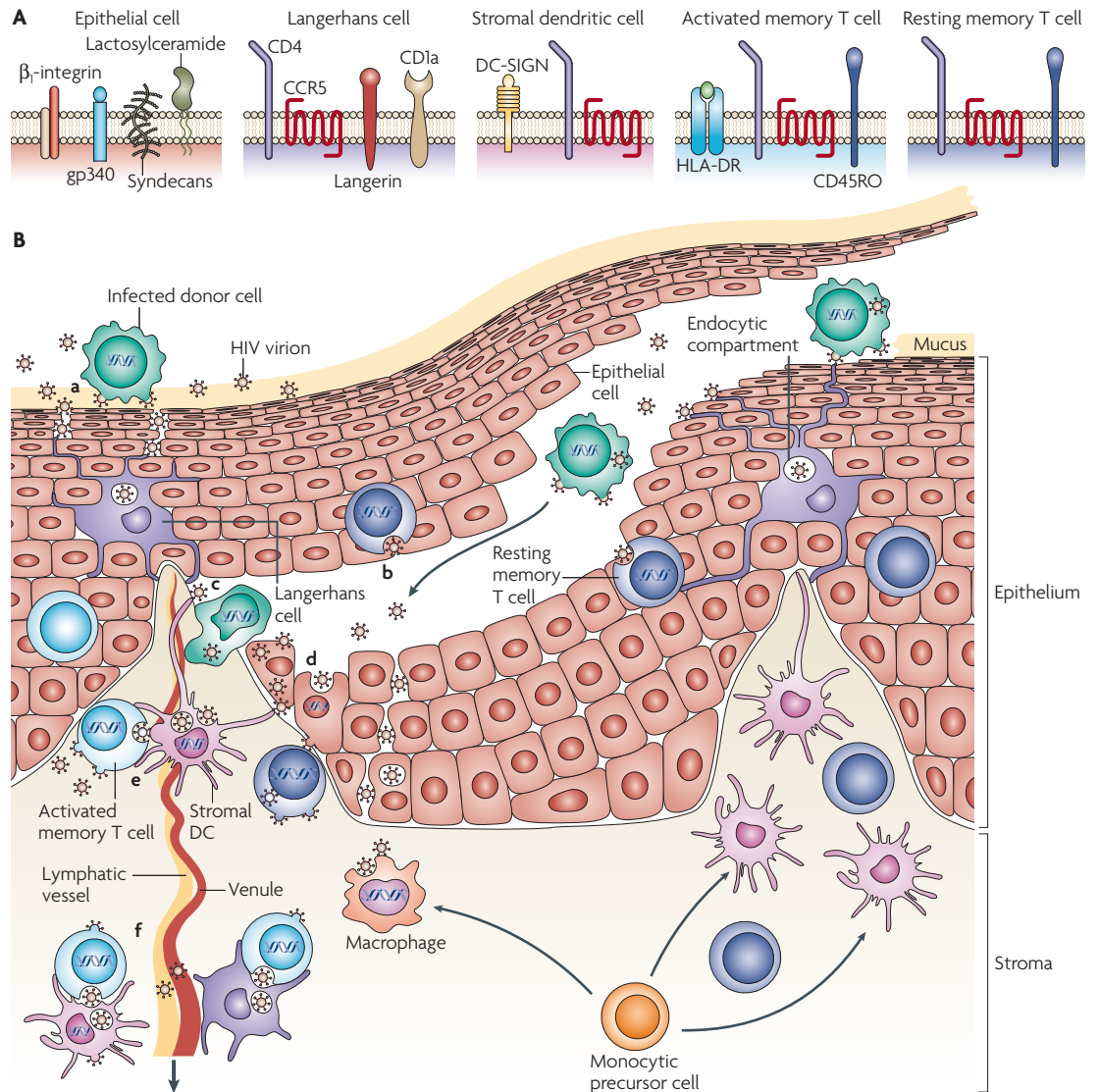
A

Epithelial cell
Lactosylceramide
β₁-integrin
gp340   Syndecans

Langerhans cell
CD4
CCR5
CD1a
Langerin

Stromal dendritic cell
DC-SIGN

Activated memory T cell
HLA-DR   CD45RO

Resting memory T cell

B

Infected donor cell
HIV virion
Endocytic compartment
Epithelial cell
Mucus
Resting memory T cell
Langerhans cell
Activated memory T cell
Stromal DC
Lymphatic vessel
Venule
Macrophage
Monocytic precursor cell
Epithelium
Stroma

Figure 2 | **Pathways of HIV invasion in the mucosa of the vagina and uterine ectocervix.** 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, which are enriched with stromal dendritic cells (DCs), reach close to the luminal surface of the mucosa. Monocytic precursor cells differentiate on arrival either into macrophages or DCs, and DCs may differentiate further into subsets. Three stromal DC subsets have been identified in human skin, distinguished by blood DC antigen 1 (BDCA1), CD1 and CD14 expression patterns[64], but their presence and susceptibility to HIV have not been determined in the mucosa. Infected donor cells and free virions may migrate along the abrasion 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, creating susceptible foci for infection. Characteristic phenotypic cell receptors and receptors relevant for HIV binding and infection are shown on the top of the figure (**A**). The possible pathways of HIV penetration are summarized in **B**. **a** | Free HIV virions or HIV-infected donor cells are trapped in mucus, resulting in penetration of the free virions into gaps between epithelial cells or attachment of HIV-infected donor cells to the luminal surface of the mucosa and secretion of virions on contact. The virions are then captured and internalized into endocytic compartments by Langerhans cells that reside within the epithelium. **b** | HIV can also fuse with the surface of intraepithelial CD4⁺ T cells, followed by productive infection of these cells. **c** | Infected donor cells or free virions can immigrate along physical abrasions of the epithelium into the mucosal stroma. There they are taken up by lymphatic or venous microvessels and transported to local lymph nodes or into the blood circulation, respectively, or they make contact with stromal DCs, T cells and macrophages. **d** | Virions can transcytose through epithelial cells near or within the basal layer of the squamous epithelium (see also Figure 3), productively infect basal epithelial cells, be internalized into endocytic compartments, or penetrate between epithelial cells. **e** | Once within the stroma, virions can productively infect stromal DCs or be internalized into the endocytic compartments of DCs and pass from the stromal DCs to CD4⁺ T cells across an infectious synapse (see also Figure 4) where massive productive infection of CD4⁺ T cells ensues. In addition, virions can productively infect resting mucosal CD4⁺ memory T cells in the stroma and possibly stromal macrophages. **f** | Productively infected CD4⁺ T cells and stromal DCs, and stromal DCs or intraepithelial LCs harboring virions in endocytic compartments, can emigrate into the submucosa and the draining lymphatic and venous microvessels. CCR5, CC-chemokine receptor 5; DC-SIGN, dendritic-cell-specific ICAM3-grabbing non-integrin.

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.

HIV encounters CD4^{+} T cells as well as LCs. LCs have dendrites that extend and retract through the intercellular spaces[31], and which can even reach up to the surface of the epithelium[32], where HIV can bind directly to them (T. Hope, personal communication). Based on observations of gut DCs[33,34], this could also 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.

Mechanical micro-abrasions of the mucosal surface induced by intercourse may also allow HIV to directly access target cells, such as DCs, T cells and macrophages, at the basal epithelium and the underlying stroma[35]. Areas above the stromal papillae, where the epithelium is relatively thin and where LCs on the epithelial-cell side (F.H., L. Ballweber and M.J.M., unpublished observations) and T cells and macrophages on the stromal side[28] congregate, appear particularly vulnerable to viral invasion. Consistent with this notion, *in vivo* simian immunodeficiency virus (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[17]. Similarly, chemical micro-abrasions from the use of certain topical microbicides and micro-abrasions due to genital ulcers caused by sexually transmitted diseases (for example, syphilis, chancroid and those caused by herpes simplex virus) are also likely to result in the exposure of vulnerable target cells in the basal epithelium and stroma[36].

Importance of cervicovaginal LCs in HIV invasion. For many years, HIV-1 invasion of the lower genital tract has been assumed to occur through the internalization of HIV-1 by LCs. This view was supported by evidence that skin LCs are susceptible to infection by HIV-1 (REFS 37–40) and that genital mucosal LCs harbour SIV virions within 24 hours of intravaginal inoculation of macaques[30]. However, soon after *ex vivo* organ culture, LCs migrate out of the epithelium[14,23,41–43]; 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 complete cervical mucosa from humans to HIV-1, emigrating DCs had efficiently captured HIV and were capable of transmitting the virus in *trans*[42]. However, it was not possible to determine whether the cells originated from the epithelium as LCs or from the underlying stroma as DCs. 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[25]. As LCs exit the epithelium at the basal side, they transport intact virions, thereby enabling the infection to spread beyond the site of viral entry (FIG. 2).

However, it is not clear if LCs in the cervicovaginal epithelium can produce and release new HIV-1 virions. HIV receptors expressed by these LCs include CD4, CCR5 and the C-type lectin receptor langerin (also known as CD207), but not CXCR4 and DC-specific ICAM3 (intercellular adhesion molecule 3)-grabbing non-integrin (DC-SIGN; also known as CD209)[25,44–47]. Antibodies that bind CD4 and CCR5 partially block the uptake of R5-tropic HIV-1 by LCs, but blocking the binding of HIV-1 to C-type lectin receptor with mannan, a mannose polymer, had little effect on uptake[25]. Although low-level CD4- and CCR5-mediated productive infection of LCs by HIV-1 in human skin explants has been shown[39,40,48], we were unable to confirm this finding in our imaging studies of vaginal LCs[25]. 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[40,48,49]. 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 DC[50–53].

The relative inefficiency of mannan in blocking the binding and endocytosis of HIV-1 by vaginal LCs was surprising[25] because C-type lectin receptors, which recognize mannose-containing carbohydrate structures, mediate viral entry in other types of DCs[46]. However, HIV-1 can bind DC subsets independent of C-type lectin receptors and CCR5 (REFS 54–56). So, although HIV virions can efficiently bind to langerin expressed by epidermal LCs for their entry into these cells[57], they appear to largely bypass binding to langerin expressed by vaginal LCs in favour of alternative endocytic routes. This distinction may be highly relevant for mucosal HIV transmission.
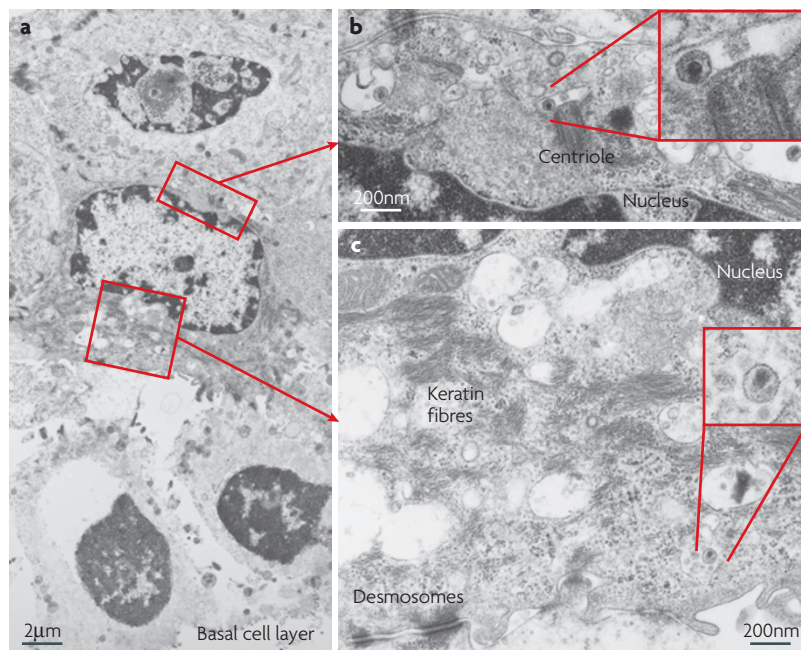

Figure 3 | **HIV-1 transcytosis *in situ* in the vaginal epithelium.** Electron micrograph showing a vaginal epithelial cell located one layer above the basal cell layer and containing intact cytoplasmic virions following 24 hours of infection with HIV-1_BaL (a procedure performed on vaginal epithelial sheets, as previously described[25]) (**a**). As shown in the higher power insets, the virions can be seen on both the apical (**b**) and basal (**c**) sides of the nucleus, signifying transcytosis. Desmosomes and keratin fibres, distinguishing features of the epithelial cell, can also be seen (**c**).

Langerin expressed by epidermal LCs can direct HIV-1 to Birbeck granules for degradation[57]. 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[58]. This is consistent with our observations that intact virions were still present in LCs that had migrated out of the vaginal epithelium 60 hours after viral challenge[25]. Therefore, it appears that HIV-1 enters vaginal LCs through a different route than when it enters skin LCs, and this results 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[46,47] and CCR5 (REFS 59,60) 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 infected DCs in the cervicovaginal stroma[14,23,41,43]. By contrast, SIV-infected DCs were present in the lamina propria of the cervicovaginal mucosa of macaques shortly after intravaginal SIV challenge[30,61], as well as in chronically infected animals[62]. Likewise, HIV-infected DCs were identified in tissue biopsies of the vaginal stroma of asymptomatic HIV-1-infected women[63].

The failure to identify infection of stromal DCs in the human explant models might be attributable to the relatively low sensitivity of the detection methods used 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[42] and massive budding of virions was observed among emigrant DCs 5 days after virus exposure[60] (FIG. 4). 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.

Therefore, stromal DCs exhibit different HIV-1 receptor expression patterns compared with LCs, potentially permitting different HIV-1 entry pathways. Much still remains to be learned about stromal DCs in the human genital mucosa in general, whether different subsets exist similar to those found in skin dermis[64], and the interaction of these DC subsets with HIV virions in particular. More sensitive *in situ* detection methods of HIV infection, as well as assays that can distinguish *de novo* virus production from endocytically engulfed virions, as recently reported[65], will be helpful in determining the exact contribution of stromal DCs to the propagation of HIV.

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[66,67]. They are also found at variable numbers within the vaginal and ectocervical squamous epithelium[66,67]. The majority of these cells are memory T cells that express higher levels of CCR5 than those that circulate in the blood[25,68–70]. One day after HIV-1 inoculation of vaginal, ectocervical and endocervical tissue cultures, infected CD4+ T cells were shown to be confined to the mucosal stroma[14,16,23,42]. This result was surprising, given the presence of CCR5+ CD4+ T cells within the squamous epithelium. However, by analysing the fate of fluorescently tagged virions as early as 2 hours after viral exposure, we observed that R5-tropic HIV-1 efficiently bound to intraepithelial vaginal CD4+ T cells, and this was followed by fusion and productive infection[25]. Therefore, infected T cells must rapidly leave the epithelium, and those found in the stroma may be the same or the 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[14,25,42], and that the initial infection of intraepithelial CD4+ T cells is probably independent of LCs[25]. The central role for genital CD4+ T cells in early infection and propagation is also evident from SIV challenge experiments in macaques[62,71,72]. Interestingly, not only does SIV productively infect activated T cells, characterized by HLA-DR and Ki67 expression, it also infects T cells that are in the resting state (HLA-DR– and Ki67– T cells)[71]. 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.M., P. Sakchalathorn, L. Ballweber and F.H., unpublished observations). In addition, the contribution of resting CD4+ T cells to viral production is substantial during the very earliest stages of infection[73]. The fact that vaginal CD4+ T cells are rapidly depleted following intravenous SIV inoculation of macaques[5,72], similar to the depletion of CD4+ T cells in the gut during acute SIV infection[74], further illustrates their high susceptibility to infection *in vivo*.

Other leukocyte HIV targets 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 cervical explant models[23,41,43], and in two reports these cells were the major cell type that was infected by R5-tropic HIV-1 (REFS 23,43). 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[60], suggesting that the expression of CCR5 by macrophages may occur during the period of activation and emigration from the mucosa[75]. By contrast, SIV-infected macrophages in genital tissues were either rare[71], or undetectable[30,61]. Likewise, macrophages in the human intestinal mucosa were reported to lack CCR5 expression and to possess low permissibility to HIV-1 infection[76]. These discrepancies illustrate a potential limitation of organ cultures. If explantation activates stromal macrophages and as a consequence increases surface CCR5 expression, this

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

Phagosomes
Vacuolar compartments that confine microorganisms after enforced endocytosis or after phagocytosis. Unless counteracted by a microbial 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.
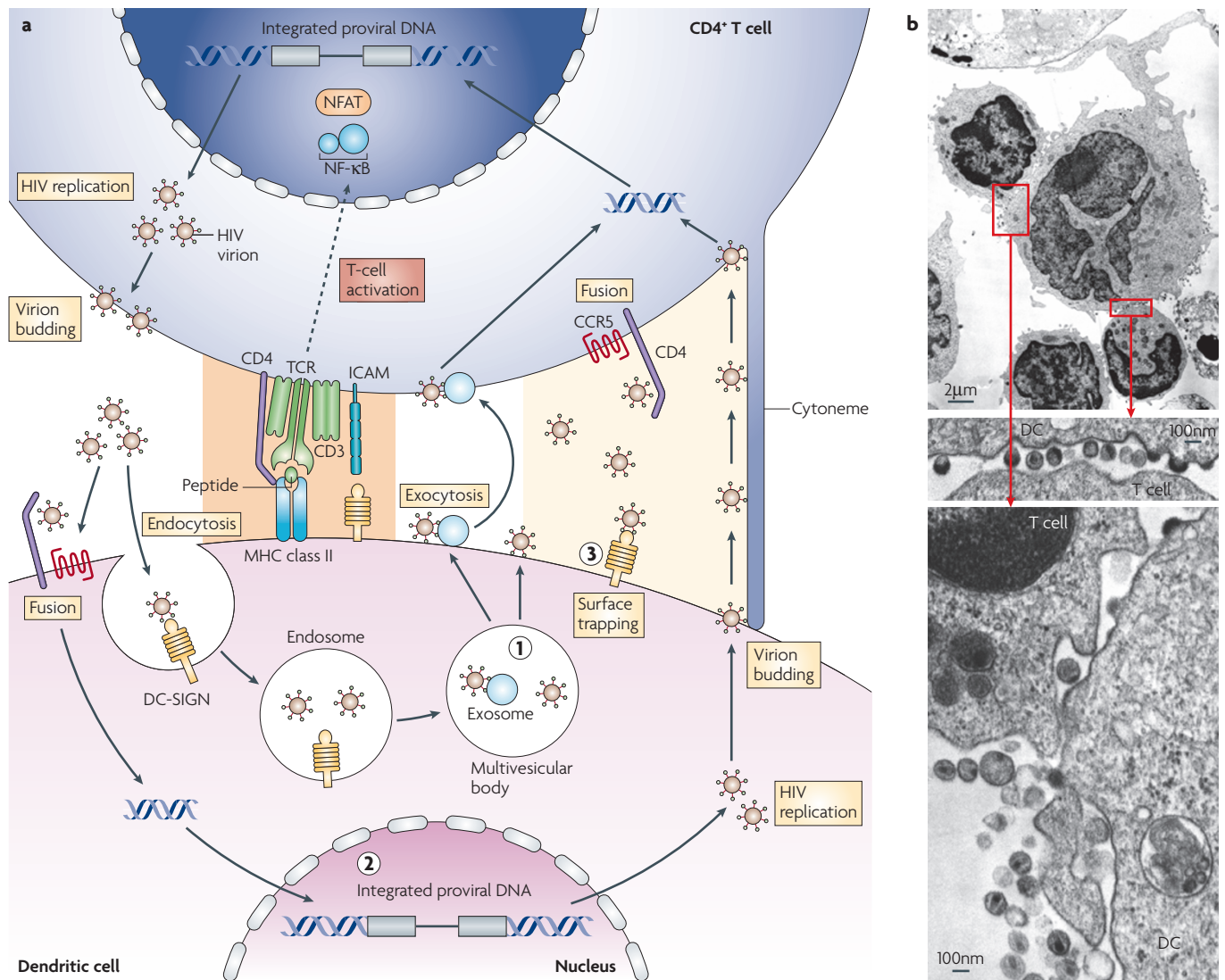
Figure 4 | **The significance of DC–T-cell interactions for HIV-1 transmission. a** | Pathways of HIV-1 passage between dendritic cells (DCs) and T cells. DCs can store HIV-1 in three forms for eventual infection of CD4+ T cells. (1) HIV can be stored as endocytosed intact virions in multivesicular bodies, following endocytic entry via C-type lectins such as DC-specific ICAM3-grabbing non-integrin (DC-SIGN; also known as CD209). (2) HIV-1 can also be stored as an integrated provirus following entry by CD4- and co-receptor-mediated fusion , which leads to productive infection of DCs. (3) HIV can be stored as surface-bound intact virions by C-type lectins, such as DC-SIGN. Passage of HIV-1 from DCs to CD4+ T cells occurs most effectively across an infectious synapse (yellow shading), formed by a concentration of HIV-1 virions 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 or by budding of newly formed virions following active viral replication. Virions trapped to surface receptors such as the C-type lectin DC-SIGN can also accumulate in 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 to exosomes as they are being released from multivesicular bodies also increases 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 with 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 (TCR). During peptide recognition, additional receptor–ligand pairs that are important for T-cell stimulation accumulate in this region and form an immunological synapse (orange shading). 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** | Example of infectious-synapse formation between DCs and T cells in the human vagina (images reproduced with permission from REF. 60 © (1999) American Society For Microbiology). 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. ICAM, intercellular adhesion molecule.

would lead to an overestimation of their susceptibility to infection *in vivo*. Of note, in addition to allowing virion fusion through their expression of chemokine receptors, monocyte-derived macrophages can also trap intact virions through syndecans[77], or even without specific envelope-receptor interactions through a process known as macropinocytosis[78]. Once captured, HIV-1 can be stored for several days and then transmitted to T cells in *trans*[79,80]. If genital macrophages similarly store infectious virions, their role in viral propagation once HIV-1 invades the stroma could be significant.

Other leukocyte populations also interact with HIV. For example, productive infection of natural killer (NK) cells has been reported[81], as well as the transmission of HIV through DC-SIGN expressed by B cells to T cells in *trans*[82]. The significance of B cells for HIV invasion in the genital mucosa remains unknown. Last, monocyte precursor cells enter the mouse dermis in large numbers following intracutaneous infection with *Leishmania major* and differentiate into stromal DCs[83]. 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 published literature suggests that HIV exploits DCs to enhance its infectivity of T cells[84]. First reported in 1992, blood-derived DCs that were exposed to HIV-1 invoked vigorous cytopathic infection of co-cultured CD4+ T cells, even though infection of the DCs could not be detected[85]. 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[86] and cervicovaginal mucosa[60]. The enhancement of HIV transmission by DCs probably occurred by facilitating T-cell activation[71,87], as well as *de novo* T-cell infection.

Four mechanisms for how DCs can augment *de novo* infection of T cells have been proposed (FIG. 4). In classic HIV *trans* infection[46], although the DCs are not productively infected they can trap and preserve the virus and subsequently transfer it to T cells across an 'infectious synapse', which is a zone of DC–T-cell contact where HIV itself and the HIV host-cell receptors are concentrated[88,89]. 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[90]. The third mechanism involves the transmission of new viral progeny from productively infected DCs across the infectious synapse to T cells[48,51–53]. In this case, the contact zone has also been termed a 'virological synapse', to indicate that the donor cell, in analogy to cell-associated HIV transmission between CD4+ T cells[91], is productively infected[92]. 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[93]. These narrow filopodial contact zones may be special cases of virological synapses[94], or may be analogous to nanotubules that are formed between immune cells[95]. In T cells, migration of HIV-1 also occurs within nanotubules[129]. Nanotubules have been shown to functionally connect DCs with other cells[95], 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[25]. No consensus has been reached over whether in *trans* infection occurs primarily from surface bound virions[46,96], internalized virions[97] or both[56]. At any rate, once HIV makes its way into the genital DC–T-cell conjugate, a profound productive infection ensues[60]. Of note, in these conjugates viral budding was not only observed from the surface of T cells but also from the surface of the DCs. Visualization of budding by electron microscopy would suggest that the cells are heavily infected with the virus. So, in genital DC–T-cell conjugates not only T cells but also DCs, which alone are generally weak producers[84], produce large amounts of viral progeny. This is consistent with findings obtained in co-cultures of monocyte-derived DCs and T cells[98]. Therefore, DC–T-cell crosstalk 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[99–101]. 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 men that are currently infected with HIV-1, an estimated 70–75% acquired it through vaginal intercourse (TABLE 1), making the male genital tract the second leading site of HIV invasion. 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[45,102–104]. Variable fractions of these cells express CD4, CCR5 and CXCR4 (REFS 45,102,103). As in the vagina, LCs do not express DC-SIGN, whereas at least some of the stromal DCs do[102]. Therefore, similar mechanisms to the ones that have been described for viral invasion of the female genital tract are likely to occur in the male genital tract.

The highly protective effect of circumcision[105,106], which has been reviewed comprehensively elsewhere[107], 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[102,103]. Consequently, the inner foreskin may be more susceptible

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.

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.

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.

to infection, a view that was supported by investigations of penile autopsy tissues[102], 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[103]. Alternatively, circumcision may reduce the risk for genital tears, abrasions and ulcer disease, resulting in decreased HIV infection risk[107,108].

Although circumcision appears to provide a protective effect against HIV infection, in circumcised males, there must be HIV entry sites other than the foreskin. 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 narrowly stratified, non-keratinized columnar epithelial cells and contains high numbers of CD4+ and CD8+ T cells, and macrophages, within the epithelium and the lamina propria[109]. Interestingly, DCs are not observed in the urethral mucosa[45,109]. The presence of *CCR5* and *CXCR4* mRNA in urethral swabs indicates that these HIV-1 co-receptors may be expressed by urethral cells[110], particularly the predominant CD4+CD45RO+ memory T cells[109]. Of note, intraurethral infusion of SIV resulted in infection of all six inoculated male macaques[111]. In men infected with HIV-1, antibiotic treatment of urethral *Neisseria gonorrhoeae* infection reduces HIV-1 shedding in semen but not the viral load in blood[112]. This finding, as well as HIV-1 shedding in the ejaculate of vasectomized HIV-positive men[113] and in the pre-ejaculatory fluid[114,115] suggest a distal viral source, pointing to the urethra as a site harbouring 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 entry portal 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. The 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 is 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[116]. 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.

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