Infection of Accessory Dendritic Cells by Human Immunodeficiency Virus Type 1

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Many details of the pathogenesis of the human immunodeficiency virus type 1 remain to be elucidated. Details of how the virus gains entry via the mucosal surface upon sexual contact or during breast feeding remain obscure. The means by which the infection travels throughout the body as well as the nature of the major reservoirs of virus infection remains, for the most part, unknown.

Recent studies raise the possibility that cells of the Langerhans/dendritic lineage play a central role in human immunodeficiency virus (HIV-1) infection and pathogenesis. It has been known for several years that veiled dendritic cells in the circulation as well as skin Langerhans are infected in people with prolonged HIV-1 infections. More recently it has been found that a large burden of viral DNA sequences is found, not only in the circulating T-cell population, but also in a population that is defined as a non-T, non-B, non-monocyte/macrophage population rich in T-helper dendritic cells.

Detailed analysis of infection of primary blood-derived T-helper dendritic cells by HIV-1 shows that such cells are the most susceptible cells in the blood to infection by this virus. The cells also produce much more virus per cell than do purified populations of other blood mononuclear cells. Moreover, primary blood-derived T-helper dendritic cells are not killed by infection by HIV-1. These cells are susceptible to lymphotropic, monocyte tropic, and primary isolates of HIV-1.

The sensitivity of primary blood-derived T-helper dendritic cells to infection by HIV-1 has been shown to be attributable to rapid uptake of virus particles as well as rapid synthesis of viral DNA. Subsequent steps of virus replication also occur more rapidly and more efficiently in populations of primary blood-derived T-helper dendritic cells than they do in purified preparations of blood-derived T cells and monocyte/macrophages.

Studies with primates using the simian immunodeficiency virus (SIV) show that dendritic cells at the surface of sexual mucosa are rapidly infected upon exposure to high concentrations of the virus. SIV is also produced in abundance in Langerhans cells located at the surface of the sexual mucosa in animals infected for prolonged periods of time. *J Invest Dermatol 99:89S–94S, 1992*

Human immunodeficiency virus (HIV-1) is a sexually transmitted disease [1]. Recent studies using the simian immunodeficiency virus (SIV) show that transmucosal infection occurs following exposure of intact male and female sexual mucosa to concentrated solutions of cell-free virus [2]. Other studies show that children of infected mothers can also be infected through breast feeding [3]. The observation that a potent class of antigen-presenting cells, cells of the Langerhans/dendritic cell lineage, are abundant at the surface of the oral and sexual mucosa raises the possibility that these cells constitute a site of primary infection by the virus. What follows is a brief review of salient features of cells of this lineage as well as a summary of what is known regarding infection of Langerhans/dendritic cells in HIV-1-infected people and in cell culture.

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Abbreviations:
DC: dendritic cells
EM: electron microscopy
FDC: follicular dendritic cells
HIV: human immunodeficiency virus
LC: Langerhans cells
LTR: long terminal repeat
PCR: polymerase chain reaction
SIV: simian immunodeficiency virus

THE LANGERHANS/DENDRITIC CELL LINEAGE

Accessory dendritic cells constitute at least four (Table I) cellular compartments (excluding dendritic epidermal T cells). Maturing dendritic cells are found as Langerhans cells in squamous epithelia, intestinal mucosa [4], airways, bile ducts, and interstitially in kidneys and hearts [5,6]. The migratory compartment of dendritic cells include the circulating veiled and blood dendritic cells in lymph and blood and interdigitating dendritic cells in the T-cell dependent areas of the lymphoid organs engaged in stimulation and growth of selected T cells from the circulating T-cell pool [7,8]. In the B-cell-dependent areas a different type of cell, called the follicular dendritic cell, traps antigen-antibody complexes and forms tight clusters with B cells [9]. The follicular dendritic cells play an important role in B-cell memory [9]. There is good evidence that the Langerhans cells in the epidermis, dendritic cells in the circulation (migratory dendritic cells), and dendritic cells in paracortical T-cell areas of the lymph nodes (interdigitating dendritic cells) are closely related to one another [7,10]. The Langerhans cells are found to migrate from the epidermis to the lymphoid organs [7,11]. It is not clear where in development the differentiation pathway that leads to the formation of follicular dendritic cells diverges from that which leads to development of Langerhans as well as circulating and interdigitating dendritic cells. It is clear that these two types of cells

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Table I. Dendritic Cells

Mucosa and Skin	Blood and Lymph	Lymphoid Organs
Langerhans cells	Blood dendritic cells	Interdigitating dendritic cells (T-cell areas)
Dendritic epidermal T cells	Veiled dendritic cells	Follicular dendritic cells (B-cell areas)

perform distinct functions and differ in expression of selected genes [7]. Table II summarizes the differences in cell markers between blood dendritic cells (DC), Langerhans cells (LC), follicular dendritic cells (FDC), and monocytes.

The common feature of all dendritic cells are their veiled phenotype, minimal phagocytic activity, and potent accessory cell function for stimulation and nurture of T cells and B cells [7,9,12].

The evidence for infection by HIV-1 of the blood dendritic cells as well as infection of other types of dendritic cells is summarized below.

HIV-1 INFECTION OF MIGRATORY DENDRITIC CELLS

The blood-derived T-helper dendritic cells from HIV-1–infected individuals contain significant amounts of HIV-1 viral DNA [13,14,15], and viral budding has been demonstrated from dendritic cells isolated from patients [13]. A recent study has found less proviral DNA in partially enriched cultures of dendritic cells from seropositive individuals as compared to purified cultures of CD4-positive T cells from the same individuals (Cameron PU, Forsum U, Teppler H, Granelli-Piperno A, Steinman RM: During HIV-1 infection most blood dendritic cells are not productively infected and can induce allogeneic $CD4^+$ T cells clonal expansion. Clin Exp Immunol 88:226–236, 1992). Blood-derived T-helper dendritic cells express the CD4 molecule, the receptor for the HIV-1 virus, as shown by immunoperoxidase and by immunogold electronmicroscopy (EM) studies [14,16]. The expression of CD4 on blood dendritic cells and Langerhans cells is enhanced by γ interferon [14,17].

Table II. Differences in Cell Markers Between Blood Dendritic Cells, Langerhans Cells, and Follicular Dendritic Cells

Determinant	Dendritic Cells	Langerhans Cells	Monocytes	Follicular Dendritic Cells
MHC				
Class I	+++	+++	++	+
Class II	+++	+++	+	+
T-cell markers				
CD1	−	+	−	−
CD3	−	−	−	−
CD4	+	+	+	−
CD8	+/−	−	−	−
TCR α/β	−	−	−	−
TCR γ/δ	−	−	−	−
Monocyte markers				
CD13	−	−	++	−
CD14	−	−	+++	+
CR, FcγR				
CR1 CD35	ND[a]	ND	+	+
CR2 CD21	−	−	−	+
CR3 CD11b (c3bi)	+	+	+	+
CD16 (FcRIII)	−	−	−	ND
CD23 (FcE)	−	+	−	+
CD32 (FcRII)	−	+	+	ND
B-cell markers				
CD18	−	−	−	+/−
CD19	−	−	−	+
CD20	−	−	−	−
CD40	+	−	−	+
NK cell markers				
CD56	−	−	−	−
CD57	−	−	−	−
IL-2R	+	+	−	−
Common Leukocyte Marker				
CD45	+	+	+	−
Adhesion molecules				
CD11a	+	+	+	−
CD18	−	−	+	+
VCAM-1	+	−	+	+
ICAM-1	+	+	+	+
VLA-β-1	+	ND	+	+

[a]Not determined.

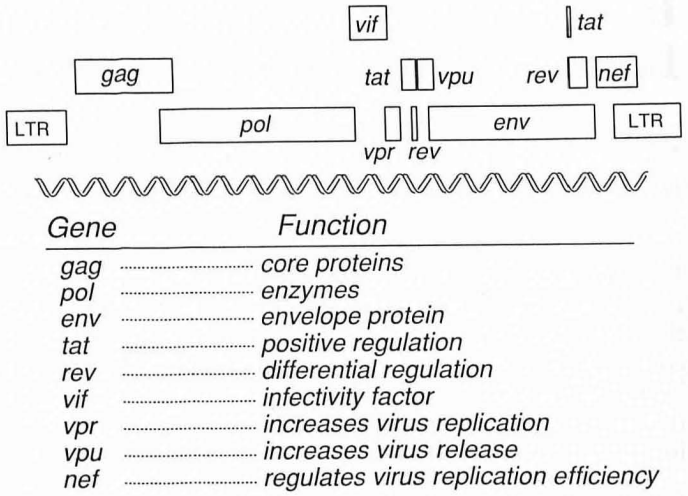

Figure 1. Genetic organization of HIV-1.

The studies of Langhoff et al (1991) have shown that blood dendritic cells *in vitro* are highly sensitive to infection with HIV-1 and support severalfold more viral production than infected bulk T cells, $CD4^+$ T cells, or monocytes [18]. Purified dendritic cells support prolific growth of both monocytropic and T-cell tropic isolates of HIV-1 as well as primary patient isolates [18].

REPLICATION OF HIV-1 IN MIGRATORY DENDRITIC CELLS

The basis for preferential infection and the high yield of virus production from cultures of primary blood–derived T-helper dendritic cells infected with HIV-1 has been investigated (Langhoff et al, submitted). Figure 1 shows the general genetic structure of the proviral form of HIV-1 and summarizes the function of the different viral genes expressed in infected leukocytes. The HIV-1 provirus encodes three structural genes, *gag, pol,* and *env.* The *gag* encodes viral structural core proteins. The pol gene encodes products necessary for synthesis of viral DNA, RNA-dependent DNA polymerase, and RNAse H. The amino terminus of *pol* specifies the protease and enzymes required for correct capsid protein processing. The carboxy terminus of this open reading frame specifies the integrase protein, an enzyme required for insertion of viral DNA sequences into the host genome. *Env* encodes proteins that are embedded in the viral outer lipid of the virus molecule (gp120 and gp41) and that determines the specific receptor interaction with the CD4 molecule on target cells.

In addition to the genes that encode virion proteins the genome of HIV-1 specifies at least six additional regulatory genes (*tat, rev, nef, vif, vpr,* and *vpu*) [19]. The tat gene encodes a transactivator function [20,21]. The *tat* protein binds to the 5′ end of the nascent RNA transcripts and accelerates the rate of initiation of mRNA as well as increasing the processivity of RNA polymerase that transcribes the HIV-1 mRNA. *Rev* encodes a protein that binds to a sequence in the 3′ portion of the primary mRNA transcript and

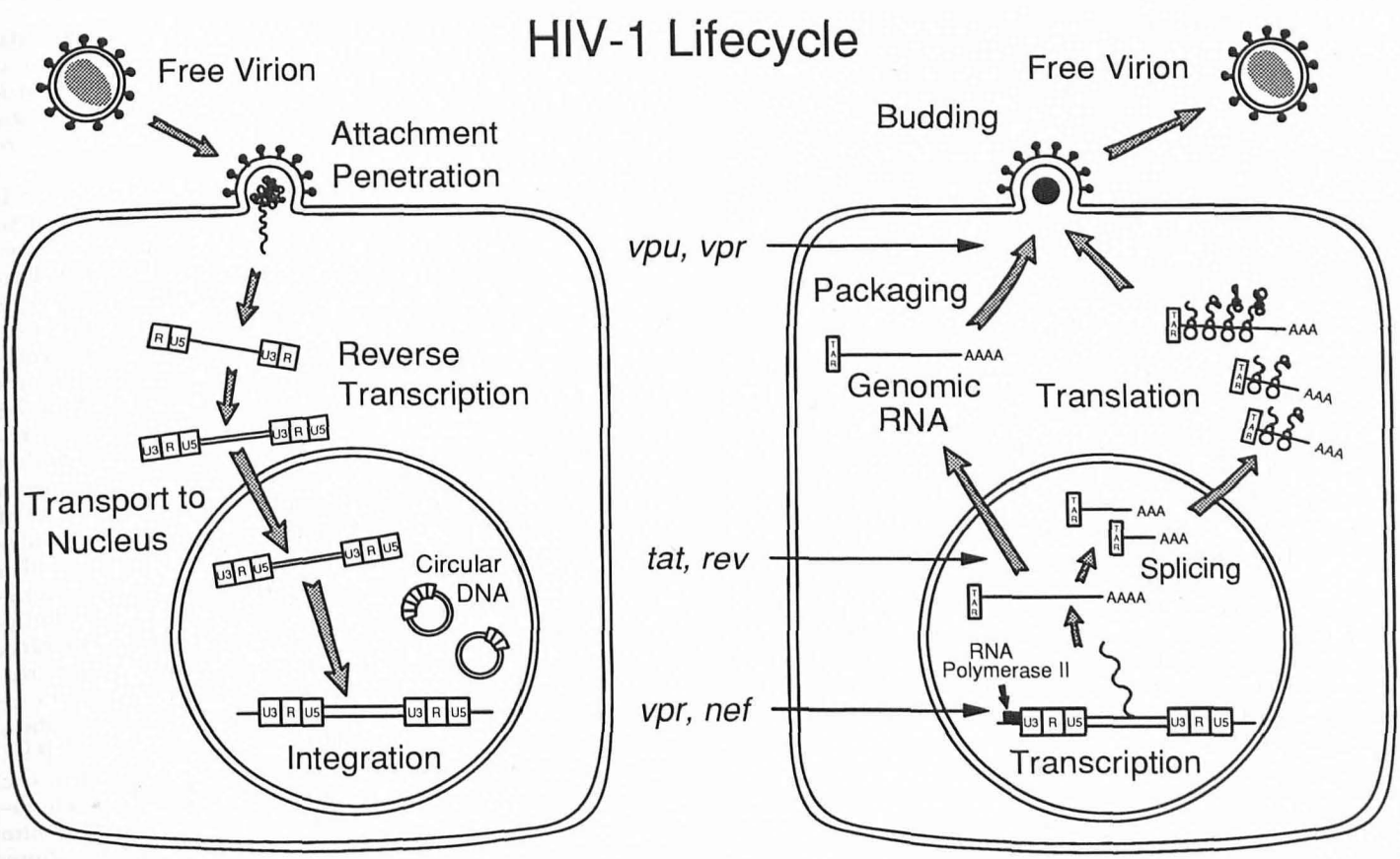

Figure 2. HIV-1 life cycle in infected cells.

permits nuclear export of unspliced and singly spliced mRNAs from the nucleus. In the absence of *rev* activity only multiply spliced mRNAs reach the cytoplasm, where they are translated [22,23]. Both *tat* and *rev* are essential genes for viral growth in lymphocytes. The *nef* coding exon begins immediately after the 3′ end of *env* [24,25]. Recent evidence indicates that *nef* may be necessary for prolific replication in the primary cells [25] and in the natural host [26].

Vif encodes a product that enhances infectivity of the virus particle [27,28]. *Vpr* [29,30] makes a protein that is found in the virus particle itself. *Vpr* is not required for virus replication in T-cell lines but does permit viruses to grow more rapidly in a variety of other cell types [31]. *Vpu* facilitates virus assembly and export [32].

The functions of these genes, *tat, rev, vpu, vpr, nef, vif,* and the regulatory elements of the long terminal repeat (LTR), have been determined in rapidly dividing tumor cell lines. Little is known regarding the function of these genes in subsets of primary leukocytes. It was recently shown that a virus defective for expression of *vpr, vpu,* and *nef* replicates in primary cultures of blood T-helper dendritic cells [18].

Figure 2 illustrates the virus life cycle in infected dendritic cells. The viral envelope glycoprotein gp120 binds to the CD4 molecule of the target cell and fuses with the cell membrane. Upon cell entry the viral RNA is converted to a double-stranded viral DNA form. This is a multistep process and includes binding of the tRNA primer, synthesis of DNA to the end of the 5′ R region, template switching to the 3′ end, and an elongation of the strand (minus strand) to the 5′ primer binding site. The synthesis of the 5′ LTR is initiated from the 3′ end of the minus strand DNA, and a second template switching followed by DNA-dependent polymerization occurs to complete the double-stranded DNA [33]. In dendritic cells this process occurs rapidly and completely. The synthesis of the double-stranded DNA in infected primary T-helper dendritic cells is completed by 20 h post-infection, much more rapidly than occurs in infected primary T cells and monocyte/macrophage cultures.

The newly made linear DNA duplex migrates to the nucleus. It is the precursor for integration. The stable association of viral and host genetic DNA is achieved by covalent linkage of the provirus DNA and the host DNA.

The linear virus DNA can also form several types of DNA circles within the nucleus. One type of circle contains two LTRs joined together end-to-end. A second type of circle contains only a single LTR formed by homologous recombination between the identical LTR sequences, which flank the full-length genome (for details see [16,33]). The importance of the circular forms for viral replication in the non-dividing dendritic cells is not known for HIV-1. For visna virus it is reported that the unintegrated forms are transcriptionally active [34]. Circular forms of HIV-1 have been shown to arise from superinfections of infected cells and may contribute to cytopathicity [35,36]. Infected cultures of dendritic cells and Langerhans cells show little cytopathic changes [18,37]. Viral mRNA is produced from integrated proviral copy.

Studies have been undertaken to determine the kinetic appearance of the viral mRNA transcripts in dendritic cells compared with the events of viral DNA synthesis and integration (Langhoff, Kalland, Haseltine, submitted). Table III shows the kinetics of viral reverse transcription, and viral mRNA expression in dendritic cells and T cells. Virus uptake and virus DNA synthesis in primary dendritic cells occurs rapidly and efficiently. Full-length copies of viral DNA appear in infected dendritic cells many hours before they appear in infected T cells. Viral mRNA species also appear in dendritic cells well before they appear in culture of infected primary T cells. The prolific virus replication at the molecular level in dendritic cells is remarkable because the high level of virus replication occurs in cells that are terminally differentiated and do not replicate [7].

Table III. Molecular Growth of HIV-1 in Blood Dendritic Cells and T Cells

	Complete Reverse Transcription		Viral mRNA Production	
	<20 h	40 h	<20 h	40 h
Dendritic cells	+	+	+	+
T cells	−	+	−	(+)

HIV-1 INFECTION OF LANGERHANS CELLS

The first evidence that HIV-1 could infect cells of Langerhans/dendritic cell lineage was the work of Tschachler and Stingl and their colleagues [38,39]. These workers reported [39] that 2–5% of Langerhans cells in the skin of HIV-1–positive individuals are infected by HIV-1 as judged by immunocytochemistry and EM. These Langerhans cells were HIV-1 culture positive [37,40]. A reduced number of Langerhans cells in the skin has also been reported in HIV-1 infected individuals, but this finding is controversial [41,42]. Recent studies of detection of proviral HIV-1 DNA by the polymerase chain reaction (PCR) has confirmed that HIV-1 infects Langerhans cells in skin [38,43,44]. Budding of mature virus from partially purified Langerhans cells infected *in vivo* has been shown by electronmicroscopy [40]. By contrast Kalter et al [45] and Kamitaki et al [46] do not find evidence of infection in skin Langerhans cells by HIV-1.

Studies of genital mucosal transmission of SIV macaques have shown that SIV infection is initiated when the virus is applied in high concentration to the sexual mucosa of male and female monkeys [2,47,48]. Miller et al find SIV antigens in cells resembling mucosal Langerhans cells shortly after application of virus to the mucosal surface. The viral antigens are initially concentrated in this type of cell. By *in situ* hybridization mucosal Langerhans cells became infected with SIV after mucosal exposure [48]. The virus doses required for mucosal infection are reported to be a thousandfold higher than those for intravenous infection [2]. Mucosal transmission is also known to occur for lentiviruses (visna-maedi) in sheep [49]. Transmission of HIV-1 by breast feeding of infants has also been proposed by Van de Perre et al [3] to occur at a rate of 36% from seropositive mothers.

It is thought that Langerhans cells in the skin undergo a process of maturation in the epidermis and migrate during this process via the dermis to the lymphatics and subsequently to the draining lymph nodes in the T-cell–dependent areas and/or through blood capillaries to the circulation [11]. Once infected in the mucosa, migration of infected cells may spread the infection to T cells in the lymph nodes. Recent studies in fact find a severalfold higher virus load in lymphoid tissue as compared to peripheral blood leukocytes [50].

HIV-1 INFECTION OF FOLLICULAR DENDRITIC CELLS

The follicular-dendritic cells form a distinct subgroup of the dendritic cells localized exclusively in the lymphoid follicles of which the B cells form the major cell component [9,51,52]. The follicular dendritic cells represent 2% of the total cell population of the follicles. In contrast to other dendritic cells, follicular dendritic cells are not thought to be bone marrow derived [53] and lack the common leukocyte marker (CD45) but have some surface markers in common with B cells (CD19) and monocyte/macrophages (CD14) [51,54]. Single cell isolates of follicular dendritic cells do not have surface CD4 expression [55,56] or mRNA for CD4 (Table II) [55].

One prominent function of follicular dendritic cells is their capacity to trap and retain antigen-antibody complexes on the long cytoplasmic extension by Fc and C3 receptors, and present these complexes to B cells [9,51,57,58]. The antigen-antibody complexes are not endocytosed and are retained for prolonged periods of time [9,59]. Follicular dendritic cells do not participate in the primary lymphocyte activation, they only retain antigen when specific antibodies are present. However, follicular dendritic cells accelerate the emergence of functional memory B cells and high-affinity antibodies [59]. Follicular dendritic cells also trap activated B cells in the germinal center by binding of the VLA-4 molecule on the B cells to NCAM-110 on follicular dendritic cells [60].

Early studies of retroviral infection of follicular dendritic cells have shown that these cells are infectable by Abelson leukemia virus and Rauscher leukemia virus [61,62] and with feline leukemia virus. HIV-1 virus particles were found associated with follicular dendritic cells in people with lymphadenopathy [63,64]. The virus particles were mainly found sequestered extracellularly in the labyrinth of the follicular dendritic cells [54,63] with occasional formation of virus suggestive of virus budding [64,65]. Subsequent studies [65–68] have demonstrated deposition of viral protein within the germinal centers primarily within the extracellular deposits of immune complexes. These studies do not rule out the possibility that the viral antigens were passively trapped as immune complexes arriving from remote extranodal sites. The functional importance of the trapping of antigen-antibody complexes is at present speculative. Klaus et al [59] have shown highly enhanced immunoglobulin responses when immunizing mice with antigen-antibody complexes rather than native antigen. The authors speculate that administration of HIV-1 antigens as a complex with the antibody targets the viral antigen to the follicular dendritic cells rather than to the cells of the monocyte/macrophage lineage. This may present one mechanism for the HIV-1–induced polyclonal B-cell activation and hypergammaglobulinemia in HIV-1–infected individuals [69].

A more definitive proof for an active virus replication in the germinal centers and in the follicular dendritic cells has been demonstration of mRNA by *in situ* hybridization [70,71]. Recent *in vitro* studies have also shown that highly purified (>90%) preparations of single-cell follicular dendritic cells are highly infectable by HIV-1 using the PCR for detection of HIV-1 DNA 4 d after infection [56]. In this case the lack of expression of the CD4 molecule by follicular dendritic cells was not limiting for the HIV-1 infection, which was also insensitive to pretreatment with anti-CD4 antibodies. The route of entry of the HIV-1 virus is unknown. An antibody mediated entry of the virus via the Fc-receptors of the follicular dendritic cells is not likely because the follicular dendritic cells retain antigen-antibody complexes on the surface [59]. HIV-1 infection of CD4-negative cells is reported to occur for certain neuronal, muscle, and intestinal cells [72,73]. The capacity of follicular dendritic cells to support long-term cell lines production is not determined.

In vivo infection with HIV-1 has profound effects on the architecture of the germinal centers. Early infection is characterized by follicular hyperplasia and disruption of the fine dendritic cell network in the follicles. With progress of the disease (follicle degeneration) follicular dendritic cells disappear and are absent in the late stage of HIV-1 infection (depleted stage) [68,71,74,75]. These *in vivo* studies raise the possibility that HIV-1 is cytopathic for follicular dendritic cells.

SUMMARY

These studies permit us to speculate that HIV is primarily an infection of the Langerhans/dendritic cell lineage. Cells of this lineage may provide the primary point of entry of the virus during sexual contact and breast feeding. Mobile Langerhans cells may permit the virus to travel from the site of primary infection to the lymph nodes and thence to the circulation. The circulating T-helper dendritic cells may provide a primary reservoir of HIV-1 infection. Virus-producing Langerhans cells at the surface of sexual mucosa may shed virus into seminal and vaginal fluids. Viewed from this perspective, infection and destruction of the $CD4^+$ T-cell population by HIV-1 may be an unfortunate secondary consequence of a primary infection of the Langerhans/dendritic cell lineage.

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