Transmission of HIV-1 by Primary Human Uterine Epithelial Cells and Stromal Fibroblasts

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Women can become infected with human immunodeficiency virus type 1 (HIV-1) after the heterosexual transmission of virus from an infected male partner. To understand the events that result in transmission of HIV-1 across the female reproductive tract, we characterized the life-cycle events of HIV-1 in primary cultures of human uterine epithelial cells and stromal fibroblasts. Epithelial cells and stromal fibroblasts released virus particles after exposure to either X4- or R5-tropic strains of HIV-1. Virus released by these cells was able to infect CD4+ T cells. When exposed to an X4-tropic strain of HIV-1, these cells supported HIV-1 reverse transcription, integration, and viral DNA transcription. When exposed to an R5-tropic strain, however, these cells released unmodified virus. These data suggest that uterine cells are targets for productive infection with X4-tropic strains and release unmodified R5-tropic viruses that would then be able to infect submucosal target cells, including T cells and macrophages.

Although heterosexual transmission is the predominant mechanism by which women acquire HIV-1 infection [1–4], our knowledge about the viral and host factors that lead to infection is limited. Identifying the cell population initially infected within the female reproductive tract (FRT) and the putative mechanisms by which HIV-1 is disseminated to distal sites is important to our understanding of the pathogenesis of HIV-1. Moreover, defining the mechanisms and conditions that either promote or inhibit HIV-1 infection within the FRT is necessary for the design and development of preventative measures.

Studies to identify cell populations within the FRT that become infected have focused on cell lines or primary cells and tissues from the lower FRT [5, 6]. Although studies using confocal microscopy have demonstrated the presence of HIV-1–positive cells in cultures of epithelial cells and stromal fibroblasts isolated from the uterus and Fallopian tubes [7], neither cell-free nor cell-associated virus has been shown to productively infect primary cervical or vaginal epithelial cells [8, 9]. Moreover, immunohistochemical studies using explants of cervical and vaginal tissue have identified macrophages, CD3+ T cells [8], and memory CD4+ T cells [10] as early primary target cells of HIV-1 infection. However, in those reports, HIV-1 infection was evaluated by use of intracellular expression of viral antigens and in situ hybridization techniques, approaches that do not detect integration of proviral DNA into the cellular genome, a hallmark of productive infection with retroviruses. The results of those studies also do not exclude the possibility that genomic viral RNA and viral proteins were simply internalized by the submucosal cells.

Although the lower FRT (cervix and vagina) is generally thought of as a primary site of HIV-1 infection, several observations suggest that the upper FRT (uterus and Fallopian tubes) may be an additional site of infection. Within minutes after in vitro fertilization, spermatozoa can be found in the uterus and Fallopian tubes...
Because spermatozoa have been shown to bind HIV-1 gp120 [12], it is conceivable that the virus could be transported to the upper FRT by piggybacking onto sperm. In other studies, both bacterial and viral sexually transmitted pathogens (STPs), including Clamydia trachomatis [13, 14], Neisseria gonorrhoeae [15, 16], and HIV-1 [17], have been found to travel freely in FRT secretions. Thus, both bacterial and viral STPs in semen are likely to reach the upper FRT within minutes of deposition in the vagina.

We previously reported that endometrial cell lines [6] and uterine primary cells [7] are susceptible to HIV-1 infection. To further investigate the possibility that uterine epithelial cells and stromal fibroblasts are primary sites for HIV-1 infection, we performed a systematic analysis of replication of HIV-1 in these cell populations. We show that a subset of both uterine epithelial cells and stromal fibroblasts express CD4, the putative HIV-1 receptor, as well as the chemokine receptors CXCR4 and CCR5. Our data show that primary human uterine cells are able to support reverse transcription (RT) and integration of HIV-1 and viral DNA transcription and can release infectious virus when infected with X4-tropic viruses. When exposed to R5-tropic viruses, however, these cells behave as viral reservoirs, sequestering and releasing unmodified virus that could then infect submucosal cells. Furthermore, cell-to-cell contact with a susceptible target cell appears to be an effective mechanism of virus transmission to submucosal leukocytes within the upper FRT.

MATERIALS AND METHODS

Source of tissues. Uterine tissue was obtained from 11 women undergoing hysterectomy at the Dartmouth-Hitchcock Medical Center. Informed consent was obtained from all patients. The menstrual stage of the endometrium was determined by examining hemotoxylin-eosin–stained paraffin sections [18] and were classified as proliferative (5 patients), secretory (5 patients), or inactive (i.e., postmenopausal; 1 patient).

Cell lines. The human CD4+ T cell line H9 (ATCC CRL1593.2) chronically infected with HIV-1inm and primary cultures of phytohemagglutinin (PHA)–stimulated peripheral blood mononuclear cells (PBMCs) infected either with HIV-1inm or HIV-1inm were used as positive controls for all assays evaluating HIV-1 infection of uterine cells. PHA blasts were prepared from the mononuclear cell fraction of blood obtained from healthy women and were cultured for 48 h in the presence of 4 µg/mL PHA (PHA-P; Sigma) before infection, as described elsewhere [7].

Antibodies. Antibodies used in the present study included an antiepithelial cell antibody (clone Ber-EP4; Dako; F0860), antivimentin (Sigma; V6630), anti-CD4–fluorescein isothiocyanate (FITC; Dako; F0766), anti–CCR5–phycoerythrin (PE; R&D Systems; FAB183F), and anti–CXCR4–PE (R&D Systems; FAB170F). Antibodies to leukocytes included anti–CD3–FITC (MHCD0301), anti–CD8–FITC (MHCD0801), anti–CD14–FITC (MHCD1401; all from Caltag Laboratories), and anti–CD45 (panleukocyte; Dartmouth Medical School Hybridoma Facility). In addition, a PE-conjugated anti-p24 monoclonal antibody (MAb; Coulter; PN604667) was used to detect the intracellular expression of HIV-1 p24 within infected cells. Isotype controls included IgG1–FITC (MG101), IgG2b–PE (MG2b04), and IgG2a–FITC (MG2a01; all from Caltag Laboratories).

Isolation of human uterine cells. Epithelial cells were isolated as described elsewhere [7]. In brief, tissues were minced into 1–2-mm fragments and were subjected to enzymatic digestion by use of a pancreatin-hyaluronidase-collagenase enzyme mixture. Epithelial cells were separated from the mixed stromal fibroblast population by use of filtration through a 20-µm pore size nylon mesh filter (Small Parts). These cells were cultured for 48 h, to allow reexpression of cell-surface receptors, and then were used for phenotyping and HIV-1 infectivity studies. Contaminating lymphocytes and macrophages were removed from both the epithelial-cell and stromal-fibroblast cultures by washing out the nonadherent cells after the 48-h incubation.

Epithelial cells were cultured in human extracellular matrix (BD Biosciences)–coated Falcon cell culture inserts, to establish confluent, polarized cultures. Confluency of the monolayers was verified by transepithelial resistance, determined by use of an EVOM electrode and volt ohmmeter (World Precision Instruments), as described elsewhere [19].

Immunophenotyping. Immunofluorescence and flow cytometry were performed by use of a FACScan flow cytometer (Becton Dickinson). Cells incubated with saturating concentrations of unconjugated MAbs were washed and incubated with FITC-conjugated goat anti–mouse IgG (Jackson ImmunoResearch Laboratories). Background levels of fluorescence were determined by incubating the cells with the appropriate isotype control. Cells were considered to be positive if >10% of the cells reacted with a particular antibody in excess of the isotype control.

HIV-1 infection and measurement of replication of HIV-1. The R5-tropic strain, HIV-1inm, was generated and titered in cultures of human macrophages. The X4-tropic strain, HIV-1inm (National Institutes of Health AIDS Repository, catalog no. 398), was generated in the H9 cells. Virus stocks were treated with 10 U/mL RNase-free DNase and 10 mmol/L MgCl2 for 30 min at room temperature. For HIV-1 infection, epithelial cells and stromal fibroblasts were grown to 70%–80% confluent, polarized cultures. Confluency of the monolayers was verified by transepithelial resistance, determined by use of an EVOM electrode and volt ohmmeter (World Precision Instruments), as described elsewhere [19].

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**HIV–1 RT.** HIV-1 viral DNA was detected by use of polymerase chain reaction (PCR) amplification using genomic DNA isolated from HIV-1–infected cells by use of the Puregene DNA isolation kit (Gentra Systems), according to the manufacturer’s instructions. HIV-1 DNA was amplified by use of the long-terminal repeat (LTR) sense primer (5′-GGCTTAAGGGAA-CACCACGGTGTTG-3′) and the HIV-1 gag antisense primer (5′-CTGAGAGAAGTCCTCCTGG-3′) [20]. The LTR/gag primers detect full-length or nearly completely synthesized viral cDNA. To control for integrity of cellular DNA and to verify equal loading, the human β-actin gene was amplified.

**Integration of HIV-1.** Integration of HIV-1 proviral DNA was detected by use of nested PCR using genomic DNA isolated from HIV-1–infected uterine epithelial cells. The first PCR was performed by use of the Alu sense primer (5′-GGCTTCCTGAGAGAAGTCCTCCTGG-3′) and the HIV-1 gag antisense primer (5′-CTGAGAGAAGTCCTCCTGG-3′) [20]. Five microliters of this PCR product was used as a template in a second nested PCR, using the LTR sense primer M667 (5′-GGCTTAAGGGAA-CACCACGGTGTTG-3′) and the antisense primer LA23 (5′-CTGAGAGAAGTCCTCCTGG-3′) [21]. This set of primers yields a 140-bp fragment.

**Transcription of HIV-1.** Transcription of HIV-1 was detected by use of RT-PCR using primers complementary to the flanking region of the common splice donor and acceptor sites of the env, tat, and rev genes. Five micromolars of total RNA extracted from an equivalent number of uninfected and HIV-1–infected epithelial cells and stromal fibroblasts was reverse transcribed with 20 U of avian myeloblastosis virus reverse transcriptase (Roche Diagnostic). Ten microliters of this reaction product was used in a PCR using the sense primer M669 (5′-GTGTGCCCCTCTGTTTGTTGACCTCGTAAAC-3′; nt 558–588) and the antisense primer LA23 (5′-GCCTATTCTGCTATGGAGTACCTC-3′; nt 5815–5792) of the HXB-2D molecular clone of HIV-1. The primers M669 and LA23 detect spliced RNA species that could arise only from newly transcribed RNA. This set of primers yields a 214-bp product from spliced RNA. The integrity of the DNA was tested by use of PCR amplification of the human β-actin gene.

**Cocultivation experiments.** H9 cells (5 × 10⁵ cells/mL) were added to wells containing uterine epithelial cells and stromal fibroblasts, on day 3 or 6 after exposure of the cells to HIV-1 env. Before cocultivation, epithelial cells and stromal fibroblasts were trypsinized and replated at a concentration of 0.1–0.2 × 10⁴ cells/well. After adherence, the cells were washed multiple times before the addition of H9 cells. The supernatant from the last wash was saved and used as an additional control. The H9 cells were cocultured with the epithelial cells or were incubated with the supernatant from the last wash for 24 h at 37°C. After this incubation, the nonadherent H9 cells were transferred to new 24-well plates and refed with fresh culture media. The cell cultures were split twice weekly and were examined 4 and 7 days later for intracellular expression of p24. Intracellular immunofluorescence was performed by fixing and permeabilizing cells by use of Fix and Perm (Caltag Laboratories), according to the manufacturer’s instructions. Uninfected cells stained with the same MAb were used as the negative control. In addition, H9 cells were incubated with anti-CD45 MAb, to verify the lack of contamination with residual CD45⁺ epithelial cells or stromal fibroblasts.

**RESULTS**

**Phenotypic characterization of human uterine epithelial cells and stromal fibroblasts.** In previous studies, we examined lineage-specific antigens and expression of HIV-1 receptors and coreceptors in tissue sections from the FRT. In those studies, cells were stained with antibodies in the absence of enzymatic digestion and were evaluated by use of confocal microscopy [7, 22]. We detected expression of Ber-EP4 on uterine epithelial cells and vimentin on stromal fibroblasts. In addition, uterine epithelial cells expressed receptors and coreceptors important for HIV-1 infection, including CD4, galactocerebroside (Gal-Cer), CXCR4, and CCR5. In the present study, FRT tissue sections were subjected to enzymatic digestion, to isolate particular cell populations before analysis of surface antigens. Homogeneity of the cells in each fraction was verified by expression of Ber-EP4 on epithelial cell cultures (90% ± 8% positive) and expression of vimentin on the stromal fibroblast cultures (90% ± 5% positive) (figure 1). Neither cell population was reactive for the panleukocyte marker CD45.

Immunofluorescence analysis revealed expression of CD4 on 42% ± 22% of the epithelial cells and 16% ± 4% of the stromal fibroblasts obtained from 3 samples from the proliferative phase. From 2 secretory-phase samples, 45% ± 15% of epithelial cells were CD4⁺, whereas only 10% of the stromal fibroblasts from a single sample were CD4⁺. CXCR4 was expressed on 13% of the epithelial cells and 8% of the stromal fibroblasts obtained from a single proliferative-phase sample, whereas only 12% ± 5% of the epithelial cells and stromal fibroblasts were CXCR4⁺ during the secretory phase (data from 2 samples). CCR5 was expressed on 20% ± 3.5% of the epithelial cells and 11% ± 9% of the stromal fibroblasts obtained from 2 proliferative-phase samples. However, CCR5 was not expressed on stromal fibroblasts from a secretory-phase sample that we examined.

Although we did not evaluate expression of GalCer on uterine epithelial cells and stromal fibroblasts after enzymatic digestion in the present study, we previously detected, by use of immunofluorescence and confocal microscopy, high levels of expression of GalCer on uterine epithelial cells during the secretory phase of the menstrual cycle [22]. GalCer is a receptor involved in HIV-1 transcytosis in the gastrointestinal tract [23].
HIV-1 Infection of Uterine Cells

Figure 1. HIV-1 receptors and coreceptors expressed by primary uterine epithelial cells and stromal fibroblasts. Primary uterine epithelial cells and stromal fibroblasts were examined for expression of surface antigen by use of flow cytometry. A fluorescence profile from patient 2349, whose sample was obtained during the proliferative phase of the menstrual cycle, is shown. The percentage of positive epithelial cells is as follows: Ber-EP4, 90%; CD4, 45%; CXCR4, 10%; and CCR5, 20%. The percentage of positive stromal fibroblasts is as follows: vimentin, 95%; CD4, 25%; CXCR4, 15%; and CCR5, 20%. The background fluorescence using an isotype control is shown (thin line).

and might be used to bind and internalize HIV-1 when expression of CD4 is low or absent.

Because primary epithelial cells normally grow as polarized monolayers with a plasma membrane separated into an apical and basolateral domain, we compared expression of Ber-EP4, CD4, CXCR4, and CCR5 on epithelial cells grown on tissue-culture dishes with expression of those grown as polarized cultures. Immunofluorescence analysis revealed no differences in the expression of any of these antigens (data not shown). Because the culture conditions of the epithelial cells did not appear to induce changes in the expression of these receptors, we chose to examine the infectivity by HIV-1 of epithelial cells grown on tissue-culture dishes with expression of those grown as polarized cultures. Replication of HIV-1 in uterine epithelial cells and stromal fibroblasts.

The early steps of replication, RT, and integration of HIV-1 and viral DNA transcription were evaluated on epithelial cells and stromal fibroblasts 2 and 3 days after exposure to virus. For these studies, isolated cell populations obtained during both proliferative and secretory phases of the menstrual cycle were analyzed. Primary cultures of uterine epithelial cells and stromal fibroblasts incubated with HIV-1IIIB demonstrated the presence of HIV-1 DNA 2 days after exposure to virus (figure 2A). In contrast, we were unable to detect HIV-1 DNA in samples infected with HIV-1_{null} (figure 2A). The PCR amplification was specific, because the 200-bp product of LTR/gag comigrated with the fragment amplified from H9 cells chronically infected with HIV-1_{null} and from PHA-stimulated PBMCs infected with HIV-1_{null}. To verify equal loading of genomic DNA in each lane, β-actin amplification from the same experimental samples is shown (figure 2A, lower panels).

Because epithelial cells lining the mucosal surfaces of the FRT are likely to be the first cell population to come into contact with HIV-1, we determined whether uterine epithelial cells exposed to X4-tropic HIV-1 strains were productively infected. We performed an integration assay using an Alu-LTR nested PCR. The first PCR was subjected to a second PCR using nested HIV-1 LTR–specific primers. As shown in figure 2B (upper panel), we detected a specific band of integrated HIV-1 proviral DNA in cultures of HIV-1–infected epithelial cells (lane 3) and infected H9 cells (lane 2) but not in cultures of uninfected epithelial cells (lane 4) or uninfected H9 cells (lane 1). To estimate the sensitivity of the integration assay, chronically infected H9 cells were mixed with uninfected cells, in proportions ranging from 100% to 0%. As shown in figure 2B (lower panel), we could detect integration of HIV-1 when the proportion of infected H9 cells was as low as 7.5%. Integration of HIV-1 DNA was not evaluated in stromal fibroblasts.

To further characterize replication of HIV-1 in cells exposed to X4-tropic strains of HIV-1, we evaluated viral transcription of the early regulatory genes tat/rev and env in both epithelial cells and stromal fibroblasts. As shown in figure 2C (upper panel), HIV-1 early transcripts were present in HIV-1–infected epithelial cells (lane 2) and stromal fibroblasts (lane 4). No HIV-1 RNA was detected in mock-infected cells (lanes 1 and 3) or in the absence of cellular RNA (lane 7). The PCR-amplified fragment comigrated with that from PHA-stimulated PBMCs (lane 6) and H9 cells used as positive controls (data not shown). To verify
the integrity of the DNA, PCR amplification of β-actin from the same experimental samples is shown (figure 2C, lower panel).

These results indicate that only X4-tropic HIV-1 isolates can complete a replicative cycle within uterine epithelial cells and stromal fibroblasts. In contrast, these cell populations do not support replication of R5-tropic HIV-1 strains.

**Release of p24 in the culture supernatant by uterine cells incubated with X4- or R5-tropic HIV-1 strains.** As shown in figure 3A, cultures of uterine epithelial cells and stromal fibroblasts infected with either X4- or R5-tropic strains of HIV-1 released p24 into the supernatants and demonstrated an increase in the amount of p24 secreted over time, beginning 2 days after infection. We also compared levels of HIV-1 p24 in the culture supernatants of epithelial cells and stromal fibroblasts isolated from 2 proliferative-phase samples (figure 3B, right panel) and 2 secretory-phase samples (figure 3B, left panel), at various days after infection with HIV-1$_{11002}$. In epithelial cells, the levels of p24 increased from day 1 to day 2 after infection and remained constant from day 3 to day 6. In stromal fibroblasts, the levels of p24 increased from day 1 to day 2 and remained constant from day 2 to day 6. There were no differences between the levels of p24 released by cultures of uterine epithelial cells and stromal fibroblasts from either the proliferative or secretory phases.

**Infectivity of the released virus.** The infectivity of the virus released from cultures of uterine cells was measured by infecting H9 cells with culture supernatant harvested 3 days after infection with HIV-1$_{11002}$. As an additional control, cultures of epithelial cells and stromal fibroblasts were extensively washed after exposure to the virus, and the media from the final wash was then used to infect H9 cells. Infection of H9 cells was determined by measuring production of p24 in the culture supernatant 7 days after infection and by measuring the percentage of p24+$^+$ cells by use of flow cytometry. As shown in figure 4A, day-3 supernatants from either epithelial cells or stromal fibroblasts contained virus that infected H9 cells. More than 95% of H9 cells incubated with day-3 supernatants from both cell populations were positive for intracellular p24 7 days after infection (figure 4B). In contrast to these findings, H9 cells incubated with
Figure 3. Release of virus by uterine epithelial cells and stromal fibroblasts infected with X4- and R5-tropic HIV-1 strains. Viral release from cultures of uterine epithelial cells and stromal fibroblasts was determined by use of p24 ELISA. A, HIV-1 p24 values (ng/mL) in the culture supernatants of epithelial cells and stromal fibroblasts at day 4 after infection (mean ± SEM). B, HIV-1 p24 values in the culture supernatants of epithelial cells and stromal fibroblasts isolated from 2 samples obtained during the proliferative phase (right panel) and 2 samples obtained during the secretory phase (left panel) of the menstrual cycle, at various days after infection with HIV-1 IIIB (mean ± SEM). AW, after wash.

The media from the final wash did not become infected (figure 4). Therefore, uterine epithelial cells and stromal fibroblasts release virus that can potentially infect submucosal leukocytes.

Transmission of infectious virus to CD4+ T cells by HIV-1-infected uterine cells after coculture. In these experiments, cultures of uterine epithelial cells and stromal fibroblasts from a secretory-phase sample infected with HIV-1 IIIB were cocultured with H9 cells, to determine whether these cell populations can transmit infection to CD4+ T cells by cell-to-cell contact. In a mixture of uterine epithelial cells or stromal fibroblasts and H9 cells, HIV-1 infection was transmitted to H9 cells by day 2 of infection, as determined by the expression of p24 in the majority of H9 cells 7 days after coculture (figure 5A, left panel). In addition, ELISA performed at the same time showed high levels of production of p24 by H9 cells (figure 5B). To rule out the possibility that H9 cells became infected by residual virus released into the culture supernatants of the epithelial cells, we added media from the final wash of the epithelial cells (before the addition of H9 cells) to a separate culture of H9 cells and then evaluated these H9 cells for infection 7 days later. H9 cells incubated with this final wash media did not develop any evidence of infection (figure 5A). This sequence of studies was repeated using HIV-1–infected epithelial cells and stromal fibroblasts on day 6 of infection, with similar results (figure 5A [right panel] and 5B). These findings demonstrate that HIV-1 transmission mediated by cell-to-cell contact can occur between primary uterine epithelial cells and stromal fibroblasts and CD4+ T cells.

DISCUSSION

Although several mechanisms that could account for the ability of HIV-1 to traverse the epithelial lining of the FRT have been postulated, the role that epithelial cells play in this process is still controversial. Epithelial cells may inhibit or prevent viral infec-
tion by acting as a physical barrier [8]; or favor viral infection by sequestering, transporting, and releasing infectious virus [9, 23–28]; and/or by developing a productive viral infection.

Using primary cultures of human uterine epithelial cells and stromal fibroblasts, we have demonstrated that the uterus is a potential site for productive infection with X4-tropic, but not R5-tropic, strains of HIV-1. Uterine epithelial cells and stromal fibroblasts infected with an X4-tropic strain of HIV-1 supported RT and integration of HIV-1, transcription of viral DNA, and viral release. Although the levels of virus that were released from cultures of either epithelial cells or stromal fibroblasts were relatively low, they were within the range of values that have been reported for epithelial cell lines of intestinal, vaginal, endometrial, or fibroblastoid origin [5, 6, 29–35]. Furthermore, the secreted virus was infectious to a CD4+ T cell line (H9 cells). H9 cells were also infected after cocultivation with HIV-1–infected epithelial cells or stromal fibroblasts. Therefore, release of infectious virus, as well as cell-to-cell contact–mediated transmission of HIV-1, may be an important mechanism of viral spread among cells within the upper FRT.

Of particular interest was the lack of RT and transcription of HIV-1 after infection of the epithelial cells and stromal fibroblasts with an R5-tropic strain of HIV-1, despite the ability of these cells to release this virus for several days after infection. These data suggest that only particular strains of HIV-1 are able to undergo replication in primary cultures of epithelial cells and stromal fibroblasts. Whether this is due to differences in expression of cellular receptors and coreceptors or to other cofactors important in viral replication is unknown at present. That unmodified virus was released from these cells after infection suggests that a transport mechanism independent of viral replication may exist. One potential mechanism might involve the use of a selective vesicular transcellular pathway that would allow the transport of intact virus from one pole of the cell to the other. This would prevent access of the internalized virus to the cytosol and would therefore preclude productive infection of the epithelial cells. Thus, transport and release of unmodified viruses by uterine cells might be an alternative mechanism of viral spread to submucosal cell populations within the upper FRT. The mechanism by which X4- and R5-tropic viruses would be shuttled to different intracellular pathways in these cells is unknown at present.
In vivo studies of human cervical tissue [10] and of nonhuman primate models [36–38] have shown that exposure to HIV-1 or simian immunodeficiency virus did not result in infection of epithelial cells. Although our observations contradict the concept that epithelial cells are refractory to infection with HIV-1, the detection of proviral DNA integrated into the epithelial cell is a clear indication that uterine epithelial cells are able to support a productive, albeit low, viral infection with X4-tropic virus strains.

Another consideration in transmission of virus within the FRT is the nature of the interaction between HIV-1–infected epithelial cells and populations of submucosal target cells. Epithelial cells and stromal fibroblasts were able to transmit HIV-1 to CD4+ T cells in coculture experiments, which then resulted in infection of the CD4+ T cells. Whether this process required cell-to-cell adhesion [39] or formation of syncytia [40] is unknown at present. Electron microscopy studies have demonstrated that, in H9 cells, cell contact–mediated transmission of HIV-1 via syncytia formation requires interaction between CD4 and gp120 and RT but does not appear to involve the release of free virus. Our finding that HIV-1 cDNA could be detected in cultures of both epithelial cells and stromal fibroblasts suggests that an episomal form of viral DNA could be transmitted, become integrated, and replicate within the target cell. Alternatively, as is the case with human T lymphotropic virus–I [41], cell contact–mediated transmission of HIV-1 may require...
polarization of the cytoskeleton at the contact area between cells and transfer of the viral genome from HIV-1–infected cells to uninfected cells.

Overall, our data suggest that viral replication in the upper FRT may occur only with certain strains of HIV-1 and may represent a mechanism for selective viral replication. Therefore, 3 potential mechanisms of virus transmission within the FRT may exist. One mechanism appears to occur only with X4-tropic strains of HIV-1 and supports the development of a productive viral infection by the epithelial cell. Infection with HIV-1(R5) an R5-tropic strain of HIV-1 in which replication is not supported, appears to lead to the gradual release of unmodified infectious virus over several days. In addition, HIV-1 infection is transmitted after cell-to-cell contact between the HIV-1–infected epithelial cells or stromal fibroblasts and the susceptible target cell. Thus, it is likely that one mechanism or all mechanisms are important in HIV-1 infection within the FRT.

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References