Human Immunodeficiency Virus Env-Independent Infection of Human CD4⁺ Cells

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CD4⁺ epithelial cells covering mucosal surfaces serve as the primary barrier to prevent human immunodeficiency virus type 1 (HIV-1) infection. We used HIV-1 vectors carrying the enhanced green fluorescent protein gene as a reporter gene to demonstrate that HIV-1 can infect some CD4⁺ human epithelial cell lines with low but significant efficiencies. Importantly, HIV-1 infection of these cell lines is independent of HIV-1 envelope proteins. The Env-independent infection of CD4⁺ cells by HIV-1 suggests an alternative pathway for HIV-1 transmission. Even on virions bearing Env, a neutralizing antibody directed against gp120 is incapable of neutralizing the infection of these cells, thus raising potential implications for HIV-1 vaccine development.

Epithelial cells that cover a large surface area are the initial site of contact between the host and human immunodeficiency virus (HIV) type 1 (HIV-1) in persons who are exposed to the virus or virus-infected cells. Therefore, epithelial cells could play an important role early in HIV-1 infection and in the initial spread of infection. The entry of virus across the epithelial barrier could significantly influence the risk of mucosal infection and systemic spread.

HIV infects CD4⁺ cells by a process of membrane fusion that is mediated by the interaction of the HIV-1 envelope glycoprotein, gp120, with two cell membrane components, CD4 and a coreceptor belonging to the chemokine receptor family (5, 6, 8, 10). Previous reports have demonstrated that some CD4⁺ human cells, including epithelial cells, are also susceptible to HIV-1 infection (9, 11, 14, 16, 24). The binding of gp120 to chemokine receptors, including CXCR4 and CCR5, or galactosylceramide (GalCer) has been postulated as the mechanism for HIV-1 infection of these cells (1, 3, 4, 7, 8, 13, 21). A few results support such a mechanism: (i) antibodies against gp120 or GalCer inhibited virus entry into some CD4⁺ epithelial cell lines (3, 13, 22); (ii) molecules that bind to CCR5 or that down-regulate GalCer blocked infection of CD4⁺ cells (7, 25); and (iii) HIV-2 could efficiently infect mink lung Mv-1-lu and feline kidney CCC cells that stably expressed CXCR4 on their cell membranes (21). However, the above results do not exclude the possibility that the infection of CD4 cells by HIV-1 may also occur through alternative mechanisms.

In this study, we tested whether HIV-1 Env⁺ infects CD4⁺ cells. We prepared a virus carrying the enhanced green fluorescent protein (EGFP) gene and with no viral envelope proteins on its surface by transfection. The prepared virus was used to infect CD4⁺ epithelial cell lines derived from mouth, kidney, cervix, and prostate gland and a fibroblast cell line. Our results indicate that CD4⁺ cells from many organs may be susceptible to HIV-1 infection in an HIV-1 Env-independent fashion.

MATERIALS AND METHODS

Human cells. Human cell lines were maintained in RPMI medium with 10% fetal bovine serum. Primary gingival epithelial cells (normal human oral keratinocytes [NHOK]) were derived from gingival tissue obtained from collections from normal donors having periodontal surgery in accordance with procedures approved by the Human Subject Protection Committee at the University of California, Los Angeles. These cells were maintained and expanded by a previously described procedure (17).

Virus preparation and titration. Thirty micrograms of plasmid pNL4-3-EGFP Env⁺ DNA alone or with plasmids containing the HIV-1_{LAI} env gene or the vesicular stomatitis virus (VSV) envelope G glycoprotein (VSV-G) gene was used to transfect 293T cells in a T175 flask by a calcium precipitation method. The transfection reagents were purchased from Promega (Madison, Wis.) (the Profection kit). The transfected cells were washed twice at 16 h posttransfection, and virus was collected at days 2 to 4 posttransfection. The collected virus supernatant was filtered through a 0.45- μ m-pore-size filter, and an aliquot was used for p24 assays. Virus stocks were stored in a -70°C Revco freezer.

Virus infection and detection of EGFP-positive cells. Cells (5×10^3 per well of 24-well culture plates or 2×10^4 per well of 6-well plates) were placed 24 h before infection. Viruses (p24 counts of 100 ng for each well of 24-well plates or 400 ng for each well of 6-well plates) were added to each well for 16 h. The viruses were removed, and the cells were washed with serum-free medium before fresh growth medium was added to the infected-cell culture. At day 6 postinfection, EGFP-positive cells were counted visually under a UV microscope or analyzed by flow cytometric analysis.

Neutralization of gp120 on HIV-1 virions by monoclonal antibody IgG1-b12. HIV-1 NL4-3-EGFP with or without HIV-1_{LAI} envelope proteins, and with a p24 count of 30 ng was mixed with 0.5 μ g of immunoglobulin G1 (IgG1)-b12 (NIH AIDS reagent) for 10 min at 37°C and then for 20 min at room temperature before infection. For the control, virus was incubated under the same conditions without antibody before infection.

MOLT4 T cells producing NL4-3-EGFP Env⁺ virus. MOLT4 cells (5×10^6) were infected with VSV-G-pseudotyped NL4-3-EGFP virus by incubating the cells with 5 ml of virus supernatant (1,000 ng of p24/ml) in the presence of Polybrene (8 μ g/ml; Sigma) at 37°C for 2 h at a multiplicity of infection of 0.5. The virus supernatant was removed, and the cells were washed twice with RPMI medium and subsequently cultured for 4 days with 10 ml of RPMI medium with 10% fetal calf serum, 100 U of penicillin/ml, and 100 μ g of streptomycin/ml. The culture medium was replaced with 10 ml of fresh medium at day 4 after infection. The culture supernatant was collected at day 8 after infection and filtered through a 0.22- μ m-pore-size filter. The p24 level of the supernatant was 1,079 ng/ml. Fifty nine percent of the cells were EGFP positive on day 8 after infection, as analyzed by flow cytometry.

RESULTS

We noted in initial experiments that HIV-1 virions formed in the absence of a functional gp120 envelope protein would still give a low level of infection for some CD4⁺ cell types. We investigated this finding further by using HIV-1 NL4-3-EGFP

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FIG. 1. Construction of HIV-1 NL4-3-EGFP Env⁻. (A) Structure of HIV-1 NL4-3-EGFP Env⁻. (B) Diagram of the preparation of NL4-3-EGFP viruses with no envelope protein, with HIV-1_{LAI} envelope proteins, or with VSV-G. LTR, long terminal repeat. (C) Reverse transcription-PCR quantification of virus stocks. Viral RNA was obtained from virus stocks with 1 ng of p24. This amount of virus represents 5×10^3 to 5×10^4 RNA molecules.

FIG. 2. Infection of CD4⁺ epithelial cell lines by HIV-1 NL4-3-EGFP Env⁻. Cells (2×10^4) were plated in each well of six-well culture plates 24 h before infection. Cells were infected as described in Materials and Methods with 400 μ g of p24 per well. At day 6 postinfection, cells from three of the cell lines, 293T, Tu139, and Tu177, infected with viruses NL4-3-EGFP Env⁻, NL4-3-EGFP Env⁻ with HIV-1_{LAI} envelope proteins, and NL4-3-EGFP Env⁻ with VSV-G, were collected for flow cytometric analysis to determine the percentage of EGFP-positive cells present. AZT (5 μ M), a reverse transcriptase inhibitor, was added to parallel cell culture dishes 30 min prior to viral infection; this concentration of AZT was maintained in the culture medium throughout the test period.

FIG. 3. Infection of CD4⁺ cells by HIV-1. (A) Infection of Tu139, Tu177, and 293T cells by HIV-1 carrying no viral envelope proteins or envelope proteins from either HIV or VSV (results from two independent experiments). The methods are described in the legend to Fig. 2. (B) Infection of cell lines HT1080, DU145, and HeLa-CD4 by HIV-1 NL4-3-EGFP-Env(-) alone or with either HIV-1_{LAI} envelope proteins or VSV-G (results from two independent experiments). Cells (5×10^3) were plated in each well of 24-well culture plates 24 h prior to infection. Viruses with a p24 titer of 100 ng were added to each well. At day 6 postinfection, EGFP-positive cells were counted visually under a UV microscope. The cells that were connected to each other, forming a positive colony, were counted as one infection event. The total numbers of infection events in each well were counted and divided by 10^4 (we estimated that the cell numbers in each well doubled after 24 h in culture) to obtain the percentage of infection. The AZT control assays were performed by adding 5 μ M AZT to the culture medium 30 min before viral infection, and this concentration of AZT was maintained in the medium after viral infection. (C) Infection of CD4⁺ prostate cell line DU145 by various doses of NL4-3-EGFP viruses with or without Env. Cells were prepared as described in panel B, and viruses with 10 to 100 ng of p24 were added to each well. At day 6 postinfection, EGFP-positive cells were counted. (D) Infection of CD4⁺ prostate cell line DU145 with HIV-1 NL4-3-EGFP Env⁻ prepared from a stably transduced CD4⁺ T-cell line, MOLT4 (see Materials and Methods). Error bars indicate standard deviations.

Env⁻, derived from HIV-1 strain NL4-3, bearing a sensitive reporter gene for EGFP, and lacking functional gp120. HIV-1 carrying the EGFP reporter gene and with a deletion of the Env proteins was generated by cotransfection. The plasmid used for transfection to generate HIV-1 NL4-3-EGFP Env⁻ was prepared by deleting part of *env* (between the two *Bgl*II sites in gp120, from nucleotides 7032 to 7612 of HIV-1 NL4-3 [National Center for Biotechnology Information accession no. M19921]), resulting in a deletion and a frameshift in the Env gp160 reading frame. Thus, gp160, the precursor of viral envelope glycoproteins gp120 and gp41, would not be produced. The *nef* sequence was also partially deleted (222 bp from the start codon), and EGFP was inserted to replace the deleted *nef*

sequence (Fig. 1A). Virus was prepared by transfection of the human 293T cell line (a human embryonic kidney epithelial cell line transformed by simian virus 40 large T antigen and adenovirus). As controls, we prepared two other viruses bearing HIV-1_{LAI} Env proteins or the VSV-G gene by cotransfecting 293T cells with pNL4-3-EGFP Env⁻ and plasmids carrying the HIV-1_{LAI} *env* gene or the VSV-G gene, respectively (Fig. 1B). VSV-G-pseudotyped viral particles have a wide target cell spectrum (18). Using semiquantitative reverse transcription-PCR, we found that all three virions were produced from transfected 293T cells with comparable efficiencies (Fig. 1C). Env is not required for virus generation, consistent with previous reports (20, 23).

FIG. 4. UV microscope detection of HIV-1-infected CD4⁺ cells. (A) CD4⁺ epithelial cell lines infected by NL4-3-EGFP Env⁻ virus with HIV-1_{LAI} envelope proteins, with no addition of envelope proteins, or with VSV-G. Photographs were taken at day 6 postinfection. Cell culturing and HIV-1 infection are described in the legends to Fig. 2 and 3. (B) EGFP-positive cells from cell culture plates of Tu177, Tu139, and DU145 CD4⁺ cells infected by NL4-3-EGFP Env⁻ virus generated from the stably transduced MOLT4 cell line. The cell culture plates were prepared as described in the legend to Fig. 3. Viruses with 20 ng of p24 in 0.5 ml of RPMI medium were added to each well of cultured cells. EGFP-positive cells were detected at day 6 postinfection.

We infected two CD4⁺ epithelial cell lines derived from an oral carcinoma, Tu139 and Tu177 (17), with these viruses. The kidney epithelial cell line 293T was also tested. HIV-1 bearing VSV-G efficiently infected all three cell lines, as assayed by flow cytometric analysis of EGFP-positive cells. At 3 to 6 days postinfection, EGFP-positive cells were also detected in the Tu139 and Tu177 cell lines infected with the other two viruses, one containing HIV-1_{LAI} Env proteins and one containing no HIV-1 Env proteins. The oral epithelial cell lines Tu139 and Tu177 showed high levels of infection, with greater than 5% of cells being positive (Fig. 2). Virus with HIV-1 Env proteins or with no Env proteins showed very low levels of infection of 293T cells, occasionally visualized by fluorescence microscopy (Fig. 3 and 4). To confirm that the observed EGFP expression was due to HIV-1 infection, zidovudine (AZT), a reverse transcriptase inhibitor, was added to a parallel set of infected cultures. The presence of AZT resulted in nearly complete elimination of EGFP-positive cells (Fig. 2). Thus, the expression of EGFP in infected CD4⁺ cells results from stable HIV-1 infection.

To assess whether CD4⁺ cells from other tissues were susceptible to HIV-1 infection, we used each of the three viruses to infect other CD4⁺ cell lines, including the fibrosarcoma cell

line HT1080 (derived from the tissue adjacent to the acetabulum; ATCC CCL-121), the prostate cancer cell line DU145, and the cervical cancer cell line HeLa and its derivative, HeLa-CD4 (with expression of the CD4 molecule). All of these cells, except for HT1080, are epithelial cell lines; HT1080 is a fibrosarcoma cell line with epithelial morphology. As described above, Tu139 and Tu177 could be infected (Fig. 3A and 4A). Significant levels of infection were also seen in two other cell lines, HT1080 and DU145, with greater than 1% of cells being infected (Fig. 3B and 4A). As expected, the HeLa-CD4 cell line was highly susceptible only to NL4-3-EGFP Env⁻ with VSV-G or HIV-1_{LAI} Env proteins (Fig. 3B).

We tested the dose response for CD4⁺ cell infection. The CD4⁺ cell line DU145 was infected with various doses of NL4-3-EGFP viruses without or with Env. As Fig. 3C shows, with increasing titers of viruses, EGFP-positive cells increased proportionately. Our results indicate that HIV-1 can infect many types of CD4⁺ cells at low but significant levels. HIV-1 without Env in our experiments (200 ng of p24/ml) demonstrated approximately 400 tissue EGFP-positive U/ml for DU145 cells, 1,300 EGFP-positive U/ml for Tu177 cells, and 2,200 EGFP-positive U/ml for Tu139 cells. HIV-1 with Env showed similar infectivity, approximately 300 EGFP-positive

FIG. 5. Time course of infection by NL4-3-EGFP Env⁻ virus. Infections of two oral cell lines were performed by the methods described in the legend to Fig. 2. After infection, cells were washed twice with serum-free medium, and new culture medium was added. After 1 h of incubation, 330 μ l (1/6 volume) of the 2 ml in the wells was harvested for the p24 assay (day 1), and 330 μ l of fresh medium was added to the wells to maintain the volume. The same procedure was followed on days 3 and 5 for p24 sample collection. The AZT control assays were performed by adding 5 μ M AZT to the culture medium 30 min prior to viral infection, and this concentration of AZT was maintained in the medium throughout the experiment. Error bars indicate standard deviations.

U/ml for DU145, 1,100 EGFP-positive U/ml for Tu177, and 1,600 EGFP-positive U/ml for Tu139 cells.

To assess the significance of Env-independent infection in more physiologically relevant cells, we derived cultured primary human oral epithelial cells from normal gingival tissue. The cells derived from gingival tissue, NHOK (17), were expanded in cultures for less than two passages. Gingival cells from two normal individuals were tested. At day 6 postinfection, 0.6 to 1.2% of the cells were EGFP positive (Fig. 3B, NHOK). The percentage of HIV-1-infected cells was higher than that seen with infection of the 293T kidney epithelial cell line but lower than that seen with infection of the oral epithelial cell lines Tu139 and Tu177. The susceptibilities of the gingival epithelial cells to HIV-1 with or without Env proteins were similar.

We also tested virus produced from a CD4⁺ T-lymphocyte cell line, MOLT4. The MOLT4 cell line was stably transduced with NL4-3-EGFP Env⁻. HIV-1 NL4-3-EGFP Env⁻ collected from this cell line was used for infection of the CD4⁻ cell lines Tu139, Tu177, and DU145. Significant levels of EGFP-positive cells were detected in all three tested cell lines (Fig. 4B). We further demonstrated a dose response for EGFP-positive cells following infection of DU145 cells (Fig. 3D).

The NL4-3-EGFP Env⁻ genome contains all the genes necessary for virus replication in susceptible cells. We predict that if infection of susceptible epithelial cells occurred by Env-independent mechanisms, we would observe ongoing spread of virus infection in the cultures. Following infection of Tu139 and Tu177, the culture medium was collected at various times postinfection and assayed for HIV-1 p24. p24 levels increased in the culture medium over time, indicating that new infectious virus particles were generated from the infected cells (Fig. 5). These results are consistent with the increasing number of

EGFP-positive cells observed over time (data not shown). The increase in p24 levels in the infected-cell culture was inhibited by the addition of AZT. Thus, these results demonstrate ongoing replication and continued spread of the virus within the cultures over time in the absence of Env.

One prediction for Env-independent infection is that neutralizing antibodies should not block infection even for HIV-1 that bears Env. We tested this prediction by examining the effect of neutralizing monoclonal antibody IgG1-b12 (NIH AIDS reagent; catalog no. 2640). The addition of IgG1-b12 efficiently decreased the infection of HeLa-CD4 cells by NL4-3-EGFP bearing the HIV-1_{LAI} envelope protein (Fig. 6A). However, the addition of this antibody did not show any neutralization of the ability of the same virus to infect CD4⁻ cells Tu139 and/or DU145 (Fig. 6B). As expected, the addition of IgG1-b12 did not neutralize infection of either Tu139 or DU145 CD4⁻ cells by NL4-3-EGFP Env⁻.

DISCUSSION

With the discovery of HIV-1 coreceptors, it appears that HIV-1 can infect cells not only via binding to CD4 and subsequent fusion but also via direct interactions between gp120 and coreceptor molecules, albeit at a lower efficiency. Although such a mechanism provides a reasonable explanation for the infection of CD4⁻ cells by HIV-1, it fails to explain our observation that gp120 is not required for some infections. Therefore, we propose a different mechanism for HIV-1 infection of CD4 cells, occurring independently of HIV-1 envelope proteins. It has been reported that cellular membrane proteins are incorporated into the viral lipid envelope during budding (12). It is possible that these cellular membrane proteins in the viral envelope interact with appropriate receptors on the surface of some target CD4⁻ cells, thus facilitating entry of the virus into the target cells. The HIV-1 Env protein, gp120, is not stable, and a significant percentage of HIV-1 virions may lose this protein from their envelopes shortly after the viral particles are released from infected cells. Without gp120, these viral particles are no longer able to infect cells via binding to CD4 molecules but may still infect cells via the route described above. Indeed, we found that virions with or without gp120 infected the tested CD4⁻ cells with equal efficiencies. A significant amount of HIV-1 could be sustained in these CD4⁻ cells and could serve as a reservoir for HIV-1. In most of our tests, virus with a p24 count of 200 ng/ml is comparable to the virus load in most AIDS patients before treatments (15). If even 0.1% of CD4⁻ cells in patients are infected, the total infected CD4⁻ cells should be greater than 10^9 .

The infection of human CD4⁻ cells by HIV-1 may be important in disease transmission, latency, and progression. Epithelial cells lining the respiratory, digestive, and genital tracts provide a protective boundary against the external environment. Mucosal epithelial cells can form tight junctions that subdivide the plasma membrane into an apical domain, which faces the luminal side, and a basolateral domain, which faces the connective tissue of the underlying lamina propria. In such polarized epithelial cells, plasma membrane proteins may differ significantly at the two surfaces. HIV-1 has been reported to bud preferentially from the basolateral surface of polarized epithelial cells (19). The release of virus from the basolateral domain may play an important role in HIV-1 transmission. However, the mechanisms of HIV-1 entry into patients through the cellular mucosal barrier remain obscure (2). The direct infection of epithelial cells and the subsequent release of virus from the basolateral domain may be important means of HIV-1 transmission.

FIG. 6. Neutralization of HIV-1 gp120 by monoclonal antibody (mAB) IgG1-b12. gp120-specific IgG1-b12 was used to neutralize NL4-3-EGFP viruses with or without HIV-1 envelope proteins. (A) Flow cytometric analysis of the infection of HeLa-CD4 cells by NL4-3-EGFP virus with the HIV-1_{LAI} envelope protein. Significant neutralization can be seen by comparing the C4 quadrants (0.9 versus 5.9% EGFP-positive cells). (B) Percentage of EGFP-positive cells following infection by IgG1-b12-treated virus relative to virus treated identically but without antibody. The percentage of EGFP-positive cells infected by virus not treated with IgG1-b12 was assigned as 100%. No EGFP-positive cells were detected on plates of HeLa-CD4 cells infected by NL4-3-EGFP Env⁻ virus, either treated or not treated with IgG1-b12, so the lane for HeLa-CD4 cells infected by NL4-3-EGFP Env⁻ is empty. Infection methods are described in the legend to Fig. 3, except that the cells were infected by IgG1-b12- or mock-treated viruses. EGFP-positive cells were counted by UV microscopic visualization. For infection of HeLa-CD4 cells, the results from UV microscopic visualization were also analyzed by fluorescence-activated cell sorting (A). Two experiments were performed, with similar results, and results from one experiment are shown. Each infection was performed in duplicate, and numbers represent the average for the two wells. The numbers of EGFP-positive cells were as follows: 1) HeLa-CD4 cells infected by NL4-3-EGFP with HIV_{LAI} Env, with IgG1-b12, 103 and 82, and without IgG1-b12, 503 and 443; Tu139 cells infected by NL4-3-EGFP with HIV_{LAI} Env, with IgG1-b12, 23 and 16, and without IgG1-b12, 8, and 18; Tu139 cells infected by NL4-3-EGFP Env⁻, with IgG1-b12, 9 and 9, and without IgG1-b12, 10 and 7; DU145 cells infected by NL4-3-EGFP with HIV_{LAI} Env, with IgG1-b12, 39 and 43, and without IgG1-b12, 41, and 45; and DU145 cells infected by NL4-3-EGFP Env⁻, with IgG1-b12, 22 and 36, and without IgG1-b12, 35, and 29.

The ability of HIV-1 to infect epithelial cells also has direct implications for vaccine development. We demonstrated that a conformationally dependent neutralizing antibody could not block HIV-1 infection of the CD4⁺ cells tested. Thus, whether neutralizing antibodies directed against the HIV-1 envelope can be fully protective for epithelial cell infection at mucosal surfaces remains to be examined.

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REFERENCES

- Bhat, S., S. L. Spitalnik, F. Gonzalez-Scarano, and D. H. Silberberg. 1991. Galactosyl ceramide or a derivative is an essential component of the neural receptor for human immunodeficiency virus type 1 envelope glycoprotein gp120. *Proc. Natl. Acad. Sci. USA* **88**:7131-7134.
- Bomsel, M. 1997. Transcytosis of infectious human immunodeficiency virus across a tight human epithelial cell line barrier. *Nat. Med.* **3**:42-47.
- Cook, D. G., J. Fantini, S. L. Spitalnik, and F. Gonzalez-Scarano. 1994. Binding of human immunodeficiency virus type 1 (HIV-1) gp120 to galactosylceramide (GalCer): relationship to the V3 loop. *Virology* **201**:206-214.
- Delezay, O., N. Koch, N. Yahia, D. Hammache, C. Tourres, C. Tamalet, and J. Fantini. 1997. Co-expression of CXCR4/fusin and galactosylceramide in the human intestinal epithelial cell line HT-29. *AIDS* **11**:1311-1318.
- Doranz, B. J., J. Rucker, Y. Yi, R. J. Smyth, M. Samson, S. C. Peiper, M. Parmentier, R. G. Collman, and R. W. Doms. 1996. A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell* **85**:1149-1158.
- Dragic, T., V. Litwin, G. P. Allaway, S. R. Martin, Y. Huang, K. A. Nagashima, C. Cayanan, P. J. Maddon, R. A. Koup, J. P. Moore, and W. A. Paxton. 1996. HIV-1 entry into CD4⁺ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* **381**:667-673.
- Edinger, A. L., J. L. Mankowski, B. J. Doranz, B. J. Margulies, B. Lee, J. Rucker, M. Sharron, T. L. Hoffman, J. F. Berson, M. C. Zink, V. M. Hirsch,

- J. E. Clements, and R. W. Doms. 1997. CD4-independent, CCR5-dependent infection of brain capillary endothelial cells by a neurovirulent simian immunodeficiency virus strain. *Proc. Natl. Acad. Sci. USA* **94**:14742–14747.
8. Fantini, J., D. Hammache, O. Delezay, G. Pieroni, C. Tamalet, and N. Yahi. 1998. Sulfatide inhibits HIV-1 entry into CD4⁺/CXCR4⁺ cells. *Virology* **246**:211–220.
9. Fantini, J., N. Yahi, and J. C. Chermann. 1991. Human immunodeficiency virus can infect the apical and basolateral surfaces of human colonic epithelial cells. *Proc. Natl. Acad. Sci. USA* **88**:9297–9301.
10. Feng, Y., C. C. Broder, P. E. Kennedy, and E. A. Berger. 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* **272**:872–877.
11. Furuta, Y., K. Eriksson, B. Svennerholm, P. Fredman, P. Horal, S. Jeansson, A. Vahlne, J. Holmgren, and C. Czerkinsky. 1994. Infection of vaginal and colonic epithelial cells by the human immunodeficiency virus type 1 is neutralized by antibodies raised against conserved epitopes in the envelope glycoprotein gp120. *Proc. Natl. Acad. Sci. USA* **91**:12559–12563.
12. Gelderblom, H., H. Reupke, T. Winkel, R. Kunze, and G. Pauli. 1987. MHC-antigens: constituents of the envelopes of human and simian immunodeficiency viruses. *Z. Naturforsch.* **42**:1328–1334.
13. Harouse, J. M., S. Bhat, S. L. Spitalnik, M. Laughlin, K. Stefano, D. H. Silberberg, and F. Gonzalez-Scarano. 1991. Inhibition of entry of HIV-1 in neural cell lines by antibodies against galactosyl ceramide. *Science* **253**:320–323.
14. Harouse, J. M., C. Kunsch, H. T. Hartle, M. A. Laughlin, J. A. Hoxie, B. Wigdahl, and F. Gonzalez-Scarano. 1989. CD4-independent infection of human neural cells by human immunodeficiency virus type 1. *J. Virol.* **63**:2527–2533.
15. Ho, D. D., T. Moudgil, and M. Alam. 1989. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. *N. Engl. J. Med.* **321**:1621–1625.
16. Howell, A. L., R. D. Edkins, S. E. Rier, G. R. Yeaman, J. E. Stern, M. W. Fanger, and C. R. Wira. 1997. Human immunodeficiency virus type 1 infection of cells and tissues from the upper and lower human female reproductive tract. *J. Virol.* **71**:3498–3506.
17. Min, B. M., J. H. Baek, K. H. Shin, C. N. Gujuluva, H. M. Cherrick, and N. H. Park. 1994. Inactivation of the p53 gene by either mutation or HPV infection is extremely frequent in human oral squamous cell carcinoma cell lines. *Eur. J. Cancer* **30B**:338–345.
18. Naldini, L., U. Blomer, F. H. Gage, D. Trono, and I. M. Verma. 1996. Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. *Proc. Natl. Acad. Sci. USA* **93**:11382–11383.
19. Owens, R. J., and R. W. Compans. 1989. Expression of the human immunodeficiency virus envelope glycoprotein is restricted to basolateral surfaces of polarized epithelial cells. *J. Virol.* **63**:978–982.
20. Owens, R. J., J. W. Dubay, E. Hunter, and R. W. Compans. 1991. Human immunodeficiency virus envelope protein determines the site of virus release in polarized epithelial cells. *Proc. Natl. Acad. Sci. USA* **88**:3987–3991.
21. Potempa, S., L. Picard, J. D. Reeves, D. Wilkinson, R. A. Weiss, and S. J. Talbot. 1997. CD4-independent infection by human immunodeficiency virus type 2 strain ROD/B: the role of the N-terminal domain of CXCR-4 in fusion and entry. *J. Virol.* **71**:4419–4424.
22. Qureshi, M. N., C. E. Barr, I. Hewlitt, R. Boorstein, F. Kong, O. Bagasra, L. E. Bobroski, and B. Joshi. 1997. Detection of HIV in oral mucosal cells. *Oral Dis.* **3**(Suppl. 1):S73–S78.
23. Sadaie, M. R., V. S. Kalyanaraman, R. Mukopadhyaya, E. Tschachler, R. C. Gallo, and F. Wong-Staal. 1992. Biological characterization of noninfectious HIV-1 particles lacking the envelope protein. *Virology* **6**:1257–1263.
24. Tan, X., and D. M. Phillips. 1996. Cell-mediated infection of cervix derived epithelial cells with primary isolates of human immunodeficiency virus. *Arch. Virol.* **141**:1177–1189.
25. Yahi, N., J. M. Sabatier, P. Nickel, K. Mabrouk, F. Gonzalez-Scarano, and J. Fantini. 1994. Suramin inhibits binding of the V3 region of HIV-1 envelope glycoprotein gp120 to galactosylceramide, the receptor for HIV-1 gp120 on human colon epithelial cells. *J. Biol. Chem.* **269**:24349–24353.