The importance of virus-associated host ICAM-1 in human immunodeficiency virus type 1 dissemination depends on the cellular context

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ABSTRACT

The primary objective of this study was to define whether the nature of virion-bound host cell membrane proteins influenced the process of human immunodeficiency virus 1 (HIV-1) capture and transmission. We pulsed cells of monocytoid lineage (established and primary) and CD4-negative epithelial cells transiently expressing DC-SIGN or LFA-1 with isogenic HIV-1 particles either devoid or bearing host-derived ICAM-1 or ICAM-3 before incubation with an indicator cell line. To our surprise, the ICAM-1/LFA-1 association was a more efficient transmission factor than the combined gp120/DC-SIGN and ICAM-3/DC-SIGN interactions. The involvement of the association between virus-bound ICAM-1 and its natural ligand LFA-1 in virus binding and carriage was confirmed when using more physiological cellular targets, i.e., human lymphoid tissues cultured ex vivo. However, the contribution of virus-anchored host ICAM-1 to the process of retention and transmission of HIV-1 could not be confirmed when using primary human cells of macrophage/dendritic lineage as transmitter cells and autologous CD4+ T lymphocytes as targets. Altogether these data underscore the complexity of factors participating in virus-cell contact and efficient dissemination of HIV-1 to target cells.

Key words: HIV • AIDS • monocytes • macrophages • T lymphocytes • dendritic cells • lymphoid tissue

Although it is now well established that entry of human immunodeficiency virus type-1 (HIV-1) into CD4+ T lymphocytes requires the formation of a fusion pore resulting from a high affinity interaction between envelope (Env) spike glycoproteins (i.e., gp120) and a complex made of CD4 and a seven-transmembrane coreceptor (e.g., CXCR4 or CCR5; reviewed in ref 1), it is becoming clear that the initial attachment step is a more complex phenomenon than initially thought. Indeed there is now convincing data showing that adsorption of HIV-1 to the cell surface is modulated by a large variety of interactions between the viral entity and the target cell surface (reviewed in ref 2). For example, heparan sulfate proteoglycans that are expressed at high levels on epithelial and endothelial cells serve as docking structures for HIV-1. Moreover, the attachment process can still occur in the absence of cell surface CD4 as
exemplified by the observation that HIV-1 can bind to a large variety of CD4-negative cell types such as follicular dendritic cells (FDC), B lymphocytes, neutrophils, and erythrocytes. It is also thought that CD4-independent interactions are responsible for ligation of HIV-1 to the surface of monocytes, macrophages, and dendritic cells (DC), which express barely detectable amounts of CD4 (reviewed in ref 1).

Some specific virus-cell interactions have been demonstrated to promote efficient transmission of HIV-1 from cell types that are naturally nonpermissive for virus infection to susceptible cells. The most convincing example is the association between the viral envelope glycoprotein gp120 and the dendritic cell-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing nonintegrin (DC-SIGN; also called CD209), which results in capture and transmission of HIV-1 to CD4+ T cells (reviewed in ref 3). DC-SIGN is a C-type lectin abundantly expressed in myeloid cell-derived DC that normally binds to ICAM-3 to promote interaction between DC and T cells. DC-SIGN expression promotes the capture of primary and laboratory isolate of HIV-1, HIV-2, and simian immunodeficiency virus (SIV) strains but does not by itself mediate virus infection. The precise mechanism through which DC-SIGN mediates transfer of HIV-1 to CD4+ T cells is not entirely elucidated. Recent findings suggest that molecules other than DC-SIGN are playing an active role in capture and transmission of HIV-1 (4–6). For example, ICAM-1 expressed on DC seems to be involved in HIV-1 transmission and the interaction between ICAM-1, and one of its physiological cognate ligands, LFA-1, contributes to cell-to-cell transmission of HIV-1 (7–9). Moreover, the adhesion molecules ICAM-1 and LFA-1 seem to play an active role in the attachment of mature HIV-1 particles to the surface of FDC (10). Experiments performed with established human lymphoid cell lines, primary peripheral blood mononuclear cells, and ex vivo lymphoid tissue culture system have indicated that several steps in HIV-1 life cycle are modulated by virion-associated host cell surface proteins such as CD28, CD44, CD62L, major histocompatibility complex class I (MHC-I), MHC-II (i.e., HLA-DR), and ICAM-1 (8, 11–17). Altogether these observations prompted us to initiate a comparative analysis in an attempt to elucidate the contribution of some specific virus-associated host proteins in HIV-1 dissemination to susceptible target cells.

To define the relative importance of the adhesion molecule ICAM-1 once embedded within HIV-1 as a virus-linked transmission factor, we used cells of monocytic lineage (established and primary), immature and mature DC, as well as CD4-negative human epithelial cells that were transiently expressing DC-SIGN or LFA-1 in combination with HIV-1 particles either lacking or bearing host ICAM-1 or ICAM-3. Next, such virus-cell mixtures were incubated with susceptible target cells consisting of reporter lymphoid cells, human lymphoid tissue cultured ex vivo, unseparated peripheral blood mononuclear cells (PBMC), or purified human CD4+ T lymphocytes. Under our experimental conditions, we found that the process of HIV-1 transmission is influenced by virion-anchored host ICAM-1 proteins but also by the cellular context and more particularly by the source of transmitter cells.

**METHODS**

**Cells**

The LuSIV reporter cell line is a CEMx174 derivative that has been stably transfected with the SIVmac239 LTR (−225 → +149) cloned upstream of the firefly luciferase reporter gene (kindly
supplied by Dr. J. E. Clements, Johns Hopkins University School of Medicine, Baltimore, Maryland; ref 18). Mono Mac 6 is an established human cell line with characteristics of mature monocytes according to morphological, cytochemical and immunological criteria (19). HEK293T cells are human embryonic kidney cells harboring the simian virus (SV40) large T antigen (20). PBMC were obtained from healthy donors and purified by Ficoll-Hypaque centrifugation. Primary human monocytes (CD14+ ) were isolated from fresh PBMC using the monocyte positive selection kit according to instructions of the manufacturer (Miltenyi Biotec, Auburn, CA). Monocyte-derived macrophages (MDM) were obtained by using freshly isolated CD14-expressing monocytes (5×10^7/well in a 48-well plate) that were cultured for 3 days in RPMI-1640 medium in the presence of macrophage colony-stimulating factor (m-CSF; 100 ng/ml). Monocyte-derived DC were differentiated from purified monocytes (i.e., CD14+ cells). Cells were cultured at a density of 10^6 cells/ml in six-well plates (3 ml per individual well). To generate immature DC (iDC), purified monocytes were cultured in RPMI 1640/10% fetal bovine serum (FBS) supplemented with granulocyte-macrophage CSF (GM-CSF; 1,000 U/ml) and interleukin-4 (IL-4; 200 U/ml) for 7 days. The culture medium was replenished with the cytokines on day 2, 4, and 6. Production of mature dendritic cells (mDC) was achieved by treating iDC for 48 h with interferon-gamma (IFN-γ; 1,000 U/ml) and lipopolysaccharide (LPS; 100 ng/ml). The final cellular phenotype of iDC and mDC was monitored by fluorescence-activated cell sorting (FACS) analysis. For example, iDC expressed high levels of HLA-DR (percentage of positive cells of 99.8 and mean fluorescence intensity of 92.2) and DC-SIGN (percentage of positive cells of 82.6 and mean fluorescence intensity of 8.0), moderate levels of CD86 (B7-2; percentage of positive cells of 79.8 and mean fluorescence intensity of 14.6), and low levels of CD14 (percentage of positive cells of 38.8 and mean fluorescence intensity of 5.5) and CD83 (percentage of positive cells of 30.9 and mean fluorescence intensity of 3.5). Expression of HLA-DR (percentage of positive cells of 99.4 and mean fluorescence intensity of 229.8), CD86 (percentage of positive cells of 96.9 and mean fluorescence intensity of 79.6), and CD83 (percentage of positive cells of 87.2 and mean fluorescence intensity of 12.3) was found to be increased in mDC. Histocultures of human lymphoid tissue were prepared as described previously (16).

**Transient transfection of HEK293T cells and production of virus stocks**

HEK293T cells were manipulated to transiently express LFA-1 and DC-SIGN by using a commercial calcium phosphate coprecipitation kit according to the instructions of the manufacturer (CalPhos Mammalian Transfection Kit, Clontech Laboratories Inc., Palo Alto, CA). Plasmids that were used include mammalian expression vectors coding for human LFA-1 (αLβ2; i.e., pCDL1 for the αL/CD11a chain and pCDB1 for the β2/CD18 integrin subunit) and DC-SIGN (i.e., pMX-DC-SIGN). pCDL1 and pCDB1 were kindly provided by Dr. T. A. Springer (Harvard Medical School, Boston, MA; ref 21) while pMX-DC-SIGN was obtained from Drs. D. Kwon and D. Littman through the AIDS Research and Reference Reagent Program (Rockville, MD; ref 22). Isogenic virus particles differing only by the absence or the presence of host-derived ICAM-1 or ICAM-3 proteins on their outer membranes were produced by calcium phosphate transfection in HEK293T cells, as described previously (14, 15). Such cells were transiently transfected with pNL4-3 (X4-tropic) or pJR-CSF (R5-tropic) in the presence or absence of either an ICAM-1 (i.e., pCD1.8; ref 23) or ICAM-3 expression vector (i.e., pIC-3; ref 24) (both molecular constructs were supplied by Dr. T. A. Springer). Virus stocks were normalized for virion content using an in-house sensitive double antibody sandwich enzyme-
linked immunosorbent assay specific for the major core viral p24 protein (16). All virus preparations underwent only one freeze-thaw cycle before initiation of infection studies.

**Antibodies**

The anti-Mac-1 antibody (clone OKM1) was kindly supplied by Dr. P. Naccache (CHUL Research Center). The monoclonal anti-ICAM-1 antibody RR1/1.1.1 was provided by Dr. R. Rothlein (Boehringer Ingelheim, Ridgefield, CO; ref 25), and the antibody specific for human ICAM-3, ICR-3, was supplied by ICOS Corporation (Bothell, WA). The two anti-LFA-1 CD11a chain antibodies TS1/22.1 and MEM30 were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and Dr. V. Horejsi (Institute of Molecular Genetics, Prague, Czech Republic), respectively. The anti-DC-SIGN antibody (clone DC28), which recognizes the repeat region of DC-SIGN and cross-reacts with DC-SIGNR, was obtained from the AIDS Repository Reagent Program (26). Phycoerythrin (PE)-conjugated goat anti-mouse immunoglobulin G (IgG) was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA).

**FACS analyses**

Expression of DC-SIGN, LFA-1, ICAM-1, and ICAM-3 on transiently transfected HEK293T cells and the cellular phenotype of iDC and mDC were monitored by flow cytometry. For staining, cells (2×10⁵) were incubated with ice-cold phosphate-buffered saline (PBS) containing 2% FBS, 0.02% sodium azide (FACS buffer), and 2 µg of the appropriate antibody per milliliter in a total volume of 100 µl. After 30 min at 4°C, the cells were extensively washed with the FACS buffer and recovered in 100 µl of FACS buffer supplemented with PE-conjugated goat anti-mouse IgG (2 µg). Cells were incubated for 30 min at 4°C, washed with FACS buffer, and analyzed with an EPICS XL apparatus (Coulter Corp., Miami, FL).

**Virus capture test and HIV-1 transmission assay**

Virus-anchored foreign ICAM-1 and ICAM-3 proteins were semiquantitatively estimated using a described previously virus capture assay (27). Briefly, the amount of immunocaptured HIV-1 particles was assessed by measuring viral p24 protein content found associated with magnetic beads coated with the anti-ICAM-1 RR1/1.1.1 or anti-ICAM-3 ICR-3 antibody. Magnetic beads coated with an isotype-matched irrelevant monoclonal antibody were used as negative controls. Transfer of HIV-1 was achieved by pulsing parental HEK293T (DC-SIGN- and LFA-1-negative), DC-SIGN⁺ HEK293T, LFA-1⁺ HEK293T, and Mono Mac 6 (5×10⁵ in 500 µl) with the tested isogenic virus stocks (100 ng of p24) for various time periods (ranging from 5 to 60 min). In some instances, cells were either left untreated or treated with the blocking anti-LFA-1 MEM30 antibody (3 µg/ml) for 20 min at 37°C before exposure to HIV-1. Cells were then extensively washed with PBS to remove untrapped virions and cocultured for 24 h with indicator LuSIV cells (1:2 ratio). Finally, cells were lysed and luciferase activity was monitored using a microplate luminometer (MLX; Dynex Technologies, Chantilly, VA). Luciferase activity is expressed as relative light units (RLU). Transmission of trapped virions to human lymphoid tissues was assessed by placing the washed virus-cell mixture on top of thin blocks of tonsils. Virus production in such HIV-1-infected tissues was quantified by measuring p24 levels present within culture medium bathing tissue blocks. For transfer experiments that were based on MDM,
the studied virus preparations were incubated with MDM in 48-well plates (1 ng of p24 in a final volume of 200 µl) for 45 min at 37°C. Cells were then washed three times with 1 ml of PBS, and PHA/IL-2-stimulated PBMC were added (5×10^5 per well in 500 µl of RPMI containing IL-2). Supernatants of such cocultured cells were harvested at different time points and quantified for p24 contents. Transmission of HIV-1 by monocyte-derived DC was performed as follows. Briefly, iDC or mDC (10^5) were incubated for 30 min at 37°C with studied virus stocks (1 ng of p24) in a final volume of 200 µl of complete culture medium. Cells were then washed vigorously three times in 1 ml of complete culture medium. Next, cells were cocultured with purified autologous CD4+ T lymphocytes, which were treated initially with PHA and IL-2 for 48 h prior initiation of the coculture, in a 48-well plate (final volume of 1 ml of complete culture medium). Virus production was estimated by measuring p24 levels over time in the cell-free culture supernatants.

RESULTS

Host-derived adhesion molecules ICAM-1 and ICAM-3 are efficiently incorporated within HIV-1

To define the relative contribution of gp120/DC-SIGN, virus-anchored host ICAM-3/DC-SIGN, and virus-associated host ICAM-1/LFA-1 interactions in HIV-1 capture and transfer to target cells, we produced viruses bearing foreign ICAM-1 or ICAM-3. Although it is well recognized that ICAM-1 is found embedded within HIV-1 (reviewed in ref 28), there is little information available about the possible insertion of host cell membrane ICAM-3 within HIV-1 virions (29, 30). To monitor the degree of incorporation of foreign ICAM-1 and ICAM-3 within mature viral entities, we employed our described previously transient transfection-and-expression system (14, 15) coupled with a virus capture assay. To this end, we transiently cotransfected HEK293T cells with an infectious molecular clone of HIV-1 (i.e., pNL4-3) and a mammalian expression vector coding for the complete human ICAM-1 or ICAM-3 (i.e., pCD1.8 and pALC-3, respectively). It should be mentioned that HEK293T cells do not endogenously express cell surface ICAM-1 and ICAM-3 molecules. Flow cytometry studies revealed that our experimental strategy resulted in roughly similar levels of ICAM-1 and ICAM-3 on the surface of HEK293T cells (Fig. 1A). Data from the virus precipitation assay indicated that progeny viruses produced by such transiently transfected HEK293T cells are efficiently captured by anti-ICAM-1 and anti-ICAM-3 antibodies linked to a solid matrix (Fig. 1B), therefore indicating that both cell surface components are inserted within budding virions.

ICAM-1-bearing viruses are more efficiently transmitted by monocytoid cells than ICAM-3-bearing virions or HIV-1 lacking both host-derived proteins

To examine whether the nature of virally embedded host constituents can influence the process of HIV-1 transmission, we developed a transmission assay relying on LuSIV. This indicator cell line permits the detection and quantification of single cycle HIV-1 infection due to Tat-mediated expression of HIV-1 LTR-driven luciferase activity, which correlates with virus infectivity (18). Given that the Mono Mac 6 monocytoid cell line is not susceptible to productive HIV-1 infection due to an almost undetectable level of CD4 (unpublished observations), our initial experiments were carried out using such cells to capture and transfer the tested virus preparations to LuSIV cells. Flow cytometric analyses indicated that Mono Mac 6 express a significant amount of
surface LFA-1, a natural counter-receptor for both ICAM-1 and ICAM-3 (Fig. 2A), but are negative for DC-SIGN (data not shown). These cells are also weakly positive for Mac-1, a second cognate ligand for ICAM-1. Mono Mac 6 cells were pulsed for the indicated time periods with similar concentrations of NL4-3 either lacking (NL4-3) or bearing ICAM-1 (NL4-3/ICAM-1) or ICAM-3 (NL4-3/ICAM-3). The results of a representative transmission experiment demonstrated that Mono Mac 6 act as weak transmitters of NL4-3 and NL4-3/ICAM-3 viruses to LuSIV (Fig. 2B). Interestingly, a very efficient time-dependent spreading of HIV-1 was obtained when using NL4-3 virions that bear host ICAM-1.

**A more efficient transmission of HIV-1 is seen when LFA-1-expressing human epithelial cells are used in combination with ICAM-1-bearing virions**

Previous observations have revealed that DC-SIGN interactions with virus-associated Env glycoproteins (i.e., gp120) promote efficient infection in trans of susceptible targets (22). To compare the capacity of DC-SIGN and LFA-1 to mediate HIV-1 transmission, we used HEK293T that transiently express DC-SIGN or LFA-1. Flow cytometry studies indicated that significant levels of surface DC-SIGN and LFA-1 were obtained when cells were transfected with similar amounts of the appropriate expression vector (i.e., 5 µg) (DC-SIGN expression vector: percentage of positive cells of 96.9 and mean fluorescence intensity of 18.8; LFA-1 expression vector: percentage of positive cells of 94.4 and mean fluorescence intensity of 12.6; Fig. 3A). The process of HIV-1 transmission was weakly increased by the presence of DC-SIGN for viruses devoid of foreign ICAM-1 (Fig. 3B). However, virus transmission was significantly more efficient when LFA-1-expressing HEK293T cells were used in combination with NL4-3/ICAM-1 viruses. To confirm that ICAM-1/LFA-1 interactions are more important than gp120/DC-SIGN interactions in HIV-1 transmission, our next series of investigations was performed by transfecting HEK293T cells with a constant amount of DC-SIGN expression vector (i.e., 5 µg) and increasing amounts of the LFA-1 expression plasmid (i.e., 0.01, 0.1, and 1 µg). This experimental strategy resulted in an increase in HIV-1 transmission depending of the amount of LFA-1 expression vector that was introduced into HEK293T cells (Fig. 3C).

Pretreatment of LFA-1+ HEK293T cells with MEM30, an anti-LFA-1 CD11a chain antibody that blocks binding to ICAM-1 (31), prevented HIV-1 transmission (Fig. 3D), indicating that ICAM-1/LFA-1 interactions are indeed critical for HIV-1 capture and transmission to target cells.

Because of the weak capacity of DC-SIGN-expressing HEK293T cells to achieve transmission of the tested virus preparations and since ICAM-3 is the natural ligand of DC-SIGN, we next tested whether acquisition of foreign ICAM-3 by virions would render such DC-SIGN-positive cells more efficient transmitters of HIV-1. Consistent with our previous data, exposure of ICAM-1-bearing virions to LFA-1-expressing cells led to an effective capture and transmission of HIV-1 to LuSIV cells (i.e., 5-fold increase) (Fig. 4). Surprisingly, HIV-1 transmission by DC-SIGN-expressing HEK293T cells was still inefficient despite the insertion of host ICAM-3 within mature HIV-1 particles.

**ICAM-1-bearing virions trapped on the surface of LFA-1-expressing human epithelial cells are efficiently transmitted to histocultures of human lymphoid tissue**

Key pathogenesis events and most viral replication take place in lymphoid tissues (reviewed in ref 32). Thus, we reasoned that the described previously HIV-1 model system based on human
tonsillar tissue blocks cultured ex vivo (33) would represent an appropriate experimental model to assess the importance of ICAM-1/LFA-1 interactions in HIV-1 transmission in a more physiological microenvironment. In this series of investigations, parental HEK293T, HEK293T/DC-SIGN+, and HEK293T/LFA-1+ cells were incubated for 45 min with NL4-3 virions either lacking or bearing host-encoded ICAM-1. After an extensive washing step to eliminate unbound virus, the virus-cell mixture was placed on top of small pieces of tonsillar tissues. Virus replication kinetics was finally tested by measuring p24 levels in the culture supernatant. Transfer of isogenic NL4-3 and NL4-3/ICAM-1 virus particles to histocultures of human lymphoid tissue was unaffected by surface expression of DC-SIGN on parental HEK293T cells (compare Fig. 5A and B). In contrast to the data with HEK293T/DC-SIGN cells, we found that transmission of HIV-1 to human lymphoid tissue cultured ex vivo was enhanced when using HEK293T/LFA-1 cells in combination with ICAM-1-bearing virions (4-fold increase; Fig. 5C). This experiment was repeated with two other donors and gave reproducible results (data not shown).

**ICAM-1-bearing virions and viruses lacking ICAM-1 are similarly transmitted by primary human cells of macrophage/dendritic lineage**

We next determined the role played by virus-anchored host ICAM-1 in transmission and propagation of HIV-1 using MDM and DC as transmitter cells. Virus transmission was comparable when MDM were pulsed with isogenic X4-tropic virions either bearing or lacking host ICAM-1 before incubation with unseparated PBMC (Fig. 6A). A similar observation was made when using R5-tropic viruses differing only by the absence or the presence of host-derived ICAM-1 (Fig. 6B). Experiments performed with iDC and mDC revealed that X4- and R5-using virions either lacking or bearing host-derived ICAM-1 were also equally transmitted to autologous CD4+ T lymphocytes (Fig. 7).

**DISCUSSION**

Although it is recognized that HIV-1 infects and replicates in many cell types and in different tissues in the body, the two most important intracellular reservoirs remain latently infected resting CD4+ T cells and macrophages (34–39). The virus is not only present under an integrated form, but infectious virus particles have been shown to be present in vivo as cell-free virus, immune-complexed virus, and cell-associated virus (40). Possible reservoirs of cell-bound HIV-1 include DC, CD8+ T lymphocytes, B cells, epithelial cells, fibroblasts, platelets, neutrophils, and erythrocytes (reviewed in ref 41). It is proposed that virus bound to these uninfected cells represents a potentially important reservoir of HIV-1 under natural situations considering that this form of virus is more stable and infectious as compared with a similar amount of cell-free virus (40, 42, 43). In fact, transmission of cell-bound virus to target cells can be several times more efficient than that of cell-free virus (44, 45). A clear example is provided by the observation that productive infection of monocytes is established with a 10-fold-lower amount of HIV-1 when DC are used to transmit HIV-1 instead of cell-free virions (43). In another study, HIV-1 was found to be 17 times more infectious for T cells once associated with CD4-negative cells (40). The precise role played by various cell-surface receptors and, more specifically, adhesion molecules in the uptake and transfer of fully competent virus via cell-association is still obscure.
Previous works clearly established that mature HIV-1 incorporates several host cell proteins, including cell adhesion molecules such as ICAM-1 that can potentially have profound effects on some specific steps of the virus life cycle such as the attachment process (reviewed in ref 28). For example, it was established that the ICAM-1-mediated increase in HIV infectivity was linked with a more productive virus entry process in primary CD4⁺ T lymphocytes (46). Indeed, the presence of host-encoded ICAM-1 on the surface of virions resulted in an augmentation of the amount of viral material released in the cytosol, a process of internalization known to result in productive HIV-1 infection. There is a paucity of data on the implication of virus-incorporated foreign adhesion molecules in HIV-1 transmission from CD4-negative cells or cell types that express little CD4 to susceptible cellular targets. Binding of a virus to a target cell is a critical event in the infectious process and, as understanding of virus-host interactions expands, it is becoming clear that the initial attachment necessitates several specific as well as nonspecific cell-surface structures for its completion.

The already known capacity of HIV-1 to incorporate ICAM-1 and the present demonstration that ICAM-3 is also associated with mature virions prompted us to define whether the mechanism of HIV-1 transmission could be influenced by these foreign adhesion molecules once found embedded within the virus. Transmitter cells used in this study consisted of human epithelial CD4-negative HEK293T cells and monocytoid Mono Mac 6 cells that express barely detectable amounts of CD4. The former cell line was manipulated to achieve transient expression of DC-SIGN and LFA-1 to compare, in a well-controlled experimental cell system, the exact contribution of gp120/DC-SIGN, ICAM-3/DC-SIGN, and ICAM-1/LFA-1 interactions in transmission of infectious virus. Transmissions studies were achieved using isogenic viruses either lacking or bearing host-derived ICAM-1 or ICAM-3 glycoprotein. The efficient transfer of fully infectious HIV-1 particles was initially measured through the use of a highly susceptible indicator cell line that permits detection of single-cycle infection events. Histocultures of human tonsillar tissue were also used to provide physiological significance to our observations. Our data demonstrate that interactions between virion-anchored host ICAM-1 and cell surface LFA-1 take over gp120/DC-SIGN and ICAM-3/DC-SIGN interactions in the process of HIV-1 dissemination, at least when transmitter cells consisted of CD4-negative epithelial cells (i.e., HEK293T) or monocytoid cells that express little CD4 (i.e., Mono Mac 6). However, the importance of the association between ICAM-1 and LFA-1 in HIV-1 transfer could not be demonstrated when using more physiological transmitter cells such as MDM, iDC, and mDC.

DC-SIGN binds to Env glycoproteins from R5- and X4-tropic, HIV-1 isolates and from HIV-2 and SIV (22, 47). DC-SIGN acts also as a major ICAM-3 receptor on DC and is playing a key role in the establishment of the initial interaction between DC and other cell types such as T cells (48). Consequently, it was really unexpected to discover that the association between ICAM-1 and LFA-1 is a more efficient virus transmission factor than the combined action of gp120/DC-SIGN and ICAM-3/DC-SIGN interactions when DC-SIGN-expressing HEK293T cells were used to accomplish the transfer of HIV-1. A weak binding of the laboratory-adapted X4 viral isolate NL4-3 to DC-SIGN is not a factor that could account for the observed phenomenon since NL4-3 was found to bind to DC-SIGN with high efficiency (47). However, several findings can help to answer this mystery. For example, the contact between ICAM-3 and DC-SIGN mediates a transient loose adhesion between DC and T cells (48), suggesting that the strength of this interaction is not of a high order of magnitude. Moreover, differences in experimental methodologies may be responsible to some extent for our data. Indeed, most published HIV-1
capture and transmission assays relied on the pulsing of transmitters cells with infectious virus for a longer period of time (i.e., greater than 2 h and sometimes even 48 h; refs 4, 10, 22, 40, 47, 49–51) than the 45 min incubation time period that was used in our work with DC-SIGN transfectants. It can be proposed that attachment of HIV-1 mediated by gp120/DC-SIGN and ICAM-3/DC-SIGN interactions require more time to resume as compared with virus attachment resulting from ICAM-1/LFA-1 interactions. This is supported by affinity and kinetic analyses revealing that ICAM-1 interacts with high affinity and a slow dissociation rate constant with LFA-1 (52). Finally, the importance of DC-SIGN interactions with ICAM-3 and external Env glycoproteins in lentivirus transmission has been recently questioned by results showing that virus dissemination was only slightly diminished by antibody-mediated disruption of ICAM-3/DC-SIGN interactions and Rhesus macaque DC efficiently transmit primate lentiviruses in the absence of DC-SIGN (5, 6, 51, 53). The participation of gp120/DC-SIGN interactions in virus transfer was also raised by a recent study showing that a significant portion of the ability of monocyte-derived DC to transmit HIV-1 is due to factors other than DC-SIGN (54).

In the present study, we demonstrate that mature HIV-1 particles can incorporate not only host ICAM-1 but also ICAM-3. Even though we did not perform HIV-1 transfer experiments with virions bearing both host cell surface constituents, we can speculate as to whether ICAM-1/LFA-1 interactions might modulate the association between ICAM-3 and DC-SIGN. It can be proposed that engagement of LFA-1 by ICAM-1 might prevent a possible interaction between LFA-1 and ICAM-3, thus favoring the interaction between ICAM-3 and DC-SIGN. This hypothesis is based on the notion that LFA-1 displays a lower affinity for ICAM-3 ($K_d$: 550 nM) compared with ICAM-1 ($K_d$: 60 nM; ref 55). Moreover, this possibility is reinforced by the higher affinity of ICAM-3 for DC-SIGN ($K_d$: between 13.92 and 129 nM) than for LFA-1 ($K_d$: 550 nM).

Although the role of HIV-1-associated foreign proteins in the mechanism of HIV-1 transmission is a research field in its infancy, the importance of cell-to-cell adhesion mediated by ICAM-1 and LFA-1 in the uptake and transfer of HIV-1 has been proposed in some studies employing specific antibodies. A work by Olinger and et al. (40) showed that an anti-CD18 antibody substantially reduced the transfer of HIV-1 from CD4-negative cells to T cells. Kacani and colleagues (43) reported that an anti-CD18 monoclonal antibody abrogated HIV-1 transmission from iDC to CD4-expressing cells of monocytoid lineage. Antibodies against ICAM-1 and LFA-1 were found to inhibit binding of HIV-1 to FDC (10), whereas efficient viral transmission from DC to T cells required ICAM-1 expression on DC (7).

Experiments conducted with MDM, iDC, and mDC suggested that interactions between virus-anchored ICAM-1 and its potential natural ligands, i.e., the integrins LFA-1 and Mac-1, are not playing a dominant role in HIV-1 uptake and eventual transfer from such transmitter cells. This could relate to the fact that the attachment process of HIV-1 can also occur through CD4-independent mechanisms involving ligation of highly glycosylated groups or basic residues found on gp120 with polyanionic sulphated chains or lectin-like domains on some specific cell surface receptors other than DC-SIGN (reviewed in ref 1). For example, gp120 have been shown to associate with the glycolipid galactocerebroside (GalCer) and its sulphated derivative (i.e., sulphatide) (56, 57), glycosaminoglycan heparan sulfate (58, 59), placental membrane binding protein (now called DC-SIGN; ref 60), and macrophage mannose receptor (MMR; ref 61). It is of interest to note that a recent study has shown that MMR plays a cardinal role in the binding
and transmission of HIV-1 to T cells by primary human macrophages (62). In addition, the mannose receptor is primarily responsible for HIV-1 binding on dermal DC (63). Besides heparan sulfate proteoglycans (HSPGs) such as syndecans serve as the main class of attachment receptors for HIV-1 on different cell types such as macrophages and endothelial cells and are thought to play a cardinal role in HIV-1 transmission (64, 65). Our data demonstrate that results from HIV-1 transmission experiments carried out in transfected cells or established cell lines cannot be extrapolated to primary human cells such as MDM and DC due to differences in cell surface constituents that can interact with HIV-1. It can also be proposed that the cellular type might also influence the rapidity with which HIV-1 is endocytosed upon an intimate contact and the fate of HIV-1 once found within the endolysosomal apparatus (51, 66). This is convincingly illustrated by the rapid internalization of DC-SIGN and MMR upon interactions between HIV-1 and DC (62, 67). In contrast, HEK293T cells display a very low efficiency of HIV-1 endocytosis (51). Thus the ability of DC-SIGN to transmit HIV-1 to susceptible cells is most likely connected with its capacity to be endocytosed.

In summary, this study provides new insight into the mechanism of HIV-1 transmission. It indicates that under some specific circumstances the process of virus dissemination is not only influenced by interactions between virus-encoded constituents (i.e., gp120) and appropriate ligands (i.e., DC-SIGN) but also by interactions between host molecules that are incorporated within HIV-1 and their natural counter-receptors (Fig. 8). This work illustrates that HIV-1 capture and transfer to susceptible target cells is a process that involves a large number of interactions between the virion and the cell surface. We present evidence that this phenomenon is influenced by the cellular context and more precisely by the identity of virus transmitter cells. Additional studies are warranted to shed light on this complex phenomenon in an attempt to develop inhibitors of HIV-1 dissemination.

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Figure 1. Immunocapture of ICAM-1- and ICAM-3-bearing HIV-1 particles. A) Flow cytometric analyses of ICAM-1 and ICAM-3 surface expression on HEK293T cells transiently cotransfected with the infectious molecular clone pNL4-3 and ICAM-1 (pCD1.8) or ICAM-3 (pIC-3) encoding vector. B) Similar amounts of each virus stock were subjected to a virus capture assay using magnetic beads coated either with an anti-ICAM-1 (clone RR1/1.1.1), anti-ICAM-3 (clone ICR3), or an isotype-matched irrelevant antibody. Amount of virus captured by each antibody was estimated with the use of a p24 test. Magnetic beads coated with the isotype-matched irrelevant antibody served as controls to determine background levels of captured viruses. Data are means ± SD from triplicate samples and represent 3 independent experiments.
Figure 2. Transmission of ICAM-1- and ICAM-3-bearing virions by cells of monocytoid lineage. **A)** Surface expression levels of LFA-1 and Mac-1 on Mono Mac 6 were defined by flow cytometry. **B)** HIV-1 transmission was performed as described in Methods. In brief, Mono Mac 6 cells were incubated for the indicated time periods with similar amounts of NL4-3, NL4-3/ICAM-1, and NL4-3/ICAM-3 virions. The virus-cell mixture was washed extensively to eliminate unbound virus and was cocultured with indicator LuSIV cells for 24 h. HIV-1 infection was determined by measuring luciferase activity. Data are means ± SD from triplicate samples and represent 3 independent experiments.
Figure 3. Virus transfer by DC-SIGN- and LFA-1-expressing cells and the effect of a blocking anti-LFA-1 antibody on viral carriage. 

A) Flow cytometric analyses of DC-SIGN and LFA-1 surface expression on HEK293T cells transiently transfected with the appropriate encoding vector (i.e., pCDL1 and pCD B1 for LFA-1 and pMX-DC-SIGN for DC-SIGN). B) HEK293T, HEK293T/DC-SIGN, and HEK293T/LFA-1 cells were incubated for 45 min with similar amounts of NL4-3 and NL4-3/ICAM-1 virions. C) 293T cells were transfected with a fixed amount of a DC-SIGN expression vector in combination with an increasing concentration of the LFA-1 encoding vector. Cells were incubated for 45 min with similar levels of isogenic NL4-3 and NL4-3/ICAM-1 virus particles. D) HEK293T and HEK293T/LFA-1 cells were incubated for 45 min with similar concentrations of NL4-3 and NL4-3/ICAM-1 viruses in the absence or presence of an anti-LFA-1 antibody that blocks ICAM-1-LFA-1 interactions. After several washes, the cell-virus mixture was cocultured with LuSIV cells for 24 h. HIV-1 infection was monitored by measuring luciferase activity. Data are means ± SD from triplicate samples and represent 3 independent experiments.
Figure 4. Role of gp120/DC-SIGN, ICAM-3/DC-SIGN, and ICAM-1/LFA-1 interactions in HIV-1 dissemination.
HEK293T, HEK293T/DC-SIGN, and HEK293T/LFA-1 cells were incubated for 45 min with similar amounts of NL4-3, NL4-3/ICAM-1, and NL4-3/ICAM-3 virions. After several washes, the virus-cell mixture was cocultured with LuSIV cells for 24 h. HIV-1 infection was monitored by measuring luciferase activity. Data are means ± SD from triplicate samples and represent 3 independent experiments.
Figure 5. Transmission of HIV-1 to histocultures of human lymphoid tissue. HEK293T (A), HEK293T/DC-SIGN (B), and HEK293T/LFA-1 cells (C) were incubated for 45 min with similar amounts of NL4-3 and NL4-3/ICAM-1 virions. After several washes, the cell-virus mixture was placed on top of blocks of tonsillar tissue. Virus production was assessed by measuring p24 levels at regular intervals in culture supernatants. Results are means values ± SD of 1 tissue block from same donor in 4 separate wells (i.e., 4 tissue blocks) and represent experiments performed with tonsil tissues from 3 donors.
Figure 6. Transfer of HIV-1 particles either lacking or bearing host ICAM-1 by MDM. Transmitter cells (i.e., MDM) were incubated for 45 min with similar amounts of virions either lacking or bearing host-encoded ICAM-1 (NL4-3/X4, A; JR-CSF/R5, B). After several washes, the virus-cell mixture was cocultured with unseparated PBMC. Virus production was assessed by measuring p24 levels at regular intervals in culture supernatants. Data are means ± SD from triplicate samples and represent 3 independent experiments.
Figure 7. Transmission of HIV-1 particles either lacking or bearing host ICAM-1 by DC. Transmitter cells (iDC, A and C; mDC, B and D) were pulsed for 30 min with X4 (A and B) and R5-tropic (C and D) viruses either lacking or bearing host-encoded ICAM-1. After several washes, the virus-cell mixture was cocultured with autologous purified CD4+ T cells. Virus production was assessed by measuring p24 levels at regular intervals in the culture supernatants. Data are means ± SD from triplicate samples and represent 3 independent experiments.
Figure 8. Possible interactions between the virion and the cell surface implicated in HIV-1 transmission. Relative contribution of depicted interactions in HIV-1 capture and transfer to susceptible target cells will be influenced by some specific features of the viral entity (e.g., presence of some specific host-derived proteins such as ICAM-1) and the type of transmitter cell.