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Efficient Interaction of HIV-1 with Purified Dendritic Cells via Multiple Chemokine Coreceptors

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Summary

HIV-1 actively replicates in dendritic cell (DC)-T cell cocultures, but it has been difficult to demonstrate substantial infection of purified mature DCs. We now find that HIV-1 begins reverse transcription much more efficiently in DCs than T cells, even though T cells have higher levels of CD4 and gp120 binding. DCs isolated from skin or from blood precursors behave similarly. Several M-tropic strains and the T-tropic strain IIIB enter DCs efficiently, as assessed by the progressive formation of the early products of reverse transcription after a 90-min virus pulse at 37°C. However, few late gag-containing sequences are detected, so that active viral replication does not occur. The formation of these early transcripts seems to follow entry of HIV-1, rather than binding of virions that contain viral DNA. Early transcripts are scarce if DCs are exposed to virus on ice for 4 h, or for 90 min at 37°C, conditions which allow virus binding. Also the early transcripts once formed are insensitive to trypsin. The entry of a M-tropic isolates is blocked by the chemokine RANTES, and the entry of IIIB by SDF-1. RANTES interacts with CCR5 and SDF-1 with CXCR4 receptors. Entry of M-tropic but not T-tropic virus is ablated in DCs from individuals who lack a functional CCR5 receptor. DCs express more CCR5 and CXCR4 mRNA than T cells. Therefore, while HIV-1 does not replicate efficiently in mature DCs, viral entry can be active and can be blocked by chemokines that act on known receptors for M- and T-tropic virus.

The ability of HIV-1 to infect cultured dendritic cells (DCs) is a subject of intense research. DCs are found at body surfaces as well as in lymphoid organs, particularly in T cell-rich regions. DCs are potent antigen-presenting cells and act as adjuvants for immune responses *in vivo*. Therefore DCs are critically positioned to pick up HIV-1 and deliver virus to CD4⁺ T cells. There is some controversy on the capacity of HIV-1 to infect cultured DCs. Efficient replication has been reported by some groups (1, 2) but not by others (3–5). More uniform findings have been reported when DCs are exposed to HIV-1 and then cultured together with T cells. Upon the interaction of the two cell types, extensive virus replication occurs (3, 4, 6–8). In this paper, we present evidence that HIV-1 efficiently interacts with purified DCs relative to T cells, as long as the analyses consider the formation of early rather than later stages of reverse transcription. We will present evidence that the development of these early transcripts represents entry of virus, and that chemokines that block M-tropic

and T-tropic coreceptors can inhibit the interaction of HIV-1 with DCs.

Materials and Methods

Cells. Mixtures of DCs and T cells were allowed to emigrate from explants of human skin and sorted into DCs and small T cells (6, 7, 9). DCs were also generated from blood monocytes using a two-step culture protocol (10, 11). In brief, E rosette negative, blood mononuclear cells were cultured 6–7 d in a mixture of GM-CSF and IL-4 each at 1,000 U/ml. The nonadherent cells then were replated in a monocyte conditioned medium (12) for 4 d. At day 11, the DCs were purified by sorting on a FACStar^{PLUS}® (Becton Dickinson and Co., Mountain View, CA). T cells were E rosetted cells that were purified by passage over nylon wool. T blasts were E rosetted cells stimulated with 1 µg/ml PHA (Burrroughs Wellcome) or 5 ng/ml SEE superantigen (Toxin Technologies) for 3 d either immediately after taking the blood, or after 7 d of culture at 5 × 10⁶ cells/ml. The medium was RPMI-1640 (GIBCO BRL, Gaithersburg, MD) with either 5–10% FCS or 1% autologous human plasma and antibiotics.

FACS®. Binding of saturating doses of mAbs to CD83 (a kind gift of Dr. Schmittling, Coulter Corp., Hiialeah, FL) and CD4 (Leu 3a; American Type Culture Collection [ATCC], Rockville, MD) was visualized with goat anti-mouse Ig and a FACScan® instrument. Binding of soluble gp120 (IIIB strain; Repligen, Cambridge, MA) was assessed by adding 1 µg/ml for 1 h on ice, followed by the 110.4 anti-gp120 mAb (13) (Genetic Systems Inc., Seattle, WA) and PE-goat anti-mouse Ig (Tago Immunologicals, Camarillo, CA). gp120 binding was carried out on blood DCs and T cells (distinguished by light scattering, references 7, 9), or on skin cells.

HIV-1. M-tropic isolates (Ba-L, JR-FL, SF162, ADA) and the T-tropic isolate IIIB were from the AIDS Resources Program. HIV-1 was grown in mitogen stimulated PBMCs except for IIIB, which was grown in the CEM T cell line. All viruses were applied in 0.1 ml to 10⁵ cells (multiplicity of infection [MOI] of 0.01–0.1) for 90 min at 37°C in either round-bottomed microtest wells or small Eppendorf tubes. To reduce the amount of HIV-1 DNA in the virus supernatants, the latter were filtered and treated with RNase-free DNase (50 U/ml; Boehringer Mannheim Corp., Indianapolis, IN) for 30 min at room temperature in 10 mM MgCl₂.

Detection of Reverse Transcripts in Leukocytes Exposed to HIV-1. To detect viral DNA, the virus-pulsed cells were washed once in PBS and lysed in lysis buffer (10 mM Tris HCl, pH 8, 1 mM EDTA, 0.001% Triton X 100/SDS and 1 mg/ml proteinase K). The samples were incubated at 60°C for 1 h and then placed in a boiling water bath to inactivate the protease. Quantitative PCR amplification was performed with one oligonucleotide of each complementary pair 5' end-labeled with ³²P. The samples were subjected to 5 min of denaturation at 94°C followed by 25 cycles of denaturation for 1 min at 91°C and polymerization for 2 min at 65°C. Amplified products were resolved on 8% nondenaturing polyacrylamide gels and visualized by direct autoradiography of the dried gels. Primers were described by Zack et al. (14) and were as follows: for R/U5 sense 5'-ggctaactaggggaaccaccgt-3', antisense 5'-ctgctagagatttccactac-3' (amplification product, 140 bp) and for LTR/gag sense 5'-ggctaactaggggaaccaccgt-3', antisense 5'-cct-

gcgtcgagagactcctctgg-3' (amplification product, 200 bp). HIV-1 copy numbers in 50,000 cells per lane were estimated by comparison with graded doses of ACH-2 cells, which contain one copy of provirus per cell (15).

Chemokines and Chemokine Receptors. Several chemokines were prepared by chemical synthesis (16) and stored frozen at 200 µM. The chemokines were used at 100 nM and applied for 30 min to cells before HIV-1 and then during exposure to virus. CCR5 cDNA, cloned into pcDNA3.1 vector using HindIII and XhoI restriction enzyme sites, was a generous gift of Drs. T. Dragic and J.P. Moore (ADARC, The Rockefeller University, New York) (17). CXCR4 cDNA, also called LESTR (18) or fusin (19), was inserted into pcDNA3 vector using EcoRI sites. For Northern analyses, we used a 380 bp CCR5 fragment, prepared by digestion with KpnI and HindIII, and a 600-bp CXCR4 fragment prepared by digestion with BamHI. The ³²P-labeled probes were synthesizing using a random primed DNA labeling kit (Boehringer Mannheim) and used at 10⁶ cpm/ml of hybridization buffer with 6 µg of RNA from the different cell types described above. Blood DCs, T cells, and T blasts were prepared from two individuals (EU3 and EU5) who are homozygous for a 32-bp deletion in CCR5 and are resistant to HIV-1 infection (20).

Results

Sources of DCs. Two types of DCs were studied with comparable results. The more abundant were DCs that develop from blood progenitors during prolonged culture in cytokines, including GM-CSF and IL-4 (10, 11). These monocyte-derived DCs were purified from residual lymphocytes by sorting, selecting for large nonadherent cells that lacked B and T cell markers (Fig. 1A). The second source was DCs that emigrate along with T cells from human skin. These also were separated into DC and T cell enriched populations by sorting (6, 7, 9). Greater than 95% of blood and skin DCs expressed the CD83 DC-restricted

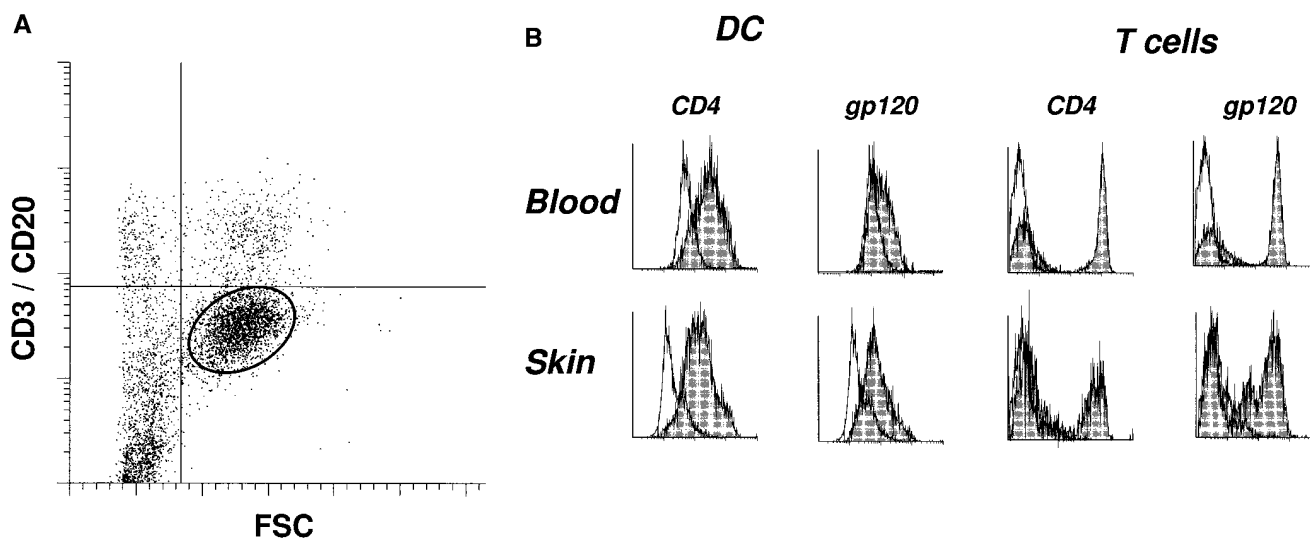


Figure 1. Expression of the CD4 receptor for HIV-1 on DCs and T cells. (A) Criteria for sorting blood-derived DCs (circle) from residual T cells. Lymphocytes (low FSC) were labeled with anti-CD3 and anti-CD20; large FSC, negative cells were isolated (FACStar^{Plus}®). (B) Expression of CD4 (mAb leu3a) and binding of soluble gp120 (HIV-1 IIIB) to DCs and T cells from blood and skin.

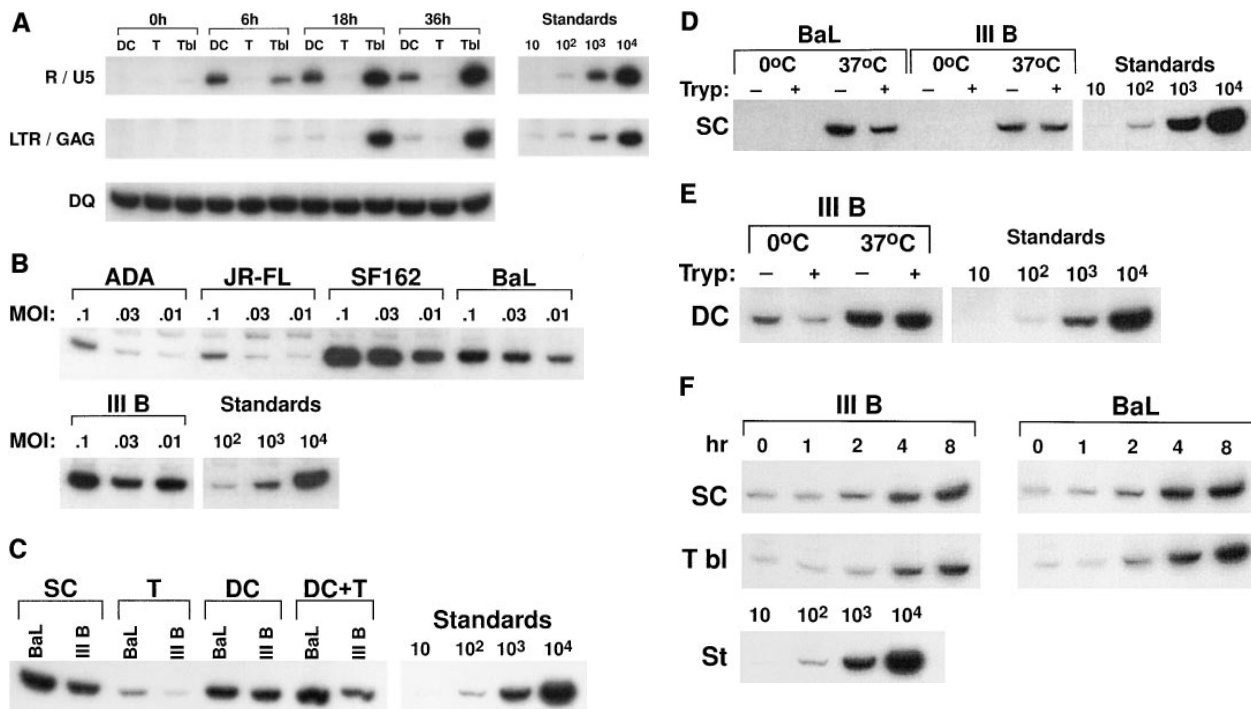


Figure 2. Efficient entry of HIV-1 into DCs but not small T cells. The results are representative of 2 or more experiments in each case. (A) Early vs. late stages of reverse transcription in blood-derived DCs. III B was added for 90 min at 37°C to DCs, purified T cells (T), and T blasts (TbI), washed, and cultured 0, 4, 18, or 36 h. Early and late (R/U5 and LTR/gag primers) transcripts were amplified by PCR (and compared to graded doses of ACH-2 cells). (B) Several isolates of HIV-1 efficiently enter blood-derived DCs. Graded doses of the denoted isolates (titled by the AIDS Resources Program) were added to DCs for 90 min, washed, and cultured 4 h before amplifying R/U5 DNA. (C) Detection of early R/U5 transcripts 9 h after adding Ba-L and III B isolates to skin leukocytes, either bulk skin cells (SC), FACS®-purified DCs and T cells, or a 1:2 DC/T mixture. (D and E) Evidence that early reverse transcripts in HIV-1 pulsed DCs represent viral entry and not simply binding to skin leukocytes (D) or blood derived DCs (E). R/U5 sequences are infrequent if virus was bound for 4 h on ice, and was trypsin insensitive (0.25%, GIBCO for 10 min at 37°C after 4 cell washes in phosphate saline) if virus was offered for 4 h at 37°C. (F) Kinetics of R/U5 sequence formation with Ba-L or III B in skin cells (SC) and T blasts. The 0 h time point was carried out 90 min after adding virus at 37°C. DNA was also analyzed 1–8 h after the virus pulse, washing, and culture.

antigen (21, not shown). Both skin and blood-derived DCs expressed CD4 and bound purified HIV-1 gp120, but CD4 and gp120 binding were much less than T cells (Fig. 1 B).

Evidence for Efficient Entry of HIV-1 into DCs. HIV-1 was applied to purified DCs and T cells from blood and skin for 90 min at 37°C, washed and recultured. At time points thereafter, DNA was amplified for HIV-1 containing sequences by PCR with described primers (14). We emphasized the R/U5 pair that detects early products of reverse transcription and the LTR/gag pair that detects the final stages.

At time 0, i.e., 90 min after adding virus to purified blood DCs at 37°C, the signals for R/U5 DNA sequences were weak or absent. HIV-1 DNA then increased steadily for 8 h to reach a plateau (Fig. 2 A) for at least 1–3 d (not shown). Only the early products of reverse transcription were abundant. At the dose of virus we employed, and relative to a standard curve derived from the ACH-2 cell line that expresses 1 copy of proviral DNA/cell (15), we observed 10^3 – 10^4 copies of R/U5 DNA in 100,000 DCs and $>10^2$ copies of LTR/gag sequences (Fig. 2 A). This signal is very high considering that we were only applied virus at <0.1 MOI. R/U5 DNA was weak or not detected with

comparable numbers of purified T cells (Fig. 2 A). T blasts were readily infected, as both R/U5 and LTR/gag containing sequences were found. The amount of these sequences increased rapidly in culture, because infection was productive in T blasts (Fig. 2 A).

Several different HIV-1 isolates entered and begin reverse transcription in DCs (Fig. 2 B). Using comparable numbers of infectious units, the interaction of Ba-L, SF162 and III B was particularly efficient. Efficient entry of these and other primary isolates was also noted in skin DCs, more than skin T cells, but only with probes for R/U5 sequences (Fig. 2 C).

Given the evidence that HIV-1 virions can contain short reverse transcripts (22, 23), we were concerned that the R/U5 sequences represented intravirion-DNA that had bound but not entered the DCs. However, entry seemed more likely for the following reasons. The R/U5 signal was weak when virus was added on ice for 4 h (Fig. 2, D and E), even though virus does bind in the cold (23–25). The R/U5 signals were weak if the cells were examined just after the 90-min virus pulse at 37°C, but increased progressively for 8 h after washing away nonbound virus (Figs. 2, A and F). In contrast, virus binding can be detected in just 3–5 min at this temperature (23–25). The R/U5 signals that developed

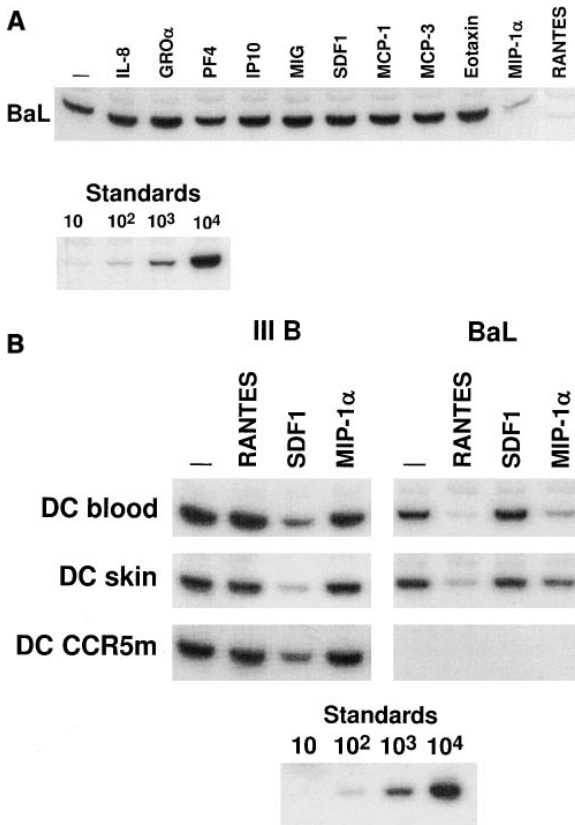


Figure 3. Role of chemokine receptors for viral entry into DCs. (A) DCs from blood were exposed to no blocker (-) or to 100 nM of the indicated chemokines for 30 min. Then Ba-L was added for 4 h before amplifying early R/U5 DNA sequences. In parallel (not shown), III B was also studied and only SDF-1 was inhibitory. (B) DCs from blood (top) or skin (middle) were exposed to Ba-L and III B in the presence of no blocker (-) or 100 nM RANTES, MIP-1 α , or SDF-1. The lower row shows R/U5 transcripts after adding Ba-L or III B to DCs from a CCR5 mutant individual.

after 4 h at 37°C also were insensitive to trypsin (Fig. 2, D and E), which releases surface-bound virus (23). We confirmed that trypsin destroys the HIV-1 binding epitope on CD4 that is identified with leu3a mAb (not shown).

Role of CD4 and Chemokine Receptors during HIV-1 Entry into DCs. The presumed entry of HIV-1 into DCs was totally blocked with recombinant soluble CD4 or the blocking leu 3a anti-CD4 antibody (not shown). The role of chemokine coreceptors was first assessed with a panel of chemokines as blockers. For both blood-derived DCs and skin DCs, the entry of Ba-L was almost completely blocked by RANTES (Fig. 3, A and B) and less completely by MIP-1 α , which are known ligands for the CCR5 coreceptor for M-tropic HIV-1 (17, 26, 27). No block of Ba-L entry was observed with SDF-1, a ligand for the CXCR4 receptor for T-tropic isolates (28, 29). Several chemokines did not block the entry of Ba-L (Fig. 3 A): the CC chemokines eotaxin, MCP-1 and MCP-3, and the CXC chemokines, IL-8, γ IP-10, Mig, and PF-4.

As mentioned, our uncloned preparation of III B which is grown in the CEM T cell line, very efficiently entered

DCs. However entry of III B into blood and skin DCs was partially blocked by SDF-1 and not RANTES (Fig. 3 B, left panels). SDF-1 binds to CXCR4 and blocks entry of T-tropic HIV-1 (28, 29). Therefore separate receptors mediate entry of M-tropic isolates and III B into DCs.

To prove that CCR5 was the major receptor for entry of M-tropic isolates into DCs, we prepared DCs from the blood of two patients with nonfunctioning mutant CCR5 (20, 30). These DCs did not permit entry of Ba-L (Fig. 3 B, right bottom) or SF162 (not shown). Entry of III B was efficient, however, and partially blocked by SDF-1 (Fig. 3 B, left bottom).

Expression of Chemokine Receptor RNA in DCs. The availability of large numbers of blood-derived DCs permitted northern blots for chemokine receptor mRNA. We used comparable amounts of RNA from FACS sorted DCs, nylon wool nonadherent T cells, E rosetted T cells stimulated with IL-2 only or with PHA plus IL-2, and total peripheral blood mononuclear cells (Fig. 4). With CCR5 probes, CCR5 mRNA was detected in DCs but not in T cells. IL-2 stimulated cells expressed CCR5 RNA. CXCR4 (also called fusin or LESTR, references 18, 19) mRNA was abundant in both DCs and resting T cells (Fig. 4), but at least 10 times more T cells were required to provide the same amount of RNA as DCs.

Discussion

In this paper, we present evidence that HIV-1 enters and begins reverse transcription efficiently in DCs, particularly when compared to T cells. Several studies on the susceptibility of purified DCs to HIV-1 in the last few years have concluded that the virus infects DCs weakly if at all (3-5, 7). Now we have obtained evidence that HIV-1 does enter DCs. The DCs we studied are fully mature as evidenced by expression of markers like CD83 and p55, high levels of CD86, a lack of Fc receptors and CD1a. Entry of HIV-1 into these cells was only manifested by the presence of the early products of reverse transcription. In prior work, the

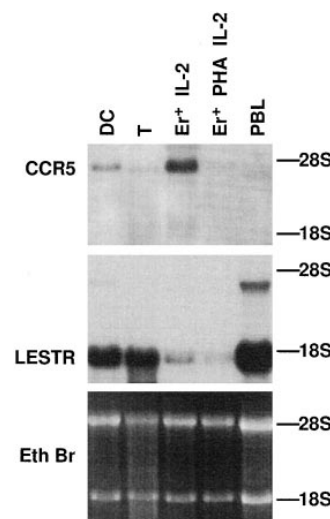


Figure 4. Northern blots for CCR5 (top) and CXCR4 (middle) mRNA in blood derived DCs, T cells, and peripheral blood cells. The exposure times were 7 d for CCR5 and 5 h for CXCR4. Results are representative of two separate experiments performed with newly synthesized CCR5 and CXCR4 cDNA probes labeled to comparable intensity and applied to 6 μ g of RNA/lane. Er⁺ cells were cultured for 8 d with IL-2 (100 U/ml) \pm PHA (1 μ g/ml) prior to RNA extraction.

presence of full length gag-containing sequences had been used to monitor infection.

We favor the idea that short R/U5 sequences indicate viral entry, rather than transcripts present in added virions, because of the need for elevated temperature and prolonged time to detect early transcripts, and once formed, their insensitivity to trypsin. Possibly reverse transcription takes place within virions after binding or endocytosis, but without fusion and true entry into DCs (31, 32). We consider this less likely since it would mean that the DCs would somehow have to provide the nucleotides and other stimuli that are required for intravirion transcription (31, 32), and that both CD4 and chemokine receptors would mediate binding to DCs but would not allow fusion and entry.

The basis for the more abundant early transcripts in DCs relative to T cells could lie at the level of viral entry or the onset of reverse transcription. We have not directly studied viral entry into DCs and T cells but only the formation of early transcripts. The biological significance of these transcripts remains to be pursued, but we know that virus-pulsed DCs initiate a vigorous infection upon coculture

with T cells. It now will be important to test if DCs harbor genomic RNA and early transcripts *in vivo*.

Several chemokine receptors can mediate HIV-1 entry. One is CCR5 that is blocked by RANTES (17, 26, 27) and deleted in select patients (20, 30). This receptor mediates entry of M-tropic isolates into DCs, as in other cell types. CXCR4 or fusin/LESTR is a second receptor that is utilized by T-tropic isolates (19) and is blocked by SDF-1 (28, 29). This receptor seems to mediate entry of IIIB into DCs, since IIIB is able to enter CCR5 deficient DCs, and entry is reduced by SDF-1 a known ligand for CXCR4. However, SDF-1 did not block entry of IIIB completely, in contrast to reports with other cells (28, 29), so other receptors may contribute to viral entry into DCs.

CCR5, the major receptor for M-tropic virus, seems dispensable given the well being of the three individuals who are known to lack CCR5. Because DCs seem important during transmission and chronic replication of immunodeficiency viruses (33), RANTES antagonists could provide a novel anti-HIV-1 therapy.

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