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HIV-1 Selectively Infects a Subset of Nonmaturing BDCA1-Positive Dendritic Cells in Human Blood

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The infection of cultured monocyte-derived dendritic cells (DCs) with HIV-1 involves CD4 and CCR5 receptors, while transmission to T cells is enhanced at least in part by the lectin DC-SIGN/CD209. In the present study, we studied BDCA-1+ myeloid DCs isolated directly from human blood. These cells express CD4 and low levels of CCR5 and CXCR4 coreceptors, but not DC-SIGN. The myeloid DCs replicate two R5 viruses, BaL and YU2, and transfer infection to activated T cells. The virus productively infects a small fraction of the blood DCs that fail to mature in culture, as indicated by the maturation markers CD83 and DC-LAMP/CD208, and the expression of high CD86 and MHC class II, in contrast to many noninfected DCs. A greater proportion of BDCA-1+ DCs are infected when the virus is pseudotyped with the vesicular stomatitis envelope VSV-G (5–15%), as compared with the R5 virus (0.3–3.5%), indicating that HIV-1 coreceptors may limit the susceptibility of DCs to become infected, or the endocytic route of viral entry used by HIV/vesicular stomatitis virus enhances infectivity. When infected and noninfected cells are purified by cell sorting, the former uniformly express HIV p24 gag and are virtually inactive as stimulators of the allogeneic MLR, in contrast to potent stimulation by noninfected DCs from the same cultures. These results point to two roles for a small fraction of blood DCs in HIV-1 pathogenesis: to support productive infection and to evade the direct induction of T cell-mediated immunity. The Journal of Immunology, 2006, 176: 991–998.

Dendritic cells (DCs) represent 1–2% of the mononuclear leukocytes in human blood, and comprise at least two major subsets that are distinguished by their reactivity with a panel of monoclonal antibodies as well as their function (1–4). The myeloid DC subset is BDCA-1+ and CD11c+, while plasmacytoid DCs (PDCs) are CD123+ and CD11c−. These two populations of DCs are reduced in the blood of HIV-1-infected patients suggesting that DCs are being targeted by HIV-1 infection (5–8), possibly at an immature developmental stage. To better understand HIV pathogenesis, the consequences of the interaction of HIV with blood DCs need further study.

In contrast, it is amply demonstrated that a fraction of immature monocyte-derived DCs (9–14) and Langerhans cells (15–17) can be productively infected with HIV-1. In addition these DCs, in the absence of overt infection, can transfer HIV-1 to receptor-positive T cells (10, 14–19). In monocyte-derived DCs, which are DCs that are differentiated from monocytes in culture by addition of GM-CSF and IL-4, the lectin DC-SIGN/CD209 is one receptor that allows the cells to sequester virus for transmission to T cells (20–24), which takes place at a virologic synapse (25, 26). Importantly, the infection of monocyte-derived DCs does not lead to maturation and in fact blocks maturation to a panel of stimuli (27). This property of the infected cells would lead to immune evasion, because it is the mature form of DC that is a powerful inducer of immunity to its captured and presented Ags, while immature DCs can induce tolerance (28–31).

In contrast to the ease with which monocyte-derived DCs can be obtained for research, blood DCs are relatively few in number and difficult to isolate in a homogeneous form. Conflicting reports exist on the extent to which bulk populations of blood DCs can be infected with HIV (11, 32, 33). Nevertheless, both myeloid and plasmacytoid DCs express the requisite receptors for HIV-1 entry, i.e., CD4, CCR5, and CXCR4 (11, 32). With respect to transmission of HIV-1 from blood DCs to T cells, one study reported that this might take place and be mediated by DC-SIGN (33), but DC-SIGN expression has been difficult to detect on freshly isolated myeloid DCs (2, 11). In this study, we take advantage of the BDCA-1 Ab to rapidly purify myeloid DCs from human blood and study the ability of these cells to replicate HIV-1, and to spread virus to T cells. We will show that HIV-1 preferentially infects a small fraction of freshly isolated myeloid DCs, particularly when HIV particles pseudotyped with vesicular stomatitis envelope (VSV-G) are used. Surprisingly, the infected DCs do not mature and exhibit very weak immunostimulatory functions.

Materials and Methods

DC isolation

DCs were isolated from the blood of normal donors, usually starting from buffy coats purchased from the New York Blood Center. Myeloid or conventional DCs were obtained from Ficoll-Hypaque-enriched mononuclear cells using the BDCA-1 isolation kit (Miltenyi Biotec). The DCs were routinely phenotyped to determine contamination with CD3+, CD19+, CD16-expressing cells. The preparations contained <0.05–1% CD3+ T cells and only traces of CD19+ and CD16+ cells. PDCs were obtained using the BDCA-4 isolation kit (Miltenyi Biotec). The enriched preparations were >75% CD123+, with most of the contaminants being CD19+ B cells.
cells. Monocyte-derived DCs were prepared as described (34) with modification. Briefly, CD14+ cells were obtained using anti-CD14 Miltenyi beads and cultured for 6 days with IL-4 (10 ng/ml; R&D Systems) and GM-CSF (100 IU/ml; Immunex). The culture medium was RPMI 1640 supplemented with 5% AB human serum (Gemini Bio-Products).

Cell surface markers of myeloid DCs

Freshly prepared DCs were phenotyped with mAbs all directly conjugated with PE and purchased from BD Pharmingen. In some experiments, the DCs were cultured for 24–48 h with IL-4 at 10 ng/ml before the FACS staining to induce the expression of DC-SIGN/CX3CR1. The DCs were also phenotyped for HIV-1 receptors expression using anti-CD4 PE, anti-CXCR4 PE, and anti-CCR5 FITC (BD Pharmingen).

Injection of myeloid DCs with HIV-1

Purified, pelleted HIV-1 BaL (R5, stock at 50% tissue culture-infective dose (TCID50) of 10^3.7/ml) and HIV-1 IIIB (X4, stock at a TCID50 of 10^3/ml) were obtained from Advanced Biotechnologies. We were provided with YU2/GFP plasmid from M. Muesing (Aaron Diamond AIDS Research Center, New York, NY) and viral particles were prepared by transfection of 293 T cells. After 50 h, supernatants were collected and for each virus we included a control, transfecting 293 cells with VSV-G and HIV minus envelope plasmids expressing G protein. We also produced HIV-1/VSV particles by co-transfecting 293 cells with VSV-G and HIV minus envelope plasmids expressing G protein and titrated these as described (35). We infected cells at a dose of 2 ng of p24 gag/10^3 cells. A total of 2 × 10^4 cells were infected for 2 h in round-bottom wells, washed four times and transferred to new wells. In some cases, AZT (National Institutes of Health AIDS Reagent Program) at 1 μM was added to the culture 20 min before infection and then kept throughout the experiment to inhibit the viral life cycle.

Detection of HIV-1 in infected myeloid DCs and in DC-T cell cocultures

To assess direct infection of DCs by HIV-1 BaL, cells were collected at several time points, fixed, and permeabilized (Cytofix/Cytoperm reagents; BD Pharmingen) and double-stained with FITC anti-HIV gag p24 (clone KC 57; Beckman Coulter) and CD11c PE, DC-LAMP/CD208 PE (Immuno-tech), the latter to define DCs that had undergone maturation (36). For cells infected with viruses expressing G protein, in general, the staining was without permeabilization. Samples were analyzed on a FACSort (BD Pharmingen) with CellQuest software. To assess transmission of virus to activated T cells, the DCs were infected with HIV-1 for 2 h, washed, and transferred to a new tube. After 4 h, cells were counted and 5 × 10^4 DCs were cocultured with 10^5 T blasts in round-bottom wells in 200 μl of medium. In some experiments, T blasts were added 48 h after the infection of the myeloid DCs. These steps were designed to remove free virions that could infect T blasts directly. Supernatants were collected and stained with PE and purchased from BD Pharmingen. In some experiments, the T cells infected with viruses expressing GFP, in general, the staining was not shown here, but see Fig. 5A, shown) CD40L-expressing cells, there were small increases in CD4, CCR5, and CXCR4, i.e., the requisite receptors and coreceptors for HIV-1 (Fig. 1C). The CXCR4 and CCR5 receptors were functional, as assessed in a chemotaxis assay. Cells migrate in response to their respective ligands, SDF-1/CXCL12 for CXCR4 and SDF-1/CXCL12 for CCR5 and RANTES (CCL5), MIP-1α (CCL3) and β (CCL4) for CCR5 (Fig. 1D). These data indicate that myeloid DCs in fresh human blood should be susceptible to HIV-1, and also that the cells only acquire the MMR and DC-SIGN markers of monocyte-derived DCs following culture in IL-4.

Productive infection of myeloid DCs by HIV

We next assessed HIV-1 infection using R5 and X4 viruses that use CCR5 and CXCR4 coreceptors, respectively. The production of p24 was analyzed at the single-cell level by FACS. With R5 viruses, positive cells started to be detected by the third day after infection and peaked at 5 days (Fig. 2A). At later time points, the DC preparation had poor viability. A very small fraction of the freshly isolated DCs showed p24 staining after exposure to X4 virus. At most, 0.1% of the cells stained for HIV gag p24 3–5 days later (data not shown), whereas 0.3–3.5% of the cells were positive for Bal. p24 (Fig. 2A). If the infected cultures were simultaneously stained for HIV-1 p24 and DC maturation markers, DC-LAMP/CX3CR1 (Fig. 2A), HIV-1 and CD86 and MHC II (data not shown here, but see Fig. 5A below), the low expression or less mature cells were preferentially infected. No p24+ cells were detected when the reverse transcriptase inhibitor AZT was added before infection, indicating that p24 expression represented viral replication within DCs (Fig. 2B, lower panels). The few contaminating resting T in the culture cells did not become gag positive upon HIV-1 infection (Fig. 2C).

The infection of DCs with GFP+ virus was further characterized to rule out that the staining seen by FACS was a consequence of autofluorescence. To this end, after 4–5 days of viral infection, cells were sorted as GFP+ and GFP− and stained for expression of gag p24 and HLA-DR. As expected, all the GFP+ cells stained diffusely for gag p24 (Fig. 2D, top left). Interestingly, the cells replicating virus showed an immature phenotype with a punctuate HLA-DR staining mostly localized in intracellular vesicles (Fig. 2D, bottom left). These data indicate that a subset of myeloid DCs can be infected with HIV-1. Infection selects for the more immature cells, and does not mature the susceptible BDCA-1+ cells.

HIV-1-infected myeloid DCs transmit virus to T cells

To assess the capacity of myeloid DCs to transmit HIV-1 to T cells, we exposed the DCs for 2 h to either of two R5 isolates, BaL, and YU2, the latter expressing G protein. The DCs were washed thoroughly and were added to CD4+ T cells immediately or after 2 days of infection. When HIV-1 p24 production was analyzed by
ELISA, infection could be detected to a similar extent when the cocultures were started at day 0 (Fig. 3, experiment 1) or at 48 h after DC infection (Fig. 3, experiment 2). To verify that most of the infection was occurring in the T cells, we used YU2-GFP virus and double-labeled for CD3 (Fig. 3B). This FACS approach showed that CD3high, CD11cdim T cells expressed GFP at a level of 2–7% of the T cells depending on individual experiments (of nine performed). There was little or no spread of infection to DCs in the DC-T coculture, because there were few infected CD11chigh cells in the FACS plots (Fig. 3B). These results indicate that myeloid DCs are capable of transmitting virus preferentially to T cells.

Comparison of virus transmission by DC subsets

To assess the efficiency of different types of DCs to transmit HIV to T cells, monocyte-derived DCs were compared with the two major subsets of blood DCs, all prepared from the blood of the same individual and infected with HIV-1 BaL in parallel. Consistently in five experiments, the monocyte-derived DCs were superior in their ability to transmit infection with R5 virus to T cells (Fig. 3C). In one other set of experiments, we compared the capacity of myeloid DCs and PDCs to be infected and spread R5 and X4 virus to activated T cells. The results indicated that the R5 virus was transmitted more efficiently than X4 virus by myeloid DCs to T cells, while the percentage of T blasts infected after exposure of PDC to R5 or X4 viruses was quite similar (data not shown). In the absence of T cells, we quantitated the amount of gag p24 released in the culture supernatants of the infected DCs by ELISA. After 5 days of infection, 10^7 monocyte-derived DCs released between 0.5 and 2 ng of p24 while myeloid DC released 0.1–0.8 ng of p24.

Infection of myeloid DCs with VSV-pseudotyped virus

HIV-1 particles can be pseudotyped by envelope glycoproteins such as VSV glycoprotein VSV-G. The VSV envelope mediates efficient entry into DCs, but only a single cycle of replication is possible because the progeny viral genomes lack an envelope. We have previously shown, using VSV-G-pseudotyped HIV-1 particles, that monocyte-derived DCs permit a single cycle of viral replication, and that the pseudotyped HIV-1 enters the cell through a pH-sensitive pathway (35), independently from the classic HIV-1 pathway that is mediated by CD4 and either the CCR5 or CXCR4 coreceptors. Thus, the use of VSV/HIV-1 allows one to study the replication potential of myeloid DCs without the restriction of HIV-1 receptor expression. In fact, the percentage of cells infected with VSV/HIV-1 was significantly higher, ranging from 5 to 14% as compared with HIV-1 BaL (range 0.3–3.5%, Fig. 4). These results indicate that the intrinsic ability of DCs to replicate virus is greater than is evident with standard infection protocols. The expression of HIV-1 coreceptors in myeloid DCs may limit their susceptibility to be infected or VSV envelope may enhance infection.

Effects of maturation stimuli on HIV-1 replication on myeloid DCs

We have shown, using monocyte-derived DC, that an exogenous maturation stimulus added to DC abrogates viral replication and also that infection itself does not drive maturation (27). We now show that analogous results can be obtained when blood DCs are matured by several maturation stimuli. Freshly prepared myeloid DCs were infected with HIV-1 and exposed to maturation stimuli at various times after infection. A total of 0.3–3% of the DCs in
culture were GFP positive, when the DCs were infected with YU2GFP HIV in the absence of maturation stimuli (Fig. 5A, top row), but if maturation stimuli were added 1 day after infection (we tested poly I:C and R848, which are TLR3 and TLR7/8 ligands, respectively, as well as a mixture of inflammatory cytokines, IL-1β, IL-6, and TNF-α), no infected cells were detected 3 days later in six separate experiments, as illustrated in Fig. 5A, middle row. If the addition of maturation stimuli was delayed 3 days, and then the stimuli added for 1–2 days, the yield of the infected cells was again reduced as shown in Fig. 5A, lower panel.

We then repeated the experiments with VSV/HIV. In the absence of maturation stimuli, the infected cells showed always an immature phenotype, with low CD86 and DC-LAMP as shown in Fig. 5B. When we added maturation stimuli at day 3, as shown in the lower part of Fig. 5B, most of the infected DCs 2 days later remained immature with low expression of CD86 and DC-LAMP. The reliability of this observation is summarized for several experiments in Table I. We verified that the cultured infected cells had markers of DCs, e.g., CD11chigh, HLA-DR and CD86 positive, and CD3 negative. Therefore, maturation stimuli shut off viral replication when added early in the viral life cycle, and when added later on (3 days), the productively infected cells remain in an immature state in terms of surface markers (see also Fig. 2D).
Blood DCs purified according to BDCA1 expression show heterogeneity being ~50% CD14+ and 50% CD14−. To assess viral replication in these subsets, the BDCA1+ selected DCs were sorted as CD14+ and CD14− and studied for their susceptibility to infection with or without maturation. As seen in Fig. 5C, both CD14-positive and -negative myDCs were susceptible to HIV-1 replication, and again maturation of DC decreased viral replication.

**HIV-1-infected blood DCs exhibit weak stimulatory function in the MLR**

Because of the substantial frequency of infected cells with VSV-pseudotyped HIV, we were able to separate infected from noninfected myeloid DCs using VSV/HIV-pseudotyped GFP virus. After 5 days of infection, cells were sorted as GFP+ (infected) and as GFP− (not infected). These sorted cells were assessed for their stimulatory activity in an allogeneic MLR over a range of DC to T cell ratios and time points. The MLR was monitored using CFSE-labeled responder cells. The GFP+ noninfected cells induced a strong MLR, with a peak of CFSE low T cells developing in just 4 days of the MLR, and at low DC to T cell ratios (Fig. 6). In contrast, the GFP− cells were virtually nonstimulatory (Fig. 6). We also verified that the infected DCs did not suppress the induction of T cell proliferation by the noninfected DCs even when a 10-fold excess of the infected DCs was added to the coculture of the noninfected cells (Fig. 6C). The infected DCs were further compromised in their capacity to secrete the immunostimulatory cytokine IL-12p70. Although 10E6 GFP+ cells upon maturation released 278 pg of IL-12p70, the infected DCs failed to produce this cytokine. IL-10 was undetectable. Addition of neutralizing anti-IL-10 Abs to the MLR with HIV-1-infected DCs did not restore T cell proliferation (data not shown). These results provide functional evidence that the interaction of HIV with myeloid DCs selects for less immunostimulatory forms of DCs and/or blocks the functional maturation of these cells.

**Discussion**

A large body of work has been directed to understanding the role of DCs as an early site of HIV-1 infection and as a driving force for subsequent CD4+ T cell infection. However, the study of DCs that are present in human blood, as opposed to the more accessible monocyte-derived cultured DCs, has been compromised by the fact that the cells are difficult to isolate. Nonetheless, there is clear evidence with new mAbs that mark DCs and their subsets (37) that the two populations of blood DCs, myeloid and plasmacytoid, are significantly reduced in number during HIV-1 infection when compared with normal individuals (5–8). Not only the percentage of DCs decreases with the progression of the disease but also their...
function as APCs seems reduced (5–7). Using bulk cultures of myeloid DC and PDC, it has been shown that these populations can be infected with HIV-1 (11, 32). The availability of mAbs to specific Ags expressed on DC subsets (37) facilitates their isolation and allows one to perform more detailed studies in highly enriched populations. Except for a recent study by Lore et al. (38), prior research has not considered the two major subsets of DCs purified by these efficient new isolation methods.

In this study, we show that myeloid DCs express CD4 and functional chemokine receptors CXCR4 and CCR5, which are known receptors required for HIV infection. Our studies, performed at the single-cell level, show that R5 HIV-1 replicates in myeloid DCs and that replication is blocked by the reverse transcriptase inhibitor AZT indicating productive infection. However, the percentage of productively infected myeloid DCs is low, in the range of 0.3–3.5%. X4 HIV-1 also seems to replicate within rare cells in this DC population. Nonetheless, a low level of infected DCs may be sufficient to facilitate virus transmission to T cells, in view of the fact that one DC can sample a large number of T cells (39, 40). Although the frequency of infection of blood myeloid DCs is low, it is possible that an immediate progenitor of the blood DC, e.g., in the bone marrow, is much more susceptible to infection and accounts for the reduction of DCs observed in the blood of HIV-1-infected individuals.

Blood DCs are able to transfer a productive infection to T cells, although we find that they are less efficient than the more frequently studied monocyte-derived DCs. Several factors may be involved in this effect. Monocyte-derived DCs have several virus attachment factors (41) that may not be present in myeloid DCs or PDCs from blood. Moreover, several C-type lectins, among them DC-SIGN, are highly expressed in monocyte-derived DCs (20, 42) and may function in enhancing infectivity by concentrating viral particles at the virological synapse (25, 26, 43).

Using HIV-1 virions that had been pseudotyped with the VSV-G envelope protein, we were able to increase the proportion of infected myeloid, BDCA-1+/H11001 DCs. The infection rate ranged from 0.3 to 3% for R5 virus to 5%, even up to 15%, for VSV HIV. VSV pseudotyping virus bypasses the CD4/CCR5 pathway used by the R5 envelope and instead infects the DCs via acidic compartments (35). Our results indicate that viral receptors are somehow regulating replication of R5 virus in myeloid DCs, or that the VSV-G envelope enhances infection.

As in our recent studies with monocyte-derived DCs, we find that HIV primarily infects cells that are less mature in phenotype (27). By simultaneously labeling the infected cells with Abs to p24 gag protein, we found that the p24+ cells predominantly expressed lower levels of the CD86 costimulator and MHC class II Ag-presenting products. Even after 3–4 days of culture, little or no CD83 or DC-LAMP, two markers of DC maturation, were detectable. When we sorted the infected DCs from noninfected DCs, using VSV-pseudotyped GFP-expressing virus, we observed that the infected cells were in addition functionally immature, being almost inactive as stimulators of the MLR. Decreased stimulation
of T cell proliferation was also reported when the enriched population of T cell unresponsiveness under steady state conditions in vivo. J. Exp. Med. 194: 769–780.


