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

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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)[3] 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 bodies 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 homogenous form. Conflicting reports exists 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

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[3] Abbreviations used in this paper: DC, dendritic cell; PDC, plasmacytoid DC; VSV, vesicular stomatitis virus; SDF-1, stromal cell-derived factor-1; MMR, macrophage mannose receptor; AZT, 3-azido-3-deoxythymidine.

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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/CD209. The DCs were also phenotyped for HIV-1 receptors expression using anti-CD4 PE, anti-CXCR4 PE, and anti-CCR5 FITC (BD Pharmingen).

Infection of myeloid DCs with HIV-1

Purified, pelleted HIV-1 BaL (R5, stock at 50% tissue culture-infective dose ($TCID_{50}$) of $10^{7.7}$/ml) and HIV-1 III B (X4, stock at a $TCID_{50}$ of 10^8/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 titered for HIV p24 gag protein. We also produced HIV-1/VSV particles by cotransfecting 293 cells with VSV-G and HIV minus envelope plasmids expressing GFP and titrated these as described (35). We infected cells at a dose of 2 ng of p24 gag/10^5 cells. A total of 2×10^5 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 (Immunotech), the latter to define DCs that had undergone maturation (36). For cells infected with viruses expressing GFP, 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 infection monitored at several time points by an ELISA for HIV gag p24 release (Beckman Coulter). Alternatively, infection was measured by FACS staining for p24 gag Ab.

Chemotaxis assays

Purified myeloid DCs, 2×10^5 in 100 μl of complete medium, were added to Transwell chambers (24-well, 5-μm pore size; Corning) with 600 μl of medium in the lower chamber, with or without chemokines. Stromal cell-derived factor-1 (SDF-1)/CXCL12 and MIP-1α/CCL3 (R&D Systems) were added at concentrations ranging from 10 to 1000 ng/ml. After 3 h of incubation at 37°C, the Transwell insert was removed. The cells in the lower chamber were collected and enumerated by collecting events for a fixed time (60 s) on a FACSCalibur.

Immunostimulatory function of cells infected with HIV/VSV

Myeloid DCs were infected with HIV/VSV and after 5 days were sorted as GFP positive (infected cells) or GFP negative (noninfected cells). To further verify that the GFP^+ cells were infected, sorted GFP-positive and negative myeloid DC were attached to printed slides (Carlson Scientific) coated with polylysine (Sigma-Aldrich) and left to adhere at 37°C for 30 min in serum-free medium. The cells were washed, fixed, permeabilized, and stained with anti-HIV gag p24 and HLA-DR for 45 min at room temperature followed by goat anti-mouse IgG Alexa 546 at a dilution of 1/800 (Molecular Probes). As a control, matching isotype IgG was applied to samples. Fluorescence was examined with a model AX70 Olympus laser scanning microscope. To assess immunostimulatory function, the sorted GFP^+ and GFP^- DCs were added in graded doses to 10^5 allogeneic T cells that had been labeled with CFSE. The MLR was assessed by CFSE dilution at days 4–8.

Results

Cell surface markers of isolated myeloid DCs

We verified that BDCA-1-purified myeloid DCs, freshly isolated from blood, expressed several typical DC markers including high levels of CD11c and HLA-DR (Fig. 1*A*). Most cells expressed moderate levels of CD86 and DEC-205/CD205 (Fig. 1*A*). A variable frequency of the DCs stained weakly for CD83 (Fig. 1*A*) or DC-LAMP/CD208 (data not shown), ranging from ~10 to 40% of the $BDCA\text{-}1^+$ fraction. BDCA-1-selected DCs were negative for DC-SIGN/CD209, MMR/CD206, and CCR7 (Fig. 1*A*). When the cells were cultured for 24 h without (Fig. 1*B*) or with (data not shown) CD40L-expressing cells, there were small increases in CD83, CD86, and HLA-DR (Fig. 1*B*, *upper row*). However, CD206 and CD209 were both strongly induced during 24 h of culture in IL-4 (Fig. 1*B*, *lower row*). The DCs expressed CD4, CCR5, and CXCR4, i.e., the requisite receptors and coreceptors for HIV-1 (Fig. 1*C*). 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 RANTES (CCL5), MIP-1α (CCL3) and β (CCL4) for CCR5 (Fig. 1*D*). 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. 2*A*). 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 IIIB p24 3–5 days later (data not shown), whereas 0.3–3.5% of the cells were positive for BaL p24 (Fig. 2*A*). If the infected cultures were simultaneously stained for HIV-1 p24 and DC maturation markers, DC-LAMP/CD208 (Fig. 2*A*), CD83 (Fig. 2*B*), or CD86 and MHC II (data not shown here, but see Fig. 5*A* below), the low expressing 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. 2*B*, *lower panels*). The few contaminating resting T in the culture cells did not become gag positive upon HIV-1 infection (Fig. 2*C*).

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. 2*D*, *top left*). Interestingly, the cells replicating virus showed an immature phenotype with a punctuate HLA-DR staining mostly localized in intracellular vesicles (Fig. 2*D*, *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 $BDCA\text{-}1^+$ 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 GFP. 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

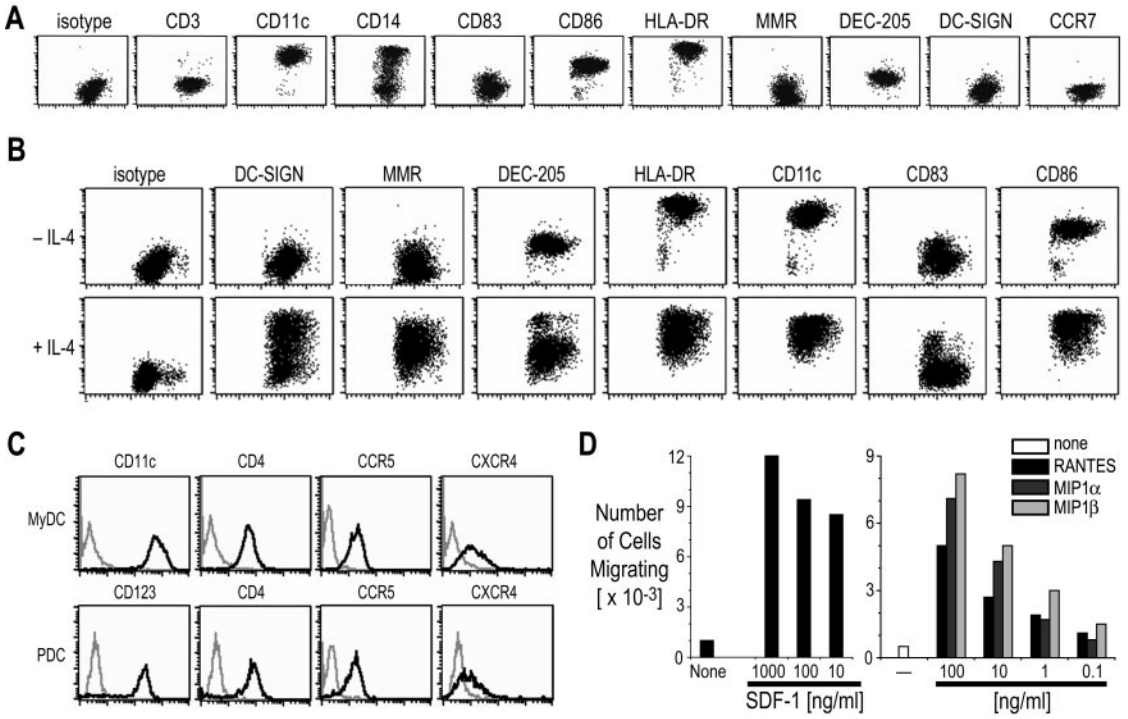

FIGURE 1. Expression of surface Ags and HIV-1 receptors in myeloid DCs (MyDCs). *A*, Purified MyDCs were stained immediately with mAbs and analyzed by FACS. *B*, Purified MyDCs were cultured for 24 h with or without 10 ng/ml IL-4. Cells were analyzed by FACS for the indicated markers. *C*, Freshly purified MyDCs and PDCs were analyzed for the expression of CD4, CCR5, and CXCR4 (black line). The gray lines represent staining with isotype-matched control Ab. *D*, The capacity of MyDCs to migrate in response to SDF-1α (CXCL12) or RANTES (CCL5), MIP-1α (CCL3), and MIP-1β (CCL4) was assessed in a chemotaxis assay.

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. 3*B*). This FACS approach showed that $CD3^{high}$, $CD11c^{dim}$ 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 $CD11c^{high}$ cells in the FACS plots (Fig. 3*B*). 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. 3*C*). 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 then 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, 10^6 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

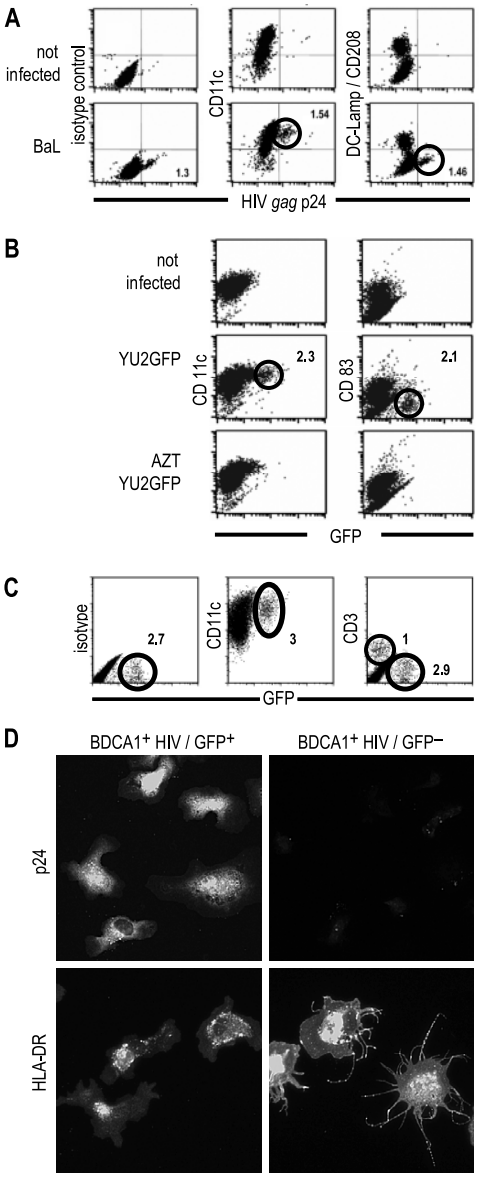

FIGURE 2. Productive HIV-1 infection of myeloid DCs. *A*, DCs were infected (BaL) or not infected for 2 h. The cells were washed and cultured for 5 days. Permeabilized cells were double-labeled for infection with anti-p24 FITC (infected cells are circled) and for other DC markers, either PE-labeled isotype control Ig, anti-CD11C, or anti-DC-LAMP PE. *B*, Myeloid DCs were pretreated or not with 1 μM AZT and then infected with YU2/GFP. After 5 days, cells were stained with anti-CD11c PE or anti-CD83 PE (surface staining) to phenotype the infected GFP-positive cells (circle). *C*, DCs were infected with YU2/GFP and stained with the indicated surface markers. Note that the few CD3 contaminating resting T cells were not infected. *D*, Myeloid DCs were infected with HIV/GFP for 4 days. Cells were sorted as GFP positive and negative and stained for p24 gag and HLA-DR. Some punctuate stain in the infected GFP-negative fraction is probably the result of internalized virions. This pattern is not present in the noninfected cells.

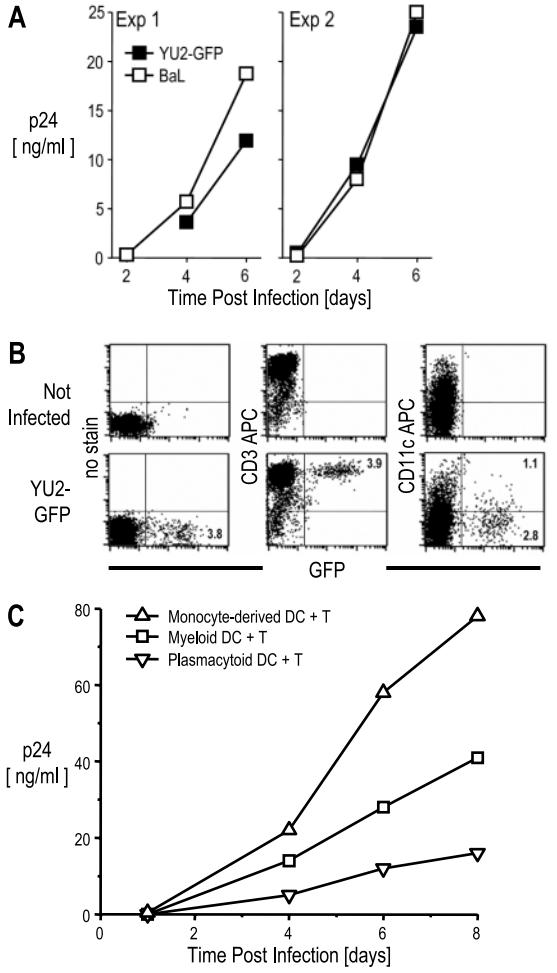

FIGURE 3. HIV-1-infected DCs transmit infection to T cells. *A*, My-DCs were pulsed with BaL or YU2/GFP for 2 h, washed, and cocultured with activated T cells. T blasts were added immediately to the virus-pulsed DCs (exp 1) or 48 h after the DC infection (exp 2). p24 release into the supernatants was quantified by ELISA. *B*, DCs were pulsed with YU2/GFP for 2 h, washed, and T blasts added after 48 h. Cultures were collected at 5 days, stained with either anti-CD3 allophycocyanin or anti-CD11c allophycocyanin, and analyzed by FACS. *C*, Efficiency of HIV transmission by different types of DCs. Immature monocyte-derived DCs (obtained after 6 days of culture) and freshly isolated blood myeloid and plasmacytoid DC were infected with BaL for 2 h, washed, and cultured for 48 h. The different DCs were then cocultured with T blasts and the kinetics of p24 release into the supernatants were measured by ELISA.

culture were GFP positive, when the DCs were infected with YU2GFP HIV in the absence of maturation stimuli (Fig. 5*A*, *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. 5*A*, *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. 5*A*, *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. 5*B*. When we added maturation stimuli at day 3, as shown in the lower part of Fig. 5*B*, 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., CD11c^{high}, 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. 2*D*)

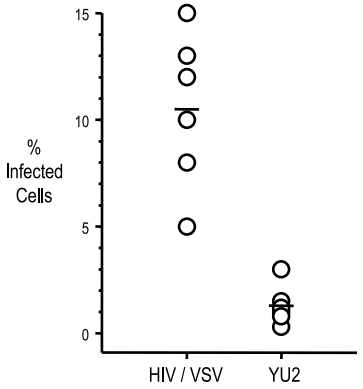

FIGURE 4. Infection of myeloid DC by HIV particles pseudotyped with VSV-G. DCs were infected with HIV-1/VSV or YU2/GFP. Productively infected cells were studied for the expression of GFP by FACS 5 days later.

Table I. *Failure of HIV-1-infected myeloid DC to mature*[a]

Experiment	Maturation Marker	% Mature GFP$^-$	% Mature GFP$^+$
1	high CD86	54	4.8
	DC-LAMP	58	3.2
2	high CD86	27	0.2
	DC-LAMP	31	0.7
3	high CD86	71	1.4
	DC-LAMP	54	1.2
4	DC-LAMP	54	0
5	DC-LAMP	50	0.36

[a] BDCA-1$^+$ blood DCs were infected with IIIV/VSV for 3 days, and then matured for 2 days with R848 (I μM) and poly I:C (30 μg/ml). At day 5, cells were permeabilized and stained with CD86 and the DC maturation marker DC-LAMP. The frequency of GFP$^+$ and GFP$^-$ cells expressing high CD86 or DC-LAMP, as in Fig. 5, are shown.

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. 5*C*, 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. 6*C*). The infected DCs were further compromised in their capacity to secrete the immunostimulatory cytokine IL-12p70. Although 10^6 GFP$^-$ cells upon maturation released 278 pg of IL-12 p70, 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

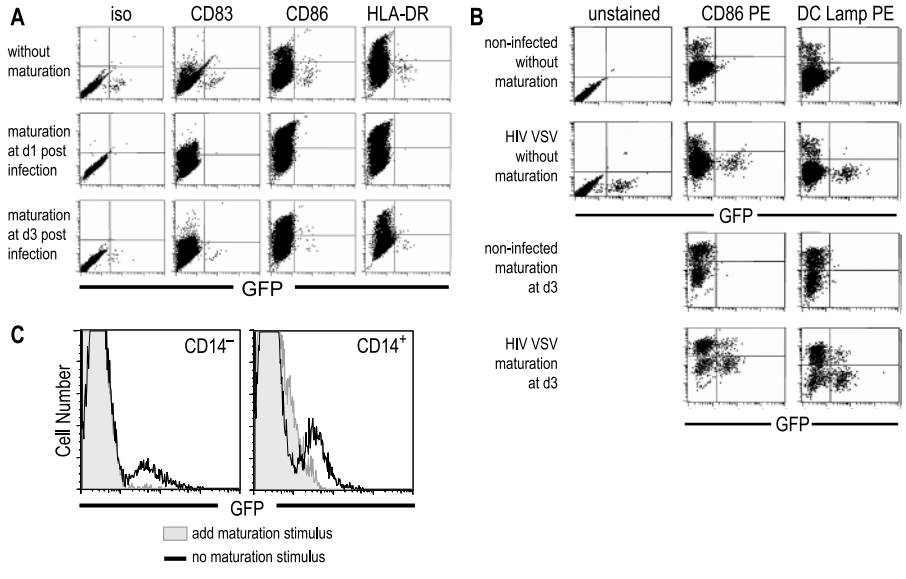

FIGURE 5. Effect of maturation stimuli added to HIV-1-infected myeloid DC. Myeloid DCs were infected with HIV/GFP. Maturation stimuli were added at the indicated time postinfection. After 5 days, cells were analyzed by FACS for expression of GFP and the maturation marker. *A*, Infection with YU2/GFP and detection of surface Ags after maturation with cytokine mixture. *B*, Infection with HIV/VSV and detection of DC-LAMP and CD86 in permeabilized cells after maturation with R 848 at 1 μM. *C*, BDCA 1$^+$ cells were sorted as CD14$^+$ and CD14$^-$ infected with HIV/GFP and after 2 days part of the cultures were matured with R 848 at 1 μM and Poly I:C at 25 μg/ml. After 5 days, cells were analyzed for GFP expression.

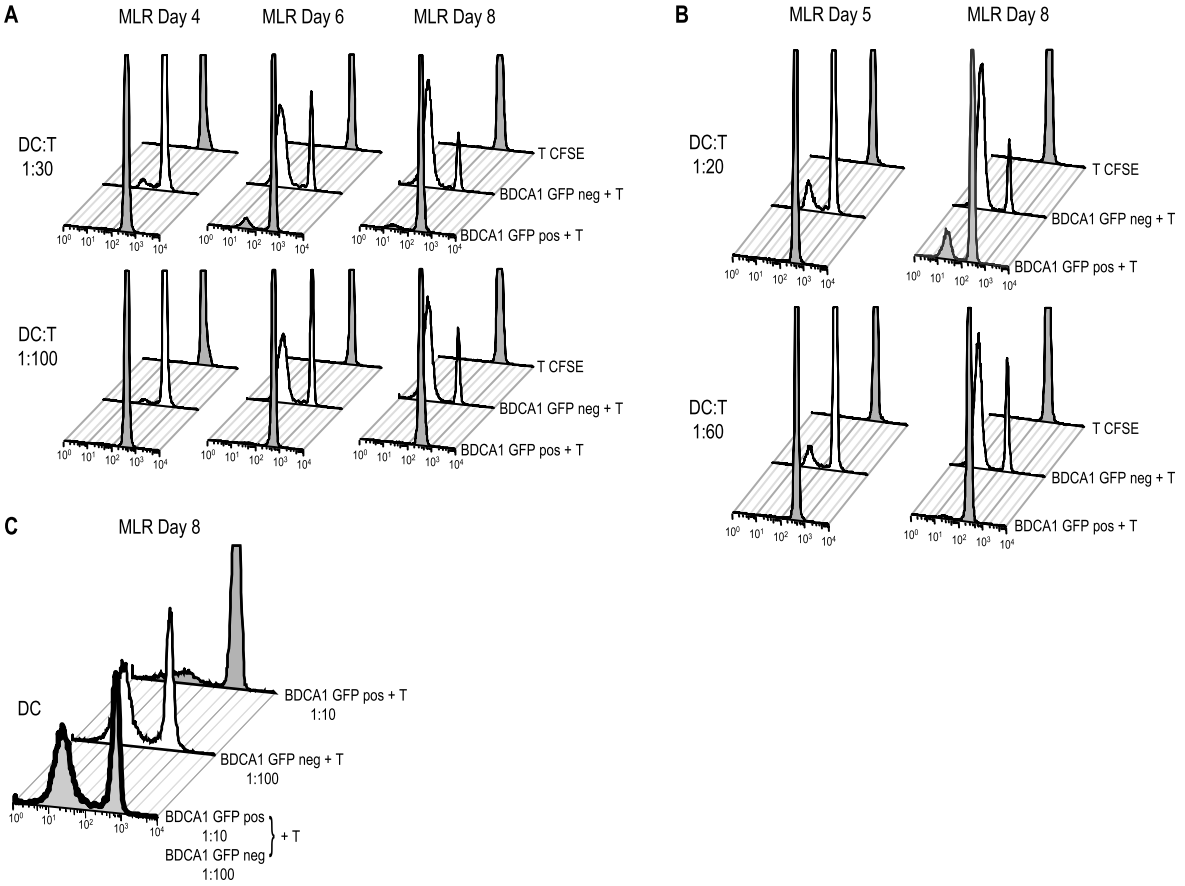

FIGURE 6. Infected myeloid DCs are weak stimulators in the MLR. Myeloid DCs were infected with HIV/VSV and sorted after 5 days as GFP^+ and GFP^-. Sorted DCs were then added in graded doses to 10^5 allogeneic CFSE-labeled CD3 T cells (DC:T cell ratios are indicated). At several time points, the presence of an MLR was documented by CFSE dilution. Shown here are two typical experiments of four performed (*A* and *B*). In *C*, GFP^+ cells were cocultured with GFP^- cells and CFSE-labeled allogeneic T cells. The MLR was documented after 8 days.

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^+ 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 HIV-1-infected monocyte-derived DC were used as stimulators in a MLR (44). These data point to a new evasion strategy of HIV-1, whereby infected cells will be poor direct stimulators of T cell-mediated immunity. Instead, the induction of immunity would require cross-presentation of HIV-1 Ags within dying infected cells, most likely T cells, by uninfected DCs, a possibility that has been documented by Buseyne et al. (45) and Larsson et al. (46).

At this time, our data do not distinguish between two possibilities. HIV-1 may select for a subset of DCs that is resistant to maturation, or as we suspect, HIV-1 itself blocks the maturation of the cells that are infected. In either case, our findings imply that presentation of HIV-1 Ag by infected blood DCs can lead to tolerance, a newly recognized property of immature DCs within lymphoid tissues (28–31). Taken together, the results in this paper indicate that HIV-1 exploits the myeloid DCs in blood not only for replication and transmission, but also for immune evasion.

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Disclosures

The authors have no financial conflict of interest.

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