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Monocyte-Derived Cultured Dendritic Cells Are Susceptible to Human Immunodeficiency Virus Infection and Transmit Virus to Resting T Cells in the Process of Nominal Antigen Presentation

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The susceptibility of monocyte-derived cultured dendritic cells (DCs) to human immunodeficiency virus (HIV) infection and their role in viral transmission in the immune response were studied in detail. We observed that highly purified cultured DCs were infected with the T-tropic Lai strain of HIV type 1 (HIV-1_{Lai}) via the CD4 receptor, and this was followed by formation of the complete provirus as detected by PCR. HIV mRNAs were transcribed at only low levels, and virus production was undetectable; however, the addition of the purified protein derivative antigen of tuberculin and of autologous resting T cells to HIV-1_{Lai}-infected DCs but not to HIV-1_{Lai}-infected macrophages led to massive HIV transmission and production. These data suggest that the interaction of infected DCs with T cells during the normal immune response could play an important role in the activation and expansion of HIV.

Dendritic cells (DCs) are widely distributed in the lymphoid and nonlymphoid tissues. They are called Langerhans cells (skin), interdigitating DCs (lymph nodes), thymic DCs, veiled cells (afferent lymph), interstitial DCs (heart, lungs, and intestine), or blood DCs, depending on their location (10, 27). DCs can efficiently take up antigen, carry it to draining lymph nodes, and present it in a processed form to resting T cells. Because other antigen-presenting cells, including B cells and macrophages, are unable to stimulate resting T cells efficiently (16), DCs appear to play a dominant role in primary immune responses.

Immunodeficiency associated with human immunodeficiency virus (HIV) infection could be related in part to the dysfunction of antigen-presenting cells, including DCs (17), and earlier studies have suggested that HIV infection results in depletion and impaired function of blood DCs (13, 14). In this context, the infectivity of the virus for DCs has been a critical issue. Recently, the presence of proviral DNA (28) and mRNA transcripts (7) was demonstrated in purified Langerhans cells, whereas purified, CD4-negative, mature blood DCs were not infected with HIV (4). However, the susceptibility of immature DCs expressing CD4 (19) is not known.

Detailed studies of the mechanism by which DCs might contribute to HIV expansion and possibly to immune suppression have been hampered by the difficulty of obtaining sufficient amounts of pure DCs. However, the recent development of methods for the in vitro differentiation of progenitor cells to DCs has enabled us to produce large numbers of these cells (5, 22, 23, 25). In this study, DCs were generated by culturing monocytes in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) (1a, 25). These cells were nonadherent large cells with the typical dendritic morphology, and they were positive for major histocompatibility complex classes I and II, LFA-1, LFA-3, ICAM-1, B7-1, B7-2, and CD40 but negative for CD3, CD20, and CD14. They possessed a potent antigen-presenting function in the allogeneic mixed lymphocyte reaction as well as in response to a bacterial superantigen, SEB (1). Unlike mature blood DCs, cultured DCs appear to be immature, and their phenotype resembles that of epidermal Langerhans cells (5, 22). They express a high level of CD1a and a low level of CD4, and they can mature in culture upon addition of tumor necrosis factor alpha (reference 25 and unpublished observations).

In order to study the susceptibility of these cultured DCs to HIV infection, we purified them with a $FACS_{vantage}$ apparatus (Becton Dickinson) set for double gating to select for $CD1a^+$, $CD3^-$, $CD14^-$, and $CD20^-$ cells with a high forward-light-scatter-to-side-angle-light-scatter ratio. This procedure depleted $CD3^+$, $CD20^+$, and $CD14^+$ cells to less than 0.01%. When these purified cultured DCs were infected with a variety of T-cell-tropic or macrophage (Mϕ)-tropic HIV type 1 (HIV-1) strains, proviral DNA was detected by PCR, but no evidence of virus production was obtained (data not shown). Treatment of cultured DCs with zidovudine or anti-CD4 monoclonal antibody (MAb) during the process of HIV infection blocked the synthesis of complete proviral DNA (Fig. 1). This indicated that HIV-1 entered cultured DCs via the CD4 receptor and completed cDNA synthesis within the DCs.

To compare the susceptibility of cultured DCs to HIV-1 infection with that of other target cells, we determined the infection frequency of various cells by quantitative PCR based on limiting-dilution analysis (2). Cells were infected with either HIV-1_{Lai} or HIV-1_{Bal} for 2 h and were washed and cultured for a further 12 h. They were lysed, and cell lysates were serially diluted for PCR amplification under conditions that allowed detection of a single provirus copy. From the number of negative amplification reactions, provirus frequencies were calcu-

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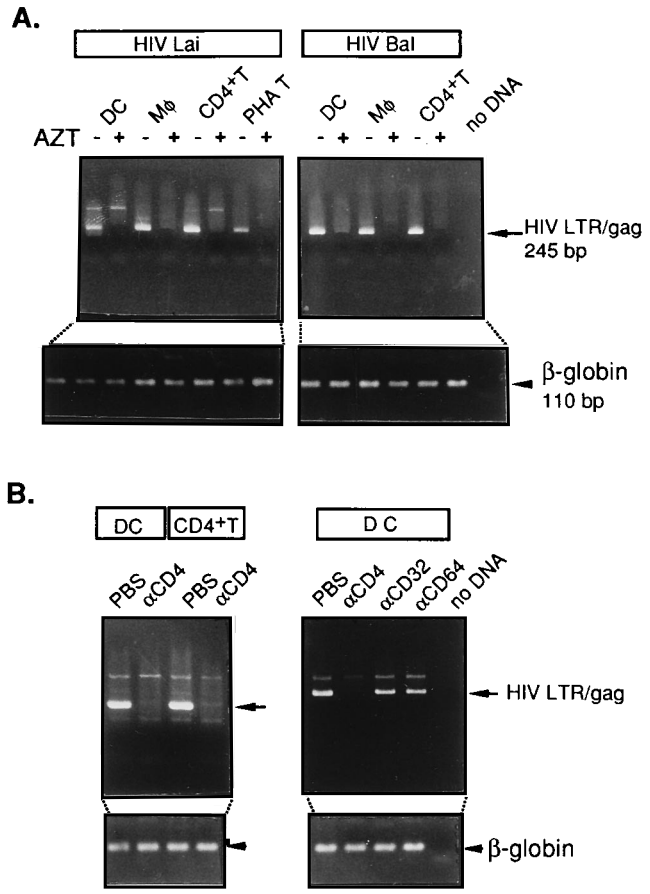

FIG. 1. HIV-1 infection of cultured DCs is blocked by zidovudine or anti-CD4 MAb. (A) Adherent peripheral blood mononuclear cells were cultured for 7 days in the presence of either GM-CSF and IL-4 or macrophage colony-stimulating factor for the generation of cultured DCs and Mϕs (designated M Mϕs), respectively. Nonadherent peripheral blood mononuclear cells were cultured for 7 days as DCs in the absence of cytokines and either enriched for $CD4^+$ T cells by depletion of CD8-, CD20-, CD14-, and CD56-positive cells (cultured $CD4^+$ T cells) or activated by phytohemagglutinin (PHA blast). Highly purified cultured DCs, M Mϕs, cultured $CD4^+$ T cells, and PHA blast were infected for 2 h with either $HIV\text{-}1_{Lai}$ or $HIV\text{-}1_{Bal}$ at the ratio of 10^5 cpm of reverse transcriptase activity per 10^6 cells in the presence (+) or absence (−) of 200 μM zidovudine (AZT). After the infection, the cells were extensively washed and then cultured for 12 h. Cell lysates of 5×10^4 cells were prepared in 10 μl of lysis buffer containing 10 mM Tris-HCl (pH 8.3), 0.5% Tween 20, 0.5% Nonidet P-40, 0.5 mM EDTA, and 100 μg of proteinase K per ml. The formation of the complete provirus was determined by PCR with HIV long terminal repeat (LTR) and *gag* primers in two steps: the first 25 cycles with JAM 62 and JAM 65 primers and the next 30 cycles with inner (nested) JAM 63 and JAM 64 primers. Their sequences were as follows: JAM 62 (LTR), 5′-GCTTCAAGTAGTGTGTGC CCGTCTG-3′; JAM 63 (LTR), 5′-GTGTGACTCTGGTAACTAGAGATCC-3′; JAM 64 (*gag*), 5′-CCGCTTAATACTGACGCTCTCGCAC-3′; and JAM 65 (*gag*), 5′-AATCGTTCTAGCTCCCTGCTTGCCC-3′. The amplification product was analyzed by electrophoresis on a 2.5% agarose gel stained with ethidium bromide (expected size of HIV DNA = 245 bp). The faint upper band is a nonspecific amplification product obtained with the outer primers. (B) DCs and $CD4^+$ T cells were treated with various MAbs (25 μg/ml) or phosphate-buffered saline for 20 min at room temperature and infected with $HIV\text{-}1_{Lai}$. After 12 h of cultivation, PCR was carried out with cell lysates as described in the legend for panel A. As a positive control for amplifiable DNA, the gene for β-globin was amplified with the primers PC03 and PC04 (24). Anti-CD4 MAb was kindly provided by H. Nakauchi (Tsukuba University, Tsukuba, Japan), and anti-CD32 and CD64 MAbs were generous gifts from H. Yagita (Juntendo University, Tokyo, Japan).

lated. Because the proviral copy number in individual cells 12 h after HIV-1 infection is known to be low (2, 8), the frequency calculation based on diluting cell lysates is expected to provide a reasonable estimation of the frequency of infection. As summarized in Table 1, the relative frequency of cultured DCs infected with $HIV\text{-}1_{Lai}$ appeared to be quite similar to that of cultured $CD4^+$ T cells and M Mϕs and much higher than that of resting $CD4^+$ T cells. In contrast, cultured DCs, like $CD4^+$ T cells, were much less susceptible to $HIV\text{-}1_{Bal}$ infection. Thus, the lack of productive $HIV\text{-}1_{Lai}$ infection of DCs seemed not to be due to a markedly reduced efficiency of reverse transcription.

TABLE 1. Cell type-dependent infection frequencies of $HIV\text{-}1_{Lai}$ and $HIV\text{-}1_{Bal}$[a]

Virus strain	Cultured DCs	Cultured $CD4^+$ T cells	Resting $CD4^+$ T cells	M-CSF macrophages	CEM cells
$HIV\text{-}1_{Lai}$	1/1,400	1/420	<1/20,000	1/2,200	1/300
$HIV\text{-}1_{Bal}$	<1/20,000	<1/20,000	ND[b]	1/100	<1/20,000

[a] For the preparation of resting $CD4^+$ T cells, fresh, nonadherent peripheral blood mononuclear cells were passed through a nylon wool column and kept frozen until use. They were thawed and enriched for $CD4^+$ T cells just as for cultured $CD4^+$ T cells (see the legend to Fig. 1). Highly purified cultured primary cells, resting $CD4^+$ T cells, and T cells from the CEM line were infected with either $HIV\text{-}1_{Lai}$ or $HIV\text{-}1_{Bal}$ for 2 h. Cells were washed extensively and cultured for a further 12 h. They were treated with 10 μg of DNase I per ml for 30 min and subsequently lysed with 20 μl of lysis buffer per 10^5 cells. These cell lysates were serially diluted, and 10 samples at each dilution were amplified with JAM 62-JAM 65 and JAM 63-JAM 64 primers by nested PCR (see the legend to Fig. 1). On the basis of the statistical distribution of PCR-negative samples, the infection frequencies of these cell types were determined (2).

[b] ND, not done.

For analysis of the relative expression of HIV mRNA in cultured DC and $CD4^+$ T cells, RNA from infected cells was isolated, reverse transcribed, and then serially diluted and amplified by PCR. HIV mRNA was detectable in a cDNA sample corresponding to only 10 cultured $CD4^+$ T cells, whereas 500 cultured DCs were necessary to yield a PCR signal (Fig. 2). In comparison, the housekeeping gene, elongation factor 1α, was transcribed in both cell types at similar levels. Thus, despite sim-

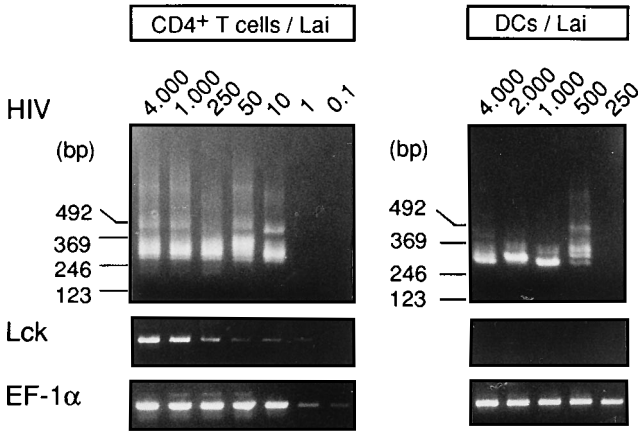

FIG. 2. Cultured DCs express a low level of multiply spliced HIV-1 mRNA. Twenty-four hours after the infection of cultured $CD4^+$ T cells and DCs with HIV-1, total RNA was isolated and cDNA was synthesized, with murine leukemia virus reverse transcriptase (Gibco BRL) and random hexamers being used as primers. These cDNAs were serially diluted and amplified by PCR with primer pairs detecting multiply spliced HIV mRNAs (outer primers, JAM 62 and JAM 69 *env*, 5′-TGGATCTGTCTCTGTCTCTCTCTCC-3′; and inner primers, JAM 63 and JAM 68 *env*, 5′-TCCACCTTCTTCTTCTATTCCTTCG-3′). In order to exclude T-cell contamination and to control for efficient cDNA synthesis, we amplified the same diluted samples with primers specific for $p56^{lck}$ (5′-GAG AACTGCCATTATCCCATAG-3′ and 5′-ATGTTTCACCACCTCTCCTCG-3′) and elongation factor 1α (EF-1α) (5′-CATCCAGGCCAAATAAGCGCC-3′ and 5′-GAAGGTTTTACGATGCATTG-3′). As a length marker for gel electrophoresis, the 123-bp ladder (Gibco BRL) was used.

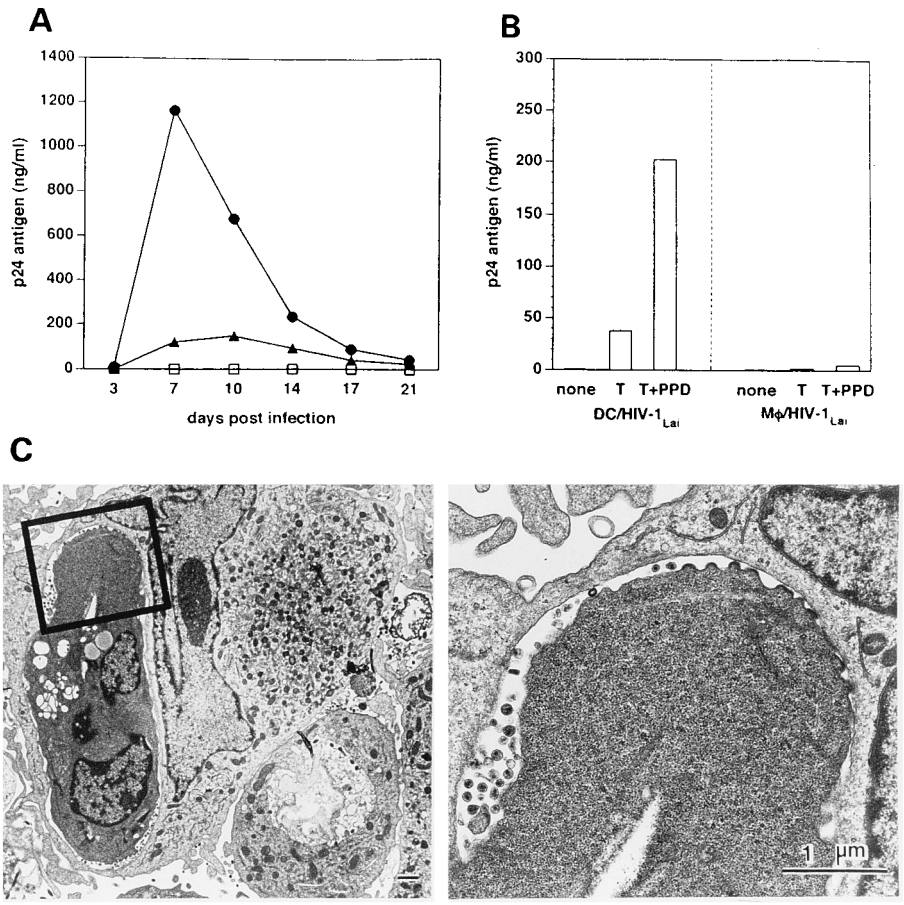

FIG. 3. Nominal PPD antigen triggers productive HIV-1 infection of T cells from HIV-1_{Lai}-infected cultured DCs. (A) HIV production was enhanced by the addition of PPD to a coculture of HIV-infected DCs and resting T cells. HIV-infected DCs (2×10^5) were cultured alone (□——□) or were cocultured with autologous resting $CD4^+$ T cells (2×10^6) in the presence (●——●) or absence (▲——▲) of 50 μg of PPD per ml. In order to support the viability of DCs during the cultivation, we added GM-CSF and IL-4 to the RPMI 1640 medium. Culture supernatant was collected on days 7, 10, 14, 17, and 21, and the amount of HIV p24 antigen in the supernatant was measured by an enzyme-linked immunosorbent assay (ELISA) that used a combination of two anti-*gag*-p24 MAbs, Nu24 and peroxidase-labeled 10B5 (kindly provided by T. Kobayashi and T. Sata). The standard deviation for triplicate measurements at each time point did not exceed 5% of the mean. (B) DCs and M Mϕs were infected with HIV-1_{Lai} and then cultured alone or cocultured with resting $CD4^+$ T cells in the absence or presence of PPD. The p24 antigen was measured by ELISA as described in the legend to panel A. (C) Activated T cells are the main producers of HIV-1 particles in the PPD-pulsed DC–T-cell clusters. Infected DCs were cocultured with T cells in the presence of PPD as described in the legend to panel A. Ultrathin sections from these cocultured cells at day 7 were prepared for study by electron microscopy. A T cell engulfed in a DC (5,000 ×) is shown on the left. At a higher magnification (right), the budding of numerous HIV particles from the cytoplasmic membrane of the T cell was observed (19,200 ×). Bars = 1 μm.

ilar infection frequencies (Table 1) and similar transcriptional activities for elongation factor 1α, the level of HIV mRNA in DCs was less than 1/10 of that in cultured $CD4^+$ T cells. This might indicate that cellular factors necessary for efficient transcription of HIV-specific RNA could be limiting in these cultured DCs.

In spite of their low levels, all of the main species of regulatory (*nef*, *tat*, and *rev*) and structural (*gag*, *pol*, and *env*) HIV mRNAs were transcribed in DCs (data not shown). To exclude minor T-cell and monocyte contaminations in the DC population sorted with the $FACS_{vantage}$, we amplified mRNAs for T-cell-specific $p56^{lck}$ and monocyte-specific CD14 by PCR with specific primers. *lck* and CD14 messages were detectable in a diluted cDNA sample corresponding to a single $CD4^+$ T cell (Fig. 2) or a single monocyte (data not shown), respectively. Because both were absent in the cDNA sample corresponding to 4,000 DCs, the HIV mRNA detected in DCs could not be due to T-cell or monocyte contamination.

DCs in the periphery migrate to the draining lymph nodes (3, 11, 12). If the latently HIV-infected DCs transmit virus to T cells in contact with them in the process of antigen presentation, such a DC–T-cell interaction would be an important step for the massive propagation of virus in lymph nodes, as previously observed in vivo (6, 20). With this assumption in mind, we studied how HIV-infected cultured DCs contributed to virus dissemination in vitro in response to a nominal purified protein derivative antigen (PPD). Cultured DCs were infected with HIV-1_{Lai} and cocultured with autologous resting $CD4^+$ T cells in the presence or absence of PPD. In order to maintain the DCs, we added IL-4 and GM-CSF to these cultures. As shown in Fig. 3A, HIV p24 antigen was barely detectable in the culture supernatant of HIV-1_{Lai}-infected DCs alone. The p24 was produced at a substantial level when HIV-1_{Lai}-infected DCs were cocultured with T cells, and its level increased markedly when PPD was added to the coculture. Treatment of DCs with zidovudine during the process of HIV infection inhibited subsequent virus production by 64 to 98%, depending on the experiment. These results suggest that virus transmission by HIV-infected DCs to T cells was derived not merely from the virus retained on the cell surface (4) but also from those produced by DCs.

Significantly enhanced p24 production was also observed in the coculture deprived of IL-4 and GM-CSF (Fig. 3B, left),

although the maximum level of p24 production was two to three times lower than that in the presence of these cytokines. Under the same conditions, virus production was barely detectable in the coculture with HIV-1_{Lai}-infected M Mϕs and autologous T cells. This was true even in the presence of PPD (Fig. 3B, right), despite the similar frequencies of HIV-1_{Lai}-infected DCs and M Mϕs (Table 1).

In order to identify the cells that contribute most to virus production, we examined cocultured cells by electron microscopy on the day when p24 levels were maximum. Numerous budding virions were observed only on large, activated T cells closely associated with or engulfed by DCs and not on any free lymphoid cells (Fig. 3C). Some viral particles were also detected along the outer membrane of DCs or in indented vesicles in the cytoplasm. These findings suggest that the activated T cells are dominant producers of HIV.

HIV-infected Mϕs have been considered to be important as a virus reservoir (26), and the role of Mϕ-tropic HIV in HIV pathogenesis was recently emphasized (18). With respect to antigen-dependent HIV transmission to T cells in vitro, however, infected DCs were much more potent than infected Mϕs and therefore could play a dominant role in HIV dissemination during normal immune responses. This might simply be reflected by their efficient antigen-presenting activity.

During antigen presentation by DCs, a variety of adhesion molecules are required for full T-cell activation. Major histocompatibility complex class II and CD4 are required for T-cell activation, and CD2, LFA-1, ICAM-1, and LFA-3 are required for both T-cell clustering and T-cell activation (9). In our coculture system, antibodies against major histocompatibility complex class II, CD4, ICAM-1, and LFA-3 suppressed HIV production (unpublished observation), raising the possibility that the DC–T-cell interaction during antigen presentation is an important step in HIV activation and transmission (21).

DC precursors in the bone marrow emigrate to local tissues, where they differentiate further into DCs in a variety of maturation stages, probably depending on the cytokines present locally. We speculate that the cultured DCs used in this study may represent one such population that is derived from the precursor of Langerhans cells. Latently HIV-infected DCs that were activated by antigen exposure or during acute inflammation (15) may potentiate HIV production in vivo by transmitting virus to T cells that are in contact with them. Utilization of cultured DCs can shed light on a further understanding of the mechanism of activation and transmission of HIV during antigen-dependent immune responses.

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