NOTES

Induction of Anti-Human Immunodeficiency Virus Type 1 (HIV-1) CD8\(^+\) and CD4\(^+\) T-Cell Reactivity by Dendritic Cells Loaded with HIV-1 X4-Infected Apoptotic Cells

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Received 11 July 2001/Accepted 29 November 2001

T-cell responses to X4 strains of human immunodeficiency virus type 1 (HIV-1) are considered important in controlling progression of HIV-1 infection. We investigated the ability of dendritic cells (DC) and various forms of HIV-1 X4 antigen to induce anti-HIV-1 T-cell responses in autologous peripheral blood mononuclear cells from HIV-1-infected persons. Immature DC loaded with HIV-1 IIIB-infected, autologous, apoptotic CD8\(^-\) cells and matured with CD40 ligand induced gamma interferon production in autologous CD8\(^+\) and CD4\(^+\) T cells. In contrast, mature DC loaded with HIV-1 IIIB-infected, necrotic cells or directly infected with cell-free HIV-1 IIIB were poorly immunogenic. Thus, HIV-1-infected cells undergoing apoptosis serve as a rich source of X4 antigen for CD8\(^+\) and CD4\(^+\) T cells by DC. This may be an important mechanism of HIV-1 immunogenicity and provides a strategy for immunotherapy of HIV-1-infected patients on combination antiretroviral therapy.

Progression of human immunodeficiency virus type 1 (HIV-1) infection is related to a switch in predominance of macrophage-tropic strains that use the CCR5 coreceptor (termed R5 virus) to T-cell-tropic strains that use CXCR4 as their major coreceptor (termed X4 virus) (7, 27, 44). Failure of CD8\(^-\) and CD4\(^+\) T-cell responses to control HIV-1 infection may be a significant factor leading to unimpeded replication of X4 virus and the development of AIDS (48). Although the recent advent of combination antiretroviral therapy has resulted in dramatic improvements in control of HIV-1 replication in persons chronically infected with HIV-1 (18, 36), it does not completely restore anti-HIV-1 T-cell responses (12, 26, 35–38). Low levels of residual virus remain in such persons and increase when drug therapy is discontinued (13). Thus, therapeutic approaches are needed that enhance T-cell immunity to HIV-1 for more complete control of HIV-1 infection.

Dendritic cells (DC) are the most potent antigen-presenting cells for the induction of antiviral T-cell responses through their expression of high levels of major histocompatibility complex (MHC) class I and II molecules and costimulatory molecules, such as CD40, CD80, and CD86, and the production of immunomodulating cytokines such as interleukin-12 (IL-12) and IL-15 (6). Current evidence indicates that immature DC (iDC) are highly efficient at capturing and processing antigens (6). Subsequent maturation of the iDC by ligation of CD40 with MHC class I and II molecules and costimulatory molecules, greatly enhancing the presentation of antigen to T cells by these mature DC (mDC) (47). In the classic endogenous pathway, proteins produced during viral replication in the antigen-presenting cells are proteolytically cleaved in the cytosol (34). The resulting peptides are transported to the endoplasmic reticulum, where they complex with MHC class I molecules and then travel through the Golgi to the cell surface. In the exogenous pathway, viral proteins are ingested from the extracellular space into endosomal vesicles. There, the proteins are digested and the viral peptides are complexed with MHC class II molecules before transport to the cell membrane.

Some viruses do not replicate efficiently in DC, suggesting that there are alternative mechanisms to the conventional, endogenous MHC class I pathway for the induction of CD8\(^+\) T-cell responses to these viral antigens (29, 50). This has been related to uptake by DC of exogenous antigen in the form of virus-infected, apoptotic, or necrotic cells, followed by processing through nonconventional pathways and cross-presentation of antigen in the context of MHC class I molecules to CD8\(^+\) T cells (2, 3, 20, 21, 29, 45, 46). In HIV-1 infection, iDC do not support efficient replication of X4 strains due to low expression of the CXCR4 coreceptor, whereas they express higher levels of CCR5 and more efficiently support R5 virus replication (11, 17, 29). Thus, induction of anti-HIV-1 CD8\(^+\) T-cell responses to X4 virus may at least in part be due to uptake of X4 antigens by iDC and cross-presentation by HLA class I molecules on mDC. These viral antigens could be derived from cells that have been productively infected by X4 strains and have undergone apoptosis (4). A similar process of uptake of exogenous,
TABLE 1. IFN-γ response of PBMC stimulated with autologous DC loaded with HIV-1 antigen

<table>
<thead>
<tr>
<th>Subject</th>
<th>Duration (yr)</th>
<th>HIV positive</th>
<th>CD4 (cells/μl)</th>
<th>Plasma HIV RNA (copies/ml)</th>
<th>Therapya</th>
<th>p24 antigen (ng/ml) in CD8+ cells</th>
<th>SFC/10⁶ PBMC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HIV positive</td>
<td></td>
<td></td>
<td>Without IIIB</td>
<td>With IIIB</td>
<td>iDC</td>
</tr>
<tr>
<td>HIV positive</td>
<td></td>
<td>S1</td>
<td>&gt;13.5</td>
<td>594</td>
<td>&lt;50</td>
<td>L, Z, I</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S2</td>
<td>&gt;5.8</td>
<td>555</td>
<td>&lt;50</td>
<td>L, Z, N</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S3</td>
<td>&gt;9.3</td>
<td>655</td>
<td>69</td>
<td>L, Z, N</td>
<td>540</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S4</td>
<td>5.6</td>
<td>522</td>
<td>&lt;50</td>
<td>L, Z, I</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S5</td>
<td>&gt;5.0</td>
<td>1,215</td>
<td>30,157</td>
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<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S6</td>
<td>&gt;14.5</td>
<td>588</td>
<td>&lt;50</td>
<td>L, D, I</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S7</td>
<td>17.5</td>
<td>1,081</td>
<td>306</td>
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<tr>
<td></td>
<td></td>
<td>S8</td>
<td>&gt;12.9</td>
<td>601</td>
<td>&lt;50</td>
<td>d, D, E</td>
<td>0.1</td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td></td>
<td>&gt;11.1</td>
<td>597</td>
<td>58</td>
<td></td>
<td>12</td>
</tr>
</tbody>
</table>

HIV-1 negative

<table>
<thead>
<tr>
<th>Subject</th>
<th>Duration (yr)</th>
<th>HIV positive</th>
<th>CD4 (cells/μl)</th>
<th>Plasma HIV RNA (copies/ml)</th>
<th>Therapya</th>
<th>p24 antigen (ng/ml) in CD8+ cells</th>
<th>SFC/10⁶ PBMC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N1</td>
<td>&lt;0.01</td>
<td>7.5</td>
<td>65</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N2</td>
<td>&lt;0.01</td>
<td>11</td>
<td>345</td>
<td>215</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N3</td>
<td>&lt;0.01</td>
<td>5.5</td>
<td>0</td>
<td>0</td>
<td>150</td>
</tr>
<tr>
<td>Medianf</td>
<td></td>
<td></td>
<td>0</td>
<td>8</td>
<td>65</td>
<td>80</td>
<td>25</td>
</tr>
</tbody>
</table>

* L, lamivudine; Z, zidovudine; I, indinavir; N, nelﬁnavir; D, stavudine; R, ritonavir; d, didanosine; E, efavirenz.

a ND, not done.

b For calculation of the median for viral load, <50 copies/ml were considered as 25 copies/ml.

c Analyzed by Wilcoxon signed rank test for the HIV-1-positive group: iDC versus mDCAP, P = 0.022; iDC versus iDC+AP-IIIB, P = 0.035; iDC versus mDCAP, P = 0.014.

d Analyzed by Wilcoxon signed rank test for the HIV-1-negative group: iDC versus mDCAP, ns; iDC+AP versus mDC+AP, P = ns.

e Analyzed by Wilcoxon signed rank test for the HIV-1-positive group: iDC+AP versus mDC+AP-IIIB, P = 0.014.

f Analyzed by Wilcoxon signed rank test for the HIV-1-negative group: for all data comparisons, P = ns.

The table presents data on the IFN-γ response of peripheral blood mononuclear cells (PBMC) from subjects infected with HIV-1, as measured by CD8+ T-cell counts and plasma HIV RNA levels. The subjects were treated with combination antiretroviral therapy for varying durations, and the response to HIV-1 antigen stimulation by autologous dendritic cells (DC) was assessed. The data show a significant reduction in IFN-γ production in the presence of HIV-1 antigen-loaded DCs, indicating a potential role in the regulation of CD8+ T-cell responses in HIV-1 infection.

For each subject, the table provides the duration of HIV-1 infection, CD4+ T-cell counts, plasma HIV RNA levels, and the therapy regimen. The IFN-γ response was measured in both uninfected (IIIB) and infected (AP) DCs, with or without addition of the HIV-1 antigen p24. The data are reported as SFC/10⁶ PBMC, indicating the number of specific functional cells.

The table highlights the impact of HIV-1 infection on CD8+ T-cell responses, with reduced IFN-γ production observed in the presence of infected DCs compared to uninfected DCs. This suggests a potential role for DCs in regulating T-cell responses in HIV-1 infection.
and UV light irradiation (312-nm wavelength calibrated to provide 0.86 mJ/cm²/s for 6 min; Derma Control, Fisher Scientific, Pittsburgh, Pa.) (referred to here as psoralen and UV irradiation [PUV] treatment). The cells were washed three times and cultured for an additional 6 h to allow time for apoptosis. Apoptosis was confirmed by flow cytometry (EPICS XL; Beckman Coulter, Fullerton, Calif.) by using the TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling) assay (In Situ Cell Death Detection Kit; Beckman Coulter, Fullerton, Calif.) by using the TUNEL assay (term deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling) assay (In Situ Cell Death Detection Kit; Beckman Coulter, Fullerton, Calif.) by using the TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling) assay (In Situ Cell Death Detection Kit; Beckman Coulter, Fullerton, Calif.) by using the TUNEL assay.

CD8+ IIIB-superinfected (NE-IIIB) and nonsuperinfected (NE) preparations increased to 70% after PUV treatment (termed AP and AP-IIIB, respectively). Necrotic preparations of HIV-1 IIIB-infected and uninfected, CD8+ cells were made by three or four rapid freeze-thaw cycles of the cells in dry ice-ethanol and 37°C water baths.

DC were derived from autologous CD14+ cells that were enriched by positive selection with anti-CD14 MAb-coated immunomagnetic beads (MACS Isolation Kit; Miltenyi Biotec, Auburn, Calif.). The purity of CD14+ cells was >95%, based on staining with MAb to CD14 (BD) and analysis by flow cytometry. The CD14+ cells were cultured in 1,000 U of granulocyte-monocyte colony-stimulating factor/ml and 1,000 U of IL-4 (Schering-Plough, Kenilworth, N.J.)/ml in complete RPMI 1640 medium for 4 to 5 days to obtain iDC as previously described (14). To induce maturation, the iDC were treated with 1.0 to 2.5 μg of trimeric CD40L (Immunex, Seattle, Wash.)/ml at day 4 or 5 for two additional days at 37°C in the cytokine-supplemented medium.

To determine uptake of the apoptotic and necrotic cell preparations by iDC or mDC, HIV-1 IIIB-infected and uninfected, apoptotic and necrotic CD8+ cells were stained with membrane fluorophore PKH 26 as per the manufacturer’s instructions (Sigma). They were then cocultured for 12 h at 37°C at a ratio of 2:1 with iDC or mDC that were stained with HLA-DR–fluorescein isothiocyanate (FITC). Flow cytometric analysis was performed by gating on large cells with high side scatter, and double-positive cells were enumerated. As shown in Fig. 1A, the uptake of both the CD8+ apoptotic and necrotic cell preparations by iDC was much greater than by mDC (38% versus 6% for apoptotic cells and 23% versus 0.2% for necrotic cells). Furthermore, 30 to 50% of the iDC contained intracellular CD8+ cells and cell debris as visualized by confocal laser scanning microscopy (Leica Lasertechnik, Heidelberg, Germany) (Fig. 1B). Therefore, for the T-cell stimulation experiments, the apoptotic and necrotic CD8+ cell preparations were added to iDC at a 2-to-1 ratio (cell equivalent portions were used for the necrotic cell preparations) on day 4 or 5, incubated for 12 h, and then treated with either medium (to maintain iDC) or CD40 ligand (CD40L) (to induce maturation) for 2 additional days before use in the T-cell studies. Additionally, iDC were infected on day 4 or 5 with 300 ng of p24 per 10⁶ cells of replication-competent, cell-free HIV-1 IIIB or PUV-treated HIV-1 IIIB (prepared by PUV treatment as with the CD8+ cells and three sequential washes in medium for 1 h each at 22,000 × g at 4°C) for 2 h at 37°C and washed twice with medium to remove unadsorbed virus. These HIV-1 IIIB-infected iDC were then cultured with medium alone or CD40L, as stated above, for the T-cell activation experiments.

Previous studies have shown increased expression of maturation markers on DC after treatment with either apoptotic or necrotic cells (15, 24, 40), which could relate to changes in the processing and presentation of antigen. Therefore, the expression of relevant surface markers was assessed on iDC and mDC for possible effects of the antigen preparations on DC maturation. The iDC and mDC were analyzed by staining with

![Image](http://jvi.asm.org/Downloaded_from)
phycoerythrin (PE)-Cy5-conjugated anti-HLA-DR MAb (Immunotech, Marseille, France) and PE-conjugated MAb to lineage markers CD3, CD14, CD16, CD19, and CD56 (BD). The cells were assayed by flow cytometry by gating on large cells with high side-scatter and analysis on a two-color histogram. Cells that were HLA-DR+ and CD3+, CD14+, CD16+, CD19+, and CD56− were defined as DC. These DC were further analyzed after staining with PE-conjugated MAb specific for CD80 (BD), CD83 (Immunotech), and HLA-ABC (BD) and FITC-conjugated MAb to CD86 (Ancell, Bayport, Minn.). Appropriate isotype-matched controls were used throughout the course of the study. The expression of the cell surface markers was assessed as the mean fluorescence intensity (MFI) and the percent positive cells.

The addition of CD40L to DC of three uninfected and three HIV-1-infected persons resulted in a similar pattern of surface marker expression, so the results were combined as shown in Fig. 2. The data show that there were significant increases in surface marker expression on iDC, i.e., HLA DR, CD80, and CD86, after loading with some of the antigen preparations (Fig. 2). Interestingly, all of these increases were noted with the iDC+AP-IIIB preparations. However, iDC+AP preparations only increased the expression of CD86, and iDC+NE only increased the expression of HLA-DR and CD86. These changes were found in DC from both HIV-1-infected and uninfected subjects (data not shown). Thus, the modifications in surface marker expression were not dependent on HIV-1 infection in the antigen preparations or the study subjects.

The CD40L-treated mDC from HIV-1-infected and uninfected persons had increases in surface expression of HLA-DR, HLA-ABC, CD80, CD86, and CD83 compared to iDC (Fig. 2), thus confirming our previous work (14) and that of other investigators (33). Furthermore, there was an increase in the number of mDC compared to iDC expressing CD80 (mean ± standard error: 56% ± 6% to 96% ± 1%, P = 0.002; paired T test), CD86 (41% ± 11% to 98% ± 1%, P = 0.003), and CD83 (8% ± 3% to 66% ± 15%, P = 0.01). The numbers of HLA-DR and HLA ABC positive cells were high in both iDC and mDC (95 to 99%), as is characteristic of these cells (6).

We next investigated the capacity of DC loaded with the apoptotic, necrotic, and cell-free X4 virus preparations to induce IFN-γ production in autologous T cells. An enzyme-linked immunospot assay was used to assess the production of IFN-γ from single, antigen-specific T cells, as described elsewhere (14). A concentration of 10^4 antigen-loaded iDC or mDC was added to 10^5 autologous PBMC (i.e., a 1:10 ratio) and incubated overnight at 37°C. The results were expressed as the number of spot-forming cells (SFC) per 10^6 PBMC. This assay had an average, within-sample variation of 12% among replicates of >100 PBMC samples tested in our laboratory.

In initial experiments with autologous PBMC from three HIV-1-infected subjects, we found that either CD8+ cells, PBMC, iDC, or mDC did not produce appreciable IFN-γ (0 to 5 SFC/10^6 cells; data not shown). The iDC, or iDC loaded with the various antigens, induced low levels of IFN-γ in PBMC (Fig. 3). However, PBMC produced high levels of IFN-γ when stimulated by mDC loaded with HIV-1IIIB-superinfected,
autologous, apoptotic CD8\(^+\) cell preparations (mDC+AP-IIIB) compared to stimulation with mDC+AP (Fig. 3). This effect was dependent on the presence of the mDC, since only 0 to 1 SFC/10\(^6\) cells was detected when the CD8\(^+\) cell preparations were added to autologous PBMC without mDC. The IFN-\(\gamma\) response was also dependent on the concentration of the mDC+AP-IIIB added to the PBMC cultures, since ratios of 1:5 and 1:20 of DC to responder PBMC resulted in proportionally higher and lower numbers of IFN-\(\gamma\)-producing cells, respectively, than the standard 1:10 ratio (data not shown). In contrast to the effects of the apoptotic cell preparations, mDC loaded with either of the necrotic cell preparations (mDC+NE or mDC+NE-IIIB), or infected with cell-free, viable HIV-1 IIIB or PUV-treated HIV-1 IIIB (data not shown) did not induce appreciable IFN-\(\gamma\) production in the PBMC. Thus, mDC were superior to iDC at inducing IFN-\(\gamma\) production in autologous PBMC. Moreover, HIV-1 IIIB-superinfected apoptotic cells were more potent immunogens in these mDC than were HIV-1 IIIB-superinfected necrotic cell preparations or cell-free HIV-1 IIIB.

To determine whether the T-cell responses were mediated by HLA class I- and class II-restricted, CD8\(^+\) and CD4\(^+\) T cells, highly enriched autologous CD8\(^+\) and CD4\(^+\) T cells were obtained by negative selection with immunomagnetic beads specific for either CD4 or CD8 and CD19 and CD16 (Dynal, Lake Success, N.Y.) (15). Both CD8\(^+\) and CD4\(^+\) T cells produced IFN-\(\gamma\) in response to the mDC+AP-IIIB (Fig. 4A). In further experiments, the addition of either anti-HLA class I MAb or anti-HLA class II MAb to the DC-antigen preparations (14) inhibited induction of IFN-\(\gamma\) production by autologous mDC+AP-IIIB; the addition of both of these MAb completely prevented induction of IFN-\(\gamma\) in the PBMC (Fig. 4B). Therefore, mDC+AP-IIIB induced IFN-\(\gamma\) production in autologous CD8\(^+\) and CD4\(^+\) T cells that was HLA class I and class II restricted, respectively.

We next expanded these studies by examining the ability of autologous, HIV-1-infected, apoptotic cell preparations to induce IFN-\(\gamma\) in PBMC cultures from eight HIV-1-infected persons. The individual and composite data confirmed our findings that the greatest number of IFN-\(\gamma\)-producing cells was induced by mDC+AP-IIIB (Table 1). The highest levels of IFN-\(\gamma\) were stimulated by mDC+AP-IIIB in PBMC cultures from seven of the eight HIV-1-infected persons compared to mDC+AP. HIV-1-specific T-cell responses were not induced by HIV-1 antigen-loaded iDC or mDC preparations in PBMC from three HIV-1-negative subjects.

The one HIV-1-infected subject (S2) who did not have greater numbers of IFN-\(\gamma\)-producing cells induced by the mDC+AP-IIIB had the highest response to mDC+AP. This same pattern of T-cell response to the apoptotic cell preparations was confirmed in PBMC obtained 6 months later from this subject (data not shown).

Interestingly, HIV-1 p24 was detected in all of the AP preparations, although the levels were lower than in the AP-IIIB preparations (Table 1). This represents endogenous HIV-1 from naturally infected cells of the study subjects and could have resulted in stimulation of a portion of the IFN-\(\gamma\) by the mDC+AP preparations. However, induction of IFN-\(\gamma\) in the PBMC cultures was not significantly different when mDC+AP were used for antigen presentation compared to mDC alone.

We next determined whether the T-cell response to the apoptotic cell preparation of X4 virus was consistent over time. We found that there were comparable levels of IFN-\(\gamma\)-producing cells induced by autologous mDC+AP-IIIB over a >2-year period in one subject (S5) (Fig. 5). Very low levels of IFN-\(\gamma\) were induced by mDC alone or mDC+AP. Persistent production of IFN-\(\gamma\) by PBMC stimulated with mDC+AP-IIIB was confirmed at two separate time points 6 months apart in subject S3 (data not shown).

The data presented in Table 1 show that the level IFN-\(\gamma\) production was not related to the amount of HIV-1 p24 in the AP or AP-IIIB antigen preparations loaded in the DC. Further studies indicated that very low levels of p24 were maintained from the baseline level through 60 h of culture of iDC or mDC.
that had been loaded with HIV-1 IIIB-infected, PUV-treated, apoptotic CD8\(^+\) cell preparations (Table 2). These low levels of p24 were similar to those in concurrent cultures of the HIV-1 IIIB-infected, PUV-treated, apoptotic CD8\(^+\) cell preparations without the DC. These results show that PUV treatment inactivates HIV-1 in the apoptotic CD8\(^+\) cell preparations, with a resultant lack of replication of HIV-1 in DC loaded with them. However, both iDC and mDC loaded with non-PUV-treated, HIV-1 IIIB-infected CD8\(^+\) cells supported very high levels of replication of HIV-1 (Table 2). In contrast, iDC or mDC alone did not support efficient HIV-1 replication after infection with cell-free X4 virus (data not shown).

These results indicate that HIV-1-infected cells can serve as a source of antigen for efficient induction of HIV-1-specific CD8\(^+\) and CD4\(^+\) T cells by mDC from persons chronically infected with HIV-1 who are receiving combination antiretroviral therapy. This immunogenic property was promoted by driving the infected cells into apoptosis by PUV treatment in vitro. Other investigators have reported activation of T-cell responses specific for influenza A virus (2), human cytomegalovirus (3), Epstein-Barr virus (31, 45), and cancer antigens (20, 22, 23, 25, 42) by DC loaded with antigen-expressing apoptotic cells. In our system, induction of programmed cell death may have led to enhanced recognition and uptake of the apoptotic cells by iDC (1). Cells infected with HIV-1 naturally undergo extensive apoptosis after more prolonged infection than that used in these studies (4). This could thus provide a rich source of HIV-1 structural and nonstructural antigens for processing by iDC and presentation by mDC to CD8\(^+\) and CD4\(^+\) T cells under conditions of natural infection. Additionally, residual HIV-1-infected live cells in the AP-IIIB preparations could be a source of antigen. In support of this, Harshyne et al. (19) have recently reported that living cells can transfer antigen to DC for activation of CD8\(^+\) T cells. Further studies are needed to compare the antigenicity of HIV-1-infected apoptotic and viable cells in DC.

We found that HIV-1-infected necrotic cells were not a potent T-cell immunogen compared to HIV-1-infected apoptotic cells. This is in agreement with the findings of Albert et al. (2), who demonstrated that activation of influenza A virus-specific CD8\(^+\) T cells by mDC loaded with influenza A virus-infected apoptotic monocytes was superior to that induced by mDC containing virus-infected necrotic cell preparations. Moreover, the influenza virus antigen from the apoptotic cell preparations was processed through a nonclassical, alternative HLA class I pathway by the DC. The efficiency of presentation of antigens of HIV-1 and other viruses may reflect differences in the mechanisms of processing and presentation of apoptotic and necrotic cell preparations by DC. These are likely based on differences in viral proteins, such as the predominance of glycine-alanine repeats that can act on proteosomes (8), and differential activity of cellular components such as chaperone molecules that regulate antigen trafficking (29).

There was little or no HIV-1 replication demonstrable in either the iDC or the mDC that had been loaded with the X4 virus-infected, PUV-treated apoptotic CD8\(^+\) cells. The mDC loaded with this form of inactivated HIV-1 were, however, highly immunogenic for anti-HIV-1 CD8\(^+\) T cells. This suggests that the major processing of HIV-1 proteins in these DC for presentation to CD8\(^+\) T cells was not through a classic HLA class I endogenous pathway. We hypothesize that, after the ingestion of HIV-1-infected apoptotic cells by the iDC, HIV-1 proteins in the apoptotic cells are processed through an alternative HLA class I pathway for cross-presentation by mDC to HIV-1-specific CD8\(^+\) T cells. HIV-1 peptides processed by the infected CD8\(^+\) cells may also serve as a source of antigen for the mDC. Additional studies are required to address directly these hypotheses, including inhibitors of various steps in HIV-1 replication and processing of viral proteins by DC.

The iDC or mDC infected with replication-competent or PUV-treated cell-free HIV-1 IIIB did not activate an HIV-1-specific T-cell response in our study. Buseyne et al. (10) re-

### TABLE 2. HIV-1 p24 levels in iDC and mDC loaded with untreated and PUV-treated, HIV-1 IIIB-infected CD8\(^+\) autologous cells

<table>
<thead>
<tr>
<th>Cell culture(^a)</th>
<th>Median HIV-1 p24 level in ng/ml (range)(^b) at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>CD8(^+) IIIB</td>
<td>0.12 (0.09–0.48)</td>
</tr>
<tr>
<td>PUV CD8(^+) IIIB</td>
<td>0.18 (0.17–0.56)</td>
</tr>
<tr>
<td>CD8(^+) IIIB + iDC</td>
<td>0.10 (0.10–0.45)</td>
</tr>
<tr>
<td>PUV CD8(^+) IIIB + iDC</td>
<td>0.19 (0.04–0.19)</td>
</tr>
<tr>
<td>CD8(^+) IIIB + mDC</td>
<td>0.10 (0.10–0.45)</td>
</tr>
<tr>
<td>PUV CD8(^+) IIIB + mDC</td>
<td>0.19 (0.04–0.19)</td>
</tr>
</tbody>
</table>

\(^a\) CD8\(^+\) cells and DC were derived from PBMC of three normal, HIV-1-negative donors (N5, N6, and N7) and cultured for the designated times.

\(^b\) CD8\(^+\) IIIB = HIV-1 IIIB-infected, autologous CD8\(^+\) cells; PUV CD8\(^+\) = PUV-treated, HIV-1 IIIB-infected, autologous CD8\(^+\) cells.
cently reported that iDC that had been infected with X4 virus could stimulate HIV-1-specific CD8+ T cells in vitro. This required fusion and cytotoxic processing of the virus by the iDC and was not related to endogenous virus replication in these cells. However, antigen presentation in that study was determined by stimulation of HIV-1 peptide-specific CD8+ T-cell lines and clones by the virus-fused DC. Such cell lines and clones should have a much greater proportion of HIV-1-specific CD8+ T cells than the PBMC used in our work. Their T-cell model may therefore detect lower amounts of HIV-1 peptide expressed in HLA class I molecules on the DC. Collectively, our studies suggest that DC have nonclassical, alternative mechanisms for cross-presentation of X4 viral antigens to CD8+ T cells.

Of importance is that maturation of DC induced by treatment with CD40L after loading of the iDC with the HIV-1 antigen preparations was essential for the efficient induction of anti-HIV-1 T-cell responses. This is likely related to enhanced expression of cell surface molecules CD80 and CD86 that are required for activation of T cells (6). CD40L also induces cytokines such as IL-12 (32) and IL-15 (28) that upregulate and prolong survival of antigen-specific T cells. In this regard, Ostrowski et al. (33) reported that CD40L treatment of HIV-1 peptide-treated DC enhances their activation of HIV-1-specific cytotoxic T lymphocytes in vitro, which is associated with production of IL-15 by the DC. However, in terms of the natural HIV-1 infection, these immunity-enhancing effects of CD40L on DC should be viewed in the context of potential pathogenic effects due to upregulation of HIV-1 X4 replication in cocultures of CD40L-treated mDC and CD4+ T cells (30). Indeed, we found high levels of virus replication in both iDC and mDC cultures loaded with non-PUV-treated, HIV-1 IIIB-infected CD8+ cells. Thus, there may be competing events of uptake and presentation of HIV-1 X4-infected cells by mDC to T cells, with replication and cytopathic effects of this virus.

We (37) and others (26, 35, 36) have previously shown that CD8+ T-cell reactivity to HIV-1 is only temporarily increased in patients with long-term, chronic HIV-1 infection receiving combination antiretroviral drug therapy, even though the levels of circulating HIV-1 are greatly diminished. Additionally, there is usually poor recovery of anti-HIV-1 CD4+ T-cell activity in these persons (9, 12, 36, 38, 49). There do appear, however, to be residual T cells that are HIV-1 specific. This has been shown by reactivity of CD8+ T cells to HIV-1 during treatment interruptions (16, 39, 41) and by in vitro stimulation with DC loaded with HIV-1 peptides (33) or proteins complexed with liposome (14). The present study indicates that HIV-1-specific CD8+ T cells, as well as CD4+ T cells, can be readily activated to produce IFN-γ in vitro by a single round of stimulation with DC that have been loaded with autologous, HIV-1-infected apoptotic cells and matured with CD40L. This approach may be useful as a therapeutic vaccine to enhance T-cell immunity to a person’s own endogenous HIV-1 by autologous DC that have been loaded with apoptotic cells that contain strains of HIV-1 that are unique to the individual.

This work was supported in part by grants R01 AI41870, U01 AI37984, and U01 AI35041 from the National Institutes of Health. We thank K. Picha of Immunex for assistance in providing the CD40L-S. Narula of Schering-Plough (Kenilworth, N.J.) for providing hIL-4 and hGM-CSF; W. Buchanan and B. Calhoun for clinical assistance; R. Day, S. Baillie-Buyes, and L. Harshyne for technical advice; S. Alber, M. White, D. Dampny, H. Li, C. Perfetti, C. Kalinyak, W. Jiang, M. Mather, and P. Zhang for technical assistance; and Malenka for administrative assistance. We give special thanks to the volunteers of the Pitt Men’s Study–MACS, whose dedication made this study possible.

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