Prim ing of Human Immunodeficiency Virus Type 1 (HIV-1)–Specific CD8+ T Cell Responses by Dendritic Cells Loaded with HIV-1 Proteins

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Proteins may serve as ideal CD8+ T cell immunogens for human immunodeficiency virus type 1 (HIV-1) if they can be delivered to and processed through the human leukocyte antigen class I pathway. This study shows that human blood monocyte–derived dendritic cells loaded with liposome-complexed HIV-1 proteins and matured with CD40 ligand can prime CD8+ T cells to HIV-1 in vitro. Whole HIV-1 protein in liposome may be an effective immunogen for HIV-1 vaccine protocols.

Generation of antigen-specific, primary CD8+ cytotoxic T lymphocyte (CTL) responses represents a challenge to the development of protein-based vaccines for prevention of human immunodeficiency virus type 1 (HIV-1) infection. The conventional mechanism for antigenic processing of extracellular proteins is through an endocytic pathway for presentation by major histocompatibility complex (MHC) class II molecules to CD4+ T cells [1]. One potential strategy to induce vigorous MHC class I–restricted CTL responses with viral proteins involves specific targeting to the cytosol of antigen-presenting cells (APCs), particularly dendritic cells (DCs), with carriers such as liposome for accessing alternative MHC class I antigen-processing pathways (termed “cross-presentation”) [2]. We have previously demonstrated that lipofectin, a cationic liposome, provides a means of delivering exogenous proteins in vitro to human DCs for more efficient induction of HIV-1 specific, memory CD8+ CTL responses than protein alone [3]. The present study investigated whether DCs loaded with protein-liposome complexes can prime anti–HIV-1 CD8+ T cell responses.

Materials and methods. To obtain immature DCs (iDCs), CD14+ monocytes were positive selected from peripheral blood mononuclear cells (PBMC) of healthy donors using anti-CD14 monoclonal antibody (MAb)–coated magnetic microbeads (Miltenyi) and cultured in AIM V medium (GIBCO) containing 1000 U/mL of recombinant interleukin (IL)–4 and 1000 U/mL recombinant granulocyte-macrophage colony stimulating factor (GM-CSF; Schering-Plough) [4]. The number of viable DCs was determined by typical DC morphology in trypan blue dye–stained preparations. The purity of the DCs was >90% on the basis of the expression of HLA-DR molecules and lack of expression of lineage markers by flow cytometry [4]. The maturation state of the DCs was determined by expression of CD80, CD83, and CD86.

The toxicity of lipofectin, composed of DOTMA (N-[1-(2,3-dioleoyloxy) propyl]–N, N, N-trimethylammonium chloride)/DOPE (dioleoyloxyphosphatidylethanolamine) (GIBCO), was determined by loading iDCs with graded doses at 37°C for 0, 2, 4, and 8 h. The viability of the DCs by trypan blue dye exclusion was >90% when lipofectin was added at 15 µg/mL for up to 8 h, and decreased at higher concentrations of liposome. Therefore, 15 µg/mL lipofectin was used in this study.

The vaccinia virus (VV) constructs contained gag p24 or p55 for BH10 and HxB2 strains of HIV-1 (VV-Gag p24 and VV-Gag p55; Therion Biologics). VV-Env was vPE11 containing the env coding region of HIV-1 BH10 minus the signal sequence (B. Moss, National Institutes of Health [NIH], Bethesda, MD). The NYCBH strain of VV (VV-Vac; Therion) was used as control virus. Synthetic peptides representing previously identified HLA class I–restricted Gag and Env epitopes were prepared by the University of Pittsburgh Peptide Synthesis Facility.

For preparation of APCs, 15 µg/mL lipofectin was mixed with 20 µg/mL HIV-1 p24 Gag or gp160 Env (IIIB; Protein
marker, CD83, was up-regulated on CD40L-treated DCs as compared to non-CD40L-treated DCs, indicating that the CD40L treatment induced DC maturation [4, 5]. Loading of iDCs or mDCs with either liposome, p24 Gag, or their combination did not alter expression of these surface molecules (data not shown).

We next established the parameters for in vitro priming by confirming the work of Zarling et al. [6], which used 1 week of priming of PBMC with HIV-1 Gag peptide-loaded, MCM-matured DCs, followed by weekly, sequential stimulations with Gag peptide-loaded PBMC to induce anti-Gag CD8+ CTL responses (data not shown). Priming with peptide-loaded iDCs did not induce CTL reactivity. In subsequent experiments, we used CD40L-matured DCs, because this treatment resulted in a more consistent maturation of the DCs [4].

Stimulation of PBMC from an HIV-1–negative subject with autologous mDCs that had been loaded with HIV-1 Gag p55 protein-liposome induced greater levels of anti–HIV-1 CD8+ T cell responses than mDCs loaded with HIV-1 Gag p55 protein alone (figure 1A). Similar priming was elicited by PUV-treated mDCs that had been loaded with Gag p55-liposome (figure 1B). The lytic reactivity was HLA class I restricted as shown by blocking of lysis by anti–HLA class I but not anti–HLA class II MAb (figure 1B). To date, we have observed that CTLs specific for Gag or Env can be consistently primed by autologous mDCs loaded with these HIV-1 recombinant protein-liposome complexes in multiple PBMC samples obtained from 7 different subjects (data not shown).

The HLA class I specificity and breadth of this primary CD8+ T cell response was further demonstrated by killing of autologous targets expressing 3 of 4 HLA A*0201 and HLA B*27 HIV-1 peptides (figure 1A). We confirmed the specificity of CD8+ T cells primed by mDC loaded with either Gag p24 or p55 and liposome for p24, p51-139 (TLNATWKKV) in 5 of 6 HLA A*0201 subjects. In further experiments, priming of CTLs from another HLA A*0201 person with mDCs loaded with gp160-liposome induced CTLs specific for HLA A*0201 epitopes gp120,311-320 (RGPGRAFVTI) and gp41,147-255 (RLVNGSLAL), but not gp120,312-319 (KLTSWTMK), gp41,184-187 (RLNATDIAV) (data not shown). Similarly, CTLs from an HLA A*03 B*07 person that were primed by mDCs loaded with Gag p24 and Env gp160-liposome complexes were specific for HLA B*07 p24,179-187 (ATPQLDNS) and HLA A*03 gp41,273-285 (RLRLDDLIVTR) epitopes, but not for the HLA B*07 p24,180-186 (SPRTNWV) epitope (data not shown).

We showed that Gag p55-liposome loaded mDCs more efficiently primed HIV-1–specific CD8+ CTLs than did monocytes or PBMC for recognition of targets expressing 4 different Gag epitopes (figure 1A). Furthermore, mDCs loaded with Gag p55-liposome were superior to iDCs for priming of CTL specific for targets expressing naturally processed Gag proteins (i.e.,
Figure 1. Priming of human immunodeficiency virus type 1 (HIV-1)–specific CD8+ cytotoxic T lymphocyte (CTL) responses by different antigen-presenting cells (APCs). Priming of HIV-1–specific CTL in peripheral blood mononuclear cells (PBMC) from a HLA A*0201, B*27 subject by 1 week of stimulation with autologous mature dendritic cells (mDCs; either untreated or psoralen UV [PUV] treated), monocytes, or PBMC loaded with Gag p55–liposome, followed by 4 sequential weekly stimulations with autologous PBMC loaded with the same antigen (A). Results are the mean of triplicate values of antigen-specific lysis. The results show positive CTL responses (≥10% specific lysis) to autologous B cell lines loaded with known CD8+ T cell epitopes for HLA A*0201 p24151–159 (TLNAWKVV), and B*27 p24263–272 (KRWIILGLNK) and p1719–27 (IRLRPGGKK), but not for HLA A*0201 p1777–85 (SLYNTVATL). The CTL activity was blocked by anti-HLA class I monoclonal antibody (MAb) but not anti-HLA class II MAb (B). E/T, effector/target cell ratio.

VV-p24 and VV-p55), as well as targets loaded with a known HLA A*0201 p24 epitope (p24151–159) (figure 2).

Discussion. We were consistently able to prime anti–HIV-1 CD8+ T cell responses with mDCs loaded with HIV-1 protein-liposome complexes but not with mDCs loaded with HIV-1 proteins alone. In vitro priming of CD8+ T cells to HIV-1 was first demonstrated by Engleman et al. [7, 8] who showed that multiple, sequential stimulations of naive CD8+ T cells with autologous or allogeneic, HLA class I–matched DCs freshly isolated from blood samples and loaded with HIV-1 Gag and Env peptides, could activate HIV-1–specific CTL. More recently, monocyte-derived iDCs transduced with an env- and nef-deleted HIV-1 vector pseudotyped by VSV G protein [9], HIV gag-encoded mRNA-transfected DCs [10], or iDCs that were matured with MCM or CD40L and loaded with HIV-1 peptides representing HLA class I epitopes [6, 11], have been used to prime anti–HIV-1 CTL in vitro. There is only one report of in vitro priming of CD8+ T cells with whole HIV-1 protein, where Nef-specific CD8+ T cells were primed by repetitive stimulation of PBMC with γ-irradiated iDCs loaded with HIV-1 Nef protein [12]. In this case, optimal T cell priming required addition of interferon (IFN)–α.

We found that priming of anti–HIV-1 CD8+ T cell responses in vitro required liposome as a delivery vehicle. We believe that this was due, in part, to the unique fusogenic property of the DOPE portion of lipofectin that enhances access of exogenous protein from the endosomes/lysosomes into the cytosol of DCs by destabilizing the endosomes and allowing the release of HIV-1 protein into the cytosol [13]. Moreover, a single stimulation of PBMC for one week with HIV-1 protein-liposome loaded mDCs, followed by multiple, weekly stimulations with protein-liposome loaded PBMC, was sufficient to induce a primary, anti–HIV-1 CD8+ T cell response. Initial stimulation with antigen-loaded mDCs was essential for priming, as iDCs, monocytes or PBMC loaded with liposome-complexed HIV-1 protein were poor primers of HIV-1 specific T cells. The mDCs expressed enhanced levels of costimulatory and HLA class II molecules, and these were not altered by addition of liposome-protein complexes. Maturation of DCs with CD40L increases surface expression of HLA and costimulatory molecules and
release of cytokines including IL-12 p70 (when combined with IFN-α stimulation) and IL-15 from mDCs that enhance antigen-specific CD8+ T cell responses [14]. Such costimulatory CD40/CD40L interactions lower the threshold of antigen required to activate primary T cell responses, and may augment survival of antigen-activated T cells.

Our data, that PUV-treated mDCs could prime anti–HIV-1 CD8+ T cell responses, are concordant with our previous findings among HIV-1–infected persons [4]. The PUV-treated DCs are likely undergoing apoptosis, which suggest that viable DCs are not essential for triggering of T cell priming. This may relate to the high levels of costimulatory molecule expression that is retained on PUV-treated DCs (X.-L.H., Z.F., and C.R.R., unpublished data).

Priming of CD8+ T cells with mDCs loaded with HIV-1 proteins and liposome elicited CTL reactivity against multiple HIV-1 HLA class I epitopes. The levels of lysis were within the range expected for CTL primed by a multi-epitope protein immunogen, using single epitope, individual peptides as targets. Lack of recognition of some HIV-1 peptides by the HIV-1 protein-primed T cells could reflect immunodominance among the peptides for their HLA class I alleles, differences in proteasomal cleavage and heterogeneity of peptide binding due to polymorphism of HLA class I alleles [2].

We reported elsewhere activation of HIV-1 specific, CD8+ T cells from HIV-1–infected persons in vitro by cross-presentation of liposome-complexed HIV-1 proteins by DCs [3]. These studies suggest that complexing of HIV-1 protein with liposome promotes processing of the protein through an alternative, cytosolic pathway for cross-presentation of peptides by HLA class I molecules to both naive and memory CD8+ T cells. Our studies, together with a recent report showing in vitro priming of Gag-specific CD4+ T cells by mDCs loaded with uncomplexed HIV-1 p24 protein [15], suggest that a protein-based antigen approach could be used to prime or boost both anti–HIV-1 CD8+ and CD4+ T cell activity in prophylactic and therapeutic vaccine protocols.

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References

In an article in the 15 January issue of the *Journal* (Huang X-L, Fan Z, Zheng L, et al. Priming of human immunodeficiency virus type 1 (HIV-1)–specific CD8$^+$ T cell responses by dendritic cells loaded with HIV-1 proteins. J Infect Dis 2003; 187:315–9), the beginning of the second line of the left-hand column on page 318 should read as “IFN-$\gamma$” [not “IFN-$\alpha$”]. The publisher regrets this error.