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Induction of Primary Human CD8+ T Lymphocyte Responses In Vitro Using Dendritic Cells

Angela L. Zarling, Julia G. Johnson, Robert W. Hoffman, and David R. Lee

The ability of two different human professional APCs, specifically macrophages (Mφ) and dendritic cells (DC), to stimulate primary responses in human CD8+ T lymphocytes was examined using both allogeneic and Ag-pulsed autologous APCs. CTL responses in CD8+ T lymphocytes isolated from HIV-uninfected donors were evaluated against six different HIV epitopes that are restricted by four different HLA alleles using autologous human PBMC-derived Mφ and DCs for primary stimulation. In a side-by-side experiment, immature DCs, but not Mφ, were able to prime a CTL response against the B14-restricted p248–298 epitope; mature DCs were also able to prime a response against this epitope. In addition, DCs were capable of priming CD8+ CTL responses against the B8-restricted p246–259–267 epitope. In contrast, Mφ were unable to prime strong CTL responses against other epitopes. Since the Ag-specific cytotoxic responses required subsequent rounds of restimulation before they could be detected, the ability of the allogeneic Mφ and DCs to directly prime CD8+ T lymphocyte responses without subsequent restimulation was examined. Similar to the aforementioned peptide-specific results, DCs were more efficient than Mφ in priming both allogeneic proliferative and cytotoxic responses in human CD8+ T lymphocytes. Collectively, these results promote an enhanced status for DCs in the primary stimulation of human CD8+ T lymphocytes. The Journal of Immunology, 1999, 162: 5197–5204.

Several studies have investigated the use of different populations of APCs for the stimulation of Ag-specific CD8+ T lymphocyte responses in vitro. Wentworth et al. (1) reported that human PBMCs activated with Staphylococcus aureus Cowan I and rabbit anti-human IgM beads in the presence of IL-4 can serve as efficient APCs for the stimulation of peptide-specific, primary CD8+ CTL responses; however, the APCs in this report were not extensively characterized in terms of the type of cell(s) and the surface expression of costimulatory molecules. Gagliardi et al. (2) demonstrated that cultured monocytes were able to prime CD8+ CTL responses against HIV epitopes. More recently, several laboratories (3–6) have demonstrated that dendritic cells (DC) have the capacity to prime human CD8+ T lymphocyte responses against viral and tumor antigenic peptides. In addition, allogeneic DCs have been reported to efficiently prime purified human CD8+ T lymphocyte responses (7, 8).

Despite these recent reports, little is known about the relative abilities of macrophages (Mφ), and both immature DCs (iDCs) and mature DCs (mDCs), to prime human CD8+ T lymphocyte responses. Human DCs have been cultured from several sources, including bone marrow, cord blood, and peripheral blood, using several different culture conditions. One laboratory has cultured CD34+ hematopoietic progenitors from human cord blood in the presence of human GM-CSF and TNF-α to generate DCs (9). Others have cultured adherent PBMCs in GM-CSF and IL-4 to generate iDCs, followed by the addition of monocyte-conditioned medium to generate mDCs (10–12). The PBMC-derived iDCs and mDCs have been suggested to be analogous to tissue-derived DCs and the more differentiated DCs found within T cell areas in secondary lymphoid organs, respectively (13). Many of the studies investigating the ability of human DCs to stimulate human T lymphocyte responses have utilized DCs from different origins, at different levels of maturity, and of different purity. The resulting populations of cells can be very heterogeneous. In this report, we have taken advantage of the general availability of human peripheral blood and the ability to generate PBMC-derived Mφ, iDCs, and mDCs from the same donor to compare the ability of each APC population to prime both Ag-specific and allogeneic human CD8+ T lymphocyte responses.

Materials and Methods

Cell lines and mAbs

The following EBV-transformed lymphoblastoid cell lines (LCLs) were used: H9B (HLA-A11,26; -B60; and -Cw1,w3), M8B (HLA-A2,11; and -B27,62 (Bw56)), 9014 (HLA-A26; -B8; and -Cw7), 9055 (HLA-A3; and -B14), 9065 (HLA-A3; -B7; and -Cw7), and 9068 (HLA-A2; -B35; and -Cw4). The latter four LCLs were obtained from the collection of the American Society for Histocompatibility and Immunogenetics. All cell lines were cultured in RPMI 1640 supplemented with 10% FCS, l-glutamine, and antibiotics.

The pan HLA class I-specific mAb W6/32 was obtained from American Type Culture Collection (Manassas, VA). Anti-CD3 (OKT3), anti-CD4 (OKT4), and anti-CD8 (OKT8) mAbs were generously provided by Dr. Marc Jenkins (University of Minnesota, Minneapolis, MN), and anti-CD83
mAb (HB-15) was kindly provided by Dr. Thomas Tedder (Duke University, Durham, NC). mAbs specific for CD80 (BB1) and CD86 (BU63) were obtained from Serotec (Raleigh, NC), and mAbs specific for CD1a (M-T102 and HL149) and CD14 (M-S130) were obtained from PharMingen (San Diego, CA).

Synthetic peptides

Peptides were synthesized using standard fluorenylmethylchlorocarbonyl chemistry on an Applied Biosystems (Foster City, CA) 432A peptide synthesizer. Six HIV and three influenza virus peptides that have been reported as HLA class I-restricted CTL epitopes were used in these studies and are listed in Table I. Their purity was assessed by reverse-phase HPLC and determined to be approximately 90%. Peptides were dissolved in DMSO (20 mg/ml) and stored at −20°C.

Binding of peptides to HLA class I molecules

A semiquantitative assay to determine the relative binding affinities of the various peptides for the relevant HLA class I molecules has been established. LCLs expressing the relevant or irrelevant HLA class I allele were acid treated, as has been previously described (1), to denature the HLA class I molecule. Various peptides for the relevant HLA class I molecules has been established. LCLs expressing the relevant or irrelevant HLA class I allele were acid treated, as has been previously described (1), to denature the HLA class I molecule. Preparations of APCs

PBMCs were purified via Histopaque (Sigma, St. Louis, MO) gradient centrifugation from blood obtained from normal healthy volunteer donors who had been previously typed for HLA class I expression (One Lambda, Canoga Park, CA). DCs were selectively expanded from this population of total PBMC based upon the protocol originally described by Bende et al. (10) and subsequently modified by Romani et al. (11, 12). Briefly, T cell-depleted, adherent PBMCs were cultured in complete medium supplemented with 1000 U/ml recombinant human GM-CSF (R&D Systems, Minneapolis, MN) or Sargramostim (pharmaceutical grade of recombinant human GM-CSF; Immunex, Seattle, WA) and 20 ng/ml recombinant human IL-4 (Genzyme, Cambridge, MA; R&D Systems); iDCs were then harvested on day 7. Alternatively, the iDCs were transferred on day 7 to new six-well plates. This was done without washing the cells in fresh medium, thereby maintaining the cytokine milieu while at the same time leaving behind any contaminating macrophages. The DC cultures were maintained for an additional 3–4 days in the presence of 25–30% monocyte-conditioned medium, which was reported to promote a terminal differentiation phase to mDCs (11, 12). Monocyte-conditioned medium was prepared as described by Romani et al. (11), except that T cells were depleted with either anti-CD2- or anti-CD3-coated magnetic beads (Dynal, Lake Success, NY).

Adherent PBMCs (2 h) were used as Mφ. These cells were sometimes sustained in culture for up to seven days in the presence of recombinant human GM-CSF or Sargramostim (100 U/ml) in six-well plates. The adherent cells were removed from plates via incubation for several hours to overnight at 4°C. Macrophages purified in this manner are essentially all viable. They are large, granular cells expressing the phenotype CD14+ CD80+ CD86+ class I bright.

Induction of peptide-specific CTLs

A population of CD8+ “enriched” lymphocytes were purified from PBMCs. Briefly, nonadherent lymphocytes were enriched for CD8+ T cells by depleting CD4+ T cells with anti-CD4-coated magnetic beads (Dynal). At this point, ~95% of the CD3+ cells remaining were CD4− CD8+. However, only about 40% of the negatively selected cells were CD3+, as B cells and NK cells also remained. In all of the allogeneic stimulation experiments, B cells were depleted using a BSA-panning technique (21).

APCs were harvested and pulsed with 50 μg/ml peptide in serum-free AIM-V medium supplemented with 3 μg/ml human β2m (10× CD8+ APC/ml) in polypropylene tubes for 2–4 h. They were then treated with 100 μg/ml mitomycin C, washed, and plated in 24-well plates (2 × 103 pulsed APCs/well) in CTL medium (RPMI 1640 plus 10% AB serum [Pel-Freeze Clinical Systems, Brown Deer, WI; Sigma], 25 mM HEPES, 50 mM KCl, 25 mM HEPES, 10 mM Hepes, and 0.5% BSA, pH 7.4) for 5 days, with restimulation every 3–4 days. Those wells responding to antigenic stimuli were determined by tetramer staining of isolated T cells.
pH 8.0, 2 mM glutamine, 0.5 mM sodium pyruvate, and penicillin-streptomycin). CD8-enriched responder cells (1 × 10^6) were added to the CTL cultures for a final volume of 1.5 ml CTL medium. In some experiments, 10 ng/ml IL-7 (R&D Systems) was also added to the cultures to encourage the growth of CTLs. On day 2, 10 U/ml recombinant human IL-2 (R&D Systems) was added.

The CTLs were harvested and restimulated every 7–11 days with either 2 × 10^6 autologous, mitomycin C-treated, peptide-pulsed Mφ (first restimulation) or 1 × 10^6 autologous, mitomycin C-treated, peptide-pulsed PBMCs (later restimulations). IL-2 (10 U/ml) was added 2 days after each restimulation. When proliferating cells in the cultures became too dense, 0.5 ml of medium in each well was exchanged for fresh medium.

Cytotoxicity

The cytotoxic activity of the peptide-primed cultures was assessed 5–7 days following restimulation by a standard ^51^Cr release assay. LCL expressing the appropriate HLA class I molecule were labeled by incubating 3 × 10^6 target cells in 200 µCi sodium ^[^51^Cr]chromate for 1–2 h at 37°C and washing three times. The labeled LCL target cells were dispersed into wells of 96-well U-bottom plates (1 × 10^5/well in CTL medium). A 20-fold excess of unlabeled K562 cells was also added to each well to reduce nonspecific lysis by NK cells. CTLs were added such that each E:T ratio was tested in triplicate. A final volume of 200 µl/well was used. In some studies, the target cells were pulsed overnight with peptide (50 µg/ml peptide in AIM-V medium) before labeling. In other experiments, peptide was added to the cultures at the time the cytotoxicity assay was set up. In each assay, targets either pulsed with an irrelevant peptide or not pulsed with peptide were also used as an indication of nonspecific CTL lysis. The plates were incubated at 37°C for 4 h, and supernatant fluids were analyzed for ^51^Cr release.

Control wells for determining spontaneous ^51^Cr release contained labeled target cells only. Maximal release was determined by adding Triton X-100 (1% final) to the target cells. The percentage specific lysis was calculated as follows: ([(mean cpm release in experimental sample) − (mean cpm of spontaneous release)] / (mean cpm of maximal release) − (mean cpm of spontaneous release)) × 100.

Allogeneic stimulation of proliferative and cytotoxic responses in CD8^+^ T lymphocytes

CD8^+^-enriched T lymphocytes (1.5 × 10^5) per well of a 96-well round bottom plate) were incubated for 3–5 days with varying numbers of allogeneic APCs in a final volume of 200 µl CTL medium. For some experiments, 15 U/ml (final concentration) of recombinant human IL-2 (R&D Systems) was added at day 2. [methyl-^3^H]Thymidine (1 µCi/well; ICN Pharmaceuticals, Irvine, CA) was added 18 h before harvest. Cells were harvested with an automatic cell harvester, and the incorporation of [^3^H]Thymidine into the cells was quantified using a liquid scintillation counter.

For primary allogeneic CTL cultures, 1.5 × 10^5 CD8^+^ T cells were cultured with 1.5 × 10^5 allogeneic APCs per well of a 24-well plate for 6 days in a final volume of 2 ml. Recombinant human IL-2 (10 U/ml) was added on day 2. Generation of cytotoxic activity was tested using ^51^Cr-labeled LCLs expressing HLA class I alleles matching those of the APC donors. Specific lysis was determined as described above. LCLs expressing irrelevant HLA class I alleles (not shared with the donor of the APCs) were included as nonspecific controls.

Results

Characterization of Mφ and DCs isolated from human PBMCs

In this study, adherent PBMCs cultured either in the presence or absence of GM-CSF are designated as Mφ. Loosely adherent T cell-depleted PBMCs cultured for 7 days in the presence of both GM-CSF and IL-4 are designated as iDCs, while iDCs that have been cultured for an additional 4 days in the presence of monocyte-conditioned medium are designated as mDCs. In the representative results shown in Fig. 1, Mφ, iDCs, and mDCs were isolated from donor 015, and the expression of cell surface markers on these cells was determined by flow cytometry. The Mφ expressed high surface levels of CD14, whereas the mDCs expressed heterogeneous levels of CD14. In some experiments using different donors, lower levels of CD14 were observed on both the iDCs and mDCs (data not shown). Surface expression of CD83 was never observed on our Mφ, occasionally observed on the iDCs, and more consistently observed on the mDCs. Generally, CD14 has been reported to be expressed on Mφ and not on DCs, whereas CD83 is thought to be exclusively expressed on DCs (10, 11, 22). Thus, the results shown in Fig. 1 suggest that our iDCs and mDCs cultures contain contaminating Mφ. In contrast, analysis of our Mφ cultures suggested that they contained either minimal or no contamination with DCs. This interpretation is also supported by analysis of surface expression of CD1a molecules, which were found on cells in our DC cultures but not in our Mφ cultures (data not shown). Other investigators have used the surface expression of CD1 molecules along with CD14 to distinguish Mφ and DCs (12, 13, 23).

The results shown in Fig. 1 also indicate that the costimulatory molecule B7.1 (CD80) was undetectable on Mφ but expressed at comparatively high levels on both iDCs and mDCs; the level of expression was usually higher on mDCs. In contrast, B7.2 (CD86) was detected on all three populations, though higher levels were observed on mDCs (Fig. 1).
Binding of HIV CTL epitopes to their respective HLA class I restriction elements

To test the ability of human Mϕ and DCs to prime Ag-specific primary responses, six different HIV CTL epitopes that are restricted to four different HLA class I alleles as well as three different influenza virus CTL epitopes restricted to three different HLA class I alleles were synthesized (Table I). Our donors were presumed to have never been exposed to HIV but to have been previously infected with influenza virus. Some of our donors are health care workers who are screened annually for exposure to HIV; all of these donors were seronegative. All of the peptides were tested for their ability to bind to LCLs that either expressed or did not express the restricting HLA class I allele, using a previously described surface stabilization assay (24–26). The semiquantitative results for the binding of all of the peptides to their respective HLA class I restriction elements are summarized in Table I. The peptides were unable to increase class I expression on HLA class I alleles but lacked expression of the HLA class I restriction element (data not shown), indicating that the binding of these peptides to their respective HLA class I restriction element is specific.

Examination of the ability of Mϕ and DCs to prime Ag-specific human CD8\(^+\) CTL responses in vitro

To compare the ability of Mϕ and DCs to prime CD8\(^+\) CTL responses, CD8\(^+\) T lymphocytes from donor 006 (HLA-B14\(^+\), HIV-seronegative) were primed with either autologous Mϕ or iDCs that had been pulsed with the B14-restricted HIV CTL epitope, p24\(^{298 \text{–} 306}\). The two primed CD8\(^+\) T lymphocyte populations were restimulated twice with peptide-pulsed autologous PBL blasts and then tested for their cytotoxic activity on peptide-pulsed or unpulsed B14\(^+\) LCL targets. As shown in Fig. 2A, cytotoxic activity was not detected in the CD8\(^+\) T lymphocytes primed with peptide-pulsed autologous Mϕ, whereas, in Fig. 2B, those that had been primed with peptide-pulsed autologous iDCs developed significant epitope-specific CTL responses. In a separate experiment from that discussed above, donor 006 (HLA-B14\(^+\), HIV-seronegative) CD8\(^+\) T lymphocytes were primed with autologous mDCs pulsed with the B14-restricted HIV p24\(^{298 \text{–} 306}\) epitope, then restimulated as indicated and tested for cytotoxicity (Fig. 2C). Although nonspecific cytotoxic activity was observed on the unpulsed B14\(^+\) LCL (9055) targets, significantly higher levels of lysis were observed for the p24\(^{298 \text{–} 306}\) peptide-pulsed targets. Together, the results presented in Fig. 2 suggest that both types of DCs are more efficient at priming Ag-specific CD8\(^+\) T lymphocyte responses than Mϕ.

To further examine the ability of iDCs to prime Ag-specific CD8\(^+\) CTL responses, CD8\(^+\) T lymphocytes from donor 015 (HLA-A3\(^+\), B8\(^+\), HIV-seronegative) were primed with autologous iDCs pulsed with the B8-restricted HIV p24\(^{259 \text{–} 267}\), the B8-restricted HIV RT\(^{pol}\) 18–26, or the A3-restricted HIV p17\(^{259 \text{–} 267}\) epitope. These primed populations were restimulated equivalently with the same APCs (described in the legend for Fig. 3) that had been pulsed with the respective peptides and then tested for epitope-specific cytotoxicity. The B8-restricted p24\(^{259 \text{–} 267}\) peptide in conjunction with autologous iDCs was able to prime epitope-specific CTL responses (Fig. 3A). In contrast, although the same APCs were used for priming and restimulation, the B8-restricted RT\(^{pol}\) 18–26 and A3-restricted p17\(^{259 \text{–} 267}\) 18–26 peptides were unable to prime epitope-specific responses. Although cytotoxic responses were generated with the RT\(^{pol}\) 18–26 peptide, they were not epitope specific (Fig. 3B). Cytotoxic responses were not detected in the cultures stimulated with the p17\(^{259 \text{–} 267}\) 18–26 peptide (Fig. 3C). The detection of significant Ag-specific cytotoxic responses to the p24\(^{259 \text{–} 267}\) 259–267 peptide but not to the other two peptides suggested that the APCs were adequate for CD8\(^+\) T lymphocyte priming and subsequent restimulation but that the latter two epitopes are not as effective as the former at generating Ag-specific cytotoxic responses. Semiquantitative binding studies (Table I) indicated that the inability of certain epitopes to stimulate peptide-specific CD8\(^+\) CTL responses did not necessarily correlate with a lower binding affinity for the presenting HLA class I allele. For example, the nonimmunogenic (in...
our assays) p17\textsuperscript{18–26} peptide binds well to HLA-A3, whereas the immunogenic p24\textsuperscript{298–306} peptide binds comparatively poorly to HLA-B14.

Although not directly compared with the ability of DCs to prime the same responses, the ability of Mϕ to prime CD8\textsuperscript{+} CTL responses against two A2-restricted HIV peptides, gp120 121–129 and RT\textsuperscript{476–484}, was also examined. As shown in Table I, Mϕ were able to prime a weak response (defined in Table I) against the former epitope but could not prime a detectable CTL against the latter epitope. Collectively, the direct comparisons in Fig. 2 and the indirect comparisons summarized in Table I suggest that DCs are superior to Mϕ in the priming of Ag-specific CD8\textsuperscript{+} CTL responses.

In addition, we examined the ability of mDCs to stimulate presumed memory CTL responses against several class I-restricted, influenza virus CTL epitopes. Strong epitope-specific CTL responses were observed 6 days after the initial stimulation with two of three of the epitopes (FLU part of Table I). These data confirm the results observed in the primary cultures that DCs are efficient APCs for Ag-specific CD8\textsuperscript{+} CTL responses.

**Comparison of the ability of DCs and Mϕ to prime allogeneic CD8\textsuperscript{+} T lymphocyte responses in vitro**

To examine the ability of Mϕ and DCs to prime CD8\textsuperscript{+} T lymphocyte responses directly without subsequent restimulation, human CD8\textsuperscript{+} T lymphocytes from donors 016 (HLA-A2,24; -B18,60; and -Cw3,w6) and 019 (HLA-A3,24; and -B7,8) were incubated with either autologous or the other donor’s (allogeneic) Mϕ or mDCs in the presence or absence of IL-2 (after 48 h). Proliferation ([\textsuperscript{3}H]thymidine incorporation) of the CD8\textsuperscript{+} T lymphocytes was measured after 96 h and is shown in Fig. 4, A and B. In both cases, the allogeneic mDCs were considerably more efficient than the allogeneic Mϕ in the generation of CD8\textsuperscript{+} T lymphocyte proliferative responses. In addition, the responses to autologous mDCs or Mϕ were low and similar to the responses of CD8\textsuperscript{+} T lymphocytes incubated in the absence of APCs. The allogeneic responses were predominately a result of HLA class I allelic differences, since the starting CD8\textsuperscript{+} T lymphocyte population contained <5% CD4\textsuperscript{+} T cell contamination. Furthermore, using flow cytometric analysis, we have never observed preferential expansion of the CD4\textsuperscript{+} T lymphocytes in our CD8\textsuperscript{+} T lymphocyte populations after stimulation with allogeneic DCs (data not shown).

To additionally compare the ability of allogeneic Mϕ and iDCs to stimulate CD8\textsuperscript{+} T lymphocyte responses, these two populations of APCs were cultured from donor 003 PBMCs (HLA-A2,3; and -B21) and used to stimulate CD8\textsuperscript{+} T lymphocytes isolated from donor 006 (HLA-A10; -B14,37; and -Cw2,w4). Similar to the previous results, the results shown in Fig. 4C demonstrate that the iDCs were more efficient than the Mϕ isolated from the same donor in stimulating an allogeneic proliferative response in the CD8\textsuperscript{+} T lymphocytes.

To further compare allogeneic stimulation with the previous peptide-specific responses, CD8\textsuperscript{+} T lymphocytes isolated from donor 032 (HLA-A24,32; -B38,44; and -Cw2,w3) were stimulated with mitomycin C-treated Mϕ or DCs cultured from donor 018 (HLA-A1,3; -B8,35; and -Cw4,w7), and cytotoxic responses were measured on an LCL target that expressed either HLA class I alleles in common with the allogeneic-stimulating APCs or irrelevant class I alleles. As shown in Fig. 5, the LCL target 9068, which expresses two HLA class I alleles in common with the allogeneic APCs from donor 018 (HLA-B35 and -Cw4) was lysed by CD8\textsuperscript{+} T lymphocytes stimulated by any of the three types of allogeneic APCs used in this report. However, the allogeneic Mϕ appeared to be less effective than the iDCs or mDCs in stimulating the CD8\textsuperscript{+} CTL responses. This trend was also observed when other LCLs that express HLA class I alleles in common with the allogeneic APCs were cultured from donor 016 (HLA-B18 and -Cw3) was lysed by CD8\textsuperscript{+} T lymphocytes stimulated by any of the three types of allogeneic APCs used in this report. However, the allogeneic Mϕ appeared to be less effective than the iDCs or mDCs in stimulating the CD8\textsuperscript{+} CTL responses.
Discussion

Although different professional APCs have been shown to be capable of stimulating Ag-specific T cell responses, the relative efficiencies of the APCs (on a per cell basis) have rarely been evaluated. Furthermore, most of these studies have focused on previously primed (secondary) T lymphocyte responses. In addition, CD4+ T lymphocyte responses have been studied more extensively than those of CD8+ T lymphocytes, due, in part, to the greater difficulty of delivering intact Ags to the class I as opposed to the class II pathway. Finally, because of the relative inconvenience of obtaining both APC populations and responding T lymphocytes from humans as opposed to mice and other laboratory animals, comparatively less is known about the APC requirements for stimulating human T lymphocytes. However, recent emphasis on vaccine development for human health, particularly for viral infection, mandates examination of the requirements for the effective stimulation of human CD8+ T lymphocyte responses.

A pioneering study by Young and Steinman (7) suggested that human PBMC-derived DCs were superior to Mφ in the stimulation of allogeneic primary CD8+ T lymphocyte proliferative and cytotoxic responses. However, the DCs used in that study were enriched by a different methodology and probably more resemble the iDCs used here. In contrast, the more recent results of Gagliardi et al. (2) and Toujas et al. (27) suggested that Mφ, iDCs, and mDCs, isolated from the same donor to stimulate either Ag-specific or allogeneic primary CD8+ T lymphocyte responses. Thus, the importance of this report lies in its comprehensive comparison of the ability of the three different APC populations, Mφ, iDCs and mDCs, isolated from the same donor to stimulate either Ag-specific or allogeneic primary CD8+ T lymphocyte responses. The data presented here provide strong evidence for the superiority of both types of DCs in this process.

The inability to generate CD8+ CTL responses against some of the HLA class I-restricted HIV CTL epitopes used in this study could be explained in a number of different ways. First, although the HIV CTL epitopes were originally defined in HIV-infected individuals, the ability of some of the epitopes to induce CTL responses during an infection may be due to the chronic course of HIV infections. In other words, large doses and repeated stimulations may be necessary to drive responses against some of the epitopes. Second, the HIV CTL epitopes were defined in HIV-infected individuals, who may differ in the HLA class I allele subtype from the donors being used in our experiments; there is precedent for class I allele subtype differences in the ability to present certain epitopes (28). Finally, the presence of different alleles at other class I loci in our donors could negatively select the appropriate CD8+ T lymphocyte response during T lymphocyte development, creating a “hole in the repertoire.”

The absence of significant numbers of helper CD4+ T lymphocytes in our system is another possible explanation for our inability to stimulate CD8+ CTL responses with certain class I-restricted HIV CTL epitopes. Recent reports (29–31) have suggested that...
CD4+ T lymphocytes provide help for CD8+ T lymphocytes by “conditioning” the APCs. Thus, when CD4+ T lymphocytes are activated through their TCR, they up-regulate CD40 ligand (CD40L) on the surface, which in turn can ligate CD40 on the surface of the APC. This leads to the up-regulation of costimulatory molecules like B7.1 (CD80) and B7.2 (CD86) on the APC (29–31) and its secretion of IL-12 in some situations (32). This “conditioning” process enhances the efficiency of the APC in the stimulation of CD8+ T lymphocytes. However, both the iDCs and the mDCs used in our studies were already found to express high levels of both CD80 and CD86 (Fig. 1). Therefore, in our system, if the inability to respond to certain class I-restricted HIV CTL epitopes was due to the lack of CD4+ T lymphocyte conditioning of the DCs, one would need to invoke the up-regulation of costimulatory molecules other than B7.1 or B7.2 on the DCs.

One problem with the results presented here is the use of heterogeneous populations of iDCs and mDCs. The flow cytometric analyses shown in Fig. 1 suggest that these populations are contaminated with CD14+ cells in our DC cultures. Others have suggested that CD14 can be expressed on some subpopulations of DCs (9, 33). In our use of this protocol to generate DCs, we have observed considerable variability among different donors with regard to the relative purity of the DC populations. Nevertheless, Mφ populations isolated from the same donor PBMCs lack detectable contamination with DCs; these Mφ populations are less stimulatory than our more heterogeneous DC populations. These results agree with an earlier report in which the addition of Mφ to DCs failed to inhibit the latter’s ability to stimulate human allogeneic responses (7). While we cannot completely rule out the possibility in our experiments that the presence of the two populations is required for optimal stimulation, it is abundantly clear that our Mφ populations are less efficient in generating primary CD8+ T lymphocyte responses. The near absence of CD14+ cells in some of our stimulatory DC populations argues that DCs are sufficient for optimal stimulation of CD8+ T lymphocytes.

These studies provide evidence that DCs are more efficient than Mφ in the primary stimulation of human CD8+ T lymphocyte responses. One obvious difference between the two populations of APCs is the expression of high levels of B7.1 on the DCs and the lack of detectable expression of this costimulatory molecule on the Mφ. Others have shown that there is a similar disparate expression of B7.1 and B7.2 on human monocyte-derived macrophages and cell lines (23, 34–36). Furthermore, others have also shown differences in the kinetics and levels of the up-regulation of the two B7 molecules on human macrophages (33, 35). While the results presented here do not directly address the role of B7.1 in the stimulation of human CD8+ T lymphocyte responses, studies in CD28-deficient mice (37) suggest that CD28 signaling is not required for CD8+ T lymphocyte responses against lymphocytic choriomeningitis virus, implicating a role for other costimulatory systems. Thus, these studies provide the foundation for the further dissection of the relative roles of costimulatory molecules like CD80/86 (38, 39), 4-1BB (40–42), OX40L (43), LFA-3 (44), or combinations thereof in the primary stimulation of human CD8+ T lymphocytes.

This system provides an ideal method for testing the immunogenicity of synthetic class I-restricted epitopes in vitro. Furthermore, these studies provide support for the ex vivo use of DCs pulsed with class I-restricted CTL epitopes to enhance CD8+ CTL responses against tumors in patients (45–47). The current clinical trials using ex vivo DCs pulsed with tumor Ags were based on similar studies in animal models (48–50). In addition, this type of approach has been used to vaccinate mice against challenges with infectious agents like lymphocytic choriomeningitis virus (51) and HSV (52). The results presented here indicate that both iDCs and mDCs are more efficient than Mφ at priming CD8+ T lymphocyte responses, whereas others have shown that DCs are superior for stimulating memory CD8+ T lymphocyte responses. Collectively, the studies provide a strong impetus for selective targeting of Ags to DCs in vaccine approaches in vivo.

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