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Induction of primary anti-HIV CD4 and CD8 T cell responses by dendritic cells transduced with self-inactivating lentiviral vectors

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Abstract

In this study, we demonstrate that a minimal self-inactivating (SIN) lentiviral vector (LV) that does not encode any human immunodeficiency virus (HIV) genes is able to induce autologous HIV-specific CD4 and CD8 T cell responses after transduction of dendritic cells (DCs). The LV-DC-primed T cells displayed HIV-specific lytic degranulation, as illustrated by acquisition of CD107a/b expression on the cell surface and release of perforin. HIV-specific cytotoxic T lymphocyte (CTL) response was consistently detected using different assays, and T cell receptors specific to three prominent HIV epitopes, SL9 (Gag peptide: SLYNTVATL), IV9 (Pol peptide: ILKEPVHGV), and MA10 (In Peptide: MASDFNLPPV) were detected using HLA-A0201 peptide-tetramers. These results demonstrate that DCs transduced with the minimal SIN-LV can efficiently induce HIV-specific CD4 and CD8 T cell responses. Since LVs are popular gene transfer tools, our results have fundamental implications for future LV applications and DC vaccine development.

Keywords

lentiviral vectors; dendritic cells; HIV; vaccine

INTRODUCTION

HIV-based lentiviral vectors (LVs) are powerful gene transfer tools and have potential for clinical applications. The replication-defective LV RNA genomes are assembled within HIV Gag and Pol proteins and pseudotyped with Vesicular Stomatitis Virus G proteins (VSV-G). The early generations of LVs encode some HIV genes. To improve safety, we and others have generated minimal self-inactivating (SIN) LVs with all of the HIV genes and many of the regulatory elements deleted from the vector backbone (1–3).

The SIN-LVs have been used to deliver cytokine, T cell costimulator and siRNA genes to modify DC immunity, and can effectively induce an anti-cancer immunity *in vivo* by over-expressing multiple highly differentiated cancer antigens in DCs (4,5). During LV gene transfer, the HIV Gag and Pol proteins in the vector particles may be processed in DCs through the MHC class II pathway or cross-presented via the MHC class I pathway (6,7). Buseyne et al. have reported that defective HIV particles with fusogenic activity can prime DCs to induce an MHC-I restricted, HIV-specific CD8 CTL response (7). However, evidence of the minimal

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SIN LV capable of inducing both CD4 and CD8 T cell responses against HIV and/or activation of HIV-specific effector functions after DC transduction has yet to be fully characterized.

In this study, we analyzed primary anti-HIV T cell immunity with autologous T cells co-cultured with DCs transduced with a minimal SIN LV encoding a self non-immunogenic thymocyte antigen, ly6e. We report that the DCs transduced with the SIN LV-ly6e are capable of priming primary autologous CD4 and CD8 T cell responses. The LV-DCs elicited a consistent CTL response towards specific HIV Gag, Pol and Int epitopes. Our results have significant implications in the usage of LV in gene transfer and the development of genetically modified DCs and HIV vaccines.

MATERIALS AND METHODS

Peptides

The HIV type 1 Gag overlapping 50 peptides and Pol overlapping 100 peptides were obtained from the AIDS Research and Reference Reagent Program (AIDS RRRP), Division of AIDS, National Institute of Allergy and Infectious Disease, National Institutes of Health. The peptides were given an arbitrary number from 1 to 49 and 1 to 100 following the position in the Gag and Pol sequence, respectively; full sequences are reported in the AIDS RRRP catalog. The lyophilized peptides were re-suspended as indicated by the manufacturers at 1 mg/ml and then stored at -80°C . To prepare the peptide mixture, the 50 Gag peptides were mixed in equal parts to a final concentration of 1 mg/ml in distilled water, and the 100 Pol peptides were mixed in equal parts to a final concentration of 1 mg/ml in PBS. The individual peptides, SL9 (Gag peptide: SLYNTVATL), IV9 (Pol peptide: ILKEPVHGV), MA10 (IN peptide: MASDFNLPPV), and Flu (M1 peptide: GILGFVFTL) were synthesized by the ICBR peptide synthesis facility at University of Florida.

Antibodies, growth factors and recombinant vaccinia viruses

The following anti-human monoclonal antibody reagents were obtained from BD Pharmingen, (San Diego, CA): FITC-labeled anti-IFN- γ , PE-labeled anti-IL-2, APC-labeled anti-TNF- α , PE-labeled anti-CD8, Cy-PE-labeled anti-CD4, purified anti-CD107a and anti-CD107b, FITC-labeled anti-active caspase 3. Recombinant human IL-2 (Ro 23-6019, TECIN), GM-CSF and TNF- α were kindly provided by Dr. Craig Reynolds of The Biological Resources Branch, Developmental Therapeutics Program, National Cancer Institute. Alternatively, recombinant human GM-CSF and IL-4 were purchased Biosource International Inc. (CA) and Research Diagnostics, Inc. (NJ). LPS was purchased from Sigma (MO). Recombinant human IL-12 was purchased from R&D Systems, Inc. and BD Pharmingen. The recombinant vaccinia virus (rVV) expressing HIV Gag (vv-gag) and nlacZ (vv-nlacZ) were obtained from the AIDS Research and Reference Reagent Program (AIDS RRRP), Division of AIDS, National Institute of Allergy and Infectious Disease, National Institutes of Health.

Lentiviral vector construction and production

The SIN lentiviral vector was constructed as described previously, which has a 3' bovine growth hormone polyadenylation signal (bGHpA) inserted behind the 3' truncated long terminal repeat (2,3,8,9). pTYF-ly6e encodes for none of the HIV genes but an irrelevant gene (Ly6e). To produce lentivirus, HEK293 cells were cotransfected with transducing vector pTYF-ly6e, packaging plasmid pHP, and envelope plasmid VSV-G (Fig 1). The supernatants were collected, filtered, concentrated, and stored in aliquots at -80°C .

Dendritic cell preparation

DC were generated as previously described (4,10). Briefly, peripheral blood mononuclear cells (PBMC) were obtained by Ficoll-Paque from HIV-seronegative donors (Civitan, Gainesville, FL). The adherent PBMCs were cultured in the presence of recombinant human GM-CSF (560 u/ml) and IL-4 (25 ng/ml) for 5 days to generate immature DC.

Lentiviral transduction of immature DCs and DC maturation

The day 5 immature DCs were plated at 5×10^5 per well in a 24-well plate containing 200 μ l of medium supplemented with GM-CSF (560 u/ml) and IL-4 (25 ng/ml). As previously described (4), transduction of DCs was carried out by adding concentrated lentiviral vectors to the cells at a multiplicity of infection (MOI) of 50–100. The cells were incubated at 37°C for 2 hr with gentle shaking every 30 min, 1 ml of DC medium was then added and the culture was incubated with the viral vectors for an additional 12 h. DC maturation was induced by adding lipopolysaccharide (LPS) at a final concentration of 80 ng/ml and TNF- α at a final concentration of 20 u/ml to the DC culture for 24 h. The DCs were further matured with IFN- γ (50 ng/ml) for 6 hr to shape the DC maturation to IL-12-producing DCs.

T cell priming

LV-DCs were washed and co-cultured with autologous PBMC at a DC:PBMC ratio of 1:50 in the presence of IL-7 (10 ng/ml), IL-12 (20 ng/ml) and IL-6 (2 ng/ml) for 2 days. The supernatants were then removed and replaced with AIM-V medium containing IL-2 (50 u/ml) and IL-7 (5 ng/ml). After 10–19 days of expansion, the T cells were analyzed for antigen specificity and cytotoxicity.

Quantitation of cytokine-producing T cells

LV-DC-primed T cells were incubated with autologous BLCL pulsed with HIV gag-pol peptides or a mock control for 1 hr at 37°C in a 5% CO₂ incubator, followed by an additional 4–5 hr in the presence of brefeldin A (BD Pharmingen). The cells were then stained with fluorochrome-conjugated antibodies to CD4 and to CD8, and Alexa Fluor 647-labeled anti-CD107a/b antibodies in phosphate-buffered saline (PBS) containing 2.5% FBS and 0.09% NaN₃ (Sigma, St. Louis, Mo.). After washing, the cells were fixed and permeabilized with a Cytotfix/Cytoperm kit (Pharmingen, San Diego, CA) and stained with fluorochrome-conjugated antibodies to IFN- γ , IL-2, and TNF- α . Six-parameter flow cytometric analysis was performed using a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems) and 2×10^6 events were collected for analysis.

Ex vivo cytotoxic T cell assays

The HIV peptide-pulsed and the mock-pulsed autologous BLCL were used as target cells in a direct *ex vivo* cytotoxicity assay. Autologous BLCL were labeled with PKH26 or PKH67. PKH26-labeled cells were either pulsed for 90 min with HIV gag-pol peptides and then washed 2 times in AIM-V, or were mock-pulsed. All cells labeled with PKH67 were mock-pulsed. Ten thousand PKH26-labeled BLCL and PKH67-labeled BLCL were incubated with T cells at different E/T ratios. After 5 hr of incubation at 37°C with 5% CO₂, the cells were analyzed by flow cytometry to evaluate the number of survived HIV-gag-pol-pulsed cells relative to the PKH67-labeled mock-pulsed cells. Paralleled assays with mock-pulsed PKH26-labeled cells served as a control for non-specific cytotoxic effects of PKH26.

Tetramer staining

The MHC-peptide tetramer assay was performed using Strep-PE conjugated HLA-A0201 gag tetramer (SL9: SLYNTVATL), HLA-A0201 pol tetramer (IV9: ILKEPVHGV) and HLA-

A0201 int tetramer (MA10: MASDFNLPPV). The HLA-A0201 flu tetramer (GILGFVFTL) served as a control. The gag, pol, int and flu tetramers were obtained from the NIH Tetramer Facility, AIDS Research and Reference Reagent Program (AIDS RRRP), Division of AIDS, National Institute of Allergy and Infectious Disease, National Institutes of Health. The LV-DC-primed T cells were stained with Strep-PE conjugated tetramer and fluorochrome-conjugated anti-CD8 and anti-CD107a/b Abs. The tetramer positive cells were measured by flow cytometry analysis.

RESULTS

HIV-specific TNF- α and IFN- γ production in T cells co-cultured with LV-DCs

Monocyte-derived immature DCs were generated from HIV-seronegative donors after culturing adherent peripheral blood monocyctic cells for 5 days in the presence of GM-CSF and IL-4. The DCs expressed high levels of CD11c, DC-SIGN and ICAM-1, moderate levels of co-stimulatory molecules, CD80, CD86, CD40, and were negative for CD123 (see ref. (4), and data not shown). LV encoding an HIV-unrelated host self antigen Ly6e, a lymphocyte antigen 6 complex, was constructed using SIN LV as illustrated in Fig 1. The DCs were transduced with LV-Ly6e at 50 MOI and 24 hr later, were treated with TNF- α /LPS and IFN- γ to induce maturation and the production of Th1 cytokine IL-12. The mature LV-DCs were co-cultured with autologous, non-adherent PBMC for 10–19 days and the expanded cells were collected for analysis.

To generate HIV-specific target cells, we pulsed autologous B lymphoblastoid cell lines (BLCL) with HIV Gag and Pol peptides (Gag-Pol, Fig. 2) or with a control (Mock, HCV peptide or the solvent only). The LV-DC-primed T cells were stimulated with the peptide-pulsed BLCL for 6 hr in the presence of brefeldin A. HIV-specific T cell response was examined by intracellular cytokine staining for TNF- α . The generation of a primary, HIV-specific, T cell response was determined by comparing T cells stimulated by Gag-Pol versus control peptides-pulsed BLCL. As illustrated in Fig. 2A, TNF- α was produced in four-fold excess in the HIV peptide-reactive T cells than the control T cells, indicating that DCs transduced with the LV elicited a primary anti-HIV T cell response.

To detect cytokine response in CD4 and CD8 T cells, the T cell subsets were analyzed by four color flow cytometry. Increased production of IFN- γ was detected in both CD4 and CD8 T cells when stimulated with BLCL pulsed with the Gag-Pol peptides but not the control peptides (Fig. 2 C, D and E). These results indicate that the LV-DCs induced an HIV-specific cytokine response in both CD4 and CD8 T cells. The HIV Gag-Pol peptide-pulsed BLCL also stimulated IL-2 production in CD4 but not CD8 T cells, suggesting an HIV-specific T helper response (not shown). To further confirm this, we repeated the above experiment using recombinant vaccinia virus (rVV) expressing HIV Gag (vv-gag) to infect BLCL as re-stimulators. We used nlacZ-encoding rVV (vv-nlacZ) as a control; nlacZ is highly immunogenic to human immune cells (unpublished observation). The result again showed that the vv-gag induced more TNF- α (Fig. 3 A and C) and IFN- γ (Fig. 3 B and D) production than did the control vv-nlacZ in both CD4 and CD8 T cells.

HIV-specific CD107a/b expression and perforin release in LV-DC-primed T cells

CD107a and CD107b are lysosomal-associated membrane glycoproteins that surround the core of the lytic granules in cytotoxic T cells (11). These proteins normally are not expressed on the surface of T cells but are found on the surface of phytohemagglutinin (PHA) activated lymphocytes (12). Upon TCR engagement and stimulation by antigens in association with MHC molecules, CD107a and CD107b are exposed on the cell membrane of cytotoxic T cells (13). Thus, the measurement of CD107a/b expression in cytotoxic T lymphocytes (CTL) is a

direct assay for the antigen-specific CTL response. To see if the HIV-specific cytokine-producing T cells display such effector phenotype, we stimulated the LV-DC-primed T cells with BLCL pulsed with HIV Gag-Pol peptides and did flow cytometry to detect CD107a/b expression. Both CD4 and CD8 T cells showed increased surface CD107a/b expression upon Gag-Pol stimulation (Fig. 4 A and B), which was correlated with an increased IFN- γ response (Fig. 4 C and D). Antigen-stimulated degranulation of CTL results in the release of perforin into the immunological synapse where they have a cytotoxic effect on the target cells (11). We also found lower levels of intracellular perforin in the T cells stimulated with HIV Gag-Pol than with the mock peptide-pulsed BLCL (not shown), suggesting that Gag-Pol induced the release of perforin in the LV-DC-primed T cells. Taken together, these results demonstrate that LV-transduced DCs are able to induce an HIV-specific T cell response with potential cytotoxic activity.

LV-DCs induce primary CTL response targeting HIV Gag-Pol antigens

The perforin release and CD107a/b acquisition on the cell surface of LV-DC-primed T cells upon stimulation by HIV peptides-loaded BLCL suggest that LV-DC-primed T cells have a potential CTL specificity targeting HIV antigens. To further confirm this specific effector killing function, we measured HIV-specific CTL activity using two different assays. We first employed a flow cytometry-based CTL assay using membrane dyes PKH67 (green) and PKH26 (red) to label control and HIV peptide-pulsed target cells (details in *Materials and Methods*). All target cells were labeled with PKH67, but target cells labeled with PKH26 were either HIV peptide-pulsed or Mock-pulsed. The ratio of survival target cells after co-culture with LV-DC-primed T cells revealed a consistent decrease of HIV-peptide pulsed PKH26-labeled target cells versus the mock-labeled target cells (Fig 5 A & B). We further illustrated HIV-specific CTL activity by the detection of active caspase 3, an early and sensitive indicator of apoptosis in target cells (14); there was a consistent increase in caspase 3 activity in HIV Gag-Pol pulsed target cells versus control (no peptide) target cells (Fig. 5 C & D). Taken together, these results uniformly demonstrate that LV-DC-primed T cells have acquired HIV-specific cytotoxic potential. Furthermore, this low HIV-specific CTL activity was detected without re-stimulation with LV-DCs.

Detection of HIV-specific MHC-I T cell receptors (TCRs)

To verify the HIV-specificity in the CD8 T cell population, we performed a MHC-I peptide tetramer assay to identify HIV-specific TCR using a Strep-PE conjugated HLA-A0201 Gag tetramer (SL9: SLYNTVATL) (15,16), HLA-A0201 Pol tetramer (IV9: ILKEPVHGV) (17), and HLA-A0201 In tetramer (MA10: MASDFNLPPV). The HLA-A0201 Flu tetramer (M1: GILGFVFTL), which corresponds to a CTL epitope in the influenza matrix protein, was used as a control. The LV-DC-primed T cells were double-stained with Strep-PE conjugated tetramer and Cy7-PE-conjugated anti-CD8 antibody. There was a clear increase in CD8+ T cell populations specific to the HIV-specific SL9, IV9 and MA10 tetramers over the Flu tetramer (Fig. 6). This is indicative of an effective primary anti-HIV response, because the Flu TCR response resides in a memory repertoire. Therefore, CD8 cells specific to the three dominant HIV MHC-I epitopes, SL9, IV9 and MA10, were effectively activated by the LV-DCs, further supporting that the LV-DC were able to induce an HIV-specific T cell response.

DISCUSSION

HIV-based lentiviral vectors are powerful tools for gene delivery into non-dividing and terminally differentiated cells. It has been used to genetically modify dendritic cells, the professional antigen-presenting cells that can prime naïve T cells (4,18–20). Therefore, LV transduction of DCs has become an important cancer vaccine modality (5).

DCs transduced with an early generation of LV encoding full-length *gag* gene effectively induce an anti-HIV primary CTL response. This is due to antigen presentation by the Class I MHC pathway leading to the priming of T cells by the LV-transduced DCs (18). In this study, we report that DCs transduced with a minimal SIN LV encoding none of the HIV proteins were able to prime T cells and induce a primary anti-HIV T cell response, including CTL activity. Our results are consistent with the finding that DCs are able to both present and cross-present antigens from fused HIV particles (7). Our analyses of different immune effector functions have consistently illustrated that the HIV-based vectors without encoding any HIV genes can initiate a primary anti-HIV immune response through both class I and class II antigen presentation pathways. This opens a new venue for HIV vaccine development by exploiting the unique properties of LVs and DCs.

MHC-I restricted exogenous HIV antigen presentation, involving pH-dependent and pH-independent receptor-mediated entry into DCs and macrophages, has been reported as a CD8 CTL response following VSV-G pseudotyped LV infection of DCs (7). We have extended the analysis with a minimal SIN LV to show that both CD4 and CD8 T cells can be effectively activated by LV-transduced DCs. In combination with genetic modifications of DCs, as previously illustrated using different cytokine and T cell co-stimulator genes, or siRNAs targeting IL-10 (4), it is possible that an effective anti-HIV immunity may be induced by LV-DC vaccines in both HIV-infected and uninfected individuals.

The cytokine response in the primed T cells determines the quantity and quality of HIV-specific T cell immunity. Upon restimulation, HIV peptide antigens and rVV-encoded HIV antigens both stimulated the production of TNF- α and IFN- γ in LV-DC-primed CD4 and CD8 T cells. The HIV antigens also stimulated the production of IL-2 in LV-DC-primed CD4 T cells. It is also evident that LV-DCs can generate T cells with HIV-specific cytotoxic potential. Although these immune effector functions are a weak primary response, they are important indicators of anti-HIV immunity upon Ag priming and boosting.

From LV-DC-primed T cells, we have detected CTL response against three prominent HIV-specific MHC epitopes, SL9, IV9 and MA10. SL9 is a highly immunogenic, epitope (SLYNTVATL) (15,16), HLA-A0201-restricted, help-independent HIV type 1 p17 Gag₇₇₋₈₅ and IV9 is an HIV type 1 Pol epitope (ILKEPVHGV) (17). CTL responses to SL9 are exceptionally prominent in chronically infected patients but rarely detected in acute infection, and SL9-specific CTL are inversely correlated to viral load in HIV patients (21–24). The HIV-1 integrase epitope MA10 is a major CD8 T cell epitope recognized by resting memory T cells of long-term nonprogressors (25).

The *in vivo* application of viral vectors is often associated with potential problems of host immunity. Our demonstration of anti-HIV immunity upon LV transduction of DCs raises an important issue regarding *in vivo* and *ex vivo* LV applications. The registered immune response to HIV antigens after LV gene transfer needs to be carefully monitored. Without clinical data, it is not clear whether repeated LV applications will lead to the activation of a humoral immune response to HIV antigens *in vivo*. Our *ex vivo* analysis indicates that it is possible that repeated injections of LV-DCs will trigger both humoral and cell-mediated immune responses to HIV antigens. Such an immune response, nevertheless, should be carefully evaluated concerning the safety of LV technology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig 1. Illustration of HIV-1-derived lentiviral vector system. (A) Genome organization of HIV-1 provirus. (B) NHP/TYF SIN vector system. pNHP is the helper plasmid which expresses gag and pol proteins for packaging the viral RNA genomes. pTYF-EF-Ly6e is a transfer vector which lacks the viral structural genes and encodes an HIV-1-unrelated gene, Ly6e, under the control of an EF1 α internal promoter. pHEF-VSV-G expresses envelope protein for pseudotyping the pTYF vectors.

Fig 2.

LV-ly6e-transduced DCs prime T cell secretion of Th1 cytokines specific to HIV-Gag-Pol peptides. DC were transduced with pTYF SIN vectors and matured by TNF- α /LPS/IFN- γ and cocultured with autologous PBMCs in the presence of IL-12, IL-6 and IL-7 for 3 days and expanded in IL-2 and IL-7- containing AIM-V medium for 11 days. The expanded T cells were restimulated with EBV-transformed autologous BLCL pulsed with HIV gag-pol peptides or mock (no peptide) for 5 hr in the presence of brefeldin. The cytokine expression was measured by intracellular staining flow cytometry. CD4 and CD8 T cells were gated for analysis of cytokine production. The TNF- α production in pan-T cells was presented in FACS profile (A) and bar comparison (B). The IFN- γ production in CD4 and CD8 T cells was presented in FACS profile (C & D) and bar comparison (E). The data are representative of 2 independent experiments.

Fig 3.

LV-transduced DCs activate T cell response to target cells infected with HIV-gag encoding vaccinia virus. LV-transduced DC were cocultured with autologous PBMC for 11 days as described in Fig. 2 legend. The expanded T cells were restimulated with EBV-transformed autologous B cells infected with vaccinia virus encoding HIV gag or nlacZ for 5 hr in the presence of brefeldin. The cytokine expression was measured by intracellular staining and flow cytometry. The TNF- α and IFN- γ production in CD4 and CD8 T cells were presented in FACS profiles after stimulations with vv-nlacZ- or vv-gag-infected B cells (A and B), and presented in bar graphs as numbers of cytokine producing cells per 10^5 T cells (C and D). The results are representative of two independent experiments.

Fig 4.

Induction of HIV-specific lytic degranulation in T cells primed with LV-transduced DC. LV-DC were cocultured with autologous PBMC as described in Materials and Methods for 11 days. The expanded T cells were restimulated with BLCL pulsed with HIV gag-pol peptides or mock (no peptide) for 5 hr. During the stimulation, Alexa 647-labeled CD107a/b Abs were added. The CD107a/b expression on the T cell surface was measured by flow cytometry. (A) FACS profile of CD107a/b expression in CD4 and CD8 T cells upon stimulation by HIV gag-pol peptides or mock pulsed BLCLs (CD107a/b positive cells circled in red). (B) Comparison of surface CD107a/b expression in CD4 and CD8 T cell populations restimulated with BLCL pulsed with Mock or Gag-Pol peptides. (C) FACS profile and (D) Bar graph comparison of

IFN- γ and CD107a/b double positive cells in CD4 and CD8 T cell populations upon stimulation by HIV gag-pol peptides or mock pulsed BLCL. The data are representative of two independent experiments.

Fig 5.

Induction of primary anti-HIV CTL response by LV-DCs. (A) Ex vivo CTL assay by flow cytometry. LV-DC expanded T cells were cocultured with BLCL pulsed with HIV gag-pol peptides or mock control. The target cells were labeled with either PKH67 or PKH26. The PKH67-labeled mock-pulsed target cells served as internal control and the PKH26-labeled target cells were pulsed with either mock or gag-pol peptides. The PKH67-labeled, mock-pulsed, target cells were mixed with either the mock-pulsed, or gag-pol peptides-pulsed PKH26-labeled target cells, and then cocultured with effector T cells for 5 hr. The relative survival of gag-pol peptide-pulsed vs mock-pulsed target cells was determined by FACS. FACS profiles of target cells at different effector/target (E/T) ratio illustrate the survived target cells (PKH26 labeled) relative to an internal control cells (PKH67 labeled). (B) Specific killing of gag-pol-pulsed target cells in an E/T ratio-dependent manner. The data is the mean value of effector cells from two donors. (C) & (D), CTL assay by measuring active caspase3 expression. Intracellular staining FACS analysis of active caspase 3 expression in target cells at E/T ratios of 40:1 or 0:1. Target cells were labeled with PKH26, which enables the gating of target cells for analysis of caspase 3 expression in target cells and the percentages of caspase 3 positive cells were determined.

Fig 6.

HIV tetramer staining of T cells primed by LV-transduced DCs. T cells from HLA-0201 donors were cocultured with DCs transduced with mock, LV-nLacZ or LV-BMLF1 for 12 days. The LVs used for DC infection included LV-nLacZ and LV-EBV-BMLF1, which encode the reporter nLacZ gene and EBV BMLF1 gene, respectively. The LV-DC-expanded T cells were stained with Cy7- or FITC-labeled anti-CD8 antibody and strep-PE conjugated HLA-A0201 gag tetramer (SL9: SLYNTVATL), HLA-A0201 pol tetramer (IV9: ILKEPVHGV), HLA-A0201 IN tetramer (MA10: MASDFNLPPV) or HLA-A0201 flu tetramer (GILGFVFTL). The tetramer and CD8 double positive cells were shown in FACS profiles (A) and (C) and representative results were compared in bar graphs (B) and (D), respectively.