

Transduction of dendritic cells by antigen-encoding lentiviral vectors permits antigen processing and MHC class I-dependent presentation

Shohreh Zarei, MS,^a Florence Leuba,^c Jean-François Arrighi, MS,^{b,c}
Conrad Hauser, MD,^a and Vincent Piguet, MD, PhD^c Geneva, Switzerland

Background: Because antigen-presenting dendritic cells (DCs) play a major role in the polarization of T cells, including T_H2 cells involved in allergy, strategies to modify DCs genetically are required.

Objective: The purpose of this investigation was to transduce murine bone marrow-derived DCs with lentiviral vectors encoding antigen to demonstrate antigen processing and MHC class I-dependent presentation.

Methods: Bone marrow leukocytes were incubated with antigen-encoding lentiviral constructs and cultured with GM-CSF, IL-4, and Flt-3 ligand. The capacity of the resulting DCs to express, process, and present antigen was tested *in vitro*.

Results: An average of 40% of DCs expressed antigen after 1 week of culture when antigen encoded by the lentiviral vector construct was green fluorescent protein. To demonstrate that transduced antigen can be presented by DCs on MHC class I, we chose the lymphocytic choriomeningitis virus glycoprotein (gp) as a model antigen, inasmuch as it is recognized by CD8 T cells from transgenic mice expressing an MHC class I-restricted T-cell receptor specific for the epitope of positions 33 through 41 of gp. DCs transduced with lentiviral construct encoding gp and matured with LPS activated transgenic T cells in an antigen-specific fashion. Using transporter associated with antigen presentation (TAP)-deficient mice, we show that presentation of the gp33-41 epitope is TAP-dependent, confirming processing of gp by the endogenous pathway.

Conclusions: These results demonstrate that CD8 T cells can recognize MHC class I epitopes processed from antigen in DCs transduced with lentiviral vectors. Lentiviral transduction of DCs and antigen presentation to CD8 T cells could be exploited for immunotherapy, because allergen-specific CD8 T cells have been shown to be suppressive in IgE-dependent allergy models. (J Allergy Clin Immunol 2002;109:988-94.)

Key words: Dendritic cells, lentiviral vectors, HIV, LCMV, antigen presentation, TAP

Abbreviations used

cPPT: Central polypurine tract
DC: Dendritic cell
EF-1: Human elongation factor 1
GFP: Green fluorescence protein
gp: Glycoprotein
Gp33: LCMV glycoprotein 33-41 peptide
LCMV: Lymphocytic choriomeningitis virus
MOI: Multiplicity of infection
PGK: Phosphoglycerate kinase
RT: Reverse transcriptase
TAP: Transporter associated with antigen presentation
TCR: T-cell receptor

There is ample evidence that T_H2 lymphocytes are involved in human allergic diseases, such as immediate IgE-dependent reactions, as well as in the cellular inflammation observed in asthma, allergic rhinoconjunctivitis, and atopic dermatitis.^{1,2} Dendritic cells (DCs) activate naive T lymphocytes and play an important role in the polarization of naive T cells toward effector cells with a type 2 lymphokine profile.³ Immune intervention that targets DCs might therefore be useful in the management of T_H2-dependent allergic disorders.

CD8 T cells recognizing antigen in the context of MHC class I have been shown to suppress IgE production.^{4,5} They appear to indirectly downregulate IgE production via induction of IFN- γ -producing T_H1 cells probably activated by DCs secreting IL-12.⁶ MHC class I-presented antigens, such as viral antigens, are processed in the endogenous processing pathway. They are synthesized in the cytoplasm, cleaved by the proteasome complex, and carried by transporter associated with antigen presentation (TAP) from the cytoplasm into the cis Golgi complex, where they are loaded to nascent MHC class I molecules. Antigen/MHC class I complexes are then transported via the endoplasmic reticulum to the cell surface for presentation to CD8 T cells.

To activate MHC class I-restricted antigen-specific T cells, murine DCs were targeted *in vitro* with lentiviral vectors so that antigen translation could occur in the cytoplasm. Because DCs represent a natural target for the lentiviruses HIV and SIV, attenuated variants constitute attractive vectors for genetically modifying DCs. In contrast to retroviral vectors, which are not able to infect non-

From the ^aDivision of Allergy and Immunology and ^bthe Division of Hematology, ^cDepartment of Dermatology, University Hospital of Geneva.

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Reprint requests: Vincent Piguet, MD, PhD, Department of Dermatology, HUG, 24 Rue Micheli du Crest, 1211 Geneva, Switzerland. E-mail: vincent.piguet@medecine.unige.ch.

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dividing cells, lentiviral vectors, including HIV- and SIV-derived vectors, are able to infect nondividing cells.⁷ Despite successful reports of transduction on human monocyte-derived DCs⁸ or human CD34-derived DCs through use of a lentiviral system,⁹ none of the studies specifically investigated antigen processing via the endogenous pathway or the involvement of the TAP machinery.

In this study, we investigated whether lentiviral transduction of bone marrow cells would permit the induction of DCs and whether transduced DCs could be matured by the inflammatory mediator LPS. We used CD8 T cells from mice transgenic for a T-cell receptor (TCR) that recognizes the lymphocytic choriomeningitis virus (LCMV) glycoprotein (gp) 33-41 peptide (Gp33) in the context of MHC class I. To show presentation on MHC class I, we transduced DCs with LCMV gp as a model antigen. We also used DCs derived from TAP-deficient mice to demonstrate the involvement of this molecule in processing of the model antigen.

MATERIALS AND METHODS

Peptide

Synthetic LCMV glycopeptide 33-41 KAVYNFATM was purchased from Neosystem (Strasbourg, France). This peptide is derived from the sequence of Gp33 (restriction elements K^b and D^b), except that C was replaced by M at position 41.¹⁰

Mice

C57BL/6 mice were purchased from Charles River (L'Arbresle, France) and used when they were between 6 to 12 weeks of age. Transgenic mice (line 327) expressing the transgenic P14 TCR (C57BL/6, V α 2 β 8) specific for the H-2D^b-restricted Gp33 have been previously described.¹¹ Transgenic mice were kindly provided by Drs R. M. Zinkernagel and H. Hengartner (Institute of Experimental Immunology, Zurich, Switzerland).

TAP-deficient C57BL/6 mice¹² were provided by Dr M. van den Broek (Institute of Experimental Immunology).

Lentiviral vectors

The 1.57-kb full-length LCMV-gp cDNA sequence was amplified by PCR from the original LCMV/pKooL plasmid (gift of Dr R. M. Zinkernagel) through use of the following primers: sLCMVgp (5'-GGATCCTAGGCTTTTGGATTGCG-3') and asLCMVgp (5'-GTCGACTCAGCGTCTTTCCAGATAGTT-3'). The amplified LCMVgp cDNA was subcloned in the pLox human elongation factor 1 (EF-1) lentiviral vector.¹³ The resulting plasmid was called *EF-1 LCMV gp* and was sequenced. The LCMV gp33-41 minigene was generated by cloning primers sgp33-41 (5'-GATCCATGAAAGCTGTGTACAATTTCCGCCACCTGTTGAGCGGC GCG-3') and asgp33-41 (5'-TCGACGCGGCCGCTCAACAGGTG CCGAAATTGTACACAGCTTTTCATG-3') in the pLox EF-1 lentiviral vector¹³ with the IRES-green fluorescent protein (GFP). The resulting construct was called *EF-1 gp33-41IRESGFP*.

Production of HIV-derived vectors pseudotyped with the vesicular stomatitis virus G envelope protein was achieved by transient cotransfection of 3 plasmids into 293T cells. Vesicular stomatitis virus G was expressed from pMDG. The HIV-derived packaging construct used was pCMV 8.91 and encodes *Gag*, *Pol*, *Tat*, and *Rev*.¹⁴ The HIV vector plasmids used in this study contain the self-inactivating modification, as previously described,¹⁵ and the woodchuck hepatitis transcriptional element.¹⁶ The phosphoglycerate kinase (PGK) GFP and EF-1 α GFP HIV vector plasmids were a

gift from D. Trono and have been described previously.¹³ The central polypurine tract (cPPT)-PGK GFP vector plasmid was a gift from L. Naldini.¹⁷ The HIV vector plasmids are derived from the original pHR' backbone⁷ and express GFP as a reporter. Vector titers were determined by transduction and flow cytometry analysis of GFP expression in HeLa cells, as previously described.¹³ Titers were between 2×10^7 and 6×10^7 HeLa transducing units per milliliter. Alternatively, titers were determined by reverse transcriptase (RT) activity, as previously described.¹⁸

Dendritic cell preparation

Bone marrow-derived DCs were generated as previously described.^{19,20} Bone marrow cells were seeded at a density of 5×10^4 cells per well and differentiated into DCs by culturing for 9 days in media supplemented with 25 ng/mL recombinant murine GM-CSF (ImmunoKontakt, Bioggio Lugano, Switzerland), 1000 U/mL of recombinant murine IL-4 (supernatant of X63/O transfectant²¹) and 10 ng/mL of recombinant murine Flt-3 ligand (R&D Systems, Abingdon, United Kingdom). On day 7, DCs were matured by adding 1 μ g/mL of LPS (Difco, Detroit, Mich) and fresh cytokines. After 48 hours, nonadherent cells were harvested and used for flow cytometric analysis or proliferation assays.

Antibodies

The following phycoerythrin-conjugated mAbs were purchased from BD Pharmingen (San Diego, Calif): anti-CD11c (hamster IgG, clone HL3) and isotype control (hamster IgG, clone A19-3). Biotin-labeled mAbs included anti-MHC class II (I-A/I-E) (rat IgG2a, clone 2G9), anti-CD40 (rat IgG2a, clone 3/23), anti-CD80 (B7-1) (hamster IgG2, clone 16-A10A1), anti-CD86 (B7-2) (rat IgG2a, clone GL1), and isotype controls (hamster IgG2, clone B81-8) (rat IgG2a, clone R35-95). Allophycocyanin-labeled streptavidin and all of the aforementioned antibodies were obtained from BD Pharmingen.

Flow cytometric analysis

Cells were incubated with the indicated antibodies and were analyzed on a FACSCalibur cytometer (Becton Dickinson, Mountain View, Calif). Living cells were identified by means of the nonpermeant DNA dye 7-amino-actinomycin D (Sigma Chemical, St Louis, Mo). Data were analyzed through use of WINMDI software written by J. Trotter at the Scripps Institute (La Jolla, Calif) and CellQuest software (Becton Dickinson).

Proliferation assay

Spleen and lymph node cells of naive transgenic mice were subjected to negative selection with mAb to CD4 (GK1.5; American Type Culture Collection, Manassas, Va) to MHC class II (M5/114.15.2; American Type Culture Collection) and complement incubation (Cedarlane, Hornby, Ontario, Canada). The resulting cells were 90% CD8⁺ as determined by flow cytometric analysis. Mature DCs were collected on day 9 and pulsed with 10^{-7} mol/L LCMV peptide for 90 minutes; this was followed by a wash step. DCs were subsequently cocultured with 0.5×10^5 purified transgenic CD8 T cells. After 72 hours of culture, cell proliferation was measured by [*methy*l-³H] thymidine incorporation after the cultures were pulsed (0.5 μ Ci per well) for 6 to 8 hours.

RESULTS

Optimizing transduction efficiency in murine DCs

Bone marrow-derived leukocytes were incubated with lentiviral vectors and cultured for 7 days with GM-CSF, IL-4, and Flt-3 ligand to generate immature DCs. To

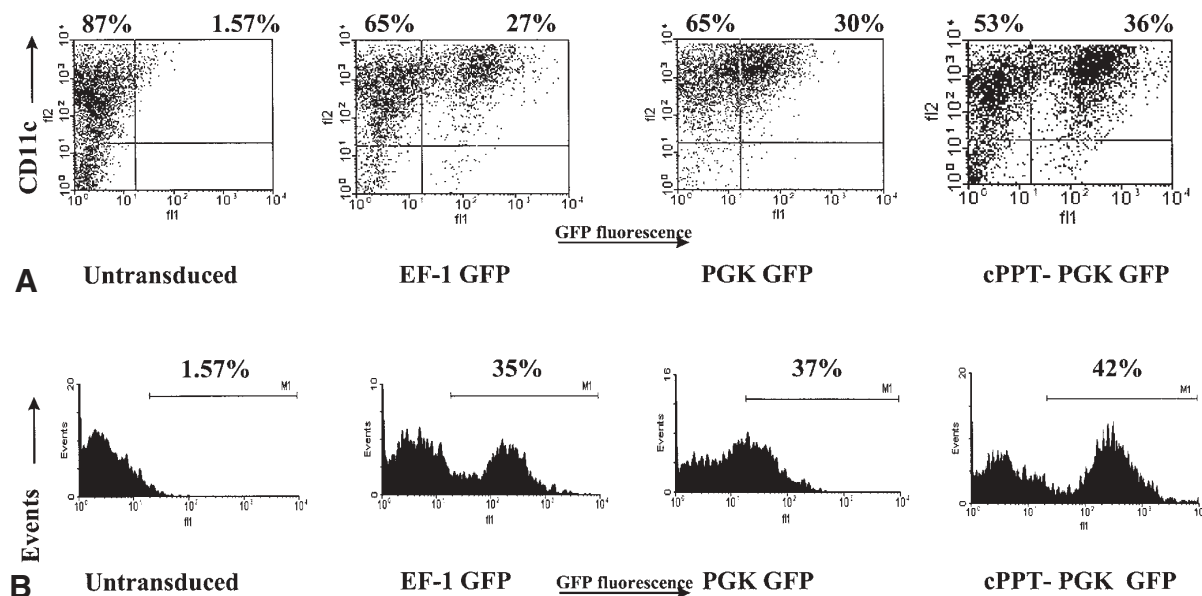


FIG 1. Transgene transfer and expression after transduction of murine DCs with lentiviral vectors. Murine bone marrow cells were transduced with lentiviral vectors containing EF-1, PGK, or PGK-cPPT promoter at an MOI of 10. **A**, Seven days after incubation with the vectors, transduced DCs were analyzed by means of flow cytometry. **B**, CD11c⁺ cell populations were gated, and monoparametric frequency histograms of GFP expression for the transduced DCs were generated. This experiment is representative of 5 independent experiments.

achieve maximal transduction of murine bone marrow–derived DCs, we evaluated 2 complementary approaches using multiplicities of infection (MOIs) from 1 to 10 and testing 3 different lentiviral constructs (Fig 1). The HIV vector plasmids differed in their internal promoter. EF-1, PGK, and a construct with the PGK promoter and a cPPT were used.¹⁷ The latter sequence plays an important role in nuclear import, thereby increasing transduction efficiency in HSCs.²² In all of the lentiviral constructs used in this study, *gag*, *pol*, *env*, *tat*, *rev*, *nef*, *vif*, *vpu*, and *vpr* were deleted. A highly efficient transduction at an MOI of 10 was achieved with the lentiviral vector encoding GFP under the control of the EF-1 promoter (EF-1 GFP construct) and with the lentiviral vector encoding GFP under the control of the PGK promoter and containing the cPPT modification (cPPT-PGK GFP vector). We obtained $39\% \pm 7\%$ (SEM of 4 independent experiments) GFP-expressing CD11c⁺ cells with the EF-1 GFP vector; this compared with $35\% \pm 4\%$ with the PGK-vector alone and $38\% \pm 2\%$ with the vector cPPT-PGK (values obtained from 4 independent experiments). GFP expression was high with the EF-1 GFP construct (mean GFP intensity, 267) and with the cPPT-PGK GFP construct (mean GFP intensity, 305); it was significantly lower with the PGK GFP construct (mean GFP intensity, 41; Fig 1). We conducted the rest of these studies using the lentiviral vectors driving antigen expression under the control of the EF-1 promoter, in view of their optimal transduction efficacy in murine bone marrow–derived DCs.

Correct phenotypical maturation of transduced DCs

To test whether lentiviral transduction of DCs affects the function of in vitro–generated murine bone marrow–derived DCs, we evaluated the capacity of immature transduced DCs to undergo maturation on LPS treatment. Several HIV genes, such as *Nef* and *Vpu*, can downregulate cell surface receptors and could affect DC maturation, but they were removed in the lentiviral vectors used in this study.²³ Bone marrow cells were incubated with the EF-1 GFP vector and then differentiated into DCs. As nontransduced DC underwent significant upregulation of MHC-II, CD40, CD80, and CD86 on LPS exposure for 48 hours, GFP-expressing DCs followed a similar maturation pattern with significant upregulation of MHC-II, CD40, CD80, and CD86 (Fig 2, *left* and *right*), suggesting that transduction of DCs did not affect their subsequent maturation.

Antigen presentation in DCs transduced with lentiviral vector encoding LCMV gp

To test whether transduced antigen can be presented by DCs on MHC class I, we chose the LCMV gp as a model antigen that can be recognized by CD8 T cells from transgenic mice expressing an MHC class I–restricted TCR specific for the epitope of positions 33 through 41 of gp. The full-length LCMV gp was subcloned into the lentiviral vector with the EF-1 promoter and called *EF-1 LCMV gp*. Titers were determined by RT

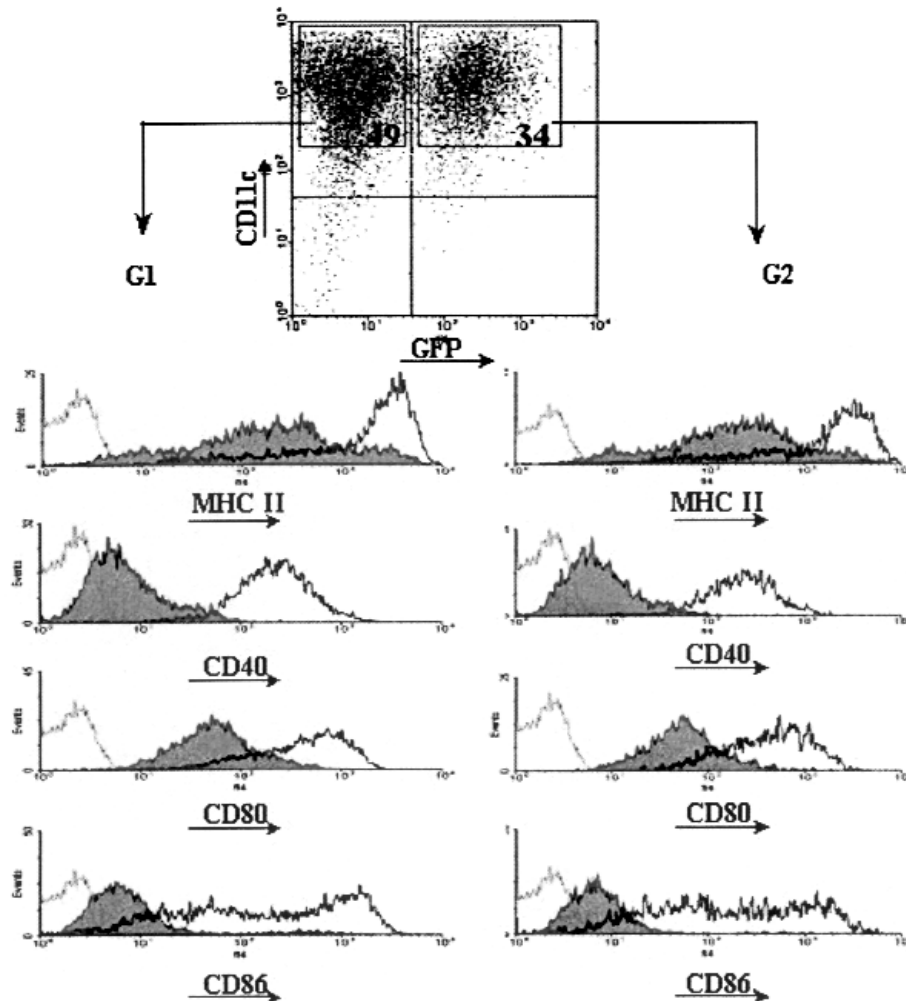


FIG 2. Transduction of DC with lentivirus vectors does not interfere with their maturation. DCs were transduced with GFP-encoding HIV-derived vectors. After maturation with LPS, surface phenotypes of transduced and nontransduced DCs from the same culture were similar when 2 different gates, G1(CD11c⁺ GFP⁻) and G2(CD11c⁺ GFP⁺), were used. This experiment is representative of 3 independent experiments.

activity and normalized through use of the EF-1 GFP lentiviral construct as a standard. DCs transduced with a lentiviral construct encoding full-length gp (EF-1 LCMV gp) and matured with LPS activated transgenic T cells in an antigen-specific fashion to a significantly higher extent than DCs transduced with a lentiviral construct encoding GFP (EF-1 GFP). Although the stimulation obtained by DCs transduced with the lentiviral construct EF-1 LCMV gp was strong, it was slightly inferior to peptide-pulsed DCs under optimal conditions (Fig 3). These experiments directly demonstrate that transduced DCs can process LCMV gp correctly and stimulate transgenic CD8 T cells in an antigen-dependent manner.

TAP dependency of antigen presentation in DCs transduced with lentiviral vectors

To demonstrate that antigen processing of the gp33-41 epitope was TAP-dependent, we incubated DCs pro-

duced from wild-type or TAP-deficient mice with lentiviral vectors expressing LCMV gp (EF-1 LCMV gp). We also developed a novel lentiviral construct with the EF-1 promoter encoding Gp33 to avoid proteolysis of the antigen by the proteasome. This construct, called *EF-1 gp33-41IRESGFP*, also contains an internal ribosomal entry site (IRES) followed by GFP, allowing the expression of both Gp33 and GFP in the transduced cells. The vector EF-1 gp33-41IRESGFP was titrated on HeLa cells, as measured by GFP expression (data not shown). Furthermore, the vector stocks were normalized by RT activity with the stocks of lentiviral vector constructs encoding full-length LCMV (EF-1 LCMV gp). Both constructs (EF-1 LCMV gp and EF-1 gp33-41IRESGFP) were used to transduce DCs from either wild-type mice or TAP^{-/-} mice. As a control, wild-type and TAP-deficient bone marrow leukocytes were transduced with the EF-1 GFP vector. In these experiments, both wild-type DCs trans-

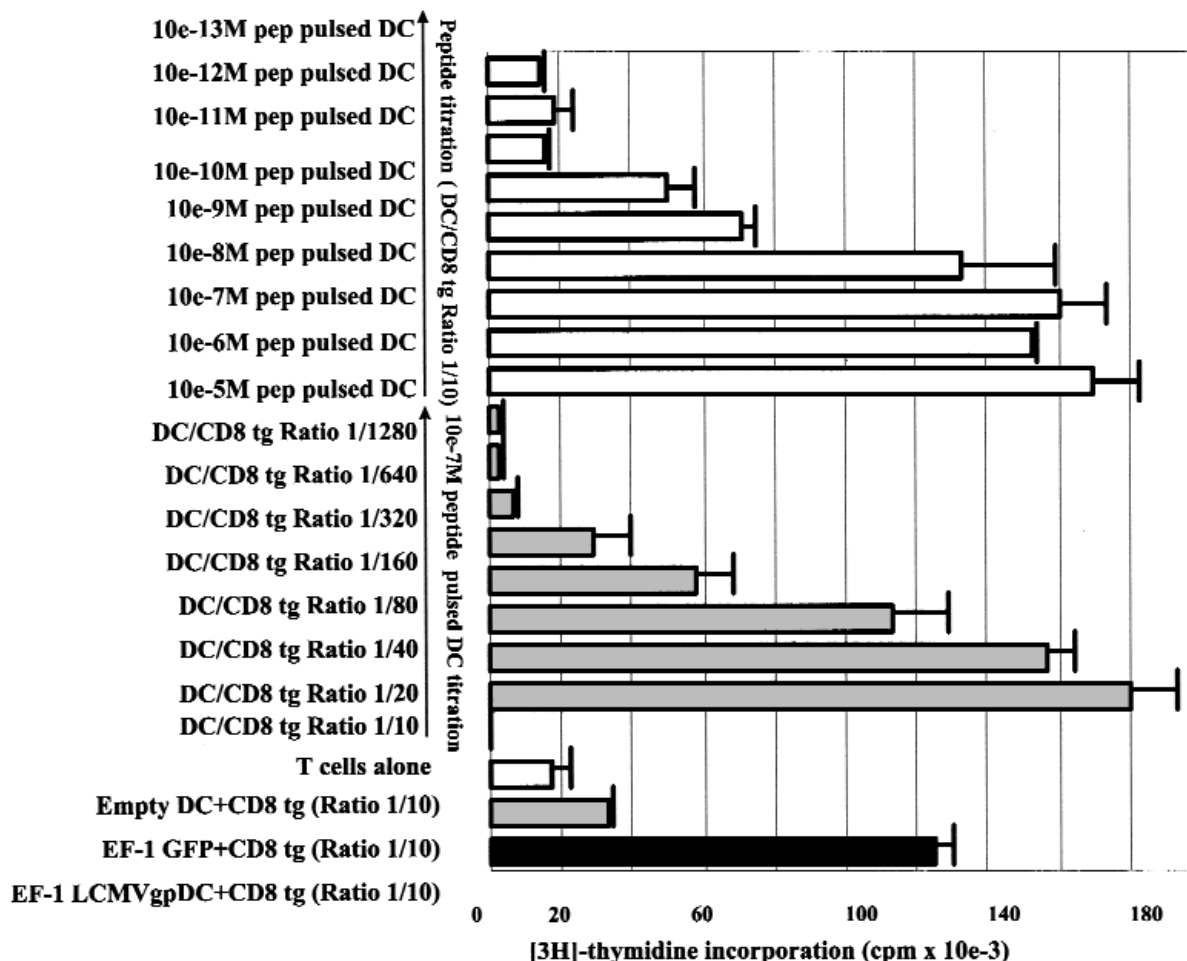


FIG 3. Transduced DCs are capable of presenting the LCMV gp to transgenic CD8 T cells specific for the Gp33 epitope. The proliferation of purified transgenic CD8 T cells incubated with DCs transduced with lentiviral vectors encoding the full-length LCMV gp (EF-1 LCMV) and control GFP (EF-1 GFP). This experiment is representative of 5 independent experiments.

duced with the EF-1 LCMV gp and wild-type DCs transduced with EF-1 gp33-41IRESGFP were capable of stimulating CD8 T cells. However, when DCs from TAP^{-/-} mice were incubated with the lentiviral vector EF-1 LCMV gp or the lentiviral vector EF-1 gp33-41IRESGFP, they were not capable of stimulating the transgenic CD8 T cells (80% to 90% inhibition in comparison with the wild-type cells; Fig 4).

In conclusion, our experiments demonstrate that the full-length LCMV antigen, as well as gp 33-41 peptide, can be efficiently transported by TAP and presented by bone marrow-derived DCs in an MHC class I-dependent manner. These results indicate that LCMV gp is cleaved by the DC proteasome producing the Gp33 epitope.

DISCUSSION

In the present study, we evaluated the capacity of lentiviral vectors as tools to deliver antigen to DCs and be presented in an MHC class I-dependent manner. Inasmuch as antigen-presenting cells such as DCs play

a key role in allergy, it is of interest to be able to genetically modify these cells in order to load them with specific antigens.

Recent advances in the ex vivo generation of nonproliferating monocyte-derived DCs^{24,25} or DCs derived from proliferating CD34⁺ progenitor cells^{26,27} suitable for clinical application have allowed the use of modified DCs in immunotherapy. In recent clinical trials, DCs were loaded either with MHC class I-specific synthetic peptides or with whole tumor cell preparations.²⁸⁻³⁰ The disadvantages of using DCs pulsed with synthetic peptides include uncertainty as to the duration of antigen presentation, restriction by the patient's haplotype, and the relatively low number of known MHC class I and MHC class II helper cell-related epitopes. As an alternative to peptide loading, one attractive method of delivering antigen to DCs involves the use of viral vectors encoding antigen.³¹ Unlike adenoviral vectors, vectors derived from lentiviruses are able to provide stable transgene integration devoid of unwanted immunogenicity in both dividing and nondividing cells.⁷

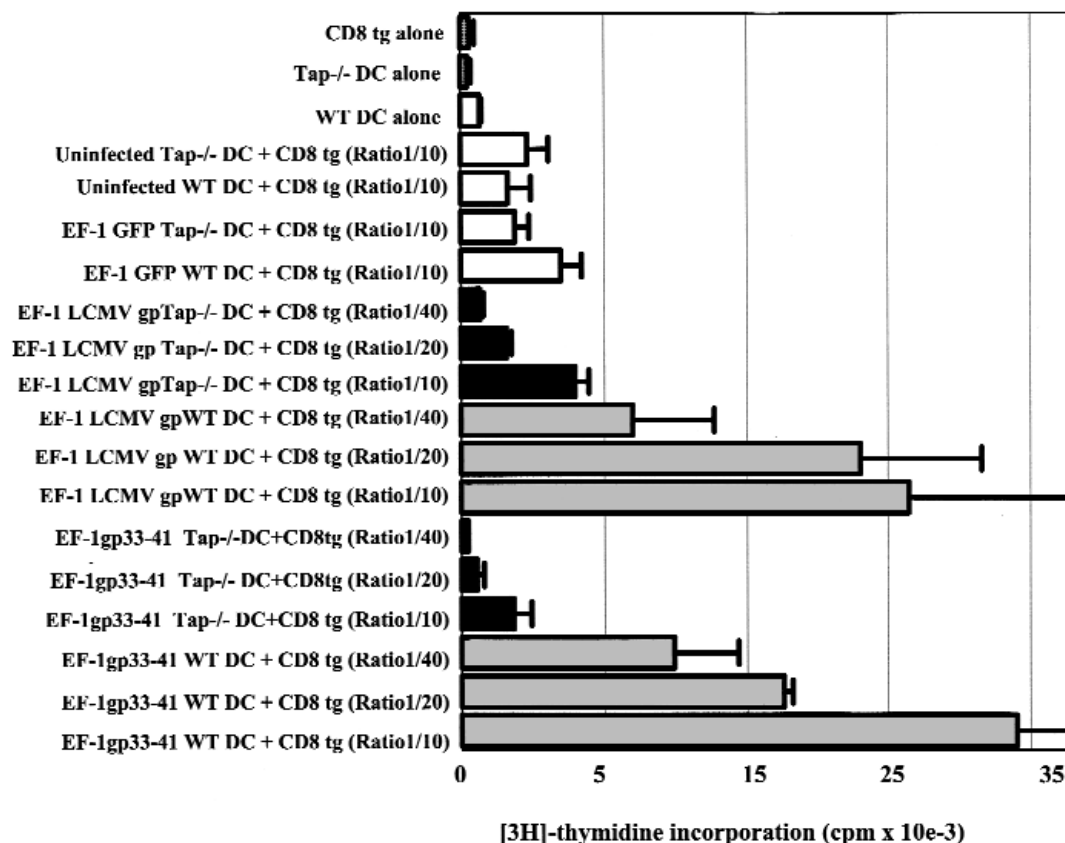


FIG 4. The presentation of the LCMV epitope by transduced DCs to CD8 T cells is TAP-dependent. Proliferation of transgenic CD8 cells incubated with wild-type and TAP-/- DCs transduced with the indicated lentiviral vectors. This experiment is representative of 3 independent experiments.

Our present study demonstrates that lentiviral vectors encoding antigens are efficient at transducing murine bone marrow-derived DCs. We identified a lentiviral construct driving antigen expression under the control of the EF-1 promoter as an optimal vector for murine bone marrow-derived DCs. Using this construct (EF-1 GFP), we obtained approximately 40% ($39\% \pm 7\%$ [SEM of 4 independent experiments]) of DCs expressing high levels of antigen after 1 week of culture when the antigen was GFP. When the antigen encoded was full-length LCMV gp, we observed that DCs transduced with vector encoding LCMV gp (EF-1 LCMV gp) was recognized by CD8 T cells from transgenic mice expressing an MHC class I-restricted TCR specific for Gp33. These results indicate correct epitope presentation. Using TAP-deficient mice, we further demonstrated that TAP was involved in the processing of the transduced antigen and that the regular endogenous processing pathway was used as with other antigens synthesized in the cytoplasm. The fact that CD8 T cells recognized both full-length LCMV gp and gp 33-41 epitope encoding viral constructs on DCs suggests that the proteasome of transfected DCs permits the generation of the dominant LCMV epitope. Previous studies showed that immunoproteasomes might not generate the same epitopes as proteasomes of nonprofessional antigen-presenting cells.³²

Our results demonstrate that CD8 T cells recognize MHC class I epitopes processed from antigen by DCs transduced with lentiviral vectors. This model offers the prospect of immune intervention with lentiviral vectors that target DCs in the management of T_H2 -dependent allergic disorders. Allergen-specific CD8 T cells suppress IgE production^{4,5} via induction of IFN- γ -producing T_H1 cells activated by DCs secreting IL-12.⁶ Therefore, targeting of DC and antigen presentation to CD8 T cells could be exploited in immunotherapy, inasmuch as allergen-specific CD8 T cells have been shown to be suppressive in IgE-dependent allergy models.

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