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Effect of Lentiviral Vectors on the Phenotypical and Functional Maturation of Human Dendritic Cells: Implications for Gene Therapy

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Abstract: Dendritic cells (DCs) play a pivotal role in orchestrating and bridging innate, adaptive, and memory immunity. For this reason, genetic modification of DCs functions is an attractive approach to treat disease, both using mature DCs (mDCs) to immunize patients, or immature DCs (iDCs) to induce tolerance. Viral vectors are efficient at transducing DCs, and we have investigated the effect of transduction with viral vectors on the phenotype and function of DCs. Adenovirus (Ad5), and Lentivirus (LV) are able to up-regulate costimulatory molecules and major histocompatibility complex (MHC) class II expression on DCs, as well as inducing production of Th1 and proinflammatory cytokines. Moreover, the function of virally infected DCs is altered. In fact, iDCs have an increased, and mDCs a decreased, ability to stimulate a mixed lymphocyte reaction (MLR). Similar results were observed when the capability of transduced-DCs to present recall-antigen was investigated. Of interest, in the context of vaccine design, these vectors were able to induce an antigen-independent T lymphocytes proliferative response. These data are relevant not only in the context of genetic manipulation of DCs for vaccine development and active immunotherapy, but also in our understanding of the response of DCs to viral infection.

Key Words: Lentiviral vectors, human dendritic cells, viral vectors.

INTRODUCTION

Dendritic cells (DCs) are known to be the most powerful antigen-presenting cell for priming T cells [1]. It addition, it is also apparent that DCs are involved in mechanisms establishing tolerance to self antigens and non-pathogenic foreign antigens. In fact, DCs located in the periphery are able to internalize both self and non-self antigens and therefore to migrate to lymph nodes [2, 3]. This process depends on their state of maturation, the stimulatory signals they have received, and the antigens they are presenting. Thus, DCs can activate the lymphocytes to respond, or to induce tolerance to the presented antigen [1].

DCs can be artificially divided into immature or mature, which are characterized by phenotypically and functionally distinct characters [4]. Immature DCs (iDCs) interact with their environment *via* non-specific phagocytosis and recognize pathogens through separate signals for antigen uptake using C-type lectin and cellular activation of pattern recognition receptors. Several signals, such as proinflammatory cytokines (tumor necrosis factor alpha [TNF-α] and alpha/beta interferon [IFN-α/β]), necrotic cells, and bacterial and viral residues, promote DCs maturation. Pathogen residues are recognised *via* inter-species conserved receptors, such as Toll-like receptors that recognize conserved microbe-associated molecules [5]. During maturation, DCs lose the ability to take up antigens, change their morphology, and migrate towards the lymphoid compartments. Once there, matured DCs (mDCs) are primed for antigen-specific naïve T-cell presentation and stimulation *via* the expression of major histocompatibility complex (MHC) class I/II and costimulatory molecules [1]. Finally, in addition to their central role in priming the naïve response, DCs also powerfully restimulate the memory T-cell response.

The dual role of DCs in inducing immunity or tolerance is a consequence of a number of factors. These include the expression of costimulatory molecules on their surface (such as CD40, CD80, and CD86) and the secretion of cytokines [3, 6]. More recently, the key role of indoleamine 2,3-dioxygenase enzyme (IDO) expression by DCs has been recognized [7]. This enzyme catabolizes tryptophan, which is essential for lymphocyte function, and has an important role in immunomodulation in peripheral sites such as the placenta, where it prevents T-cell-mediated rejection of allogeneic foetuses [8]. DCs that express high levels of IDO cannot activate T-cell responses, and thus are capable of inducing tolerance to antigens that they express. (IFN-γ) is a powerful inducer of IDO activity in DCs. Recently, it has been shown that cytotoxic T-lymphocyte-associated antigen 4-fusion immunoglobulin (CTLA-4Ig) [9] and surface bound CTLA-4 [10] act to up-regulate IDO expression in DCs, possibly by increasing IFN- γ production.

The ability of DCs to regulate the balance between tolerance and immunity offers considerable opportunities for their use in therapy. Activated DCs (mDCs) can be used to induce immunity to pathogens or tumor-related antigens, while iDCs, which are tolerogenic, may have a role in

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controlling autoimmune disease or transplantation. Such strategies may also involve the genetic modification of DCs, either to express an antigen of interest or to modulate the function of the cells.

At present, the most efficient method for genetic modification of DCs is to use viral vectors, though there are a number of nonviral strategies that are being developed. In particular, adenoviral (Ad) or lentiviral vectors have all been shown to be effective in transducing DCs. However, the transduction of DCs by these vectors may be associated with alterations in the phenotype and function of the cells. Viruses can activate iDCs to become mDCs by several pathways, [11-14] in particular through the presence of double-stranded (ds) RNA in virally infected cells.

Several groups have studied viral vector-DCs interaction. Most of these reports can be divided roughly into those that study genetic modification of DCs to induce tumor- or pathogen-specific cellular responses and those that evaluate the interaction of DCs with viral vectors in order to predict, understand, and limit the potential immune response following *in vivo* gene transfer. Here we compare the effect of two different viral vectors on the phenotype and function of iDCs and mDCs. In general terms, viral vectors that are successful at transducing DCs also activate the cells, as determined both by alterations in the surface phenotype and secretion of cytokines. DCs transduced with these vectors have an altered phenotype and function, with iDCs becoming capable of stimulating a two-way mixed lymphocyte response (MLR), but mDCs having a reduced ability to stimulate allogeneic T cells. These data have important consequences for the therapeutic application of DCs that have been genetically modified using viral vectors.

MATERIALS AND METHODS

DC Preparation and Cultures

Blood samples were obtained from 5 healthy volunteers enrolled among the staff members, after informed consent. Monocytes were separated from peripheral blood mononuclear cells, and isolated using the MACS CD14 isolation kit (Miltenyi Biotech). Monocytes were cultured for 5 days with 200 ng/ml of recombinant human GM-CSF and 10 ng/ml recombinant human IL-4 (Schering-Plough Research Institute). These cells were termed immature dendritic cells (iDC). To achieve the maturation, iDC were cultured with 20 ng/ml of LPS (Sigma) for 48 hours.

Adenovirus Production

The adenovirus serotype 5 vector (Ad5-eGFP, carrying the enhanced green fluorescence protein-1; Stratagene, Italy) were amplified and titered using a standard plaque assay on 293T cells as previously described [15]. For transduction, 10^4 DCs were incubated with adenovirus vectors (Ad5-eGFP at various multiplicities of infection [MOIs]) in 100 μl optiMEM I (Invitrogen) for 2 to 3 hours, at which time the volume was increased to 0.5 ml by addition of DCs culture medium. As control, an adenoviral vector containing no GFP insert (Ad0) was used.

Lentivirus Production

The HIV-based constructs were propagated by a 3 plasmids cotransfection technique in 293T cells as described elsewhere [16] and according to the manufacturer's instructions (Invitrogen). Briefly, the lentiviral transfer vectors used in this study are the third generation HIV-based lentiviral vectors, in which most of the U3 region of the 3′ LTR was deleted, resulting in a self-inactivating 3′-LTR or SIN. Lentiviral (LV) vectors were prepared by transient transfection of 293T cells using a standard calcium phosphate precipitation protocol. The viral supernatants were harvested 72 hours post-transfection, filtered and concentrated. The pellets were then resuspended in an appropriate volume of cold PBS. The transduction of DCs was performed as previously described [17].

Flow Cytometry and Antibodies

The phenotype of transfected or untransfected DCs was assessed by flow cytometry 5 days after transduction. Preliminary experiments were performed in order to determine the time after infection when the maximal expression of eGFP was detected. To this end, mAb specific for CD11c, CD1a, CD14, CD40, CD80, CD86, CD83, HLA-DR, ICOS-L, and irrelevant molecules (anti-CD19; all from Becton Dickinson) were used.

Assessment of eGFP Reporter-Gene Expression

Following transfection, *eGFP* reporter-gene expression was determined using flow cytometry or an inverted fluorescent microscope as previously described [18].

ELISA

In order to determine cytokine production following viral transduction, IL-1β, IL-12, IL-6, TNF-α, IL-8, IFN-γ, IL-4, and IL-10, concentration in cell supernatants were measured by ELISA after collecting supernatants at 4 days after transduction. All ELISA kits were from Bender MedSystems.

Mixed Lymphocyte Reactions

Allogeneic $CD4^+$ T cells were isolated and purified by positive selection with magnetic beads coated with mAb to CD4 (MACS, Miltenyi Biotec). Accessory cell contamination was assessed by measuring T-cell proliferation in the presence of PHA) in a 48-hour assay. The purified cells (1×10^5/well) were cultured in 96-well flat-bottom plates in the presence of 10^4 irradiated transfected or untransfected allogeneic DCs as stimulators for 5 days. Proliferation was measured by a 18-hour pulse with [^{3}H]thymidine (0.5 μCi, around 1.85×10^5 Bq; Amersham Biosciences). Dry filters with scintillation fluid were counted in a gamma counter (Beckman-Coulter).

Antigen-Specific Proliferation Assays

Transfected or untransfected DCs and autologous $CD4^+$ T lymphocytes were obtained as described above. DCs were pulsed with recall antigens: *Candida albicans* bodies at $3x10^5$/ml, and purified protein derivative (PPD) from *Mycobacterium tuberculosis* (Statens Serum Insitut, Copenhagen, Denmark) at a final concentration of 5 μg/ml. Proliferative responses were measured by culturing $3x10^4$ pretreated DCs in the presence of 10^5 $CD4^+$ autologous T lymphocytes in 0.2 ml of complete medium, in 96-well flat-bottom microtiter plates. Cultures were pulsed with

[^{3}H]thymidine on day 5 and harvested 18 h later. Dry filters with scintillation fluid were counted in a gamma counter (Beckman-Coulter).

Statistical Analysis

All statistical analyses were performed by using Prism 4 software. In detail, statistical evaluation of data was performed with Student *t* test for simple comparison between 2 means. Otherwise, the ANOVA test (analysis of variance) was used for multiple comparisons. A *P* less than 0.05 was considered statistically significant. All data shown are representative of at least 3 experiments.

RESULTS

Adenoviral and Lentiviral Vectors are Able to Transduce Human DCs with Different Efficiency

In order to compare the ability of the two viral vectors to transduce DCs, either iDCs or mDCs (matured by treatment with LPS) were infected with Ad5, or LV vectors encoding eGFP at different MOIs (Fig. **1A**). The transfection efficiency was then determined by flow cytometry analysis of *e*GFP cell expression. As shown in Fig. (**1B**) and in Table **1**, Ad5 and LV were capable of transducing DCs, even if with a reduced ability to transduce mDCs when compared with

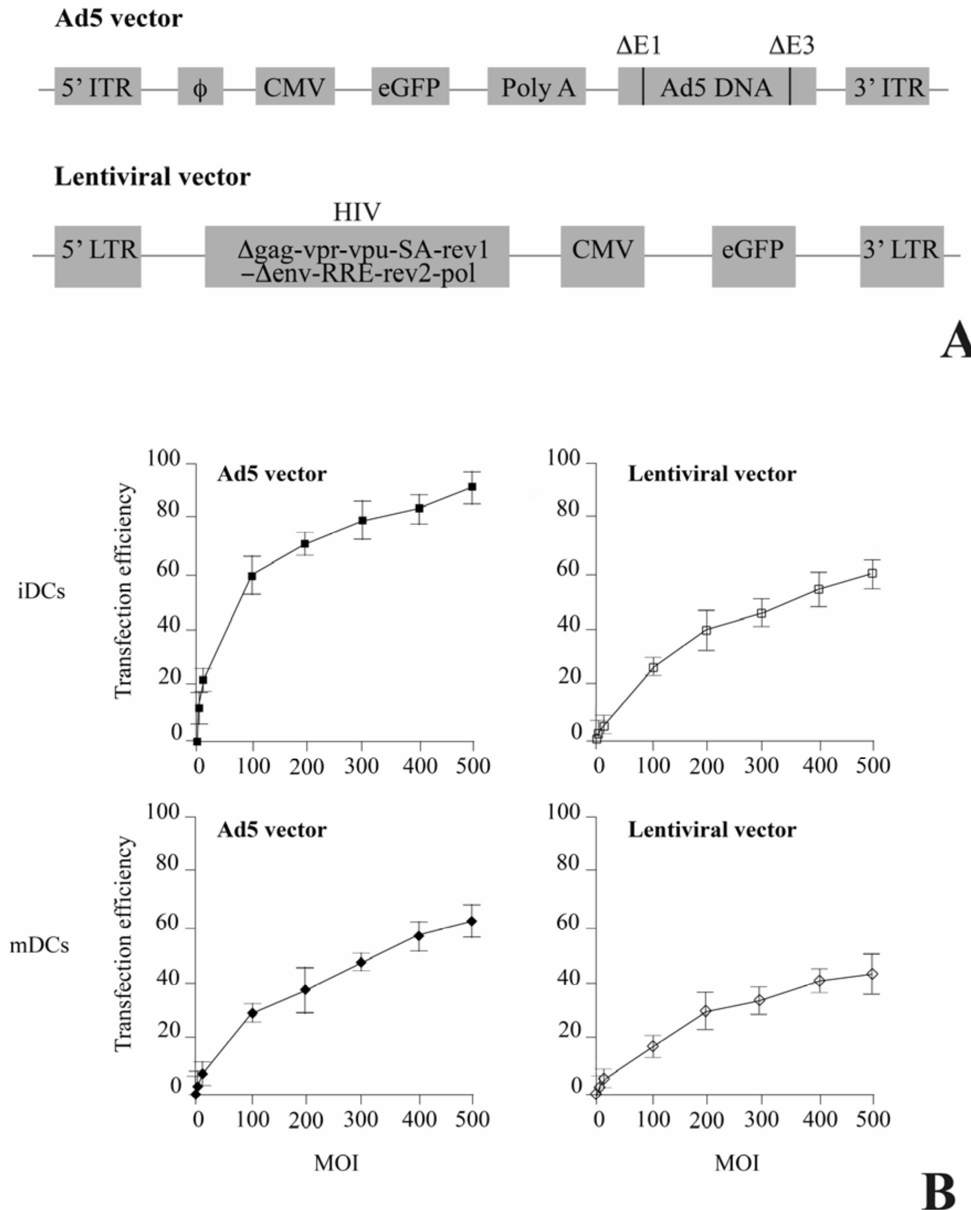

Fig. (1). Viral vectors are able to transduce DCs. Panel **A**. We used Ad5, and LV constructs to generate replication-deficient viruses encoding (e)GFP. ITR: inverted terminal repeat; Φ: encapsidation signal; CMV: Cytomegalovirus; HIV: Human Immunodeficiency Virus; LTR: long terminal repeat.

Panel **B**. DCs were transduced with Ad5, and LV vectors encoding eGFP at the MOI indicated either as iDCs (top row) or following stimulation with 20 ng/mL LPS for 48 hours (bottom row). The transfection efficiency was assessed after 5 days using flow cytometry to measure eGFP expression. Results are expressed as the percentages of eGFP-positive ± mean standard deviation of triplicate determinations.

Table 1. Adenoviral (Ad5) and Lentiviral (LV) Vectors Transduce Human DCs with Different Efficiencies. A Comparison with Some Literature Results is Shown

	% of Transfection Efficiency of iDCs MOI					% of Transfection Efficiency of mDCs MOI				
	100	**200**	**300**	**400**	**500**	**100**	**200**	**300**	**400**	**500**
Ad5	60±15.5	73.3±8.8	78.9±15.6	82.2±11.1	92±12.2	28.8±7.8	35.5±16.7	46.7±6.7	55.5±11.2	63.3±12.2
LV	26.6±8.8	40±17.8	48.8±11.1	55.6±13.3	61.2±11.3	15.6±7.8	27.8±14.2	33.3±10.2	41.1±8.9	43.2±14.5
Ref. [18]										
Ad5	60±10	n.d.	n.d.	n.d.	90±10	35±12	n.d.	n.d.	n.d.	60±15
LV	30±10	n.d.	n.d.	n.d.	60±10	15±10	n.d.	n.d.	n.d.	30±10
Ref. [28]										
Ad5	20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ref. [29]										
LV	from 69 to 93.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected;

iDCs. Of note, Ad5 was the most efficient vector, showing higher transfection efficiencies related to lower MOI (60% of iDCs were GFP positive for an MOI=100, whereas to obtain the same result with LV we could reach an MOI=500). On the other hand, to obtain a 40% of mDCs transduction we used an MOI of 200 and 400 respectively for Ad5 and LV.

Phenotypic Changes of iDCs Following Viral Transduction

In order to determine the effect of viral vectors transduction on the expression of surface markers, the surface immunophenotype 4 days after transduction of iDCs with both vectors was assessed. As shown in Fig. (**2**), transduction of iDCs with Ad5 vectors resulted in marked up-regulation of CD83, and MHC class II, and the costimulatory molecules CD40 and CD80. In addition, ICOS-L and CD86 were also slightly up-regulated. The cells therefore adopted a phenotype similar to that of mDCs, as has been previously reported [14, 18]. This was seen both with vectors encoding *e*GFP and with a control vector (Ad0) containing no insert, indicating that the up-regulation observed was not a consequence of GFP expression. Interestingly, exposure of mDCs to Ad5 failed to modify the expression of these molecules (data not shown). Following transduction of LV vectors, CD83, MHC class II, and costimulatory molecules on both iDCs (Fig. **2**) and mDCs were up-regulated (data not shown). As additional control, incubation of DCs in medium and buffers used to prepare viruses did not affect the phenotype of the cells (data not shown). Finally, the differentiation of monocytes in DC was verified by expression of cell surface markers: CD14 was down-regulated and remained low in both immature and mature DC, whereas CD1a and CD11c were up-regulated (data not shown).

Table **2** summarises the results obtained and compares them with some studies published previously.

Cytokine Modulation Following Viral Transduction

Recently, it has been reported that transduction of DCs with viral vectors results in an increase of the secretion of cytokines [14, 18]. Similar up-regulation of cytokines, such as enhanced secretion of IL-1β, IL-12, IL-6, IL-8, and IFN-γ, in both iDCs and mDCs was observed (Fig. **3**). Transduction of DCs with LV vectors resulted in an increase in secretion of IL-12, TNF-α, and IFN-γ, in both iDCs and mDCs. In addition, Ad5 and LV had an apparent stimulatory effect on IL-4 (even if not statistically significant) and IL-10 production on iDCs, whereas mDCs seemed not to be affected by viral transduction (data not shown). These results on the pattern of cytokines produced after viral transduction demonstrated that viral vectors did not alter the potential capability of both iDCs and mDCs to produce relevant co-stimulatory cytokines upon subsequent stimulation.

Immunostimulatory Capacity of DCs is Altered Following Viral Transduction

In order to test the functional consequences of viral transduction of DCs, either iDCs or mDCs were incubated with viral vectors at various MOIs, and then used as stimulators in an MLR. As expected, untransduced iDCs were poor at stimulating an MLR; however, when they were transduced with Ad5 their ability to activate allogeneic T cells was markedly increased (Fig. **4A**), as previously reported [20]. These differences, even if observed for MOI>10, were significant at an MOI of 500 (p<0.05). These data are consistent with our observations on the up-regulation of MHC class II and costimulatory molecules on iDCs following Ad5 transduction. Conversely, mDCs that are known to be potent

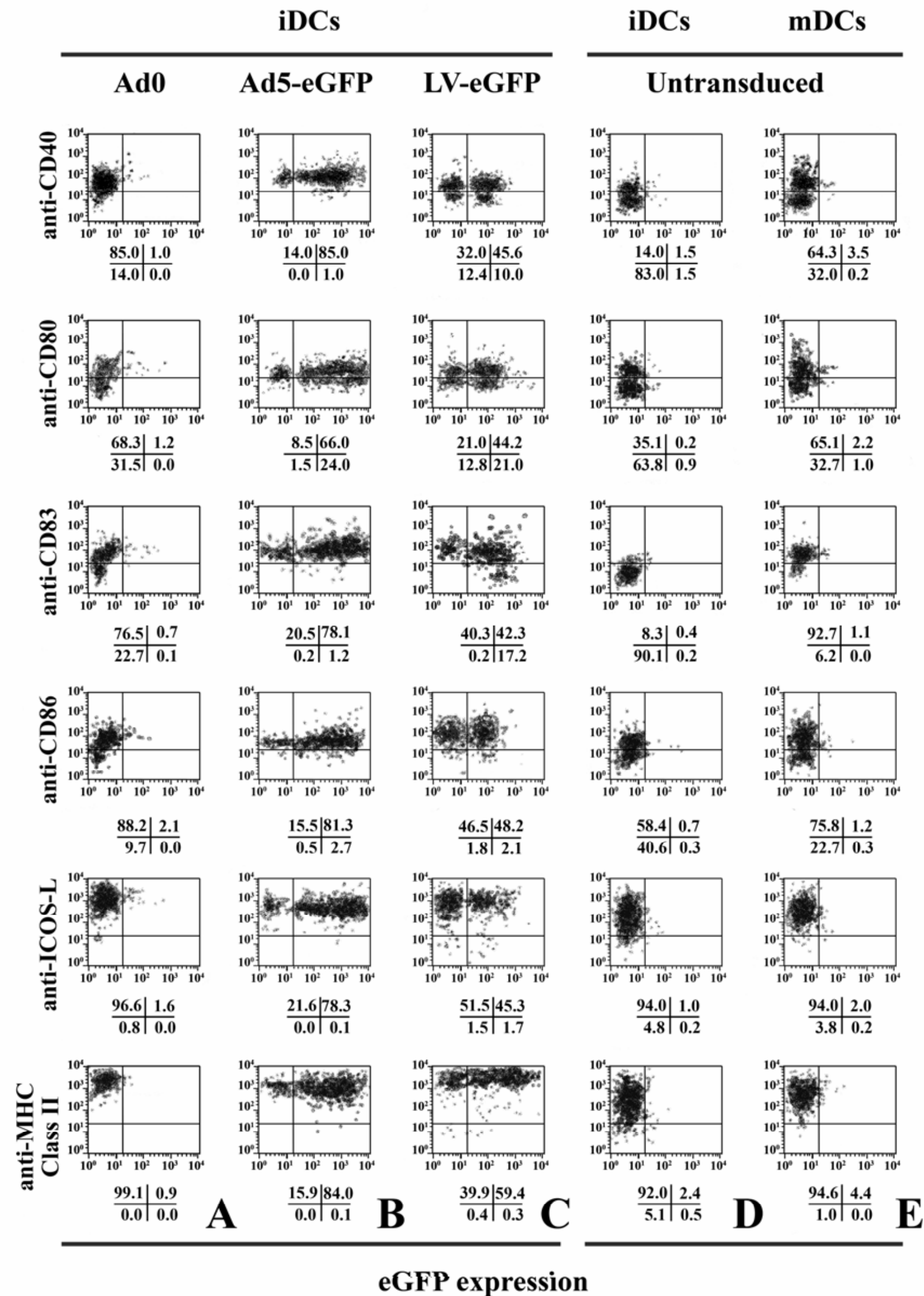

Fig. (2). Change in phenotype of iDCs following viral transduction. Following transduction of iDCs with Ad0 (*column* **A**; MOI 500), Ad5-eGFP (*column* **B**; MOI 500), or LV-GFP (*column* **C**; MOI 500), the phenotype of the cells was analyzed using 2-color flow cytometry for the expression of eGFP (x axis) and the surface marker indicated (y axis). As a control, untransfected cells were either unstimulated (iDCs, *column* **D**) or activated with 20 ng/mL LPS for 48 hours (mDCs, *column* **E**). The results shown are representative of 3 experiments. The percentage of cells in each quadrant of the flow cytometry profiles is shown in the diagram beneath each profile. The transduction of mDCs was not able to induce a significative modification of cellular immunophenotype (data not shown).

stimulators of the MLR, when transduced with Ad5 loose their immunostimulatory capabilities. This effect was statistically significant for an MOI of 100 and 500 ($p<0.05$). The effect of LV transduction on the antigen-presenting functions of iDCs and mDCs was less evident. On the other hand, there was an increase in the capacity of iDCs to stimulate an MLR (MOI=500, $p<0.05$), and a reduced one of mDCs (MOI=500, $p<0.05$).

Viral Vectors Increase the Ability of DCs to Stimulate Ag-specific T Cells

Because viral vectors seem to positively regulate the expression of co-stimulatory molecules, cytokine secretion and MLR-proliferation induction, the ability of these vectors to affect the antigen presenting capability of DCs to T cells was investigated. Thus, iDCs and mDCs were transduced, pulsed

Table 2. Analyses of Immunophenotypical Changes of iDCs after Viral Transduction. A Comparison with Some Literature Results is Shown

Immunophenotypical Changes of Transfected iDCs (MOI=)						
	CD40	**CD80**	**CD86**	**CD83**	**IcosL**	**MHC Class II**
Ad5	+	+	+	+	inv.	+
LV	+	+	+	+	inv.	+
Ref. [18]						
Ad5	+	+	+	+	±	±
LV	+	+	+	+	±	±
Ref. [28]						
Ad5	+	+	+	+	n.d.	±
Ref. [14 and 29]						
LV	n.d.	n.d.	+	n.d.	n.d.	n.d.
Ref. [30]						
LV	inv.	inv.	inv.	inv.	inv.	inv.

+: indicate an increasing of surface expression of the receptor analysed respect to the control (not tarsnduced) cells;
±: indicate a slight increasing of surface expression of the receptor analysed;
n.d.: not detected;
inv.: unchanged.

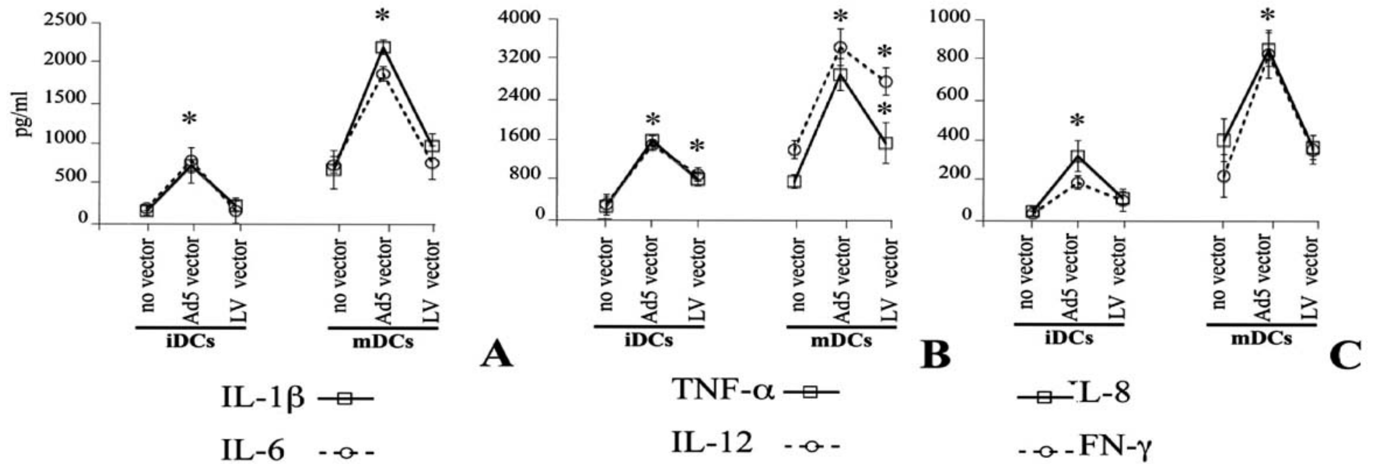

Fig. (3). Cytokine production following viral vector transduction. DCs that were either unstimulated (iDCs) or activated (mDCs) were transduced with Ad5 and LV viral vectors (MOI 500) on day 5 or left untransduced and the supernatants were collected on day 10. The levels of IL-1β, IL-6 (Panel **A**), TNF-α, IL-12 (Panel **B**), IL-8, and IFN-γ (Panel **C**) were determined using ELISA. The results are the mean of triplicate wells ± the standard deviation (SD). * indicates when $P<0.05$ when ANOVA analysis and simple Student *t* test were carried out to compare multiple means and 2-pairing means, respectively.

with recall antigens (*C.albicans* or PPD), and then added to purified autologous CD4$^+$ T cells stimulated as specified in Methods section. In the absence of antigen, as expected untransduced iDCs did not induce any significant proliferation of T cells, as did CD4$^+$ T cells alone. On the contrary, in the presence of recall antigens, Ad5-transduced iDCs showed a strong capability to induce antigen-specific T cell proliferation (Fig. **4B** and **4C**). Similar results were obtained when *C.albicans* or PPD was used as recall antigen (Fig. **4B** and **4C**). Of note, in the absence of antigens Ad5- and LV-transduced iDCs were still able to induce a (non-specific) proliferation of autologous T cells, showing a statistically significant induction of CD4$^+$ T cell proliferation (Fig. **4B** and **4C**) (Student's t test, p= 0.04 when the growth condition untrasduced iDCs was compared to Ad5-transduced iDCs in the absence of antigens, and p= 0.013 when compared to LV transduced iDCs in the absence of antigens). On the other hand, results obtained when untransduced or transduced mDCs were used displayed a different behaviour related to the amount of LV vector utilised (Fig. **4B** and **4C**). The

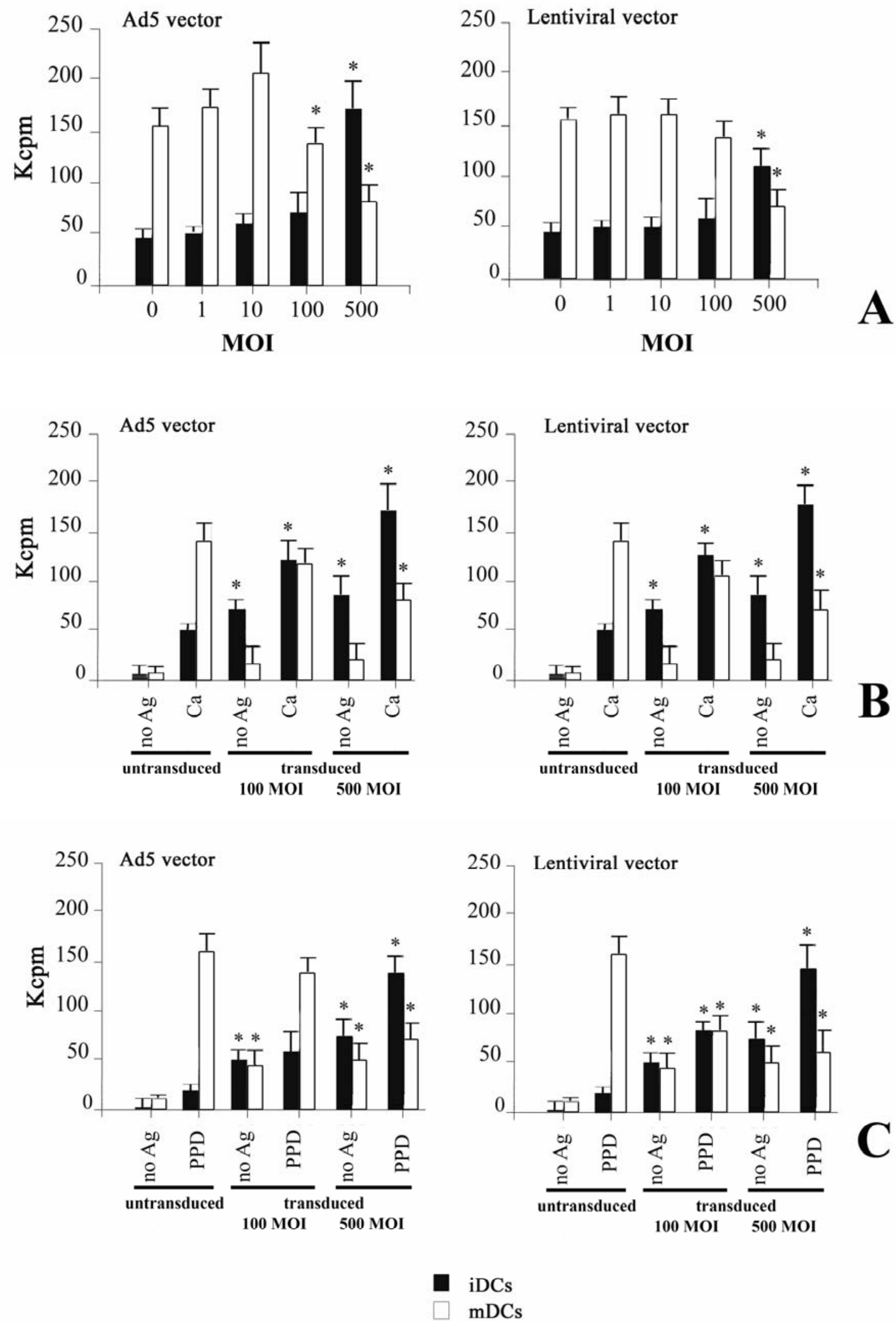

Fig. (4). Viral vectors are able to modify the ability of DCs in inducing T cell proliferation. Panel **A**. The functional consequence of viral transduction was tested in MLRs to evaluate the capacity of 10^4 DCs to stimulate 10^5 allogeneic T cells. DCs (either iDCs, or mDCs) were transduced with Ad5, or LV at the MOI indicated and then used as stimulators of allogeneic T cells in an MLR. ^{3}H-thymidine incorporation was analyzed on day 5. The results are shown as the mean ± SD. * indicates when $P<0.05$ when ANOVA analysis and simple Student *t* test were carried out to compare multiple means and 2-pairing means, respectively.
Panel **B** and **C**. The functional effect of viral transduction was also tested in an antigen-specific T cell proliferation assay. ^{3}H-thymidine incorporation was analyzed on day 5. The results are shown as the mean ± SD. * indicates when $P<0.05$ when ANOVA analysis and simple Student *t* test were carried out to compare multiple means and 2-pairing means, respectively.

proliferative response to recall antigens was slightly increased for MOI lower than 100 (data not shown). On the contrary, LV vectors at the highest MOI (100 and 500) utilized in these experiment demonstrated a reduced capability of mDCs to induce the proliferation of antigen-specific T lymphocytes when compared to untransduced recall antigen pulsed mDCs.

DISCUSSION

Given the central role of DCs in initiating and regulating both innate and adaptive immune responses, genetic manipulation of DCs is a potential therapeutic strategy. However, in designing these approaches it is important to know the effect of the gene therapy vector on the phenotype and function of the DCs. To this end, we have therefore compared the changes in phenotype and function of DCs following transduction with adenoviral and lentiviral vectors.

As reported previously, both Ad5 vectors and lentiviral vectors [17, 18, 20, 21] are efficient in the transduction of DCs, even if Ad5 vectors are somewhat more efficient.

In accordance to previous data [13, 18, 19, 22], we observed up-regulation of the expression of CD83, MHC class II, and costimulatory molecules by iDCs following transduction with Ad5 vectors. We also detected a modulation in the secretion of cytokines by both iDCs and mDCs. This is an interesting observation, as cytokines can play a role in regulating several aspects of immune and inflammatory responses. In addition, the effect of viral vectors on cytokine production is a feature of interest for the apparent contrast among published data. Some earlier reports suggested no change in cytokine production by DCs infected with adenovirus, [23, 24] but more recent studies have suggested up-regulation of many type-1 and pro-inflammatory cytokines in this system [18, 25]. Data from work in mice are also contradictory, with some reports suggesting IL-12 production post adenoviral infection [26]. However, others show production of IL-6, IL-15, IFN-γ and TNF-α in response to adenovirus infection but without IL-12 and IL-10 production [22, 27]. These differences could be related to small but critical differences in the techniques used to generate the DCs or in the timing of infection and of secretion. Of note, a similar up-regulation of Th1 cytokines was previously seen in murine DCs following Ad5 transduction [22]. This is a key finding as Th1 cytokines have important implications for the use of genetically modified DCs *in vivo*.

Similar, though less marked, iDC activation (in terms of cell-surface phenotype and cytokine secretion) was also seen following transduction with HIV-based lentiviral vectors. On the contrary, no increase in secretion of any of the cytokines tested was seen in mDCs following lentiviral transduction. Again, our data do not agree with an earlier report [22]; although it should be noted that changes in DCs phenotype were only seen at high MOIs [25].

The main pathway involved in the activation of iDCs to mDCs is that mediated through NF-kB [26]. However, there are several potential ways in which NF-kB can be activated. During an immune response to viral infection, DCs can be activated in response to dsRNA. This response is partially dependent on the cytosolic dsRNA-binding enzyme protein kinase R and does not require signaling through toll-like receptor 3 (TLR3), a surface receptor for dsRNA [11]. Alternatively, plasmacytoid DCs have been shown to respond to wild-type influenza virus by a pathway that requires endosomal recognition of single-stranded RNA virus through TLR7- and MyD88-mediated signalling [12].

Adenovirus-mediated activation and maturation of DCs was recently attributed to the high levels of TNF-α expression by murine bone marrow-derived DCs, comparable to levels observed with LPS exposure [28]. Adenovirus-induced TNF-α production was found to be necessary for DC maturation but was not dependent on the MyD88 signaling pathway. It was proposed that integrin-mediated PI3K induction of NFkB activates an autocrine TNF-α pathway required for DC maturation in response to Ad. While our observations with human DCs are consistent with this, in as much as we saw the high production of TNF-α following Ad5 infection, our data seem to indicate that activation of dsRNA-triggered antiviral pathways may also be important.

The phenotypic changes seen following viral transduction are reflected in functional alterations. Thus, as might be expected from the up-regulation of costimulatory molecules and MHC class II expression, iDCs transduced with Ad5 or LV vectors showed an increased ability to act as stimulators in an MLR. In contrast, and somewhat surprisingly, mDCs that have been transduced with both vectors show a reduced ability to stimulate an MLR. This has been reported previously [18, 20] and it has been suggested that this effect is due to viral immunodominance and/or the expression of immunomodulatory viral proteins [20]. In addition, it has been proposed that up-regulation of IDO observed during viral vectors transductions [18], may participate in feedback pathways that serve to regulate the induction of the immune response.

In conclusion, we have shown that transduction with viral vectors (Ad5 and LV), has dramatic consequences for DCs in terms of their phenotype, the activation pathways induced, and their function. Of particular interest in the context of vaccine design is the possibility to induce an antigen-independent T lymphocytes proliferative response by these vectors. This aspect could be advantageous in the context of tumors or aggressive pathogens, as these vectors could lead to initiate potent and durable immune responses. On the other hand, the decreased capability of transfected-mDCs to stimulate the antigen-specific proliferation of T cells could be considered in order to obtain a tolerogenic effect (specially with LV-vectors). Finally, these data could be relevant since vaccine development using DC functions through genetic manipulation may offer several applications for active immunotherapy of cancer and chronic infections.

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To Andrea Merlo, a very good friend.

REFERENCES

[1] Banchereau, J.; Briere, F.; Caux, C.; Davoust, J.; Lebeque, S.; Liu, Y.J.; Pulendran, B.; Palucka, K. Immunobiology of dendritic cells. *Annu. Rev. Immunol*., **2000**,*18*, 767-811.

[2] Lechler, R.; Ng, W.F.; Steinman, R.M. Dendritic cells in transplantation-friend or foe? *Immunity,* **2001**, *14*(4), 357-368.

[3] Thompson, A.G.; Thomas, R. Induction of immune tolerance by dendritic cells: implications for preventative and therapeutic

immunotherapy of autoimmune disease. *Immunol. Cell. Biol.,* **2002**, *80*(2), 509-519.
[4] Colonna, M.; Pulendran, B.; Iwasaki, A. Dendritic cells at the host-pathogen interface. *Nat. Immunol.,* **2006**, *7*(4), 117-120.
[5] Underhill, D.M.; Ozinsky, A.; Hajjar, A.M.; Stevens, A.; Wilson, C.B.; Bassetti, M.; Aderem, A. The toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. *Nature,* **1999**, *401*(6755), 811-815.
[6] Ardavin, C.; Amigorena, S.; Reis, C.; Sousa, C. Dendritic cells: immunobiology and cancer immunotherapy. *Immunity,* **2004**, *20*(1), 17-23.
[7] Grohmann, U.; Fallarino, F.; Puccetti, P. Tolerance, DCs and tryptophan: much ado about IDO. *Trends Immunol.,* **2003**, *24*(3), 242-248.
[8] Munn, D.H.; Zhou, M.; Attwood, J.T.; Bondarev, I.; Conway, S.J.; Marshall, B.; Brown, C.; Mellor, A.L. Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science,* **1998**, *281*(5380), 1191-1193.
[9] Grohmann, U.; Orabona, C.; Fallarino, F.; Vacca, C.; Calcinaro, F.; Falorni, A.; Candeloro, P.; Belladonna, M.L.; Bianchi, R.; Fioretti, M.C.; Puccetti, P. CTLA-4-Ig regulates tryptophan catabolism *in vivo*. *Nat. Immunol*., **2002**, *3*(11), 1097-1101.
[10] Fallarino, F.; Grohmann, U.; Hwang, K.W.; Orabona, C.; Vacca, C.; Bianchi, R.; Belladonna, M.L.; Fioretti, M.C.; Alegre, M.L.; Puccetti, P. Modulation of tryptophan catabolism by regulatory T cells. *Nat. Immunol.,* **2003**, *4*(12),1206-1212.
[11] Diebold, S.S.; Montoya, M.; Unger, H.; Alexopoulou, L.; Roy, P.; Haswell, L.E.; Al-Shamkhani, A.; Flavell, R.; Borrow, P.; Reis e Sousa, C. Viral infection switches non-plasmacytoid dendritic cells into high interferon producers. *Nature,* **2003**, *424*(6946), 324-328.
[12] Diebold, S.S.; Kaisho, T.; Hemmi, H.; Akira, S.; Reis, E.S.C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science,* **2004**, *303*(12), 1529-1531.
[13] Rea, D.; Schagen, F.H.; Hoeben, R.C.; Mehtali, M.; Havenga, M.J.; Toes, R.E.; Melief, C.J.; Offringa, R. Adenoviruses activate human dendritic cells without polarization toward a T-helper type 1-inducing subset. *J. Virol.,* **1999**, *73*(12), 10245-10253.
[14] Hirschowitz, E.A.; Weaver, J.D.; Hidalgo, G.E.; Doherty, D.E. Murine dendritic cells infected with adenovirus vectors show signs of activation. *Gene Ther*., **2000**, *7*(7), 1112-1120.
[15] Bett, A.J.; Haddara, W.; Prevec, L.; Graham, F.L. An efficient and flexible system for construction of adenovirus vectors with insertions or deletions in early regions 1 and 3. *Proc. Natl. Acad. Sci. USA,* **1994**, *91*(19), 8802-8806.
[16] Naldini, L.; Blomer, U.; Gallay, P.; Ory, D.; Mulligan, R.; Gage, F.H.; Verma, I.M.; Trono, D. *In vivo* gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science,* **1996**, *272*(5259), 263-267.
[17] Gruber, A.; Kan-Mitchell, J.; Kuhen, K.L.; Mukai, T.; Wong-Staal, F. Dendritic cells transduced by multiply deleted HIV-1 vectors exhibit normal phenotypes and functions and elicit an HIV-specific cytotoxic T-lymphocyte response *in vitro*. *Blood,* **2000**, *96*(4), 1327-1333.
[18] Tan, P.H.; Beutelspacher, S.C.; Xue, S.-A.; Wang, Y.H.; Mitchell, P.; McAlister, J.C.; Larkin, D.F.; McClure, M.O.; Stauss, H.J.; Ritter, M.A.; Lombardi, G.; Gorge, A.J. Modulation of human dendritic-cell function following transduction with viral vectors: implications for gene therapy. *Blood,* **2005**, *105*(10), 3824-3832.
[19] Miller, G.; Lahrs, S.; Pillarisetty, V.G.; Shah, A.B.; De-Matteo, R.P. Adenovirus infection enhances dendritic cell immunostimulatory properties and induces natural killer and T-cell-mediated tumor protection. *Cancer Res.,* **2002**, *62*(23), 5260-5266.
[20] Jonuleit, H.; Tuting, T.; Steitz, J.; Brück, J.; Giesecke, A.; Steinbrink, K.; Knop, J.; Enk, A.H. Efficient transduction of mature $CD83^+$ dendritic cells using recombinant adenovirus suppressed T cell stimulatory capacity. *Gene Ther*., **2000**, *7*(3), 249-254.
[21] Negre, D.; Mangeot, P.E.; Duisit, G.; Blanchard, S.; Vidalain, P.O.; Leissner, P.; Winter, A.J.; Rabourdin-Combe, C.; Mestali, M.; Moullier, P.; Darlix, J.L.; Cosset, F.L. Characterization of novel safe lentiviral vectors derived from simian immunodeficiency virus (SIVmac251) that efficiently transduce mature human dendritic cells. *Gene Ther*., **2000**, *7*(19), 1613-1623.
[22] Morelli, A.E.; Larregina, A.T.; Ganster, R.W.; Zahorchak, A.F.; Plowey, J.M.; Takayama, T.; Logar, A.J.; Robbins, P.D.; Falo, L.D.; Thomson, A.W. Recombinant adenovirus induces maturation of dendritic cells *via* an NF-kappaB-dependent pathway. *J. Virol*., **2000**, *74*(20), 9617-9628.
[23] Rea, D.; Laface, D.; Hutchins, B.; Kwappenberg, K.; Melief, C.J.; Hoeben, R.C.; Offringa, R. Recombinant adenovirus-transduced human dendritic cells engineered to secrete interleukin-10 (IL-10) suppress Th1-type responses while selectively activating IL-10-producing $CD4^+$-T cells. *Hum. Immunol.,* **2004**, *65*(18), 1344-1355.
[24] Newton, K.R.; Sala-Soriano, E.; Varsani, H.; Stephenson, J.R.; Goldblatt, D.; Wedderburn, L.R. Human dendritic cells infected with an adenoviral vector suppress proliferation of autologous and allogeneic T cells. *Immunology,* **2008**, *125*(8), 469-479.
[25] Miller, G.; Lahrs, S.; Pillarisetty, V.G.; Shah, A.B.; DeMatteo, R.P. Adenovirus infection enhances dendritic cell immunostimulatory properties and induces natural killer and T-cell-mediated tumor protection. *Cancer Res.,* **2002**, *62*, 5260-5266.
[26] Philpott, N.J.; Nociari, M.; Elkon, K.B.; Falck-Pedersen, E. Adenovirus-induced maturation of dendritic cells through a PI3 kinase-mediated TNF-alpha induction pathway. *Proc. Natl. Acad. Sci. USA,* **2004**, *101*(16), 6200-6205.
[27] Andreakos, E.; Smith, C.; Monaco, C.; Brennan, F.M.; Foxwell, B.M.; Feldmann, M. Ikappa B kinase 2 but not NF-kappa B-inducing kinase is essential for effective DC antigen presentation in the allogeneic mixed lymphocyte reaction. *Blood,* **2003**, *101*(3), 983-991.
[28] Perreau, M.; Mennechet, F.; Sterratrice, N.; Glasgow, J.N.; Curiel, D.T.; Wodrich, H.; Kremer, E.J. Contrasting effects of human, canine, and hybrid adenovirus vectors on the phenotypical and functional maturation of human dendritic cells: implications for clinical efficacy. *J. Virol.,* **2007**, *81*(8), 3272-3284.
[29] Yang, L.; Yang, H.; Rideout, K.; Cho, T.; Joo, K.I.; Ziegler, L.; Elliot, A.; Walls, A.; Yu, D.; Baltimore, D.; Wang, P. Engineered lentivector targeting of dendritic cells for *in vivo* immunization. *Nat. Biotechnol*., **2008**, *26*(3), 326-334.
[30] Chen, X.; He, J.; Chang, L-J. Alteration of T cell immunity by lentiviral transduction of human monocyte-derived dendritic cells. *Open Retrovirol.,* **2004**, *1*, 37.

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