

# Comparison Between Sendai Virus and Adenovirus Vectors to Transduce HIV-1 Genes Into Human Dendritic Cells

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Immuno-genotherapy using dendritic cells (DCs) can be applied to human immunodeficiency virus type 1 (HIV-1) infection. Sendai virus (SeV) has unique features such as cytoplasmic replication and high protein expression as a vector for genetic manipulation. In this study, we compared the efficiency of inducing green fluorescent protein (GFP) and HIV-1 gene expression in human monocyte-derived DCs between SeV and adenovirus (AdV). Human monocyte-derived DCs infected with SeV showed the maximum gene expression 24 hr after infection at a multiplicity of infection (MOI) of 2. Although SeV vector showed higher cytopathic effect on DCs than AdV, SeV vector induced maximum gene expression earlier and at much lower MOI. In terms of cell surface phenotype, both SeV and AdV vectors induced DC maturation. DCs infected with SeV as well as AdV elicited HIV-1 specific T-cell responses detected by interferon  $\gamma$  (IFN- $\gamma$ ) enzyme-linked immunospot (Elispot). Our data suggest that SeV could be one of the reliable vectors for immuno-genotherapy for HIV-1 infected patients. **J. Med. Virol.** 80:373–382, 2008. © 2008 Wiley-Liss, Inc.

**KEY WORDS:** viral vector; immuno-genotherapy; AIDS

## INTRODUCTION

Introduction of highly active anti-retroviral therapy (HAART) has improved the clinical course of patients infected with human immunodeficiency virus type 1 (HIV-1) dramatically. However, there are many obstacles to the long-term administration of anti-retroviral drugs, such as metabolic disorders, emergence of drug resistant viruses, and high medical expenses. The combination of therapeutic vaccines and HAART could not only reduce the adverse effects of HAART but also decrease the medical expenses especially in developing countries.

Cellular immune responses play a crucial role in controlling the replication of HIV-1 [Yang et al., 1997;

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Matano et al., 1998; Brander and Walker, 1999; Jin et al., 1999; McMichael and Rowland-Jones, 2001]. One of the possible strategies for treating HIV-1 infection is to enhance the cellular anti-viral capacity. Since dendritic cells (DCs) have high ability of antigen presentation, they have been used as stimulators of T-cell responses by inserting HIV-1 antigens [Engelmayer et al., 2001; Stubbs et al., 2001; Tsunetsugu-Yokota et al., 2003; Mwau et al., 2004].

Sendai virus (SeV), one of the members of *Paramyxoviridae*, is an enveloped virus with a nonsegmented negative-strand RNA genome. It causes severe respiratory disease in mice but is nonpathogenic for humans. SeV vector has been developed and shown to have high gene transduction efficiency and protein expression in different cell lineages [Kato et al., 1996; Kawana-Tachikawa et al., 2002]. In addition, the cytoplasmic replication of SeV precludes the integration of its genetic information into cellular genome. Recently, the second generation SeV vector deficient in F gene (dF-SeV) has been developed [Li et al., 2000]. The dF-SeV has been proved not to cause secondary infection. SeV and dF-SeV vectors could be promising systems to introduce HIV-1 genes into DCs for stimulating HIV-1 specific T-cell responses in primates and humans [Kano et al., 2002; Takeda et al., 2003; Kato et al., 2005].

Adenovirus (AdV), vaccinia virus, and retrovirus have been used as viral vectors for gene transfer into DCs [Engelmayer et al., 1999; Rea et al., 1999; Bonini et al., 2001; Rouas et al., 2002]. In this study, we compared the efficiency of inducing green fluorescent protein (GFP) and HIV-1 gene expression in human monocyte-derived DCs between AdV vectors and new viral vectors based on SeV. We showed that SeV vector transduced DCs efficiently and elicited HIV-1 specific T-cell responses. Our results suggest the potential use of SeV vector for immuno-gene therapy for HIV-1 infected patients.

## MATERIALS AND METHODS

### Viral Vectors

SeV carrying GFP (SeVGFP) [Agungpriyono et al., 2000], HIV-1 NL4-3 Gag (SeVGag), and HIV-1 SF2 Env (SeVEnv) [Yu et al., 1997] were propagated in 10-day-old embryonated chicken eggs. The fusion protein (F)-defective SeV (dF-SeV) [Li et al., 2000] was propagated in the monkey kidney cell line expressing Sendai virus F gene product (LLC-MK2/F7) [Li et al., 2000] because the replication capacity of dF-SeV was incompetent. SeV particles were purified by 50% sucrose (w/v)/10 mM Tris-HCl and 30% sucrose (w/v)/10 mM Tris-HCl density centrifugation, dialyzed against Dulbecco's phosphate buffered saline (PBS) (Sigma, St. Louis, MO), and stored at  $-80^{\circ}\text{C}$ . The titers of the vector stocks, determined on LLC-MK2 [Kato et al., 1996], were as follows; SeVGag:  $5.2 \times 10^8$  CIU/ml, SeVEnv:  $6.4 \times 10^7$  CIU/ml, SeVGFP:  $5.2 \times 10^8$  CIU/ml, GFP/dF-SeV:  $3.1 \times 10^9$  CIU/ml, SeV without inserts:  $5.2 \times 10^8$  CIU/ml.

Recombinant AdV used in this experiment was derived from AdV type 5 and was replication-deficient

with deletion of E1 and E3 genes. The AdV carrying HIV-1 genes (*gag-pol*, *env*, *rev*, and RRE) was generated with AdV Expression Kit (TakaraBio, Shiga, Japan) according to the manufacturer's protocol. Each HIV-1 gene was inserted into the expression cassette of pAxCawt cosmid vector equipped with CAG promoter and rabbit beta-globin polyadenylation signal. All HIV-1 sequences were derived from SF2 strain [Levy et al., 1986]. 5' half and 3' half of *EcoRI* fragments from SF2 provirus were subcloned pUC19, generating pUC19-9B/R7 and pUC19-9B/R6, respectively. HIV-1 *gag-pol* gene was obtained from pUC19-9B/R7 after deleting the sequence upstream of *gag*. *NarI* site was introduced next to *gag* initiation codon by PCR using primers *gag/NarI-S* (5'-CAGGCGGCAAGGAGAGAGATGGGTGC-GAG-3') and *gag/ApaI-AS* (5'-CCTTTTTCTCTAGGGG-CCCTGC-3') (restriction sites are underlined). PCR-amplified fragment was returned to *NarI* and *ApaI*-digested pUC19-9B/R7, generating pUC19-GP. The 4.5 kb *NarI-NdeI* fragment containing the HIV-1 *gag-pol* gene was inserted into the *SwaI* site of pAxCawt cosmid vector to create AdVGP. HIV-1 *rev* responsible element (RRE) was generated from pUC19-9B/R6 by PCR using primers *rev/pfMI-S* (5'-GCCATAGAATG-GCCAAGGCAAAGAGAAGAGTGG-3') and *rev/BamHI-AS* (5'-GGGATCCCAAGGCACAGCAGTGGTTGC-3'). The PCR fragment was inserted between *pfMI* and *BamHI* site of pUC19-GP, and thus placed downstream of *gag-pol* gene. The consequent 4.9 kb *NarI-BamHI* fragment containing the HIV-1 *gag-pol*-RRE sequences was inserted into the *SwaI* site of pAxCawt cosmid vector to create AdVGPR. HIV-1 *rev* gene was made by two-step PCR from *StuI-XhoI* fragment of pUC19-9B/R6. The 1st-PCR primer set was *rev/1st-S* (5'-CTCAGGACAGTCAGACTCATCAAGCTTCTCTATCAAAG-CAACCCGCTCC-3') and *rev-AS* (5'-GGCTATTCTT-TAGTTCTGAAATCCAATACTGCA-3'), and the 2nd-PCR primer set was *rev/2nd-S* (5'-GGATGGCAGGAA-GAAGCGGAGACAGCGACGAAGAGCTCCTCAGGA-CAG-3') and *rev-AS*. The PCR fragment was digested with *SphI* and *SpeI*, and inserted into the *SwaI* site of pAxCawt to create AdVRev. The absence of PCR errors was confirmed for all PCR-amplified fragments by sequencing. The 2.1 kb *MluI-XhoI* fragment of pUC19-9B/R6 containing the HIV-1 *env* gene was inserted into the *SwaI* site of pAxCawt cosmid vector to create AdVEnv. Recombinant AdV, Ax1w1 [Miyake et al., 1996] bearing no insert and AdVGFP [Miyake et al., 1996] expressing GFP, were kindly provided by Dr. Izumu Saito and Yumi Kanegae (University of Tokyo, Tokyo, Japan). AdV was propagated in 293 cells [Graham et al., 1977], purified by two rounds of CsCl density centrifugation [Kanegae et al., 1994], dialyzed against PBS containing 10% glycerol and stored at  $-80^{\circ}\text{C}$ . The titer of the viral stocks was determined by a plaque-forming assay on 293 cells. The titers of each virus were as follows; AdVGPR:  $7.6 \times 10^9$  pfu/ml, AdVEnv:  $1.6 \times 10^{11}$  pfu/ml, AdVRev:  $2.7 \times 10^{11}$  pfu/ml, AdVGFP:  $5.9 \times 10^{10}$  pfu/ml, Ax1w1:  $4.7 \times 10^{11}$  pfu/ml. Vector stocks were tested for the

absence of replication-competent AdV as described [Ishii-Watabe et al., 2003].

### Cell Lines and Media

Monkey kidney cell line (LLC-MK2) was cultured in minimal essential medium (MEM) (Sigma) supplemented with 100 U of penicillin/ml, 100 U of streptomycin/ml, and 10% heat-inactivated fetal calf serum (FCS). Two hundred ninety three cells were cultured in Dulbecco's modified eagle medium (DMEM) (Sigma) supplemented with 100 U of penicillin/ml, 100 U of streptomycin/ml, and 10% FCS.

### Generation of DCs

Immature myeloid DCs were generated from human peripheral blood mononuclear cells (PBMCs) using previously described methods [Nagayama et al., 2003]. Briefly, PBMCs of healthy adult volunteers were collected in heparinized tubes, subjected to density centrifugation over Ficoll-Paque Plus (Amersham Pharmacia Biotech, Piscataway, NJ), and washed twice with PBS. These PBMCs were plated on 10 cm PRIMARIA tissue culture dish (Becton Dickinson Labware, Franklin Lakes, NJ) and kept at 37°C for 30 min to remove nonadherent cells. Floating cells were removed gently by rinsing with 10 ml of PBS three times and the remaining adherent cells were cultured overnight in 6 ml of RPMI 1640 medium at 37°C. Cells were washed three times again on the next day with 10 ml of PBS and the remaining adherent cells were cultured for 7 days in DC medium [6 ml of RPMI 1640 supplemented with 100 U of penicillin/ml, 10 mg of streptomycin/ml, and 10% FCS, 300 ng of recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) (Wako, Osaka, Japan), and 300 ng of recombinant human interleukin-4 (rhIL-4) (Wako)]. After 7 days, DCs were collected with a scraper. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (Wako) was added to the DC medium at a final concentration of 50 ng/ml on day 7 in some experiments and cultured for another 2 days to generate mature DCs. The purity of DCs was >95% based on the expression of CD1a and CD11c and lack of expression of T-cell, B-cell, NK-cell, and monocyte lineage markers by flow cytometry (data not shown).

### Infection of DCs With Viral Vectors

$5 \times 10^5$  DCs were infected with SeV or AdV vectors for 1 hr at 37°C in a final volume of at least 500  $\mu$ l of serum-free RPMI 1640. After the infection, DCs were washed with serum-free RPMI 1640 medium and cultured in 24 well plates with 1 ml of the DC medium.

### SDS-PAGE and Western Blot Analysis

DCs infected with SeV (SeVGag or SeVEnv) at a multiplicity of infection (MOI) of 2 or with AdV (AdVGFR, AdVEnv, AdVRev) at an MOI of 1,000 were harvested after 24 or 48 hr of infection, respectively. After washing with PBS, the cells were resuspended in

RIPA buffer (10 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1% NP-40, 0.1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 1 mM EDTA, complete mini as 1 $\times$  protease inhibitor cocktail [Roche, Basel, Switzerland]), and kept on ice for 10 min. The suspension was spun for 5 min at 9,000g to remove cell debris. The amount of protein in the cell lysate was determined by protein assay kit (Bio-Rad Laboratories, Hercules, CA). Cell lysates containing 30  $\mu$ g of cellular protein were loaded onto a 10–20% Ready Gels J (Bio-Rad Laboratories) and electrophoretically transferred to immobilon polyvinylidene difluoride transfer membrane (Millipore, Billerica, MA). Western blot analysis was performed using Lumi-Light plus Western Blotting Kit (Roche) according to the manufacturer's instructions. Briefly, the membrane was blocked in 1% blocking solution at 4°C overnight. The membrane was incubated with mouse monoclonal antibodies against p24 (Advanced Biotechnologies, Inc., Columbia, MD) and gp120 (Immuno Diagnostics, Inc., Woburn, MA) of HIV-1 for 1 hr. The blots were then washed four times with 1 $\times$  TBST and incubated with anti-mouse IgG conjugated with horseradish peroxidase (Roche). Proteins were illuminated by Lumilight Plus (Roche) and detected with Lumi Imager (Roche). Quantification was done by densitometric analysis with the Lumi Analyst software (Roche).

### Immunostaining and Flow Cytometry

GFP expression and viability of DCs infected with SeVGFP, dF-SeVGFP, or AdVGFP were analyzed by flow cytometry. To determine the viability,  $5 \times 10^5$  DCs in about 300  $\mu$ l of media were stained with 10  $\mu$ l of propidium iodide (PI) (50  $\mu$ g/ml; SIGMA). Events were acquired on a FACS-Caliber (Becton Dickinson) and analyzed with CellQuest software (Becton Dickinson) and Flow Jo software version 4.1 (Tree Star, Asland, OK).

To determine the effects of transduction on the expression of DC surface marker, immature DCs (imDCs) were cultured with the DC medium for 48 hr, mature DCs (mDCs) were cultured with the DC medium plus TNF- $\alpha$  for 48 hr. DCs transduced with SeV or AdV were cultured with the DC medium for 24 or 48 hr, respectively. Those DCs were stained with antibodies at 4°C for 20 min and then washed three times with PBS. Those cells were analyzed by flow cytometry after fixing 1% paraformaldehyde. The antibodies we used were as follows: fluorescein isothiocyanate-anti-Lineage (Lin-FITC) (CD3, CD14, CD16, CD19, CD20, CD56) (Becton Dickinson), phycoerythrin (PE)-anti-CD1a (Immunotech, Marseilles, France), PE-anti-CD83 (Immunotech), PE-anti-HLA-ABC (Dako), Peridinin chlorophyll protein (PerCP) -anti-CD4 (Becton Dickinson), PerCP-anti-HLA-DR (Becton Dickinson), allophycocyanin (APC)-anti-CD14 (Immunotech), APC-anti-CD40 (PharMingen), APC-anti-CD11c (PharMingen), Biotin-conjugated anti-CD86 (Becton Dickinson). Streptavidin-FITC (Becton

Dickinson) was employed as secondary reagents. FITC- (PharMingen, San Diego, CA), PE- (Dako Glostrup, Denmark), Per CP- (Becton Dickinson), APC- (Becton Dickinson) conjugated species- and isotype-matched, mAbs were used to determine the level of background staining.

### Elispot Assay

We performed enzyme-linked immunospot (Elispot) assay to know the efficiency of HIV-1 specific T-cell induction by DCs infected with AdV or SeV vector. First, we developed mDCs as described above from PBMCs of two HIV-1-infected patients. On day 7, we infected those mDCs with AdV vector at an MOI of 1,000 or SeV vector at an MOI of 2 for 1 hr, or just added overlapping peptides (*gag*, *env*). We used the overlapping peptides derived from consensus B sequence since both patients were infected with subtype B HIV-1. We did not check the AdV sero-status of these two patients. Both patients were on HAART and have undetectable viral load (<50 copies/ml). CD4 counts of patients 1 and 2 are

408/ $\mu$ l and 336/ $\mu$ l, respectively. We used those mDCs as stimulators in Elispot assay. PBMCs from each patient were used as effectors cells. The protocol of Elispot assay was described previously [Furutsuki et al., 2004].

## RESULTS

### Sendai Viral Vectors Transduce DCs at Lower MOIs Than Adenoviral Vectors

We infected imDCs with SeVGFP, dF-SeVGFP or AdVGFP at different MOIs (Fig. 1) in order to know which MOI is the best for these three vectors. We stained these cells with PI to evaluate the expression of GFP in viable cells. In SeVGFP, the expression of GFP reached the maximum (32.5%) at an MOI of 2 and the mean fluorescent intensity (MFI) of GFP showed around 1,400 at all MOIs. However, as shown by the fraction of PI-positive cells, SeVGFP killed around 30% of DCs even at an MOI of 0.5. The staining pattern of DCs infected with dF-SeVGFP was similar to that of SeVGFP. In AdVGFP, both the percentage of GFP-positive cells and the MFI of

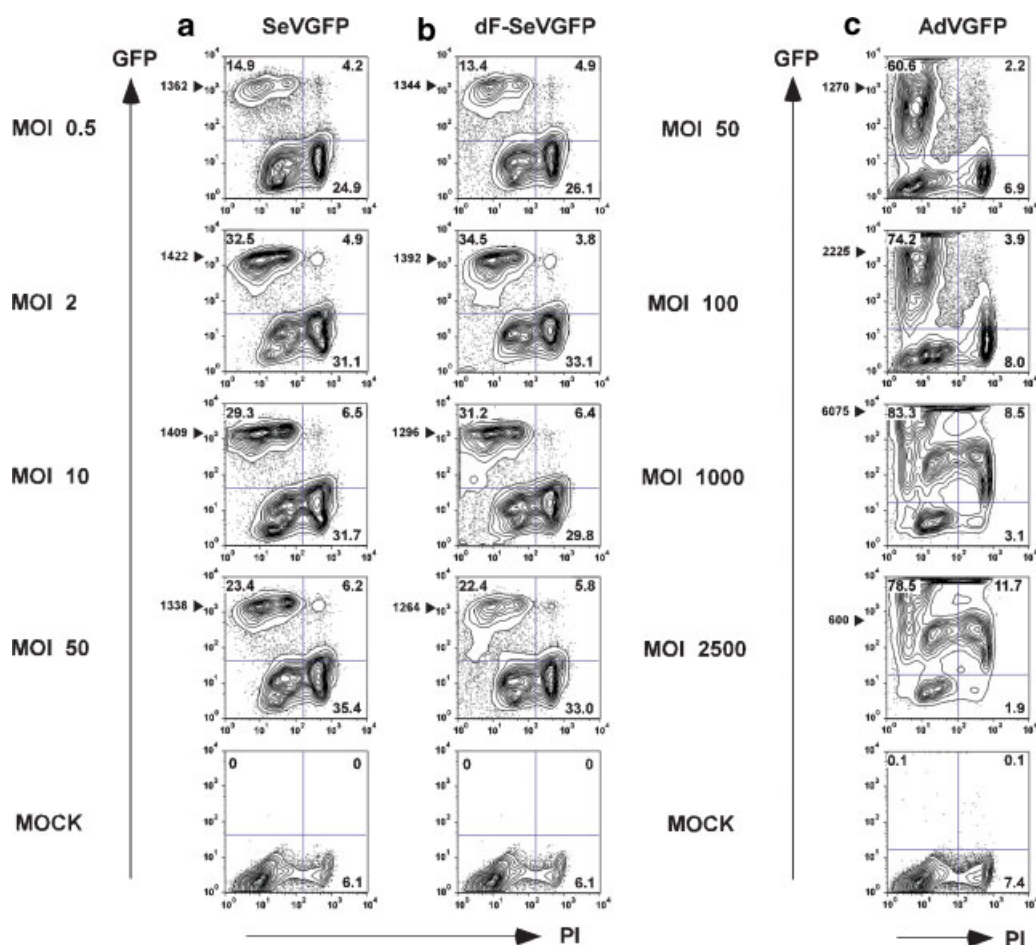


Fig. 1. Comparison of different MOIs for the maximum gene expression by SeV (a), dF-SeV (b), and AdV (c) vectors 48 hr after infection. Cell viability was determined by staining with PI. GFP expression and PI staining were analyzed by flow cytometry. The percentages of GFP- and PI-positive cells are shown on each corner. Arrowheads indicate MFI of GFP-positive cells within PI-negative fraction. The numbers in each panel represent the mean value of three independent experiments.

GFP increased up to an MOI of 1,000. Although PI-positive cells in AdV increased according to MOIs, the percentage of PI-positive cells was less than 14% even at the highest MOI: 2,500. From these results, SeV vector is likely to transduce DCs at much lower MOIs than AdV vector, but kill more DCs than AdV. We chose an MOI of 2 for SeVGFP and dF-SeVGFP, and an MOI of 1,000 for AdVGFP in the subsequent experiments.

**Sendai Viral Vectors Showed Maximum Transduction Level Earlier Than Adenoviral Vectors**

We next examined the time course of GFP expression (Fig. 2). We detected GFP-positive cells as early as 8 hr after infection in all three vectors. The proportion of GFP-positive cells reached the maximum level (around

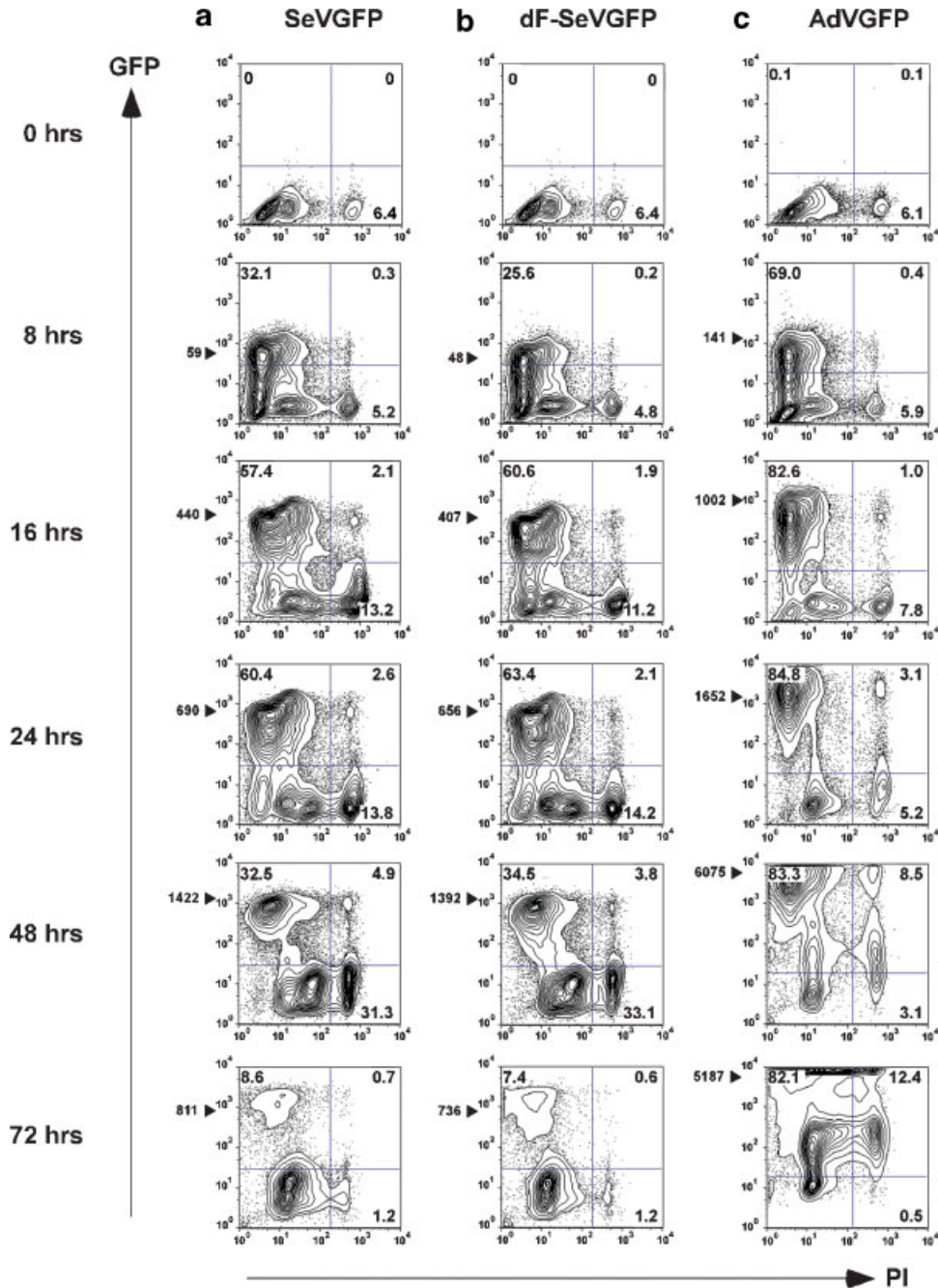


Fig. 2. The time course of the maximum gene expression by SeV (a), dF-SeV (b), and AdV (c) vectors. DCs were infected with SeV and dF-SeV at an MOI of 2 and were infected with AdV at MOI of 1,000 and then cultured for 8–72 hr. Cell viability was determined by staining with PI. GFP expression and PI staining were analyzed by flow cytometry. The percentages of GFP- and PI-positive cells are shown on each corner. Arrowheads indicate MFI of GFP-positive cells within PI-negative fraction. The numbers in each panel represent the mean value of three independent experiments.

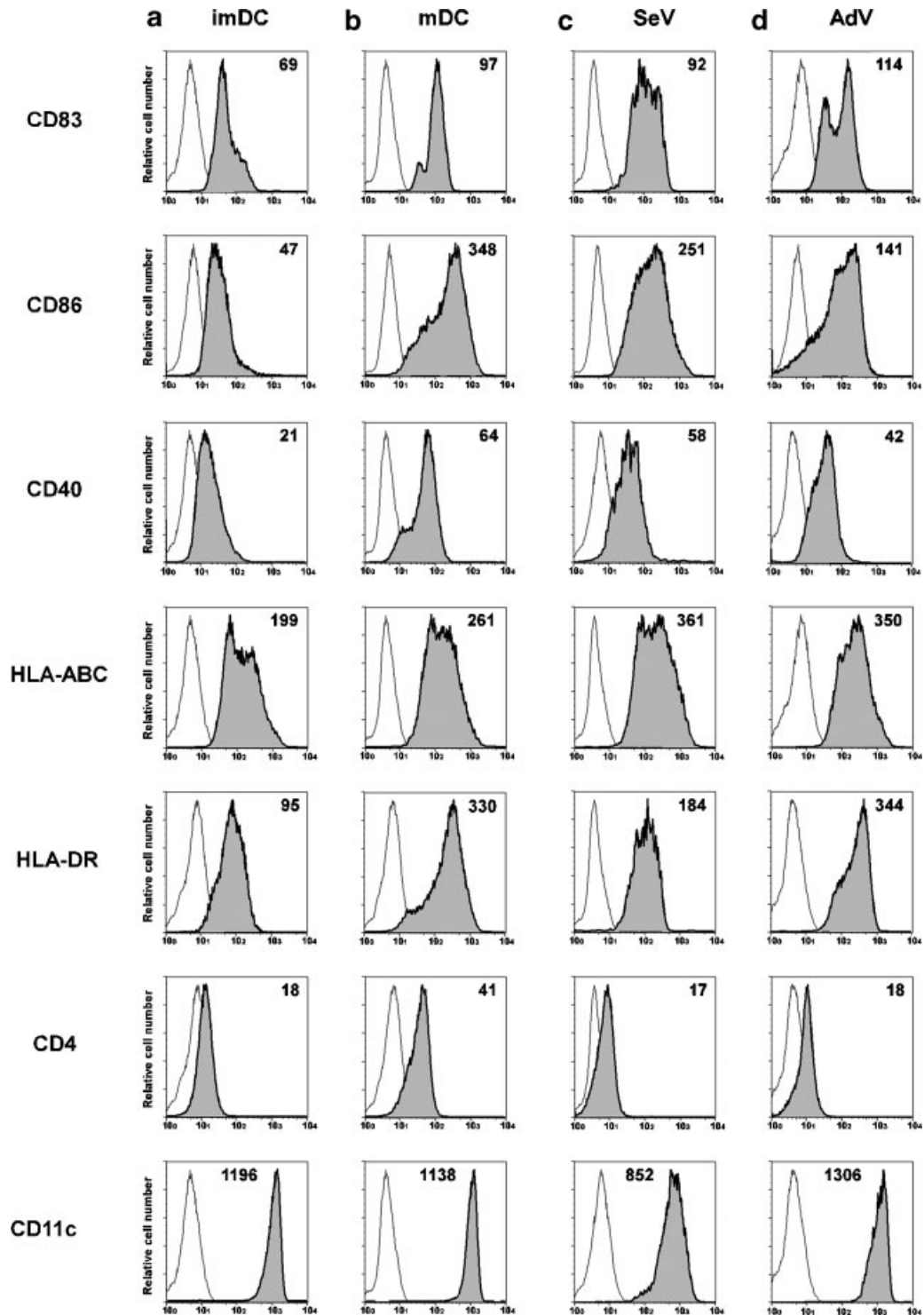


Fig. 3. Infection of DCs with SeV and AdV vectors modified the expression of cell surface markers. DCs infected with SeV vector containing no inserts at an MOI of 2 (c) and AdV vector containing no inserts at an MOI of 1,000 (d) were maintained in the DC medium for 48 hr (AdV vector) and 24 hr (SeV vector). DCs were incubated with (b) or without (a) TNF- $\alpha$  for 48 hr, respectively. These DCs were analyzed by flow cytometry with FITC, PE, PerCP, APC-conjugated

antibodies for expression of CD86, CD83, HLA-ABC, HLA-DR, CD4, CD40, CD11c. The open profiles represent isotype-matched mAb controls. MFIs are indicated on the right corner in each panel. The background values of all experiments were less than 15. The numbers in each panel represent the mean value of three independent experiments.

60%) 24 hr after infection with SeVGFP or dF-SeVGFP. The proportion of GFP-positive cells decreased to around 30% at 48 hr, although the MFI of GFP showed the maximum at 48 hr. AdVGFP, on the other hand, showed the maximum level of both GFP-positive cells and MFI of GFP 48 hr after infection.

### Sendai and Adenoviral Vectors Changed Phenotype of imDCs Following Viral Transduction

In order to determine the effect of transduction on imDCs with these vectors, we examined the surface markers of cells after transduction. The phenotype of imDCs and mDCs are shown in Figure 3a,b, respectively. We infected imDCs with SeV or AdV vectors and cultured them for 24 or 48 hr, respectively (Fig. 3c,d). As compared with the phenotype of uninfected imDCs, DCs infected by SeV and AdV vectors showed up-regulation of a maturation marker CD83, the major histocompatibility complex (MHC) classes I and II molecules (HLA-ABC and HLA-DR), and costimulatory molecules CD40 and CD86. Incubation of DCs in medium and buffers used to prepare vectors did not affect the phenotype of the cells (data not shown). These results indicate that SeV as well as AdV vector infection induced DC maturation in terms of cell surface phenotype.

### Both SeV and AdV Vectors Elicited HIV-1 Specific T-Cell Responses

To evaluate protein expressions, we developed five viral vectors carrying HIV-1 structural proteins (Fig. 4a). We infected DCs with these vectors under the optimal conditions we concluded from the results shown above. Gp120 expression by SeV vector was 3.8 times higher than that by AdV vector (Fig. 4b, compare lanes 3–7). Since 3.2 and 5.0 kb are the maximum gene sizes for SeV and AdV vector, respectively [Sakai et al., 1999; the manufacturer’s protocol of AdV Expression Kit], we inserted HIV-1 *gag* gene (about 1.5 kb) in SeV and *gag-pol* gene (about 4.9 kb) in AdV vector. Both *cis*-acting RRE sequence and *trans*-acting Rev protein were necessary for Gag protein expression by AdV vector (Fig. 4b, compare lanes 1–2). Rev expression is not required for SeV-mediated Gag or Env expression

because SeV replicates in the cytoplasm. In the presence of Rev protein, AdV vector expressed similar levels of Gag protein to SeV vector (Fig. 4b, compare lanes 1–6). Although SeV Gag did not have HIV-1 protease sequence, a band was detected near the size of p24. It was not a nonspecific band derived from SeV because we could not detect the band with other SeV constructs, such as SeV Env (data not shown). Gag might be processed by some proteins of SeV.

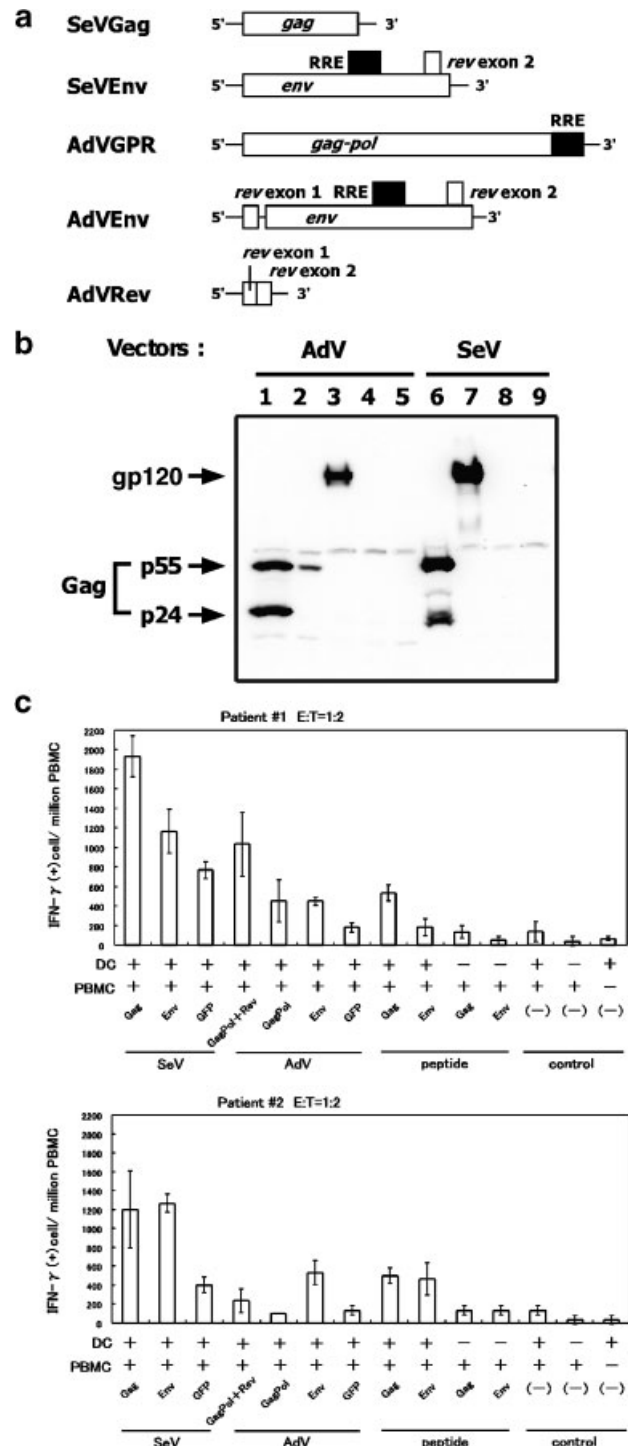


Fig. 4. **a**: Schematic structures of five viral vectors. *gag*, HIV-1 gag gene; *env*, HIV-1 env gene; *gag-pol*, HIV-1 gag and pol genes; *rev*, HIV-1 rev gene; RRE, HIV-1 RRE; GFP, green fluorescent protein. **b**: The expression of HIV-1 structural proteins by AdV and SeV vectors. DCs infected with AdV vector at an MOI of 1,000 and SeV vector at an MOI of 2 were harvested for 48 or 24 hr after infection, respectively. Thirty microgram of lysate was subjected to immunoblot analysis using anti-Gag p24 or anti-Env gp120 mAbs. The other two independent experiments showed similar results. Lane 1, coinfection with AdVGPR and AdVRev; Lane 2, AdVGPR without AdVRev infection; Lane 3, AdVEnv; Lane 4, AdV without inserts; Lane 5, mock; Lane 6, SeVGag; Lane 7, SeVEnv; Lane 8, SeV without inserts; Lane 9, mock. Arrows on the left indicate positions of gp120, Gag p55, and p24. **c**: The results of IFN- $\gamma$  ELISPOT assays in two HIV-1 infected patients. Autologous DCs infected with SeV vector or AdV vector, or just added overlapping peptides (*gag*, *env*) were used as stimulators. PBMCs from the same patients were used as effectors cells. Results are shown as mean  $\pm$  SEM of three independent assays.

After developing mDCs from frozen PBMCs of two HIV-1 infected patients, we infected these mDCs with SeV or AdV vector and used them as stimulators for interferon  $\gamma$  (IFN- $\gamma$ ) Elispot. Both SeV and AdV vectors elicited HIV-1 specific T-cell responses, although some nonspecific responses were also detected (Fig. 4c).

## DISCUSSION

DCs are efficient antigen presenting cells that are critical for induction of primary T-cell responses. At present the most useful method for genetic manipulation of DCs is to use viral vectors. As reported previously, AdV vector is efficient at the transduction of DCs [Tan et al., 2005]. SeV is also one of the reliable vectors for immunotherapy and has several unique features, such as cytoplasmic localized replication cycle and brief contact time for cellular uptake. In this study, we analyzed the capacity of SeV as a vector in terms of transducing GFP and HIV-1 genes into human DCs. We showed that SeV vector transduced GFP genes efficiently into monocyte-derived imDCs. DCs infected with SeV and dF-SeV vectors expressed high amount of GFP gene 24 hr after infection at an MOI of 2 (Fig. 2a,b). The expression level of HIV-1 structural gene, *env*, by SeV vector was higher than that by AdV. These results proved the high ability of gene expression by SeV. However, the proportion of GFP positive cells did not increase according to MOI. About 30% of cells were still GFP-negative 48 hr after infection even at an MOI of 50 (Fig. 1a,b). This could be caused by the disruption of sialic acid which is the receptor for SeV.

Both SeV and dF-SeV vectors killed nearly 30% of target DCs at the lowest MOI: 0.5. One of the reasons for this phenomenon is likely to be apoptosis. Several studies reported that SeV is able to induce apoptosis in viral host cells [Tropea et al., 1995; Bitzer et al., 1999]. This cytopathic effect might enhance specific T-cell responses by cross-presentation of DCs. Presentation by DCs derived from virus-infected apoptotic and necrotic cells could activate T-cells efficiently [Arrode et al., 2000; Herr et al., 2000; Larsson et al., 2001; Tabi et al., 2001]. In order to apply SeV in a clinical setting, further studies about cytopathic effect by SeV vector will be required.

AdV vector is known to require high MOI to achieve high transduction rates [Diao et al., 1999]. Our study also demonstrated that much higher MOI was needed in AdV than SeV to transduce DCs. One of the reasons for this phenomenon could be insufficient expression of coxsackievirus and AdV receptor (CAR) [Stockwin et al., 2002] on DCs. CAR is the primary receptor for AdV type 5, and the AdV used in this study was derived from AdV type 5. However, MFI of GFP in AdV vector increased according to MOI. AdV might be able to use other receptors to infect DCs. Several studies have shown that AdV can infect cells through integrins or MHC molecules [Huang et al., 1996; Hong et al., 1997]. Recently, AdV vector containing Ad5/35 chimeric fiber protein was reported as a useful vector for the cells lacking in sufficient CAR expression [Mizuguchi and Hayakawa,

2002]. This chimeric vector would be useful for DCs because the receptor of Ad5/35 vector is CD46, which is expressed on DCs.

When imDCs capture antigens, they mature while migrating to T-cell areas in the lymph nodes [Banchereau and Steinman, 1998]. DC maturation is critical for strong T-cell binding and stimulation [Lipscomb and Masten, 2002]. Our results showed that SeV vector infection induced DC maturation of human monocyte-derived DCs as well as AdV vector infection. However, the expression levels of CD86 and CD40 were lower as compared to those of mDCs. CD40 expression leads to increased DC survival and stimulates cytokine production [Caux et al., 1994; Wong et al., 1997]. CD86, a ligand for CD28 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), stimulates T-cell proliferation and generation of CTL [Lanier et al., 1995]. In order to achieve further up-regulation of those molecules, the addition of a maturation factor, such as TNF- $\alpha$ , should be considered.

DCs infected with SeV as well as AdV elicited HIV-1 specific T-cell responses detected by IFN- $\gamma$  Elispot (Fig. 4c). Elispot by SeV GFP showed about 800 SFC/million PBMC which was obtained from patient #1. One possibility of this nonspecific response is antigenic cross-reactivity. SeV belongs to the genus *Respirovirus* of the *Paramyxoviridae* family. *Respirovirus* includes human parainfluenza virus type 1 (hPIV-1) and 3 (hPIV-3). hPIV-1 is the most common cause of pediatric laryngotracheobronchitis (croup), which means many people are infected by hPIV-1 in early life. Previous studies showed SeV and hPIV-1 shared sequence homology and antigenic cross-reactivity [Gorman et al., 1990; Lyn et al., 1991; Smith et al., 1994]. The high nonspecific response by SeV GFP could be caused by cross-reactive immunity induced by previous exposure to human hPIV-1.

In conclusion, our results showed that SeV vector had high ability of gene transduction. SeV vector induced the maturation of DCs in terms of their phenotype and stimulated HIV-1 specific T-cell responses, which is beneficial in vaccination. Though further studies will be required to improve vector design, SeV vector has a potential to be used for immuno-genetherapy.

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