Helper-Dependent Adenoviral Vectors Efficiently Express Transgenes in Human Dendritic Cells but Still Stimulate Antiviral Immune Responses

Michael D. Roth, Qingwen Cheng, Airi Harui, Saroj K. Basak, Kohnosuke Mitani, Teresa A. Low and Sylvia M. Kiertscher

*J Immunol* 2002; 169:4651-4656; doi: 10.4049/jimmunol.169.8.4651
http://www.jimmunol.org/content/169/8/4651

References

This article cites 35 articles, 18 of which you can access for free at: http://www.jimmunol.org/content/169/8/4651.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Helper-Dependent Adenoviral Vectors Efficiently Express Transgenes in Human Dendritic Cells but Still Stimulate Antiviral Immune Responses

Michael D. Roth, Qingwen Cheng, Airi Harui, Saroj K. Basak, Kohnosuke Mitani, Teresa A. Low, and Sylvia M. Kiertscher

Adenoviral (AdV) vectors can be used to transduce a wide range of human cells and tissues. However, pre-existing immunity to AdV, and enhancement of this immunity after repeated administration, limits their clinical application. This may be especially relevant when vectors are loaded into APCs. Helper-dependent AdV (Hd-AdV), in which viral coding regions are replaced by human stuffer DNA, offers a new approach for limiting antiviral responses. To evaluate their immunogenicity, human dendritic cells (DCs) were infected with either an Hd-AdV or a conventional replication-deficient E1-deleted AdV (E1-AdV) and were evaluated for their capacity to stimulate antiviral T cell responses. Hd-AdV proved to be 50- to 275-fold more effective than E1-AdV at expressing the lacZ transgene in human DCs. PCR demonstrated similar transduction efficiencies, but RT-PCR revealed much higher expression of transgene mRNA after transduction with Hd-AdV. Functionally, DCs transduced with Hd-AdV stimulated the proliferation of autologous T cells to the same level as DCs transduced with E1-AdV. Identical viral-specific T cell responder frequencies were observed and T cells stimulated with either type of AdV-transduced DC lysed viral-infected target cells. Disrupting transcription of vector-based genes had no effect on T cell activation, suggesting that responses against both vectors were directed against preformed components of the viral capsid. We conclude that Hd-AdV vectors can be used to obtain higher transgene expression in human DCs but that they still evoke a vector-related immune response similar to that generated by E1-AdV.


Dendritic cells (DCs) are potent APCs that efficiently process Ags and stimulate Ag-specific immunity (1). Several methods have been evaluated for loading DCs with target Ags, including pulsing them with MHC-specific peptides, soluble proteins, or apoptotic cells or transducing them with vectors encoding the protein of interest (1–4). In this respect, adenoviral (AdV) vectors offer a simple and effective approach (1, 5). Close to 100% transduction efficiency can be achieved and expression levels are significantly higher than those obtained by other methods. A single injection of AdV-transduced DCs, modified to express Ags such as MART-1 or HER-2/neu, stimulates protective antitumor immunity in mice (6, 7). However, little is known about the immunologic consequences of the AdV vector itself or the capacity for AdV-transduced DCs to stimulate antiviral responses. AdV is a ubiquitous human pathogen and pre-existing immunity can reduce vector potency in vivo, limit the length of transgene expression, or even produce destructive, potentially lethal, inflammatory side effects (8–10).

Early-transcribed AdV genes are involved in viral replication and also down-regulate MHC expression and Ag presentation (11). To diminish virulence and immunosuppressive effects, replication-incompetent AdV vectors lacking the E1 and/or E3 genes have been used in most DC vaccine models (1, 5, 6, 12, 13). However, cells transduced with these vectors still express viral proteins and host responses to them have been shown to modify AdV therapy in animal models (14, 15). In contrast, helper-dependent AdV (Hd-AdV), a so-called gutless vector, lacks viral coding regions in the final infectious particle (16, 17). When injected in vivo into the mouse, Hd-AdV vectors produce a higher level and longer-lasting transgene expression than that associated with first- or second-generation AdV vectors (18, 19). These features correlate with a reduced antiviral immune response. It is unclear whether the same effects will occur in humans (20, 21), but the use of Hd-AdV vector to modify DCs might allow high-level transgene expression but limit unwanted stimulation of host antiviral T cell responses.

In this study, human monocyte-derived DCs were infected with either conventional replication-deficient E1-deleted AdV (E1-AdV) or an Hd-AdV carrying the same marker transgene. DCs transduced by these two vectors were directly compared for their expression of the transgene product (β-galactosidase (β-gal)) and their ability to stimulate antiviral T cells. Hd-AdV proved to be 50- to 275-fold more efficient than E1-AdV at expressing β-gal protein. However, both vectors stimulated the same frequency of AdV-specific T cells, suggesting that pre-existing antiviral immunity is directed primarily against the viral capsid and not viral genes transcribed de novo. Our results suggest that host responses...
to AdV vectors, including Hd-AdV, should be carefully considered for their impact on human DC-based immunotherapy.

Materials and Methods

Culture media and Ad reagents

Complete medium consisted of RPMI 1640 (Irvine Scientific, Santa Ana, CA) supplemented with 10% heat-inactivated human AB serum (Omega Scientific, Tarzana, CA), 10 mM HEPES (Calbiochem, La Jolla, CA), 100 U/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml fungizone (Life Technologies, Rockville, MD). Ads used for DC phenotyping included fluorochrome-conjugated anti-HLA-DR (BD Biosciences, San Jose, CA) and anti-CD16 (BD Pharmingen, San Diego, CA). Ads used to purify CD3+ T cells (anti-CD14, anti-CD16, and anti-CD19) and in vitro-cultured DCs (anti-CD3, anti-CD19, and anti-CD56) were obtained from BD Pharmingen (no azide, low endotoxin). Fluorochrome-conjugated Abs against human CD4, CD8, TNF-α, and IFN-γ were obtained from BD Biosciences. Sheep anti-mouse Ig-conjugated immunomagnetic beads were obtained from Dynal (M450 Dynabeads; Lake Success, NY).

AdV vectors

A purified E1-AdV type 5 containing the green fluorescent protein (GFP) reporter gene under control of a CMV enhancer/promoter (E1-AdV/GFP) was kindly provided by Dr. A. J. Berk (Molecular Biology Institute, University of California, Los Angeles, CA). Viral stocks of recombinant E1-AdV type 5 that either lacked a reporter construct (E1-AdV/RK5) or contained the Escherichia coli lacZ reporter gene under control of a CMV enhancer/promoter (E1-AdV/β-gal) were propagated in 293 cells, purified by centrifugal concentration, dialyzed, and stored at −80°C as previously described (2). An Hd-AdV type 5 vector containing the LacZ reporter gene under control of the same CMV enhancer/promoter (Hd-AdV/β-gal) was constructed using an E1-deleted helper virus with lox sites flanking the packaging signals (AdΔC68Lac; Merck, Whitehouse Station, NJ) and a 293 cell line expressing Cre recombinase (293-Cre4 cells; Miami University, OH). The reaction was stopped by the addition of ice-cold staining buffer and counters from the same donor at a 1:20 ratio in round-bottom 96-well plates. After 5 days of culture, cells were pulsed with 1.25 µCi/well [3H]thymidine (Amersham Pharmacia) and harvested the next day onto glass fiber strips using a semiautomated cell harvester (Brandel, Gaithersburg, MD). Proliferation, as determined by [3H]thymidine uptake, was measured by scintillation counting and reported as the average cpm for five replicates in each well. In some experiments, mAb against HD-A20 (mouse IgG2b, 10 µg/ml) were added at the start of the assay to block Ag-specific or CD8-specific T cell proliferation, respectively (23).

Preparation and transduction of human monocyte-derived DCs

PBMCs were obtained from healthy normal donors by density-gradient centrifugation and DCs were prepared by culturing the adherent fraction with 800 U/ml GM-CSF (Immunex, Seattle, WA) and 500 U/ml IL-4 (PeproTech, Rocky Hills, NJ) at 37°C with 800 U/ml penicillin, 100 µg/ml streptomycin, 10 mM HEPES (Calbiochem, La Jolla, CA), 100 µM 2-mercaptoethanol (Sigma-Aldrich, St. Louis, MO) at an initial concentration of 2 µg/ml after the first hour, and intracellular staining for IFN-γ and TNF-α was performed according to the manufacturer’s protocol (BD Biosciences). Cells were counterstained with fluorochrome-conjugated anti-CD4 and anti-CD8 mAb and fixed in 1% paraformaldehyde. Flow cytometry data were obtained using a FACSCalibur flow cytometer and CellQuest software.

Chromium release assay for detection of AdV-specific CTLs

Purified CD3+ T cells were stimulated in vitro at a 20:1 ratio with either autologous HD-AdV-transduced DCs or AdV1-AdV-transduced DCs using the same protocol as described above for the induction of AdV-specific CD4+ and CD8+ T cells. After 1 wk in culture, T cells were collected, washed, and used as effector cells in a 4-h chromium release assay. HLA-matched M202 cells that had been transduced 18 h previously with E1-AdV/RKS at a MOI of 40 were loaded for 1 h with 100 µCi of [51Cr] and used as target cells at a final concentration of 2.5 × 10^6 targets/well in U-bottom 96-well microtiter plates. Sufficient effector T cells were added to produce E:T ratios of 50:1, 25:1, and 12.5:1 in a total volume of 200 µl of complete

HDLA-DR expression and their β-gal content was determined by fluorescence of metabolized FDG. For more sensitive quantitation, 1 × 10^6 transduced cells were suspended in lysate buffer (100 mM potassium phosphate, pH 7.4, 1.4 µM DTT) and subjected to three rounds of freeze/thaw. A sample of lysate was mixed with β-gal reaction buffer (Clontech, Palo Alto, CA) and enzyme activity was measured after 30 min at room temperature using a luminometer (model BG-1; GEM Biomedical, Sparks, NV). Results are reported in light units. Cells transduced with E1-AdV/GFP were examined by FACS analysis for GFP-related fluorescence and results are reported as mean fluorescence intensity.

PCR and RT-PCR

Genomic DNA was extracted from control and transduced DCs using a Genomic Prep kit (Amersham Pharmacia, Piscataway, NJ). Total RNA was extracted by the TRizol method (Life Technologies). For PCR, 0.01 µg of DNA was used as a template and amplified with 25 cycles consisting of 45 s at 92°C, 45 s at 50°C, and 45 s at 72°C. For RT-PCR, 1 µg of total RNA was used to synthesize cDNA with a cDNA Cycle Kit (Invitrogen, Carlsbad, CA), and 1 µl of the reverse transcriptase product was used as a template to perform PCR as described above. β-Gal was detected by amplifying the fragment from 2884 bp to 3366 bp as the primer sequence, and GAPDH was detected by amplifying the full-length cDNA. PCR or RT-PCR products were resolved on a 1.2% agarose gel and visualized with a UV Image Store 7500 (UVP, Upland, CA). Expression of the GAPDH gene was used to control for loading, and signal intensities were quantified by densitometry using NIH Image 1.62 software to obtain a β-gal/GAPDH ratio. Using this approach, serial dilutions of genomic DNA or total RNA demonstrated a linear relationship between starting copy number and measured signal intensity over a ninefold range of dilutions.

Proliferation assay

T cells were purified from PBMCs by labeling with anti-human CD14, CD16, and CD19 mAb and depleting labeled cells with M450 Dynabeads. Control DCs, or DCs transduced with the various AdV constructs, were cocultured with purified T cells from the same donor at a 1:20 ratio in 96-well round-bottom plates. After 5 days of culture, cells were pulsed with 1.25 µCi/well [3H]thymidine (Amersham Pharmacia) and harvested the next day onto glass fiber strips using a semiautomated cell harvester (Brandel, Gaithersburg, MD). Proliferation, as determined by [3H]thymidine uptake, was measured by scintillation counting and reported as the average cpm for five replicates in each well. In some experiments, mAb against HD-A20 (mouse IgG2b, 10 µg/ml) were added at the start of the assay to block Ag-specific or CD8-specific T cell proliferation, respectively (23).

Detection of AdV-specific CD4+ and CD8+ T cells

DCs were transduced with either E1-AdV/β-gal or Hd-AdV/β-gal (125 multiplicity of infection (MOI)) and 1 day later were activated with 1 µg/ml CD40 ligand (generously provided by Immunex) and 500 U/ml IFN-γ (PeproTech, Rocky Hills, NJ) for an additional 24 h. Two days after transduction, DCs were washed and used to stimulate autologous T cells at a 1:20 ratio in round-bottom 96-well plates containing complete medium supplemented with 10% FBS and IL-7 (Biosource International, Camarillo, CA). After 1 wk of stimulation, T cells were collected, washed, and challenged for 5 h at a 1:10 ratio using either autologous control DCs (no AdV vector) or DCs transduced with E1-AdV/RKS (250 MOI). Cells were cultured in fresh medium containing 1 ng/ml IL-12 (PeproTech) and 3 µg/ml anti-CD28 Ab to maximize cytotoxicity (BD Pharmingen). Briefly, 800–1500, (Brandel, Gaithersburg, MD). Proliferation, as determined by [3H]thymidine uptake, was measured by scintillation counting and reported as the average cpm for five replicates in each well. In some experiments, mAb against fluorochrome-conjugated anti-CD4 and anti-CD8 mAb and fixed in 1% paraformaldehyde. Flow cytometry data were obtained using a FACSCalibur flow cytometer and CellQuest software.

Downloaded from http://www.jimmunol.org/ by guest on July 19, 2017
medium. After a 4-h culture at 37°C, plates were centrifuged and the amount of chromium released by cell lysis was determined by removing 100 μl of supernatant from each well and counting in a gamma counter. Spontaneous release of chromium was obtained from wells containing only target cells, and the maximal release of chromium was determined from wells lysed with 0.5% Nonidet P-40 detergent. Cytotoxic activity was expressed by the following formula: % lysis = 100 × [(experimental release − spontaneous release)/(maximal release − spontaneous release)]. Assay wells were run in triplicate and results are presented as the mean ± SD.

**Statistical analysis**

Data for each group are represented as the average value ± SD for the indicated number of replicate determinations. Differences between groups were determined by two-tailed Student’s t tests with significant differences documented at p values of ≤0.05.

**Results**

**Hd-AdV produces higher transgene expression than E1-AdV**

Hd-AdV/β-gal does not replicate independently in 293 cells, and therefore titers for it and E1-AdV/β-gal were based on detection of β-gal 293 cells (BFU) and not on plaque-forming activity. Viral preparations were also examined for OD260 to determine the number of intact viral particles, the values of which correlated directly with the measured BFU. Their capacity to express β-gal protein in human monocyte-derived DCs was evaluated at MOIs of 125, 250, and 500 BFU (Fig. 1A). These MOIs had minimal effects on DC recovery or viability, whereas MOIs in excess of 1000 were observed to be cytopathic. DCs transduced with Hd-AdV expressed higher β-gal enzyme activity under every condition with an average 150-fold increase over E1-AdV at the 500 MOI (range, 52- to 275-fold increase). Flow cytometry of FDG-loaded cells confirmed enhanced transgene expression at the single-cell level (Fig. 1B).

**Higher transgene expression by Hd-AdV occurs as a result of enhanced transcription**

To determine whether higher β-gal expression was the result of more efficient transduction (vector loading) or a higher level of transduction, genomic DNA and total mRNA were extracted from control and transduced DCs and were analyzed by semiquantitative PCR (Fig. 2A) and RT-PCR (Fig. 2B), respectively. When transduced using the same BFU, identical levels of lacZ transgene were detected from DCs exposed to either vector. In contrast, levels of mRNA encoding for β-gal were always higher in cells exposed to the Hd-AdV. These results suggest that both vectors exhibit equal transduction efficiencies, but that higher transcriptional activity and/or mRNA stability accounts for the higher β-gal levels in cells exposed to Hd-AdV.

**AdV-transduced DCs stimulate autologous T cell proliferation that correlates with viral load**

To evaluate the impact of viral transduction on T cell stimulation, DCs were transduced with E1-AdV/β-gal at various MOIs and were cocultured with purified autologous T cells. β-Gal expression was used as a measure of viral transduction efficiency (Fig. 3A) and resulting T cell proliferation was measured by radiolabeled thymidine incorporation (Fig. 3B). Both β-gal activity and T cell proliferation increased in a concentration-dependent manner as the vector MOI was increased. To determine whether this effect was specific for the β-gal insert or related to the viral components of the vector, experiments were repeated using an E1-AdV/GFP (Fig. 3, C and D) or E1-AdV/RR5 (data not shown). The same dose-dependent increase in proliferation was observed, suggesting that transduced DCs process and present viral Ags and stimulate the proliferation of viral-reactive T cells. T cell proliferation was blocked by the addition of anti-CD86 mAb, consistent with this hypothesis, and the addition of mAb against β2-microglobulin partially reduced T cell proliferation, suggesting proliferative responses by both CD4+ and CD8+ T cells (data not shown).

**DCs transduced with the same MOI of Hd-AdV and E1-AdV activate similar antiviral T cell responses**

Working under the hypothesis that viral gene products synthesized de novo act as an important source of viral Ags, we expected that E1-AdV-transduced DCs would stimulate significantly greater T cell proliferation than Hd-AdV-transduced DCs. However, both sets of DCs stimulated the same level of T cell proliferation (Fig. 4). To evaluate this further, intracellular cytokine staining was used to identify the frequency of viral-reactive T cells. Fresh T cells were stimulated for 1 wk in vitro with either Hd-AdV-transduced DCs or E1-AdV-transduced DCs and then were challenged...
with either control DCs or DCs transduced with the E1-AdV/RR5 vector. This approach allowed us to discriminate viral-specific T cell activation (as measured by challenge with AdV/RR5-transduced DCs) from nonspecific activation (as measured by challenge with autologous DCs alone). Intracellular production of TNF-α and IFN-γ was determined by flow cytometry as a sensitive measure of Ag-specific activation (Fig. 5). As with the proliferation assay, DCs transduced with the same MOI of either vector stimulated similar frequencies of AdV-specific T cells. The response frequencies were high, consistent with the activation of pre-existing antiviral immunity. Both CD4+ T cells and CD8+ T cells responded to E1-AdV/RR5-transduced DCs, suggesting efficient loading of vector-related Ags into both the MHC class I and II pathways (Fig. 5). The generation of AdV-specific cytolytic activity was confirmed using a standard chromium release assay. Again, both Hd-AdV-transduced DCs and E1-AdV-transduced DCs resulted in the stimulation of cytolytic activity against viral-transduced target cells (19.8 ± 3.8% vs 10.4 ± 1.5% lysis at a 50:1 E:T ratio and 11 ± 1.7% vs 5.1 ± 2.4% lysis at a 25:1 E:T ratio, respectively). The stimulation of a primary immune response to β-gal (as compared with the memory response to AdV) was not expected in this short-term assay and no cytokine-producing T cells or CTLs were detected when challenged with DCs loaded with β-gal protein (data not shown).

FIGURE 3. Increasing virus concentration (MOI) results in DCs that express higher levels of transgene and stimulate greater T cell proliferation. Monocyte-derived DCs were transduced at MOIs ranging from 125 to 500 using either E1-AdV/β-gal (A and B) or E1-AdV/GFP (C and D) and were evaluated for both transgene expression (A, luminescence assay; C, FACS analysis) and their capacity to stimulate the proliferation of autologous T cells (B and D). Control DCs were not transduced with AdV. Representative experiment, n = 4 for E1-AdV/β-gal and n = 2 for E1-AdV/GFP.

FIGURE 4. DCs transduced with Hd-AdV/β-gal express higher levels of transgene but stimulate the proliferation of autologous T cells to the same level as DCs transduced with E1-AdV/β-gal. Monocyte-derived DCs were transduced at an MOI of 500 using either E1-AdV/β-gal or Hd-AdV/β-gal and 48 h later were used to stimulate autologous T cells at a DC:T cell ratio of 1:20. Expression of β-gal by transduced DCs was determined by luminescence assay (A), and resulting T cell proliferation was determined by [3H]thymidine uptake after 6 days of coculture (B). Representative experiment, n = 3.

FIGURE 5. Single-cell analysis confirms that E1-AdV and Hd-AdV stimulate a similar frequency of AdV-specific CD4+ and CD8+ T cells. Monocyte-derived DCs transduced with either E1-AdV/β-gal (A) or Hd-AdV/β-gal (B) were used to stimulate autologous T cells at a 1:20 ratio for 7 days. AdV-reactive T cells were then detected by challenging with either autologous control DCs (containing no AdV) or DCs transduced with E1-AdV/RR5, an E1-AdV vector containing no β-gal transgene. After a 5-h challenge, cells were fixed, permeabilized, and stained with a combination of FITC-labeled anti-IFN-γ and anti-TNF-α mAb. The percentages of T cells expressing cytokine in response to either control DCs or E1-AdV/RR5-transduced DCs were enumerated by FACS analysis. Representative experiment, n = 2.
Antiviral T cell responses were directed against preformed viral components and not viral genes transcribed de novo

Theoretically, the vector components that Hd-AdV/β-gal, E1-AdV/β-gal, and E1-AdV/RR5 share in common are limited to viral capsid and the noncoding packaging signals. To confirm the role of preformed viral components as the source of responsible Ags, we disrupted gene transcription in both the HD-AdV and the E1-AdV vectors using UV irradiation. To do this, Hd-AdV and E1-AdV were exposed to 6 J/cm² of UV light before using them to transduce DCs. This dose was sufficient to disrupt vector-related DNA and reduce the capacity for gene transcription by 3 logs (Fig. 6A). However, irradiation of the vectors in this manner had no effect on the capacity for transduced DCs to stimulate the proliferation of autologous T cells.

Discussion

AdV-based gene therapy provides an important approach for enhancing the Ag-presenting activity of DCs and for targeting the subsequent immune response against defined tumor Ags (2–4, 7, 12). However, this technique simultaneously loads viral material into DCs and enables them to stimulate antiviral T cells (20, 24). Recombinant viruses lacking the E1 and/or E3 genes may actually enhance this effect by circumventing viral-induced immunosuppression and allowing effective viral Ag presentation (11). In this study, an E1-AdV and an Hd-AdV, both expressing lacZ under control of the same CMV promoter/enhancer, were compared for their ability to transduce human monocyte-derived DCs and for their subsequent impact on the stimulation of antiviral T cells. The gutless vector proved to be exceptionally potent at expressing transgenic protein in DCs, but deletion of the viral genome failed to eliminate its antiviral stimulatory effect.

The level of β-gal protein in DCs transduced by Hd-AdV/β-gal averaged 150-fold higher than that produced by an equal MOI of E1-AdV/β-gal. Our results are consistent with work by Parks et al. (25), which demonstrated enhanced transgene expression when A549 lung cancer cells were transduced with Hd-AdV in vitro. By comparing different Hd-AdV constructs, they concluded that differences in expression were due to an enhancer effect mediated by noncoding regions contained in the stuffer DNA. In our studies, we used semiquantitative PCR and RT-PCR to confirm that both vectors load the same amount of transgene DNA, but that Hd-AdV produces higher levels of transgene mRNA. Other approaches for increasing AdV-based transgene expression in DCs have been reported, but they rely on modifying the viral capsid to increase viral loading (26, 27). The use of Hd-AdV to transduce DCs may be advantageous in this respect, producing high levels of expression but limiting the loading of viral Ags.

When DCs were loaded with E1-AdV at different MOIs, there was a direct correlation between viral load and their capacity to stimulate T cell proliferation and generate AdV-specific T cells. This proliferative effect was not induced by transgene expression, in that similar responses were observed with E1-AdV/β-gal, E1-AdV/GFP, or E1-AdV/RR5. Furthermore, it was blocked by the addition of anti-CD86 or anti-β1-microglobulin Ab, suggesting a specific response by both CD4⁺ T cells and CD8⁺ CTLs to AdV Ags. The generation of AdV-specific CTLs was confirmed by standard chromium release assays. Olive et al. (28) recently reported the presence of memory antiviral T cells in fresh peripheral blood from 20 of 22 randomly tested donors. Similarly, proliferative responses to AdV-transduced DCs, but not control DCs, were observed in every one of our subjects. AdV is a ubiquitous pathogen and both memory T cell responses and circulating Ab appear to exist in most healthy individuals (28, 29). More importantly, antiviral responses are boosted in a dose-dependent manner after treatment with E1-AdV in vivo (9, 30). The amount of viral Ags present in DCs is therefore likely to predict the magnitude of the host antiviral response.

Work by Yang et al. (15) suggested that immune responses to E1-AdV in vivo are directed against viral gene products expressed de novo. Based upon this, many investigators speculated that Hd-AdV would circumvent antiviral immunity. This viewpoint was reinforced by studies in which administration of Hd-AdV to immunocompetent mice produced long-lasting expression with limited toxicity (18). However, significant antiviral responses against Hd-AdV have been detected, and factors other than a lack of antiviral immunity may contribute to the prolonged in vivo expression associated with Hd-AdV (31, 32). Maione et al. (19) reported that injection of Hd-AdV produced the same titer of neutralizing antiviral Ab as did injection of the same amount of E1-AdV. Escape from antiviral immunity was achieved by lowering the dose of Hd-AdV vector. Other work by the same group found that stability of Hd-AdV expression was strain dependent, with long-lasting responses in C57BL/6 mice, but not in BALB/c mice (32). Histology and molecular marker analysis correlated the loss of gene expression with a mononuclear infiltrate, particularly CD8⁺ cells. It appears from these studies that Hd-AdV can induce an antiviral response depending upon dose, route, and strain-specific factors.

In this report, DCs transduced with an Hd-AdV produced the same degree of antiviral T cell activation as did DCs transduced with E1-AdV. This was confirmed by intracellular cytokine expression, which also demonstrated a balanced response by both
CD8+ and CD8- T cells. Viral Ags were efficiently processed and presented regardless of whether the vector carried viral DNA. This was investigated further by infecting DCs with UV-inactivated vectors. These transcription-incompetent virions produced the same T cell responses as their functional counterparts, confirming that antiviral responses were directed almost exclusively against preformed components of the viral capsid. Our results are in agreement with work by Molinier-Frenkel et al. (21), who determined that CD8+ CTLs from E1-AdV-immunized patients recognize capsid components, and Smith et al. (20), who demonstrated that CTLs resulting from E1-AdV-transduced DCs can lyse infected targets in the absence of viral gene transcription. Fiber knob, penton base, and hexon components all appear capable of eliciting potent human immune responses (21, 33).

We conclude from our studies that Hd-AdV offers an effective approach for achieving high-level transgene expression in human DCs. However, by packaging the transgene into a viral capsid, Hd-AdV still delivers potent viral Ags for processing and presentation by DCs. Although this raises concerns about the generation approach for achieving high-level transgene expression in human potent human immune responses (21, 33).

Modified with an adenovector to express a model tumor antigen. Gene Ther. 7:1263.