Improved Adenoviral Vector for Vascular Gene Therapy
Beneficial Effects on Vascular Function and Inflammation

Hu Sheng Qian, Keith Channon, Valentina Neplioueva, Qing Wang, Mitchell Finer, Lisa Tsui, Samuel E. George, James McArthur

Abstract—First-generation, E1-deleted adenoviral vectors (ΔE1-AV) can transduce the vascular endothelium with high efficiency, but their use is limited by the resulting acute endothelial injury and the long-term development of intimal hyperplasia. To reduce the impact of viral proteins on the gene-modified cells, a second-generation adenoviral vector with an additional pair of deletions in the E4 region was developed. To determine whether this ΔE1/ΔE4-AV vector would be useful for vascular gene transfer, we directly compared the efficiency of gene transfer to uninjured rabbit carotid arteries using either an ΔE1/ΔE4-AV or an ΔE1-AV vector encoding β-galactosidase. Both vectors efficiently transduced vascular endothelium; however, the ΔE1/ΔE4-AV vector gene–modified vessels showed higher β-galactosidase expression 10 days after gene transfer. Importantly, the ΔE1/ΔE4-AV vector produced substantially less endothelial cell activation, less inflammation, and reduced neointimal hyperplasia compared with the ΔE1-AV vector–treated vessels. The ΔE1-AV vector–transduced vessels also demonstrated significantly impaired endothelium-dependent relaxation whereas the ΔE1/ΔE4-AV vector did not impact vasomotor function, even at doses of virus in 5-fold excess of the amount required for >90% transduction of the endothelium. We conclude that the ΔE1/ΔE4-AV vector is superior to the ΔE1-AV vector for vascular gene therapy because of the prolonged transgene expression, reduced vascular inflammation, reduced intimal hyperplasia, and maintenance of normal vasomotor function. (Circ Res. 2001;88:911-917.)

Key Words: vascular gene therapy n adenoviral vectors

Recombinant E1-deleted adenoviral vectors (ΔE1-AV) have proven to be a powerful tool for vascular gene transfer and shows promise for gene therapy of restenosis,1–8 vascular thrombosis,9 and vein graft failure.10–16 However, adenoviral gene transfer may be limited by vector-induced acute and chronic inflammatory responses that injure the endothelium and promote intimal hyperplasia.17,18 The leaky expression of early genes in the first-generation ΔE1-AV allows low-level transcription of viral late genes and expression of viral proteins that result in virus-mediated cytopathology, as well as a virus-specific host immune response.19–24 To address these problems, first-generation E1-deleted adenoviral vectors have been modified to further reduce adenoviral protein expression.25–30 One such approach combines an E1 deletion with additional deletions in the E4 region. The E4 region encodes a series of 6 proteins that are involved with viral DNA replication and viral RNA transport. To this end, Wang et al30 created the E1- and E4-deleted adenoviral vector in which only the E4 orf4 remained. Intravenous administration of equivalent doses of the ΔE1-AV and ΔE1/ΔE4-AV vectors initially produce similar levels of transgene expression; however, the mice that receive the ΔE1/ΔE4-AV vector demonstrate greatly reduced virus-induced hepatitis and inflammatory cell infiltrates as well as prolonged transgene expression.31,32

We undertook the present study to establish whether vascular gene therapy with the ΔE1/ΔE4-AV vector would avoid the impairment of vessel function and intimal hyperplasia observed with first-generation AV vectors. Our results show that the ΔE1/ΔE4-AV–treated vessels demonstrated prolonged transgene expression, markedly reduced endothelial activation, vascular inflammation, and intimal hyperplasia, and had little or no detrimental impact on endothelial vasomotor function relative to ΔE1-AV vector–treated vessels. These improved characteristics represent a significant advance for vascular applications of adenoviral gene transfer.

Materials and Methods

Adenovirus

The ΔE1-AV and ΔE1/ΔE4-AV vectors encoding the phosphoglyceratekinase (PGK)-promoter β-galactosidase (β-gal) transgene expression cassette were generated as previously described.30 Both the ΔE1/ΔE4 and ΔE1 vectors viruses used in the described studies retain the E3 region genes. The ΔE1/ΔE4-AV vectors were produced

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in 293 cells that expressed the E4-orf6 gene under the control of a cAMP-inducible promoter. The virus was purified by two rounds of CsCl gradient centrifugation, dialyzed against PBS containing 10% glycerol, and stored in PBS containing 10% glycerol and 50 mg/mL human serum albumin. The frequency of replication-competent adenovirus has been determined to be $<10^{-10}$ in ΔE1ΔE4-AV preparation and $<10^{-11}$ in the ΔE1ΔE4ΔE4-AV preparations of virus (data not shown). Particle titer was determined by using optical density on a spectrophotometer (Beckman DU640). The particle titer for the ΔE1-AV and ΔE1ΔE4-AV preparations were $4 \times 10^{12}$ vp/mL and $6 \times 10^{12}$ vp/mL, respectively. Infectious titer was determined by infecting 293 cells with an increasing number of virus particles and quantifying lacZ expression with a fluorescein di-β-D-galactopyranoside (FDG)–based fluorescence-activated cell sorter assay. The functional titer for the ΔE1-AV and ΔE1ΔE4-AV preparations were $1 \times 10^{12}$ fp/mL and $6.7 \times 10^{12}$ fp/mL, respectively. The total to functional particle ratios for the preparations of ΔE1ΔE4-AV and ΔE1ΔE4ΔE4-AV used in these studies were 40:1 and 90:1, respectively.

### Animals and Gene Delivery
Male New Zealand white rabbits (2 to 2.5 kg) were maintained on a normal diet. Anesthesia was induced with ketamine and xylazine subcutaneously as previously described. All animal care and procedures were approved by Duke University Institutional Animal Care and Use Committee and compiled with the Guide for the Use and Care of Laboratory Animals (NIH publication 80-23, revised 1985).

For gene transfer to the carotid artery, a midline incision was made, and the external carotid artery was exposed. The vessel was clamped, and an arteriotomy was performed. Approximately 200 μL of virus solution was infused per vessel. This volume was sufficient to expand the vessel to physiological dimensions. The solution was allowed to dwell for 15 minutes and was removed. The artery was then flushed with Dulbecco’s modified Eagle’s (DME) medium, and the arteriotomy was closed. The clamps were then removed, and circulation was restored. High-titer viral stock was diluted with DME medium/virus storage medium to ensure equal composition of virus solutions at different viral titers. Virus doses given to each artery were $1 \times 10^9$, $5 \times 10^9$, and $2.5 \times 10^8$ fp. Sham infections were performed with DME/virus storage medium alone.

### Vessel Harvesting and Analysis
Vessels were harvested at time points indicated in the figures as previously described. Briefly, animals were anesthetized and heparinized (700 IU intravenously), and the carotid arteries were dissected free. Animals were then killed with 100 mg/kg intravenous pentobarbital. Vessels were immediately excised and washed in PBS. Segments from each vessel were processed for various analyses. They were (1) immediately frozen at $-80$°C for β-gal quantification, (2) equilibrated in 30% sucrose and frozen in OCT (Miles Laboratories) for immunohistochemistry, (3) fixed in 10% formalin and paraffin embedded for histological stains, or (4) were hung fresh without delay in organ baths for vasomotor studies.

### Histological Methods
#### LacZ Staining
Vessels were equilibrated in 30% sucrose in PBS briefly and then frozen in liquid nitrogen. Snap-frozen vessel segments were cut into 6-μm sections and stained with 5-bromo-4-chloro-3-indolyl-b-D-galactosidase (X-Gal) solution for 4 hours at room temperature. Total protein was also extracted and quantified for β-gal protein by using an ELISA kit and for total protein by using a Bradford protein assay. β-Gal protein was measured in nanograms of β-gal per milligram of total vessel protein.

### Immunohistochemistry
Briefly, 6-μm frozen sections were cut and dried at room temperature and then equilibrated in PBS. Blocking solution (1.5% horse serum in PBS) was applied for 1 hour at room temperature or overnight at 4°C. Antibodies were diluted in blocking solution at the manufacturer’s recommended concentration and were applied to tissue sections for 1 hour. Immunohistochemistry for lymphocytes was performed by using primary antibodies directed against rabbit CD18 (from Serotec). Vascular cell adhesion molecule-I (VCAM-1) and intercellular adhesion molecule-I (ICAM-1) staining was performed with monoclonal antibodies raised against rabbit VCAM-1 and ICAM-1 (a generous gift of Dr. M. Cybulsky, Harvard University, Boston, Mass.).

### Vessel Morphology
Paraffin-embedded sections were treated with Voerhoff’s stain or with Masson’s trichrome stain (Sigma) to visualize the vessel structure. Neointimal thickness was measured with the NIH Image software analysis program.

### Image Analyses
For quantification, image analyses were performed. Stained sections were visualized by using an Olympus IX70 inverted microscope, and images were captured by using Adobe Premiere. The immunohistochemical staining results were quantified by using NIH Image 1.61 software. Two arteries per animal were exposed to virus in each treatment group at each dose: $1 \times 10^9$ fp (ΔE1-AV n = 10 vessels, ΔE1ΔE4-AV n = 8 vessels), $5 \times 10^9$ fp (ΔE1-AV n = 42 vessels, ΔE1ΔE4-AV n = 48 vessels), and $2.5 \times 10^8$ fp (ΔE1-AV n = 18 vessels, ΔE1ΔE4-AV n = 18 vessels). Briefly, cross sections for each vessel were taken and quantified in 4 to 6 different portions of the vessel at ×5 magnification. The neointimal thickness was determined on trichrome-stained vessel sections and expressed as millimeters per high power field. Four to 6 points were measured for each portion per section.

### Vasomotor Studies
Studies to assess vasorelaxation after adenoaviral gene delivery was performed as previously described. Briefly, 5-mm arterial rings were mounted in 30-mL organ baths containing oxygenated (95% O₂, 5% CO₂) Krebs-Henseleit buffer at 37°C. Briefly, cumulative dose-response curves to phenylephrine ($10^{-9}$ to $10^{-12}$ mol/L) were established; vessels were then submaximally precontracted with phenylephrine (usually $3 \times 10^{-7}$ mol/L), and endothelial function was evaluated by vascular relaxation in response to acetylcholine ($10^{-4}$ to $10^{-1}$ mol/L). Statistical significance was assessed by ANOVA.

### TaqMan PCR Analyses
Quantification of adenoaviral DNA in the rabbit carotid artery after gene transfer was determined by using TaqMan polymerase chain reaction (PCR). DNA extraction of the treated vessel was performed by using the Qiagen tissue DNA extraction kit. Fifty nanograms of genomic DNA was analyzed with TaqMan PCR by using adenovirus primers located upstream of the E4 region of adenovirus. The following primer set was used to produce a 400-bp amplicon: forward primer, 5'-ACAACCTCAAAGTGCATACTC-3'; reverse primer, 5'-CTGATACGGTTGCTGCA-3'. The probe used was 6FAM-CTTGTCCTGCAACAATCTAATA-TP (Applied Biosystems). A final reaction volume of 50 μL consisted of 1× TaqMan Buffer A; 0.4 μmol/L each primer; 50 mmol/L probe; 0.2 mmol/L each dATP, dCTP, and dGTP; 0.4 mmol/L dUTP; 4.5 mmol/L MgCl₂; 2.5 units of AmpliTaq GOLD polymerase; and 0.5 units of AmpErase UNG (Applied Biosystems). A plasmid containing the adenovirus amplicon sequence was diluted in a TRNA diluent (25 μg/mL) and used as a positive control series at 10 000, 1 000, 100, 10, 1.0, and 0.1 copies per replicate, and these were run in triplicate. Each reaction was run under the following conditions: 50°C for a 2-minute hold; 95°C for 10-minute hold; then 40 cycles of 95°C for 15 seconds and 60°C for 1-minute in an ABI PRISM 7700 Sequence Detection System unit (Applied Biosystems). The results were analyzed with the Sequence Detection System (version 1.6.3) software’s default settings (baseline 3 to 15; threshold set to 10× the standard deviation of the baseline). By using the determination that 71-ng genomic DNA is equivalent to 10 000 cellular.
genomes, the results are expressed in adenovirus DNA genome copies per cell.

Late Gene Expression by PCR
HeLa cells were seeded in 6-cm dishes at a density of 1×10^6 cells and transduced with ΔE1-AV and ΔE1/ΔE4-AV vectors encoding lacZ at functional MOI of 10 and were harvested 4 hours, 24 hours, or 48 hours later. RNA was isolated from cell pellets, and cDNA was generated by reverse transcriptase (RT)-PCR by using first-strand synthesis with random hexamer primers (SuperScript kit by Gibco BRL). The following primers were designed for amplification of the adenovirus L3 and L5 gene transcripts: 5'-GGACAATTATTGTTCTAAA-3' (L3 forward); 5'-TGTTTGGGTTATCATCAG-AATT-3' (L3 reverse), 5'-GAGGACTAAGGATTTGATT-3' (L5 forward), 5'-CGTGAGATTTGGATAAG-3' (L5 reverse). The PCR reactions were set up with the SuperScript kit with the following conditions: 94°C for 5 minutes, 40 cycles of 94°C for 45 seconds, 60°C for 45 seconds, 72°C for 1 minute, and 72°C for 10 minutes. The PCR products were run out on a 2% agarose gel for analysis.

Results

ΔE1/ΔE4-AV–β-Gal–Transduced Rabbit Carotid Arteries Demonstrate Improved Transgene Expression
Exposure of uninjured rabbit carotid arteries to 5×10^9 fp of ΔE1-AV or ΔE1/ΔE4-AV vector encoding lacZ efficiently transduced the intima with >80% of the endothelial cells expressing β-gal 3 days after gene transfer (Figure 1a through 4d). To further quantify β-gal expression, an ELISA was performed on whole-vessel lysates 3, 10, and 28 days after gene transfer (Figure 1e). No significant difference in gene expression was observed at 3 days between the two vectors, which confirmed that the titers and infection conditions were essentially equivalent between the ΔE1-AV and ΔE1/ΔE4-AV vectors. The number of β-gal–positive endothelial cells declined considerably by 10 days after gene transfer; however, there was a clear histologic difference between ΔE1-AV– and ΔE1/ΔE4-AV–treated arteries with greater staining in the latter. β-Gal protein ELISA measurements of the transduced arteries indicated that β-gal expression was 4-fold higher in the ΔE1/ΔE4-AV–treated vessels at the interim time point of 10 days (Figure 1e). By day 28, β-gal staining and protein expression were negligible in vessels treated with either AV vector (<1%) (data not shown). This decline in protein expression was paralleled by a decrease in the number of vector genomes (Figure 2). Maximal genome copy numbers for ΔE1-AV–transduced vessels were observed at day 3 with a significant loss in genomes observed 10 and 28 days after gene transfer. The number of vector genomes was approximately 4-fold higher in ΔE1/ΔE4-AV–transduced vessels than ΔE1-AV–transduced vessels at days 10 and 28, consistent with the prolonged β-gal protein expression determined by histology and ELISA.

Endothelial Activation and Vascular Inflammation
To compare the degree of endothelial activation induced by the two vectors, arterial cryosections were compared for ICAM and VCAM expression by using immunohistochemistry (Figure 3). Clear differences in these markers were observable between sham-treated, ΔE1/ΔE4-AV–treated, and ΔE1-AV–treated arteries 3, 10, and 28 days after gene transfer. To quantify the observed differences, image analysis was performed. This revealed that ICAM and VCAM expression was significantly higher in the ΔE1-AV–treated arteries compared with ΔE1/ΔE4-AV–treated arteries. These differences were statistically significant (P<0.01) and were observed at all 3 virus doses. Recombinant first-generation adenoviral vectors have been shown to induce the infiltration of neutrophils, lymphocytes, and monocytes. Cellular infiltration into the ΔE1/ΔE4-AV–transduced vessels was assessed by using immunohistochemistry.
with CD18, a marker for neutrophils and monocytes (Figure 4a through 4c), and CD43, a pan T-lymphocyte marker (Figure 4d through 4f). Substantially fewer CD18 and CD43 positive cells were present in ΔE1/ΔE4-AV–treated arteries, relative to ΔE1-AV. This difference was quantified by both cell counting (data not shown) and image analysis (Figure 4g and 4h). As was the case for adhesion molecule expression, the ΔE1/ΔE4-AV vector induced greater inflammatory cell infiltration than did the sham treatment but significantly and substantially less than that observed with the ΔE1-AV vector.

**Studies of Vasomotor Function**

Exposure of vessels to ΔE1-AV vectors has been shown to impair endothelium-dependent relaxation in a titer-dependent manner. Vasomotor function of ΔE1/ΔE4-AV vector–treated carotid arteries was evaluated in organ baths at 3, 10, and 28 days after gene transfer (Figure 5). The ΔE1-AV vector significantly impaired endothelium-dependent relaxation at all time points (Figure 5). In contrast, the ΔE1/ΔE4-AV had surprisingly little impact on endothelium-dependent relaxation. Phenylephrine-induced contraction and relaxation were examined at 3, 10, and 28 days after gene transfer (Figure 5). The ΔE1-AV vector significantly impaired endothelium-dependent relaxation at all time points (Figure 5). In contrast, the ΔE1/ΔE4-AV had surprisingly little impact on endothelium-dependent relaxation. Phenylephrine-induced contraction and relaxation were examined at 3, 10, and 28 days after gene transfer (Figure 5).
sodium nitroprusside–induced relaxation were the same in all vessels, regardless of treatment (data not shown).

**Intimal Thickening**

In addition to inducing endothelial cell activation, inflammatory cell infiltrates, and vessel dysfunction, the first-generation ΔE1-AV provoked a profound thickening of the intima. In vessels receiving the 5 × 10^6-fp ΔE1-AV, intimal thickening was readily apparent as early as 10 days after gene transfer (Figure 6f). This intimal thickening progressively increased at day 28 after transduction (Figure 6h and 6j). A similar but less robust intimal thickening was observed at the lower dose of ΔE1-AV of 1 × 10^6 fp (Figure 6b, 6d, and 6i). The ΔE1/ΔE4-AV–treated vessels demonstrated significantly less neointimal thickening at all doses and time points examined compared with their ΔE1-AV–treated counterparts (Figure 6a, 6c, 6e, 6g, 6i, and 6j). As expected, with increasing doses, ΔE1/ΔE4-AV produced more intimal thickening. However, even at the highest dose, the ΔE1/ΔE4-AV induced less neointimal thickening than 5-fold lower doses of the ΔE1-AV.

**Reduced Adenovirus Late Gene Expression in the ΔE1/ΔE4-AV–β-Gal Vector**

These results demonstrate that ΔE1/ΔE4-AV–β-gal–transduced vessels experienced reduced vector associated pathology than the ΔE1-AV–β-gal–treated vessels. Leaky expression of adenoviral late genes has been shown to be responsible for stimulating an immune response against the transduced cells and inducing cell death of the transduced cells. Indeed, we have observed that the ΔE1/ΔE4-AV–β-gal vector late gene expression is reduced in ex vivo cell cultures. HeLa cells were transduced with either the ΔE1/ΔE4-AV–β-gal or ΔE1-AV–β-gal vectors or wild-type adenovirus (Figure 7) and the presence of L3 or L5 late gene transcripts examined by using RT-PCR 4, 24, and 48 hours later. The ethidium bromide–stained gels of the PCR products indicated that the L3 transcripts were reduced in both the ΔE1-AV–β-gal and ΔE1/ΔE4-AV–β-gal transduced cells compared with wild-type adenovirus-infected cells. These gels were then Southern blotted and probed with a probe specific to the L3 hexon gene. L3 gene expression was detected in the Southern blot analysis in the ΔE1-AV–β-gal vector–transduced cells (Figure 7, bottom panel) but was still undetectable in the ΔE1/ΔE4-AV–β-gal–transduced cells. L5 gene expression was reduced in the ΔE1/ΔE4-AV–β-gal–transduced cells compared with both ΔE1-AV–β-gal vectors and wild-type adenovirus-transduced cells. Reduced late gene expression in ΔE1/ΔE4–AV–transduced vessels may be responsible for the greater persistence of vector genomes and β-gal protein over time and the lower levels of inflammation and intimal thickening observed in the ΔE1/ΔE4-AV vector–transduced vessels.
published studies examining the hepatic gene transfer after E4-AV–treated vessels is consistent with the previously
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to a release of growth factors and chemokines that in turn
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response. Endothelial injury promotes endothelial activation,
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Thus, we
treated cells. This may be attributable to the observed residual
inflammation, which although is substantially less than the
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E1-AV vector, is nevertheless greater than that observed in
sham-treated vessels. The residual inflammation may be the
result of continued low-level viral antigen expression despite
the multiple lethal mutations. Although no L3 gene (hexon protein)
expression and greatly reduced L5 (fiber protein) gene expression
is observed in ΔE1/ΔE4-AV–transduced cells in vitro, this low level of late gene expression may result
in the expression of the immunogenic adenovirus fiber protein. This may in turn be responsible for the observed loss of
adenoviral genomes from the ΔE1/ΔE4-AV–transduced arteries over time. The gutless or helper-dependent adenoviral vectors
that are deleted of all genes encoding adenoviral proteins26,33,34 would not experience this problem. It should be
noted however that detectable levels of ΔE1-AV are frequently found to contaminate preparations of the gutless
vectors, and it is unclear how this would impact vessel biology. An immune response to the xenoprotein β-gal may
also be contributing to the observed residual inflammation. Previous studies using recombinant adenoviruses expressing
either species-homologous genes35 or animals that are tolerant to the foreign transgenes27,36 have suggested that the
observed antiviral immune responses stem from both viral- and transgene-specific responses. Furthermore, these studies
suggest that reducing either the viral or transgene component can significantly improve the persistence of transgene
expression. The impact of the β-gal transgene on vascular biology however is unclear as our previous observations with a
first-generation empty vector suggests that it promoted similar levels of inflammation as an identical vector containing the
β-gal transgene.18
The demonstrated improvements in reduced inflammatory
responses, neointimal thickening, impairment of vessel func-
tion, and improved persistence in transgene expression with
the ΔE1/ΔE4-AV vector are encouraging. The biology of this
vector suggests that the ΔE1/ΔE4-AV vector may be particularly
well suited in the treatment of vascular conditions requiring only transient gene expression, for example, the
genetic modification of vein grafts. We hypothesize that antiproliferative and/or anti-inflammatory transgenes should
give substantially better results in vein graft models with the
ΔE1/ΔE4-AV vector than in an ΔE1-AV vector. Further studies are underway to examine this possibility.

Discussion
First-generation E1 gene–deleted adenoviral vectors lack
essential immediate-early genes—encoding transcription factors
that are required for viral DNA replication and the expression of viral late proteins. Although these deletions render the recombinant adenovirus replication incompetent and greatly reduce its cytopathogenicity, transgene persistence is limited by virus- and immune-mediated cytopathology resulting from residual low-level expression of the viral late gene products. Viral late gene expression is reduced in the ΔE1/ΔE4-AV vectors.

Transduction of rabbit carotid arteries with an ΔE1/
ΔE4-AV vector encoding lacZ produced superior persistence of β-gal expression and a significant reduction in vessel inflammation, intimal hyperplasia, and vessel dysfunction compared with the corresponding ΔE1-AV vector. We have previously shown that first-generation adenovirus induces tier-dependent impairment in endothelium-dependent relaxation. The functional impairment was likely a result of the host inflammatory response, because arteries from pan-
cytopenic rabbits had entirely normal vasomotor function even after high doses of 2×1015 fp AV vector.15 Thus, we believe that the normal vasomotor profile of ΔE1/ΔE4-AV–
treated vessels is a reflection of the virus’ reduced toxicity and the corresponding diminished vascular inflammation. The reduced intimal thickening in the ΔE1/ΔE4-AV vector–
treated vessels is likely a benefit of the reduced inflammatory response. Endothelial injury promotes endothelial activation, platelet deposition, and inflammatory cell infiltration, leading to a release of growth factors and chemokines that in turn stimulate vascular smooth muscle growth and migration into the intima.

The reduced inflammatory response observed in the ΔE1/
ΔE4-AV–treated vessels is consistent with the previously
published studies examining the hepatic gene transfer after intravenous delivery of the ΔE1/ΔE4-AV vector in mice.32 However, in the latter studies, the ΔE1/ΔE4-AV vector–
treated mice demonstrated significantly prolonged hepatic transgene expression (>7 months) compared with the
ΔE1-AV vector (<2 months). In the present studies, we observed only improved transgene expression with the ΔE1/
ΔE4-AV vector at 10 days after gene transfer. Essentially, no transgene expression was observed at 28 days with either vector. Similar results were obtained by 3 different methodologies: lacZ-staining of the vessels, anti–β-gal ELISA, and PCR detection of the AV vectors. The drop-off in viral genomes, determined by using PCR, indicates that the loss of β-gal–staining resulted from the clearance of the transduced cells. This may be attributable to the observed residual inflammation, which although is substantially less than the
ΔE1-AV vector, is nevertheless greater than that observed in sham-treated vessels. The residual inflammation may be the
result of continued low-level viral antigen expression despite
the multiple lethal mutations. Although no L3 gene (hexon protein) expression and greatly reduced L5 (fiber protein) gene expression is observed in ΔE1/ΔE4-AV–transduced cells in vitro, this low level of late gene expression may result
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References

Figure 7. Reduced adenovirus late gene expression in the
ΔE1/ΔE4-AV–β-gal vector. HeLa cells were transduced with either the ΔE1/ΔE4-AV–β-gal or ΔE1-AV–β-gal vectors or wild-
type adenovirus at a MOI of 10 fp. At the indicated times, the cells were lysed and RNA prepared and analyzed by using
RT-PCR and gel electrophoresis for the presence of L3 or L5 late gene transcripts or as a control, actin transcripts. To
increase the sensitivity of the assay, the L3 RT-PCR gel was blotted and probed with a probe specific for hexon sequences. No hexon transcripts were detected in the ΔE1/ΔE4-AV–β-gal-
transduced cells.


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