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Patricia Lemarchand, M Jones, I Yamada, R Crystal. In vivo gene transfer and expression in normal uninjured blood vessels using replication-deficient recombinant adenovirus vectors.. *Circulation Research*, 1993, 72 (5), pp.1132-1138. <10.1161/01.RES.72.5.1132>. <inserm-05117017>

HAL Id: inserm-05117017

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Submitted on 17 Jun 2025

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Rapid Communication

In Vivo Gene Transfer and Expression in Normal Uninjured Blood Vessels Using Replication-Deficient Recombinant Adenovirus Vectors

Patricia Lemarchand, Michael Jones, Izumi Yamada, and Ronald G. Crystal

Replication-deficient recombinant adenovirus vectors do not require target cell replication for transfer and expression of exogenous genes and thus may be useful for in vivo gene therapy in the endothelium. To evaluate the feasibility of adenovirus-mediated gene transfer in vivo in normal intact blood vessels, adenovirus vectors containing the *Escherichia coli lacZ* gene or a human α_1 -antitrypsin (α_1 AT) cDNA were injected in vivo into the lumen of an occluded vessel segment of sheep jugular vein and/or carotid artery. After 15 minutes of incubation, circulation was restored; the vessels were harvested 1–28 days later and evaluated for gene transfer and expression. Three days after in vivo exposure to the *lacZ* adenovirus vector, the endothelium of jugular veins and carotid arteries expressed β -galactosidase. Exposure of jugular veins and carotid arteries in vivo to the α_1 AT adenovirus vector resulted in the expression of α_1 AT mRNA transcripts detected by Northern analysis and in the synthesis and secretion of α_1 AT detected by ex vivo [35 S]methionine labeling. Expression with the adenovirus vectors was efficient and easily detectable 1–14 days after injection, with maximum expression at 7 days. Expression was no longer evident at 28 days. Thus, adenovirus vectors are capable of transferring exogenous genes to the endothelium of normal arteries and veins with expression for at least 2 weeks, suggesting that these vectors have the potential for a variety of cardiovascular experimental and clinical applications. (*Circulation Research* 1993;72:1132–1138)

KEY WORDS • adenovirus • gene expression • gene transfer • endothelium

Gene therapy is a medical intervention to alter the genetic program of cells for therapeutic purposes.¹ The endothelium of arteries and veins represents an important potential target for gene therapy by virtue of its large surface area and proximity to the circulation.^{2–14} If genes can be transferred to the vascular endothelium, it should be possible to modify the function and integrity of the blood vessel wall and to deliver therapeutic proteins into the circulation. In this context, gene transfer to the endothelium may be applicable to a variety of inherited and acquired diseases.

Theoretically, two strategies could be used to achieve the goal of gene therapy using the endothelium in vivo. First, the gene can be transferred into endothelial cells in vitro, followed by reintroduction of the modified cells into the vessel wall of the recipient.^{2–5} Second, the gene can be transferred in vivo directly into the vessel wall.^{7,11,13–16} Although the in vitro approach is readily achieved by a variety of approaches, the in vivo strategy has a major technical hurdle based on the inherent biology of the endothelium in its resting state in vivo. In

this regard, endothelial cells replicate slowly, limiting the use of gene transfer vectors that depend on cell proliferation to express the exogenous gene.^{17,18}

The replication-deficient recombinant adenovirus (Ad), which does not require host cell proliferation to express the exogenous gene, offers a potential solution to this problem. In a recent study, we demonstrated that Ad vectors will mediate transfer of genes to intact human vessels ex vivo, with a high level of expression of the transferred gene in the endothelium.¹⁹ Other useful characteristics of Ad vectors are as follows: 1) They can be manipulated to accommodate exogenous genes of at least 7.5 kb. 2) Live Ads have been used safely as human vaccines in more than 5 million individuals. 3) There is no known association of human malignancy with Ad infection.^{20–24} Human Ad vectors have been approved by the National Institutes of Health recombinant DNA advisory committee for human gene transfer trials to treat the respiratory manifestations of cystic fibrosis.²⁵

With the knowledge that human endothelial cells are susceptible to in vitro gene transfer with Ad vectors, the present study is directed at evaluating the feasibility of Ad-mediated gene transfer in vivo in normal intact vessels. To accomplish this, we have used direct injection of the Ad vectors into the lumen of jugular veins and carotid arteries of sheep. Two model genes were evaluated. The *Escherichia coli lacZ* gene, coding for β -galactosidase (β -gal), was used as an example of a gene coding for an intracellular protein. The human

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Received January 4, 1993; accepted February 2, 1993.

α_1 -antitrypsin (α_1 AT) cDNA, coding for the plasma antiprotease α_1 AT, was used as a model of a protein secreted into the circulation. The data demonstrate that the Ad vectors are capable of in vivo gene transfer and expression in the endothelium of normal jugular veins and carotid arteries for at least 2 weeks.

Materials and Methods

Adenovirus Vectors

The Ad vectors used are replication-deficient recombinant Ad based on Ad type 5, in which the majority of the E1 and E3 regions are deleted and a cassette containing a recombinant exogenous gene and promoter is inserted at the E1 region.^{20,21} For details concerning the general design, assembly, production, and propagation of the recombinant Ad vectors, see References 19–21 and 26. Three different Ad vectors were used: 1) Ad.RSV β gal carries the *E. coli lacZ* gene encoding for β -gal, an intracellular protein that can be detected with a simple in situ histochemical assay.²⁷ Ad.RSV β gal uses the long terminal repeat of the Rous sarcoma virus as a promoter, followed by the SV40 nuclear localization signal and the *E. coli lacZ* gene.^{19,26} 2) Ad- α_1 AT, carrying the human α_1 AT cDNA, codes for α_1 AT, a secreted glycoprotein. Ad- α_1 AT uses the Ad2 major late promoter as a promoter, followed by the human α_1 AT cDNA.²⁰ 3) AdCFTR, used as a control, is a vector containing the Ad2 major late promoter as a promoter, followed by the normal human cystic fibrosis transmembrane conductance regulator (CFTR) cDNA.²¹ The preparations of Ad.RSV β gal were evaluated by colorimetric analysis to ensure that they were not contaminated with β -gal,^{26,28} and the preparations of Ad- α_1 AT were evaluated by enzyme-linked immunosorbent assay (ELISA) to ensure there was no contaminating α_1 AT.²⁰

Cell Culture and In Vitro Infection

To estimate the time needed for exposure of the endothelium to the Ad vectors to ensure gene transfer, human umbilical vein endothelial cells were exposed to Ad.RSV β gal for various periods of time and then evaluated for gene transfer and expression. Human umbilical vein endothelial cells were cultured as described previously.¹⁹ After cells were grown to 80% confluence, duplicate cultures were incubated with Ad.RSV β gal at 100 plaque-forming units (pfu) per cell for 1–30 minutes. The cells were then washed five times with phosphate-buffered saline (PBS) to eliminate the virus remaining on the cell surface and incubated with virus-free media for 24 hours. As controls, parallel cell cultures were incubated with no virus or with Ad.RSV β gal for 24 hours. After 24 hours, the presence of the *lacZ* gene product was determined in duplicate by staining the endothelial cells with the X-Gal reagent (5-bromo-4-chloro-3-indolyl β -D-galactopyranoside, Boehringer Mannheim Corp., Indianapolis, Ind.).²⁷ Expression of the *lacZ* gene was considered positive when a nuclear-dominant blue color was observed.

In Vivo Gene Transfer

The in vivo study was carried out with female and male sheep weighing 20–25 kg (mixed Rambouillet, Dorset, Suffolk breed; 4–6 months old). Gene transfer was attempted in a total of 26 sheep. Two animals died

and were not evaluated: one during surgery of a cardiac arrhythmia and one of aspiration pneumonia at 1 day. Twenty-four sheep were evaluated for administration of the Ad vectors into jugular veins, and 22 were evaluated for administration into carotid arteries. The administrations of Ad vectors to the lumen of veins and arteries were performed under general anesthesia and sterile surgical conditions. Anesthesia was induced with intravenous sodium pentobarbital (25 mg/kg) and diazepam (10 mg) and maintained with 1–2% isoflurane with oxygen via an endotracheal tube and a volume-cycled ventilator. Bilateral longitudinal cervical incisions exposed the right and left external jugular veins and common carotid arteries. The periadventitial tissues of the common carotid arteries were infiltrated with papaverine (30 mg/ml, 0.5–1 ml) and lidocaine hydrochloride (10 mg/ml, 0.5–1 ml) to prevent vasospasm. All branches of both arteries and veins, between and including the superior and inferior laryngeal branches, were ligated and divided. Bovine lung sodium heparin (300 units/kg) was injected intravenously for systemic anticoagulation while the vessels were occluded. The vessels were encircled with silastic tourniquets, isolating a 5-cm segment of each. Blood was evacuated from the vessels, and the medium containing the Ad vector ($1-4 \times 10^{10}$ pfu in 800 μ l into the external jugular veins and $0.5-3 \times 10^{10}$ pfu in 400 μ l into the carotid arteries) was introduced by direct injection into the lumen of the occluded vessel segment. For each animal, the lumen of the right jugular vein and/or carotid artery was injected with Ad.RSV β gal or Ad- α_1 AT, while the lumen of the contralateral vessel was used as a control and was injected with AdCFTR. After 15 minutes of incubation (a time determined by preliminary in vitro studies, see “Results”), the medium containing the remaining Ad vector inside the occluded vessel segment was aspirated, the tourniquets were removed, and circulation was restored. To aid in identification of the segments of the vessels exposed to the Ad vectors, the regions of the vessels that had been exposed to the vectors were delineated by suture markers placed circumferentially. The cervical incisions were closed, and the animals were allowed to recover. Prophylactic antibiotics, gentamicin (3 mg/kg) and penicillin G (9×10^5 units), were administered twice daily for 3 days.

The vessels were harvested 1–28 days later under general anesthesia as described above. After exposure of the vessels, the animals received systemic heparin. The carotid arteries and external jugular veins were excised before euthanasia. To evaluate the specificity of the local administration of the vectors, in selected animals, tissue samples of cervical muscle, thyroid, liver, lung, heart, spleen, brain, both kidneys, and gonads were excised after death.

Evaluation of Gene Transfer and Expression

To evaluate in vivo gene transfer and expression at the histological level, 3 days after in vivo administration of the Ad.RSV β gal vector (or the control Ad vector AdCFTR), jugular vein and carotid artery segments were fixed (2 hours at 4°C) in 2% formaldehyde and 0.2% glutaraldehyde in PBS, pH 7.4. Samples were then stained for 6 hours in X-Gal solution as described above. After staining, samples were postfixed in the

same fixative, embedded in paraffin, cut into 5- μ m sections, and counterstained with nuclear fast red.

To evaluate *in vivo* Ad-mediated gene transfer and expression at the mRNA level, the expression of α_1 AT mRNA transcripts was evaluated by Northern analysis at 1–28 days after *in vivo* administration of Ad- α_1 AT (or the control vector AdCFTR). The lumen of each vessel was filled with guanidium thiocyanate homogenization buffer (5 minutes at 23°C), the buffer was removed, and total RNA was extracted, purified, and analyzed with 32 P-labeled human α_1 AT cDNA probe or, as a control, γ -actin cDNA probe as described previously.¹⁹

To evaluate *in vivo* Ad-mediated gene transfer and expression of a secreted protein, the biosynthesis and secretion of human α_1 AT in the vessels was evaluated *ex vivo* by [35 S]methionine labeling. At 1–28 days after *in vivo* administration of Ad- α_1 AT (or the control vector AdCFTR), each vessel was removed and perfused *ex vivo* with methionine-free media (5 ml at 37°C) containing [35 S]methionine (2.5 mCi, 1,233 Ci/mmol, New England Nuclear, Boston).¹⁹ After 24 hours, the perfusing medium was collected and evaluated by immunoprecipitation with an anti-human α_1 AT antibody, sodium dodecyl sulfate–acrylamide gel electrophoresis, and autoradiography as previously described.¹⁹

Results

When incubated *in vitro* with Ad.RSV β gal for 1 minute, human umbilical vein endothelial cells exhibited gene transfer and expression in 10% of the cells (Figure 1). The relative number of endothelial cells expressing β -gal increased with time of exposure of the cells to the vector. Based on the observation that a reasonable proportion (58%) of the cells expressed β -gal when the vector was in contact with the cells for 15 minutes, this time was chosen as a time of exposure of the veins and arteries to the Ad vectors for the *in vivo* studies.

To evaluate the feasibility of *in vivo* gene transfer with direct intraluminal exposure of the Ad vectors in blood vessels and to identify the cell types expressing the exogenous gene, the Ad vector containing the *lacZ* gene (and as a control, AdCFTR) was administered into the lumen of jugular veins, and β -gal expression in the vein was evaluated 72 hours later. After administration of AdCFTR, X-Gal staining revealed no blue coloration and thus no β -gal activity (Figures 2A and 2B). In contrast, after administration of Ad.RSV β gal, X-Gal staining showed extensive blue coloration of the interior of the portion of the vessel exposed to the vector, indicative of β -gal activity in cells lining the lumen (Figures 2D and 2E). Microscopic examination of the veins showed normal morphology after exposure to either AdCFTR and Ad.RSV β gal. Strikingly, there was β -gal activity only in the veins exposed to the Ad.RSV β -gal vector, and the β -gal expression was limited to the endothelial cells (Figures 2C and 2F).

In the carotid arteries exposed to the Ad vectors, the results were similar. After administration of AdCFTR, X-Gal staining revealed no blue coloration and thus no β -gal activity (Figures 3A and 3B). In contrast, after administration of Ad.RSV β gal, X-Gal staining showed extensive blue color of the interior of the vessel exposed to the vector, indicative of β -gal activity in cells lining the lumen (Figures 3D and 3E). Microscopic examina-

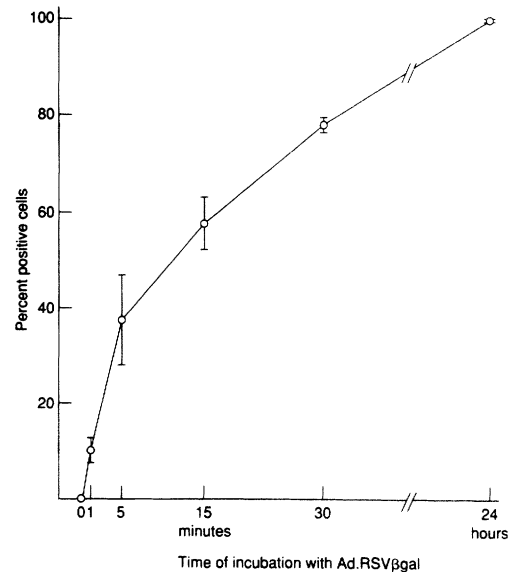


FIGURE 1. Graph showing the minimum time of exposure of the endothelium to adenovirus vectors to permit optimal transfer of the exogenous gene. Transfer and expression of the *Escherichia coli lacZ* gene in human endothelial cells infected *in vitro* with adenovirus vector Ad.RSV β gal was evaluated as a function of incubation time with Ad.RSV β gal. Human endothelial cells were incubated with Ad.RSV β gal for various periods of time, washed with phosphate-buffered saline to eliminate the remaining virus, and incubated with media. After 24 hours, cells were stained by X-Gal, and the positive cells (i.e., nuclear-dominant blue color) were counted and expressed as percent positive cells of 500 total cells per well. The data represent duplicate assays of three separate experiments (mean \pm SEM).

tion of the arteries showed normal morphology after exposure to either AdCFTR or Ad.RSV β gal. As with the veins, there was β -gal expression only in the arteries exposed to Ad.RSV β gal, and the β -gal expression was limited to the endothelial cells (Figures 3C and 3F).

In three sheep in which veins and arteries were exposed to Ad.RSV β gal *in vivo*, tissues from cervical muscle, liver, lung, heart, spleen, brain, both kidneys, both gonads, and thyroid (used as a positive control) were excised after death and stained with X-Gal in parallel with the vessels. None of these tissues showed any β -gal activity, except the thyroid, as expected, which showed light cytoplasmic blue coloration that was due to the presence of endogenous β -gal (not shown).

After *in vivo* exposure to Ad- α_1 AT, the endothelial cells in both jugular veins and carotid arteries expressed the human α_1 AT cDNA. Northern analysis was performed with an α_1 AT cDNA probe, and no α_1 AT mRNA transcripts were observed in veins 1 day after exposure to AdCFTR (Figure 4, lane 1). In contrast, human α_1 AT mRNA transcripts of the expected size were detected in veins exposed to Ad- α_1 AT at 1, 7, and 14 days after administration of the vector (lanes 2–4). By 28 days, no α_1 AT mRNA transcripts could be detected (lane 5). Similarly, no α_1 AT mRNA transcripts were observed in carotid arteries 1 day after *in vivo* exposure to AdCFTR (lane 6). In contrast, human α_1 AT mRNA transcripts of the expected size were detected in

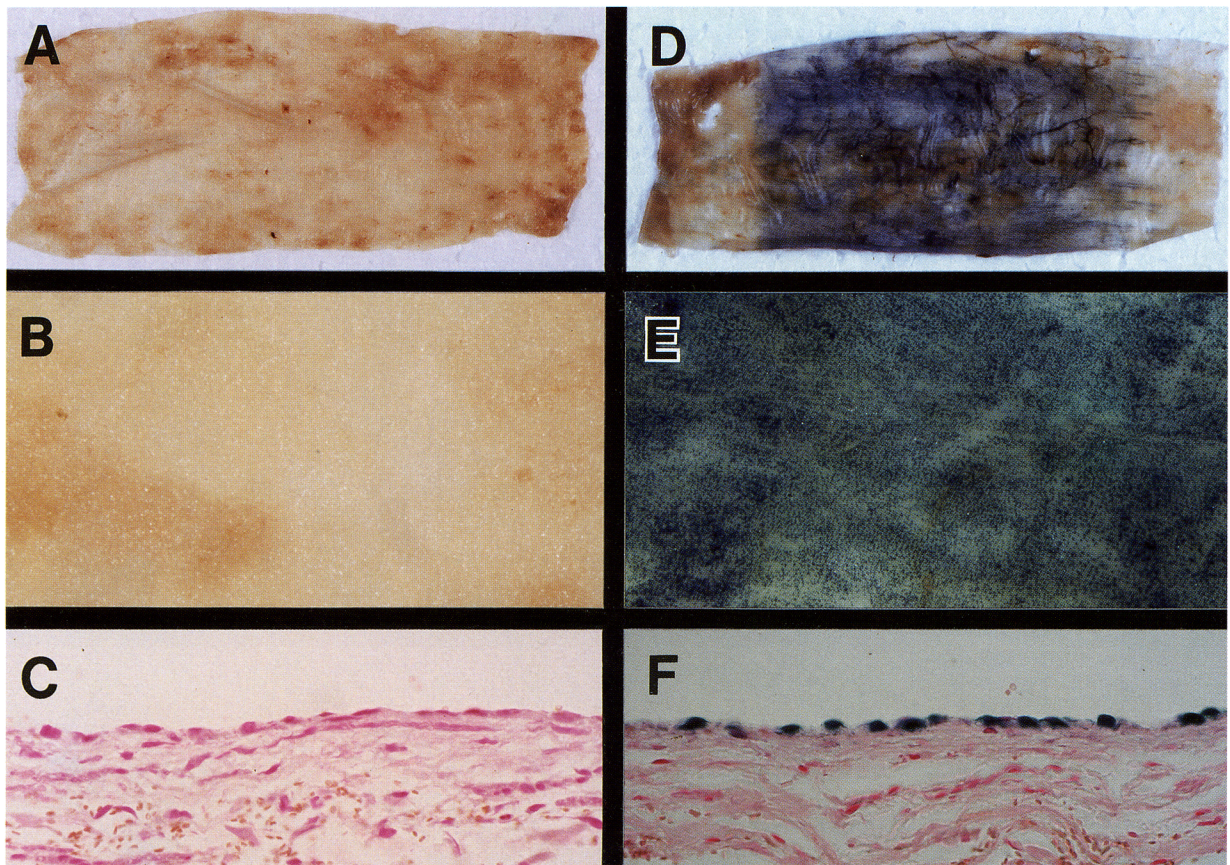


FIGURE 2. Photomicrographs showing adenovirus-mediated transfer and expression of the lacZ gene in vivo in sheep jugular veins. The jugular veins were clamped and exposed to adenovirus vector Ad.RSV β gal or AdCFTR (as a control) for 15 minutes, the clamps were released, and the blood flow was reestablished. The vessels were evaluated 3 days later. Panel A: Jugular vein exposed to AdCFTR. Stain, X-Gal. Macroscopic view, $\times 1.4$. Panel B: Same conditions as in panel A. Magnification, $\times 16$. Panel C: Same conditions as in panel A. Counterstain, nuclear fast red. Microscopic view, $\times 320$. Panels D–F: Same conditions as in panels A–C, respectively, but infected with Ad.RSV β gal. In panel D, note the demarcation to the left and right where the vein was occluded and thus not exposed to the vector.

veins exposed to Ad- α_1 AT at 1, 7, and 14 days after exposure to the vector (lanes 7–9). As with the veins, no α_1 AT mRNA transcripts could be detected 28 days after exposure to Ad- α_1 AT (lane 10). Control γ -actin mRNA transcripts were at similar levels in all samples (lanes 11–20).

Evaluation of the jugular veins for synthesis and secretion of human α_1 AT demonstrated that veins exposed to AdCFTR did not secrete human α_1 AT (Figure 5, lane 1). In contrast, de novo biosynthesis and secretion of human α_1 AT was easily detectable in veins exposed to Ad- α_1 AT 1 day after exposure to the vector (lane 2). The labeled secreted protein was specifically human α_1 AT, as shown by blocking of the anti-human α_1 AT antibody using excess amounts of unlabeled human α_1 AT (lane 3). The biosynthesis and secretion of human α_1 AT by the veins exposed to Ad- α_1 AT was detected at 7 and 14 days after exposure to Ad- α_1 AT (lanes 4 and 5) but was undetectable by day 28 (lane 6). Similarly, evaluation of the carotid arteries for synthesis and secretion of human α_1 AT demonstrated that the arteries exposed to AdCFTR did not secrete human α_1 AT (lane 7). In contrast, biosynthesis and secretion of human α_1 AT was demonstrated in arteries exposed to Ad- α_1 AT 1 day after exposure to the vector (lane 8).

This labeled secreted protein was specifically human α_1 AT, as shown by blocking of the anti-human α_1 AT antibody with excess amounts of unlabeled human α_1 AT (lane 9). The biosynthesis and secretion of human α_1 AT by the arteries exposed to Ad- α_1 AT was easily detected at 7 and 14 days after exposure to Ad- α_1 AT (lanes 10 and 11) but not at 28 days (lane 12). To evaluate if human α_1 AT could be detected in the serum of animals that received Ad- α_1 AT, sheep serum was evaluated by ELISA before and 1 day after administration of Ad- α_1 AT in the jugular vein. There was no detectable human α_1 AT in the tested serum (detection threshold, 3 ng/ml).

Discussion

The endothelium of arteries and veins represents an excellent target for in vivo gene transfer. It is easily accessible and, because of its intimacy with the circulation, provides the ideal site to produce secreted proteins for therapeutic use in the local and/or systemic circulation. The present study demonstrates that it is feasible to transfer genes in vivo to the endothelium of normal arteries and veins with replication-deficient recombinant Ad vectors. Subsequent to exposing the endothelium of large vessels in vivo to an Ad vector for 15

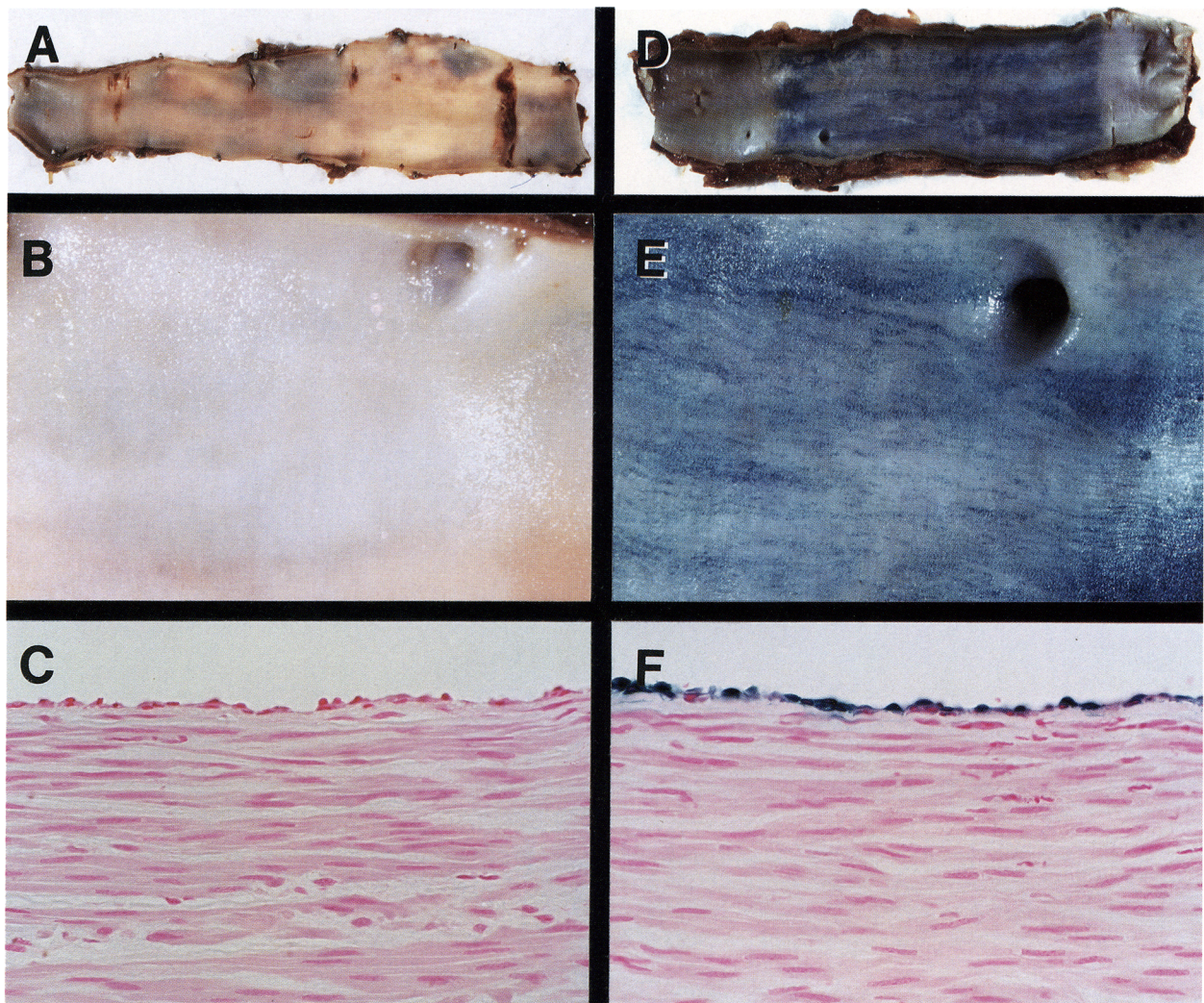


FIGURE 3. Photomicrographs showing adenovirus-mediated transfer and expression of the *Escherichia coli lacZ* gene in vivo in sheep carotid arteries. The carotid arteries were clamped and exposed to adenovirus vector *Ad.RSVβgal* or *AdCFTR* (as a control) for 15 minutes, the clamps were released, and the blood flow was reestablished. The vessels were evaluated 3 days later. Panel A: Carotid artery exposed to *AdCFTR*. The patches of light blue-gray coloration represent blood in the vessel wall. Stain, X-Gal. Macroscopic view, $\times 1$. Panel B: Same conditions as in panel A. Magnification, $\times 15.4$. Panel C: Same conditions as in panel A. Counterstain, nuclear fast red. Microscopic view, $\times 320$. Panels D–F: Same conditions as in panels A–C, respectively, but infected with *Ad.RSVβgal*. Note in panel D the demarcation to the left and right where the vessel was occluded and thus not exposed to the vector; the light blue-gray color in these areas represents blood in the vessel wall.

minutes, there is effective transfer and easily detectable expression of the exogenous gene contained within the vector for at least 2 weeks, suggesting that Ad-mediated gene transfer could be used for a variety of potential therapies for human diseases. The data also suggest that endothelial cells have receptors for Ad, although direct quantification of receptor number and avidity have not been made. These results are consistent with our previous study,¹⁹ in which the same Ad vectors were used to transfer exogenous genes to human umbilical vein endothelial cells in vitro and to the intact umbilical vein ex vivo and in which the peak of expression of the transferred gene was also at 7 days. In the present study, we could not detect by ELISA human α_1 AT in the serum of the sheep that received *Ad-α₁AT* 1 day after administration of *Ad-α₁AT* in the jugular vein, likely because of the dilution of the secreted α_1 AT into the blood mass of

the sheep (approximately 2 l) and the detection threshold of the ELISA.²¹

There are a number of advantages of Ad vectors over other approaches to in vivo gene transfer to blood vessels. First, although not evaluated in this study, it is likely that the Ad DNA is not integrated in the genome of the target cell.^{22,23} This has advantages for gene therapy in that insertional mutagenesis is not a concern, and it may provide flexibility for dosing therapeutic genes in acute acquired diseases. Second, the Ad does not require target cell proliferation to express the exogenous gene, in contrast to retrovirus vectors in which target cell replication is required. In this regard, because the endothelium replicates very slowly in vivo, to achieve efficient gene transfer, retrovirus vectors necessitate local injury (such as with an intravascular balloon or high injection pressure) to promote endothe-

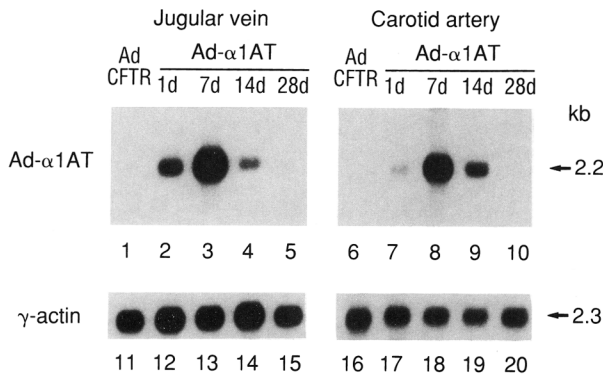


FIGURE 4. Adenovirus (Ad)-mediated transfer and expression of the human α_1 -antitrypsin (α_1 AT) cDNA in vivo in the sheep jugular vein and carotid artery evaluated by Northern analysis. CFTR, cystic fibrosis transmembrane conductance regulator. One to 28 days after administration of Ad- α_1 AT or AdCFTR (as a control vector), the vessels were excised, and RNA was extracted and hybridized with an α_1 AT cDNA probe (lanes 1–10) or a γ -actin cDNA probe (lanes 11–20). Each lane contains 2 μ g per lane of total RNA extracted from one single vessel (5-cm length): lane 1, jugular vein 1 day after exposure to AdCFTR; lane 2, jugular vein 1 day after exposure to Ad- α_1 AT; lane 3, jugular vein 7 days after exposure to Ad- α_1 AT; lane 4, jugular vein 14 days after exposure to Ad- α_1 AT; lane 5, jugular vein 28 days after exposure to Ad- α_1 AT; lanes 6–10, identical to lanes 1–5 but in carotid artery; lanes 11–20, identical to lanes 1–10 but evaluated with a γ -actin probe. The sizes of the transcripts are indicated in kilobases (kb).

lial cell proliferation.^{7,13,29} Plasmid-based vector systems, such as plasmids in association with liposomes, do not require target cell proliferation, but these strategies have a very low level of efficiency.¹⁴ In contrast, the present study demonstrates that replication-deficient recombinant Ad vectors can mediate efficient and easily detectable gene transfer to the normal endothelium in vivo with no damage from the vector itself. In this regard, gene transfer and expression was observed in the endothelium up to 14 days after exposure of the blood vessels to the Ad vectors, and microscopic examination of the vessels infected with the Ad.RSV β gal vector showed that most of the endothelial cells were successfully transfected, with no attendant damage from the vector.

Although expression with the Ad vectors was efficient and at easily detectable levels, it was maximum at 7 days and then declined, such that expression was no longer evident at 28 days. There are several hypotheses that may explain this decline. First, although endothelial cell replication is slow in vivo, it is conceivable that target cell turnover may play some role. Although the surgical intervention was limited, it could have caused mild vascular injury and stimulated the endothelial cells to proliferate at a faster rate than normal. However, from 1 to 28 days after injection of the Ad vectors, we never observed any damage to the endothelium, loss of endothelium, or intimal hyperplasia, suggesting that the endothelium was not damaged. Second, the chronicity of expression was evaluated with a vector coding for human α_1 AT. Since this is a secreted protein and the

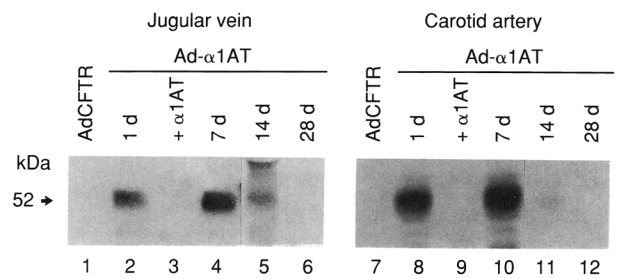


FIGURE 5. Adenovirus (Ad)-mediated transfer and expression of the human α_1 -antitrypsin (α_1 AT) cDNA in vivo in the sheep jugular vein and carotid artery evaluated by α_1 AT biosynthesis ex vivo. CFTR, cystic fibrosis transmembrane conductance regulator. Shown are sodium dodecyl sulfate-acrylamide gel analyses of supernatants of sheep vessels incubated 24 hours with [³⁵S]methionine at different time points after in vivo exposure to Ad- α_1 AT (or the control vector AdCFTR) and immunoprecipitated with an anti- α_1 AT antibody before sodium dodecyl sulfate-acrylamide gel electrophoresis and autoradiography. Conditions were as follows: lane 1, jugular vein 1 day after exposure to AdCFTR; lane 2, jugular vein 1 day after exposure to Ad- α_1 AT; lane 3, same as lane 2 but with immunoprecipitation carried out with excess unlabeled α_1 AT; lane 4, same as lane 2 but 7 days after exposure to Ad- α_1 AT; lane 5, same as lane 2 but 14 days after exposure to Ad- α_1 AT; lane 6, same as lane 2 but 28 days after exposure to Ad- α_1 AT; lanes 7–12, same as lanes 1–6 but in carotid artery.

study was carried out in experimental animals with an intact immune system, it is possible that some of the decreased expression over time results from immunity directed against the heterologous protein or against the modified cells. Finally, the expression of the exogenous gene may be suppressed inside the cell, in a fashion analogous to that observed with retrovirus exogenous gene expression in cells modified ex vivo and then transplanted into experimental animals.³⁰ If further study demonstrates that the expression of the exogenous gene does only last for approximately 2 weeks, repetitive administration will have to be used to achieve chronic expression.

Importantly, the studies with Ad.RSV β gal vector demonstrate that administration of an Ad vector in an occluded vessel results in localized endothelial cell expression of β -gal, with no expression of the *lacZ* gene in other organs, including the gonads, 3 days after administration of the Ad vector. This supports the hypothesis that administration of Ad vectors to a specific organ, such as limited segments of blood vessels, will not result in the systemic spread of gene expression, which is an important safety consideration relating to in vivo gene therapy. This observation is similar to that made in a study of gene transfer to the liver with these vectors: when adult rats were given the Ad vector directly to the liver via intraportal injection, gene transfer and expression were limited to the liver.³¹ In contrast, systemic administration of Ad vectors to newborn mice results in the exogenous gene expression in multiple organs including lung, liver, intestine, heart, and skeletal muscle.^{32,33}

In the context of the observations in the present study, the adaptation of replication-deficient recombi-

nant Ad vectors for in vivo gene transfer to the endothelium of arteries and veins should be useful in a variety of applications for the understanding of vascular biology and for developing a variety of new therapeutics for genetic and acquired diseases.

Acknowledgments

We would like to thank C. Danel and H.A. Jaffe, Pulmonary Branch, National Heart, Lung, and Blood Institute, for helpful discussions; the staff of the Laboratory of Animal Medicine and Surgery, National Heart, Lung, and Blood Institute, for their assistance; and S. Dame for editorial assistance.

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