Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 6482–6486, July 1992
Medical Sciences

Adenovirus-mediated transfer of a recombinant human α_1-antitrypsin cDNA to human endothelial cells

PATRICIA LEMARCHAND*, H. ARI JAFFE*, CLAIRE DANEL*, MARIA C. CID†, HYNDA K. KLEINMAN†, LESLIE D. STRATFORD-PERRICAUDET‡, MICHEL PERRICAUDET‡, ANDREA PAVIRANI§, JEAN-PIERRE LECOCQ§, AND RONALD G. CRYSTAL*¶

*Pulmonary Branch, National Heart, Lung, and Blood Institute, and †Laboratory of Developmental Biology, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892; ‡Institut Gustave Roussy, Centre National de la Recherche Scientifique, UA 1301, Villejuif, France; and §Transgene SA, Strasbourg, France

Communicated by Maurice B. Burg, March 30, 1992

ABSTRACT **To evaluate the feasibility of using a replication-deficient recombinant adenovirus to transfer human genes to the human endothelium, human umbilical vein endothelial cells were infected *in vitro* with adenovirus vectors containing the *lacZ* gene or a human α_1-antitrypsin (α_1AT) cDNA. After *in vitro* infection with the *lacZ* adenovirus vector, cultured endothelial cells expressed β-galactosidase. In parallel studies with the α_1AT adenovirus vector, infected cells expressed human α_1AT transcripts, as evidenced by *in situ* hybridization and Northern analysis, and *de novo* synthesized and secreted glycosylated, functional α_1AT within 6 hr of infection, as shown by [^{35}S]methionine labeling and immunoprecipitation. Quantification of the culture supernatants demonstrated 0.3–0.6 μg of human α_1AT secreted per 10^6 cells in 24 hr, for at least 14 days after adenovirus vector infection. To demonstrate the feasibility of direct transfer of genes into endothelial cells in human blood vessels, *lacZ* or α_1AT adenovirus vectors were placed in the lumen of intact human umbilical veins *ex vivo*. Histologic evaluation of the veins after 24 hr demonstrated transfer and expression of the *lacZ* gene specifically to the endothelium. α_1AT adenovirus infection resulted both in expression of α_1AT transcripts in the endothelium and in *de novo* synthesis and secretion of α_1AT. Quantification of α_1AT in the vein perfusates showed average levels of 13 μg/ml after 24 hr. These observations strongly support the feasibility of *in vivo* human gene transfer to the endothelium mediated by replication-deficient adenovirus vectors.**

Endothelial cells are attractive cell targets for gene therapy because of their accessibility and immediate contact with the bloodstream, permitting delivery of the gene and the resulting gene modifications as a therapeutic strategy for protein delivery to systemic or local circulations (1–10). One major problem in accomplishing this is related to the slow rate of endothelial cell proliferation *in vivo*, estimated to be only ≈1% of the cells per day (11). One strategy for direct gene transfer to endothelial cells is to utilize a vector that does not require host cell replication for gene expression. Replication-deficient recombinant adenovirus (Ad) vectors fulfill this criterion (12, 13). Other advantages of these vectors are that despite common human Ad infection there is no known association of human malignancies with Ad infection, the Ad genome can be manipulated to accommodate foreign genes up to 7.0–7.5 kilobases (kb), live Ad has been safely used as a human vaccine, and there are examples in animals and humans of infection of endothelial cells by natural Ad (12–19).

To assess the feasibility of using a replication-deficient recombinant Ad to transfer genes to human endothelial cells, we have utilized two recombinant Ad vectors, one containing the *Escherichia coli lacZ* gene (as a histologic marker gene to demonstrate specific endothelial cell expression), and the other containing the human α_1-antitrypsin (α_1AT) cDNA (as a prototype human gene that directs the synthesis of a secreted protein not expressed by endothelial cells) (13, 20–22). Since humans are the only natural targets of human Ad infection, we have focused on human endothelial cells as targets for gene transfer with the Ad vectors. Two models were used: (*i*) *in vitro* infection of primary endothelial cell cultures and (*ii*) intact human umbilical veins perfused with the Ad vectors. While the first permits detailed evaluation of the expression of the transferred gene, the latter represents a model of *in vivo* gene transfer to human endothelium in circumstances where the architecture is as close to the human *in vivo* situation as possible *ex vivo*. With both models, the data clearly suggest that *in vivo* human gene transfer to endothelial cells is indeed feasible with a recombinant Ad.

METHODS

Ad Vectors. The replication-deficient recombinant Ad vectors were based on Ad type 5 (13, 15). Portions of the E1 and E3 regions were deleted, and a cassette containing a recombinant exogenous gene and its promoter was inserted at the site of the E1 deletion. For details on the general design, assembly, production, and propagation of recombinant Ad vectors, see refs. 12, 13, 15, 16, and 20. Ad-α_1AT contained the Ad type 2 major late promoter driving a human α_1AT cDNA (13, 20). Ad.RSVβgal contained the long terminal repeat of the Rous sarcoma virus as a promoter followed by the simian virus 40 nuclear localization signal and the *lacZ* gene [encoding β-galactosidase (β-gal)] (21, 38). Ad-CFTR, used as a control, contained the Ad 2 major late promoter and a human cystic fibrosis transmembrane conductance regulator (CFTR) cDNA (23). All preparations of Ad-α_1AT were evaluated by ELISA to ensure that there was no contaminating human α_1AT (13).

Cell Culture and *in Vitro* Infection. Human umbilical vein endothelial cells were obtained (24) and cultured (25) as described, but with the addition of heparin (5 units/ml) to the medium. Identification of endothelial cells was confirmed by positive staining with anti-factor VIII-related antigen (von Willebrand factor) antibody (Dako) in an immunoperoxidase technique (24, 26). After cells were grown to 80% confluence, they were infected with Ad-α_1AT, Ad.RSVβgal, or Ad-CFTR at 100 plaque-forming units (pfu) per cell. The cells were incubated for 90 min with medium containing the virus

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: α_1AT, α_1-antitrypsin; Ad, adenovirus; β-gal, β-galactosidase; CFTR, cystic fibrosis transmembrane conductance regulator; pfu, plaque-forming unit; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside; NE, neutrophil elastase.

¶To whom reprint requests should be addressed.

and 2% bovine calf serum; the incubation was then continued with the addition of the medium described above.

***lacZ* Expression in Endothelial Cells *in Vitro*.** The presence of the *lacZ* gene product was determined by staining the endothelial cells with 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal; Boehringer Mannheim) (27). After *in vitro* infection with the Ad vectors for 24 hr, the endothelial cells were fixed (5 min, 4°C) in 2% formaldehyde/0.2% glutaraldehyde/phosphate-buffered saline, pH 7.4. Cells were then stained (2 hr, 37°C) in 5 mM $K_4Fe(CN)_6$/5 mM $K_3Fe(CN)_6$/2 mM $MgCl_2$ with X-Gal (200 μg/ml). Expression of the *lacZ* gene was considered positive when a nuclear-dominant blue coloration was observed.

α_1AT Gene Expression in Endothelial Cells *in Vitro*. Expression of α_1AT mRNA was evaluated by *in situ* hybridization and by Northern analysis. For *in situ* hybridization, endothelial cells were incubated with ^{35}S-labeled human α_1AT cRNA antisense and sense probes (13). After hybridization, the cells were evaluated by autoradiography (4 days) and counterstained with Wright–Giemsa stain. For Northern analysis, total RNA was extracted, purified, electrophoresed in formaldehyde/agarose gels, blotted onto nylon membranes (Nytran, Schleicher & Schuell) (28), and probed with either a ^{32}P-labeled human α_1AT cDNA probe (13) or, as a control, a γ-actin cDNA probe (29).

De novo synthesis and secretion of α_1AT were evaluated by culturing the endothelial cells in 6-cm plates, in 2 ml of methionine-free medium containing [^{35}S]methionine (500 μCi; 1233 Ci/mmol, New England Nuclear; 1 Ci = 37 GBq). After 24 hr the supernatants were collected and evaluated by immunoprecipitation with an anti-human α_1AT antibody (Boehringer Mannheim), SDS/PAGE, and autoradiography (13).

Glycosylation of the newly synthesized α_1AT by the endothelial cells was evaluated by adding tunicamycin [an inhibitor of core glycosylation (30)] (10 μg/ml; Boehringer Mannheim) or swainsonine [an inhibitor of the distal oligosaccharide-processing enzyme Golgi mannosidase II (30)] (5 μg/ml; Boehringer Mannheim) for 6 hr prior to labeling and throughout the 24-hr [^{35}S]methionine labeling period. The ability of the newly synthesized human α_1AT to inhibit its natural substrate, neutrophil elastase (NE), was evaluated by incubating the supernatant of Ad-α_1AT-infected cells with 0.05–1.5 μM active NE (30 min, 25°C) prior to immunoprecipitation (13).

The early time course of α_1AT secretion by the Ad-α_1AT-infected endothelial cells was evaluated by [^{35}S]methionine labeling (13). Endothelial cells (80% confluent, 6-cm plates) were infected with Ad-α_1AT. For periods from 0 to 24 hr after infection, the cells were pulsed for 2 hr with [^{35}S]methionine (250 μCi) in 1 ml of methionine-free medium. Supernatants were then collected and evaluated as above.

The amount and chronicity of human α_1AT secretion by Ad-infected endothelial cells were determined in parallel 6-cm plates for up to 14 days with an ELISA capable of detecting α_1AT at $\geq$3 ng/ml (13). At intervals following Ad infection, the medium was replaced and supernatants were collected 24 hr later. The cells were then removed with trypsin/EDTA (Biofluids, Rockville, MD) and cell number and viability (always >95%) were determined by trypan blue exclusion.

***Ex Vivo* Infection of Endothelial Cells in Human Umbilical Veins.** Human umbilical cords obtained after normal vaginal deliveries were placed in Hanks' balanced saline solution (HBSS, 4°C) for 2 to 24 hr. The vein (standard length of the umbilical cord, 12 cm) was flushed with HBSS (40 ml, 37°C) to rinse out the blood. Each piece of cord was then placed in HBSS, the Ad vector (10^{10} pfu/vein, in 2 ml of medium containing 2% bovine calf serum) was added to the lumen of the vein, and the cord was clamped at both ends. Each vein was incubated (30 min, 37°C) with Ad-α_1AT, Ad.RSVβgal, Ad-CFTR, or medium only. The vein was then cannulated at both ends and perfused (Peristaltic Pump P-1; Pharmacia) with a recirculating total of 15 ml of the same medium used for the *in vitro* endothelial cell cultures (2 ml/min, 5% CO_2, 37°C) for 24 hr.

***lacZ* and α_1AT Gene Expression in Endothelial Cells in Human Umbilical Veins.** To evaluate the veins for the transfer and expression of the *lacZ* gene after infection with Ad.RSVβgal (or controls) for 24 hr, the cords were fixed for 2 hr (2% formaldehyde/0.2% glutaraldehyde/phosphate-buffered saline) and then placed in X-Gal staining solution for 6 hr. After staining, samples were postfixed in the same fixative, embedded in paraffin, cut into 5-μm sections, and stained with hematoxylin or with hematoxylin and eosin. Identification of endothelial cells was confirmed by positive staining with an antibody to factor VIII-related antigen (24, 26).

Expression of α_1AT mRNA was evaluated by *in situ* hybridization and Northern analysis, 24 hr after Ad infection. For *in situ* hybridization, cords were fixed in 4% paraformaldehyde and frozen. Cryostat sections (7–10 μm) were evaluated with ^{35}S-labeled human α_1AT cRNA antisense and sense probes as described above, and counterstained with hematoxylin and eosin. For Northern analysis, the lumens of umbilical veins were filled with 4 M guanidinium thiocyanate (28) (4 min, 23°C), the buffer was removed, and total RNA was extracted, purified, and analyzed (13, 28, 29).

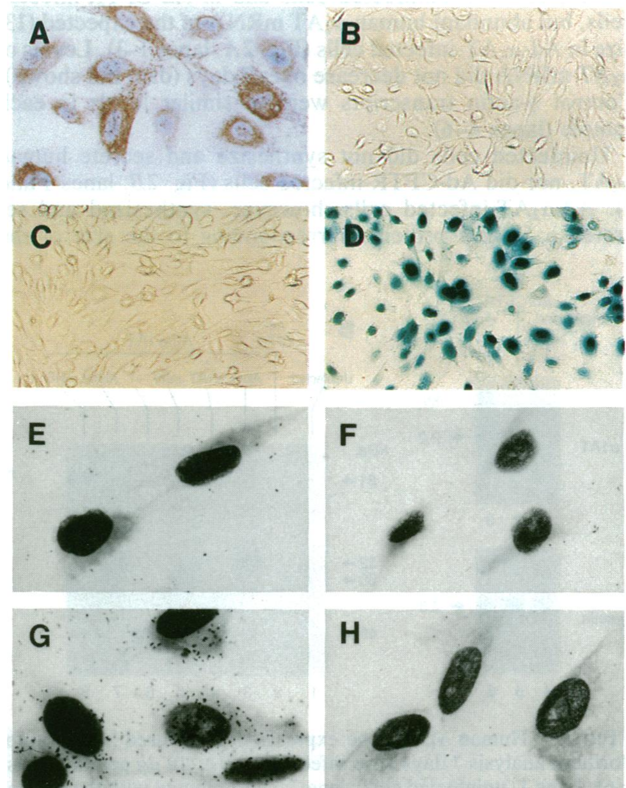

FIG. 1. Ad-mediated transfer of the *lacZ* (β-galactosidase) and α_1AT genes to human umbilical vein endothelial cells *in vitro*. (*A*) Factor VIII-related antigen expression (brown color) in endothelial cells, shown with an anti-factor VIII-related antigen antibody. (×315.) (*B*) β-Galactosidase activity (blue color) in uninfected cells. (X-Gal stain, ×100.) (*C*) Same as *B*, but infected with the control vector Ad-CFTR. (*D*) Same as *B*, but infected with Ad.RSVβgal. (*E*) *In situ* hybridization with an α_1AT antisense probe, uninfected cells. (×500.) (*F*) Same as *E*, but infected with Ad-CFTR. (*G*) Same as *E*, but infected with Ad-α_1AT. (*H*) Same as *G*, but with an α_1AT sense probe.

Ex vivo biosynthesis and secretion of human α_1AT by the endothelial cells in umbilical veins were evaluated by [^{35}S]methionine labeling 18 hr after the beginning of the Ad infection. The vein was perfused with methionine-free medium (5 ml, 37°C) containing [^{35}S]methionine (2 mCi). After 6 hr the perfusing medium was collected and evaluated by immunoprecipitation with an anti-human α_1AT antibody and SDS/PAGE as described above. The amount of human α_1AT secreted into the perfusates of the veins over 24 hr was quantified by ELISA (13).

RESULTS

Identification of endothelial cells *in vitro* was confirmed by positive staining with antibody to factor VIII-related antigen (Fig. 1*A*). Uninfected cells or cells infected *in vitro* with the control vector Ad-CFTR did not express β-gal activity (Fig. 1 *B* and *C*), but cells infected *in vitro* with Ad.RSVβgal did (Fig. 1*D*). The percentage of positive cells averaged 88% (2634 positive cells of 3000 cells evaluated); no positive cells were seen in the controls. *In situ* hybridization with an α_1AT antisense probe demonstrated no human α_1AT mRNA in uninfected cells or in Ad-CFTR-infected cells (Fig. 1 *E* and *F*). In contrast, Ad-α_1AT-infected cells expressed human α_1AT transcripts, with autoradiographic grains over most cells (Fig. 1*G*). Parallel analysis with an α_1AT sense probe showed only background grains (Fig. 1*H*). Consistent with the *in situ* data, Northern analysis confirmed the absence of α_1AT mRNA in uninfected cells and in Ad-CFTR-infected cells, but abundant human α_1AT mRNA of the expected (13) size in Ad-α_1AT-infected cells (Fig. 2*A*, lanes 1–3). Levels of α_1AT mRNA did not decrease over 7 days (data not shown). Control γ-actin transcripts were at similar levels in each sample (lanes 4–6).

Uninfected cells did not synthesize and secrete human α_1AT, nor did Ad-CFTR-infected cells (Fig. 2*B*, lanes 1 and 2). Ad-α_1AT-infected cells, however, synthesized and secreted a 52-kDa protein, corresponding to the size of the mature form of human α_1AT (lane 3) (13, 31). Further evidence that this protein was specifically α_1AT was demonstrated by blocking the anti-human α_1AT antibody with excess amounts of unlabeled human α_1AT (lane 4). The α_1AT secreted by Ad-α_1AT-infected endothelial cells showed all characteristics of human α_1AT (13), including the following: (*i*) in the presence of swainsonine, a molecular mass of 50 kDa (lane 5), a size typical of the premature α_1AT in the Golgi (31); (*ii*) in the presence of tunicamycin, the expected molecular mass of nonglycosylated α_1AT (31), 45 kDa (lane 6); and (*iii*) when the supernatant was incubated before immunoprecipitation with NE, the α_1AT complexed with NE (81 kDa), demonstrating that α_1AT was functional (lanes 7 and 8).

Secretion of human α_1AT was detected as early as 6 hr after the initiation of Ad-α_1AT infection, with the amount of ^{35}S-labeled α_1AT in the supernatant increasing with time (Fig. 2*C*). After the first 24 hr, the amount of α_1AT secreted by the Ad-α_1AT infected cells was relatively constant for the length of the experiment (14 days), as measured by ELISA, ranging from 0.3 to 0.6 μg per 10^6 cells per 24 hr (Fig. 2*D*). In contrast, uninfected cells or cells infected by Ad-CFTR did not produce detectable human α_1AT.

To evaluate the feasibility of Ad-mediated gene transfer to human endothelial cells in circumstances where the natural architecture is preserved, the Ad vectors were used to infect human umbilical vein endothelial cells from their luminal surface. Uninfected or Ad-CFTR-infected veins showed no β-gal activity (Fig. 3 *A* and *B*). In contrast, 24 hr after Ad.RSVβgal infection, X-Gal staining showed extensive blue coloration on the interior surface of the vein, indicative of β-gal activity in cells lining the lumen (Fig. 3 *C* and *D*). Microscopic examination showed that the endothelial cells were still attached to the wall and displayed normal morphology. Immunohistochemical staining using an antibody to human anti-factor VIII-related antigen demonstrated that those cells that displayed β-gal activity also contained factor VIII-related antigen (data not shown).

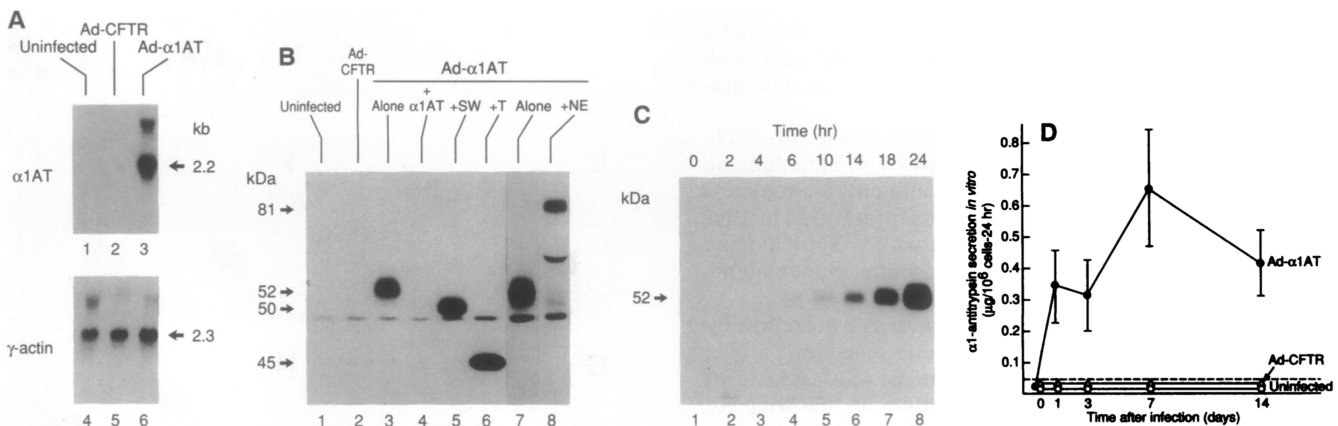

FIG. 2. Human α_1AT gene expression in cultured human umbilical vein endothelial cells following *in vitro* infection with Ad-α_1AT. (*A*) Northern analysis 7 days after infection. RNA (10 μg per lane) was hybridized with an α_1AT cDNA probe (lanes 1–3) or a γ-actin probe (lanes 4–6). Lane 1, uninfected cells; lane 2, cells infected with the control vector Ad-CFTR; lane 3, cells infected with Ad-α_1AT; lanes 4–6, identical to lanes 1–3 but evaluated with a γ-actin probe. Transcript sizes are indicated. Bands migrating slower than the indicated transcripts most likely represent, in lane 3, alternative polyadenylylation, and in lanes 4–6, nuclear RNA. (*B*) Form and function of the human α_1AT synthesized by the endothelial cells. Shown are SDS/PAGE analyses of anti-α_1AT immunoprecipitates of supernatants of endothelial cells incubated 24 hr with [^{35}S]methionine following 24-hr infection with Ad-α_1AT. Lane 1, uninfected cells; lane 2, cells infected with Ad-CFTR; lane 3, cells infected with Ad-α_1AT; lane 4, same as lane 3 but with immunoprecipitation carried out with excess unlabeled α_1AT; lane 5, same as lane 3 but cells were incubated with swainsonine (SW); lane 6, same as lane 3 but in presence of tunicamycin (T); lanes 7 and 8, activity of the secreted α_1AT: lane 7, cells infected with Ad-α_1AT; lane 8, same as lane 7 but with NE (0.5 μM) added to the supernatant prior to immunoprecipitation. Sizes of the secreted α_1AT and of the α_1AT–NE complex are indicated. (*C*) Time course of secretion of α_1AT. At various times after Ad-α_1AT infection, [^{35}S]methionine was added for 2 hr and the supernatant was collected over a 2-hr period 0–24 hr after Ad-α_1AT infection. Size of the secreted α_1AT is indicated. (*D*) Quantitation of human α_1AT secreted. Human α_1AT levels in the supernatants were quantified by ELISA over a 24-hr period, beginning 1 day before infection (time 0) and 1, 3, 7, and 14 days after infection. As controls, parallel cultures of uninfected cells and of Ad-CFTR-infected cells were evaluated. Data are the average of five experiments (±SEM). Detection threshold of the ELISA is 3 ng/ml.

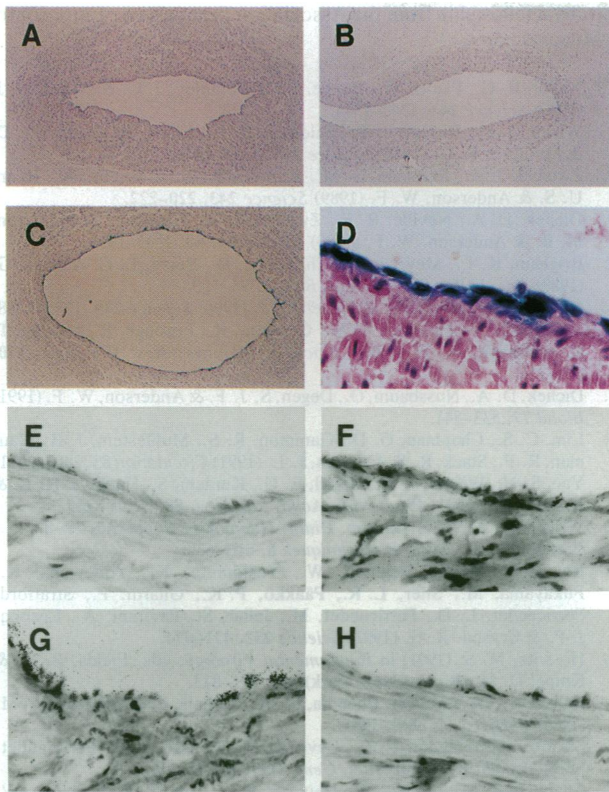

FIG. 3. Ad-mediated transfer of genes to endothelial cells in intact human umbilical veins *ex vivo*. Veins were exposed for 24 hr to Ad.RSVβgal, Ad-α_1AT, or Ad-CFTR and then evaluated for β-gal (*A–D*) or by *in situ* hybridization for α_1AT mRNA (*E–H*). (*A*) Uninfected vein (X-Gal and hematoxylin stain, ×25.) (*B*) Same as *A*, but infected with Ad-CFTR. (*C*) Same as *A*, but infected with Ad.RSVβgal. (*D*) Same as *C*, but high-power view and hematoxylin and eosin stain. (×315.) (*E*) Uninfected vein, evaluated with α_1AT antisense probe. (×200.) (*F*) Same as *E*, but infected with Ad-CFTR. (*G*) Same as *E*, but infected with Ad-α_1AT. (*H*) Same as *G*, with α_1AT sense probe.

In situ hybridization with an antisense α_1AT probe showed no α_1AT mRNA in uninfected or Ad-CFTR-infected veins (Fig. 3 *E* and *F*). In contrast, human α_1AT transcripts were detected in the internal layer of Ad-α_1AT-infected veins (Fig. 3*G*). Parallel analysis with a sense α_1AT probe showed only background grains (Fig. 3*H*). Northern analysis confirmed the absence of α_1AT mRNA transcripts in uninfected veins or in Ad-CFTR-infected veins. However, in Ad-α_1AT infected veins there were α_1AT transcripts of the expected (13) size (Fig. 4*A*, lanes 1–3). Control γ-actin transcripts were at similar levels in each sample (lanes 4–6).

Uninfected or Ad-CFTR-infected veins did not synthesize or secrete α_1AT (Fig. 4*B*, lanes 1 and 2). In contrast, biosynthesis and secretion of human α_1AT were demonstrated in Ad-α_1AT-infected veins (lane 3). This protein was specifically α_1AT, as shown by blocking of the anti-human α_1AT antibody with excess amounts of unlabeled human α_1AT (lane 4). Quantification of α_1AT in the perfusing medium 24 hr after introduction of Ad-α_1AT into the vein showed average levels of 13 μg/ml (Fig. 5).

DISCUSSION

Endothelial cells represent an ideal target for transfer of genes coding for secreted therapeutic proteins, since the target cell population is enormous, with an estimated 10^{12} endothelial cells covering a 10^3-m^2 surface area (32). To achieve the goal of gene therapy using endothelial cells, two

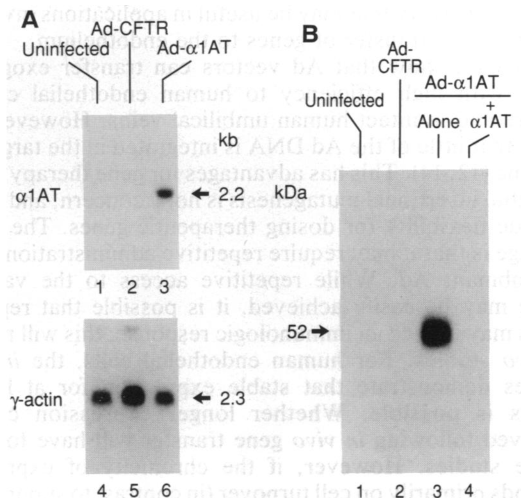

FIG. 4. Human α_1AT gene expression in human umbilical veins infected *ex vivo* with Ad-α_1AT. (*A*) Northern analyses. RNA (5 μg per lane) was hybridized with an α_1AT probe (lanes 1–3) or a γ-actin probe (lanes 4–6). Transcript sizes are indicated. Lane 1, uninfected vein; lane 2, vein infected with Ad-CFTR; lane 3, vein infected with Ad-α_1AT; lanes 4–6, identical to lanes 1–3 but with γ-actin probe. Bands migrating slower than the indicated transcripts in lanes 4–6 most likely represent nuclear RNA. (*B*) Biosynthesis and secretion of human α_1AT. Shown are SDS/PAGE analyses of anti-α_1AT immunoprecipitates supernatants of veins perfused for 24 hr with Ad-α_1AT and with [^{35}S]methionine in the last 6 hr. The 52-kDa α_1AT is indicated. Lane 1, uninfected vein; lane 2, vein infected with Ad-CFTR; lane 3, vein infected with Ad-α_1AT; lane 4, same as lane 3 but with immunoprecipitation carried out with excess unlabeled α_1AT.

strategies have been employed: (*i*) *in vitro* gene transfer (1–5, 7–10), with subsequent reintroduction of the modified endothelial cells by seeding of vascular grafts or stents (2–4, 33), or direct injection (1); and (*ii*) direct *in vivo* gene transfer (6, 9, 34). The major technical hurdle is based on the inherent biology of the endothelium in its resting state *in vivo*. Endothelial cells replicate slowly, limiting the use of gene transfer vectors that depend on cell proliferation for expression of transferred genes (11, 35). Ad does not require host cell proliferation to express the exogenous gene (12, 13), a

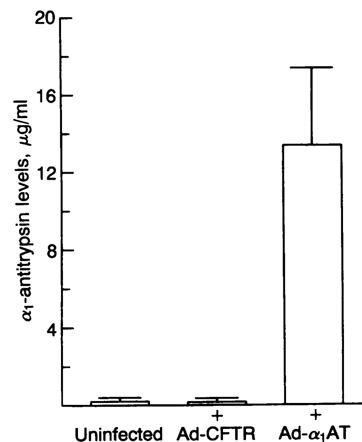

FIG. 5. Amounts of human α_1AT produced by human umbilical veins infected by Ad-α_1AT. Measurements were made in the perfusate by ELISA 24 hr after introduction of Ad in the lumen of the veins. Shown are the α_1AT levels for uninfected veins, Ad-CFTR-infected veins, and Ad-α_1AT-infected veins. Data represent the average of six experiments (±SEM). There is a small amount (<0.2 μg/ml) of human α_1AT in the controls, most likely from trapped α_1AT from human plasma from the donor.

biologic property that may be useful in applications involving direct *in vivo* transfer of genes to the endothelium.

Our data show that Ad vectors can transfer exogenous genes with high efficiency to human endothelial cells in culture and in intact human umbilical veins. However, it is likely that little of the Ad DNA is integrated in the target cell genome (12, 14). This has advantages for gene therapy in that potential insertional mutagenesis is not a concern, and it may provide flexibility for dosing therapeutic genes. The disadvantage is that it may require repetitive administration of the recombinant Ad. While repetitive access to the vascular space may be easily achieved, it is possible that repeated doses may induce an immunologic response; this will require *in vivo* studies. For human endothelial cells, the *in vitro* studies demonstrate that stable expression for at least 2 weeks is possible. Whether longer expression can be achieved following *in vivo* gene transfer will have to await future studies. However, if the chronicity of expression depends primarily on cell turnover (in contrast to suppression or loss of the exogenous gene), then the slow rate of endothelial cell turnover is in favor of this vector system. Consistent with this concept, Ad-CFTR has been used to transfer the human CFTR cDNA to the bronchial epithelium *in vivo* (23). Northern analysis of lung RNA revealed CFTR transcripts for up to 6 weeks, despite the fact that bronchial epithelium also replicates slowly *in vivo*.

α_1AT is normally produced by the liver and functions as an antiprotease that protects the lung from destruction by NE, a powerful proteolytic enzyme (13, 22, 36). In hereditary α_1AT deficiency, mutations of the α_1AT gene result in a deficiency of α_1AT in the circulation and hence insufficient α_1AT to protect the lung from NE (36). As a result, the alveoli of the lower respiratory tract are slowly destroyed, culminating in progressive emphysema. The endothelium is an ideal target for gene therapy for α_1AT deficiency, since the modified endothelial cells would secrete human α_1AT directly into the circulation, where it would diffuse into the alveolar interstitium, providing protection against NE.

Under normal circumstances, human endothelial cells do not express the α_1AT gene. The present study shows that a modified Ad is capable of transferring an α_1AT cDNA into human endothelial cells, conferring upon the cells the ability to synthesize and secrete α_1AT capable of combining with NE. The secreted α_1AT is glycosylated in a normal fashion, an important consideration in the context that nonglycosylated α_1AT has a half-life in the circulation 50-fold shorter than the glycosylated form (37). Further, endothelial cells have the capacity to synthesize and secrete large amounts of α_1AT, as indicated by the observation that the vein within a 12-cm segment of human umbilical cord infected from the luminal side with 10^{10} pfu of Ad-α_1AT could produce 13 μg of α_1AT per ml of perfusate over 24 hr. The endothelial cells had normal morphology, and high levels of α_1AT mRNA transcripts were detected by *in situ* hybridization, suggesting that the levels of α_1AT achieved in the vessel perfusates were due to continuous synthesis and secretion of α_1AT and not to release of α_1AT after cell death. Given the enormous surface area of the endothelium, direct endothelial cell transfer of the α_1AT gene is a feasible strategy for therapy of inherited α_1AT deficiency, particularly if the gene transfer were carried out in the pulmonary arteries and capillaries—i.e., at the site where the molecule is needed. Further, in the context of the level of α_1AT secreted in 24 hr by a short segment of blood vessel, Ad-mediated transfer of exogenous genes should be applicable to a wide variety of genes to treat human disorders.

We thank R. L. Bowman (Laboratory of Cell Biology, National Institutes of Health) for advice and assistance. P.L. is supported, in part, by a fellowship from the Association Française de Lutte contre la Mucoviscidose.

1. Nabel, E. G., Plautz, G., Boyce, F. M., Stanley, J. C. & Nabel, G. J. (1989) *Science* **244**, 1342–1344.
2. Wilson, J. M., Birinyi, L. K., Salomon, R. N., Libby, P., Callow, A. D. & Mulligan, R. C. (1989) *Science* **244**, 1344–1346.
3. Zwiebel, J. A., Freeman, S. M., Kantoff, P. W., Cornetta, K., Ryan, U. S. & Anderson, W. F. (1989) *Science* **243**, 220–222.
4. Dichek, D. A., Neville, R. F., Zwiebel, J. A., Freeman, S. M., Leon, M. B. & Anderson, W. F. (1989) *Circulation* **80**, 1347–1353.
5. Brigham, K. L., Meyrick, B., Christman, B., Berry, L. C. & King, G. (1989) *Am. J. Respir. Cell Mol. Biol.* **1**, 95–100.
6. Nabel, E. G., Plautz, G. & Nabel, G. J. (1990) *Science* **249**, 1285–1288.
7. Zwiebel, J. A., Freeman, S. M., Cornetta, K., Forough, R., Maciag, T. & Anderson, W. F. (1990) *Biochem. Biophys. Res. Commun.* **170**, 209–213.
8. Dichek, D. A., Nussbaum, O., Degen, S. J. F. & Anderson, W. F. (1991) *Blood* **77**, 533–541.
9. Lim, C. S., Chapman, G. D., Gammon, R. S., Muhlestein, J. B., Bauman, R. P., Stack, R. S. & Swain, J. L. (1991) *Circulation* **83**, 2007–2011.
10. Yao, S.-N., Wilson, J. M., Nabel, E. G., Kurachi, S., Hachiya, H. L. & Kurachi, K. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 8101–8105.
11. Wright, H. P. (1970) *Thromb. Diath. Haemorrh. Suppl.* **40**, 79–87.
12. Berkner, K. L. (1988) *Biotechniques* **6**, 616–630.
13. Rosenfeld, M. A., Siegfried, W., Yoshimura, K., Yoneyama, K., Fukayama, M., Stier, L. R., Pääkkö, P. K., Gilardi, P., Stratford-Perricaudet, L. D., Perricaudet, M., Jallat, S., Pavirani, A., Lecocq, J.-P. & Crystal, R. G. (1991) *Science* **252**, 431–434.
14. Horwitz, M. S. (1991) in *Fundamental Virology*, eds. Fields, B. N. & Knipe, D. M. (Raven, New York), pp. 771–813.
15. Ballay, A., Levrero, M., Buendia, M.-A., Tiollais, P. & Perricaudet, M. (1985) *EMBO J.* **4**, 3861–3865.
16. Stratford-Perricaudet, L. D., Levrero, M., Chasse, J.-F., Perricaudet, M. & Briand, P. (1990) *Hum. Gene Ther.* **1**, 241–256.
17. Chanock, R. M., Ludwig, W., Heubner, R. J., Cate, T. R. & Chu, L.-W. (1966) *J. Am. Med. Assoc.* **195**, 446–452.
18. Rushton, B. & Sharp, J. M. (1977) *J. Pathol.* **121**, 163–167.
19. Friedman, H. M., Macarak, E. J., MacGregor, R. R., Wolfe, J. & Kefalides, N. A. (1981) *J. Infect. Dis.* **143**, 266–273.
20. Gilardi, P., Courtney, M., Pavirani, A. & Perricaudet, M. (1990) *FEBS Lett.* **267**, 60–62.
21. Mastrangeli, A., Danel, C., Rosenfeld, M. A., Stratford-Perricaudet, L., Perricaudet, M., Pavirani, A., Lecocq, J.-P. & Crystal, R. G. (1992) *Am. Rev. Respir. Dis.* **145**, A688 (abstr.).
22. Carlson, J. A., Rogers, B. B., Sifers, R. N., Hawkins, H. K., Finegold, M. J. & Woo, S. L. C. (1988) *J. Clin. Invest.* **82**, 26–36.
23. Rosenfeld, M. A., Yoshimura, K., Trapnell, B. C., Yoneyama, K., Rosenthal, E. R., Dalemans, W., Fukayama, M., Bargon, J., Stier, L. E., Stratford-Perricaudet, L. D., Perricaudet, M., Guggino, W. B., Pavirani, A., Lecocq, J.-P. & Crystal, R. G. (1992) *Cell* **68**, 143–155.
24. Jaffe, E. A. (1984) in *Biology of Endothelial Cells*, ed. Jaffe, E. A. (Nijhoff, Boston), pp. 1–13.
25. Grant, D. S., Tashiro, K.-I., Segui-Real, B., Yamada, Y., Martin, G. R. & Kleinman, H. K. (1989) *Cell* **58**, 933–943.
26. Fu, Y.-M., Spirito, P., Yu, Z.-X., Biro, S., Sasse, J., Lei, J., Ferrans, V. J., Epstein, S. E. & Casscells, W. (1991) *J. Cell Biol.* **114**, 1261–1273.
27. Dannenberg, A. M. & Suga, M. (1981) in *Methods for Studying Mononuclear Phagocytes*, eds. Adams, D. O., Edelson, P. J. & Koren, H. S. (Academic, New York), pp. 375–395.
28. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, O. D., Smith, J. A., Seidman, J. G. & Struhl, K., eds. (1987) *Current Protocols in Molecular Biology* (Wiley, New York).
29. Gunning, P., Ponte, P., Okayama, H., Engel, J., Blau, H. & Kedes, L. (1983) *Mol. Cell. Biol.* **3**, 787–795.
30. Elbein, A. D. (1987) *Annu. Rev. Biochem.* **56**, 497–534.
31. Curiel, D. T., Chytil, A., Courtney, M. & Crystal, R. G. (1989) *J. Biol. Chem.* **264**, 10477–10486.
32. Jaffe, E. A. (1985) *Ann. N.Y. Acad. Sci.* **454**, 279–291.
33. Eskin, S. G., Meidell, R. S., McNatt, J., Buja, L. M., Sturgis, L. T., Gerard, R. D., Ferguson, J. J., Sambrook, J. F. & Willerson, J. T. (1991) *Circulation* **84**, II-399 (abstr.).
34. Canonico, A. E., Conary, J. T., Christman, B. W., Meyrick, B. O. & Brigham, K. L. (1991) *Clin. Res.* **39**, 219A (abstr.).
35. Miller, D. G., Adam, M. A. & Miller, A. D. (1990) *Mol. Cell. Biol.* **10**, 4239–4242.
36. Crystal, R. G. (1990) *J. Clin. Invest.* **85**, 1343–1352.
37. Casolaro, M. A., Fells, G., Wewers, M., Pierce, J. E., Ogushi, F., Hubbard, R., Sellers, S., Forstrom, J., Lyons, D., Kawasaki, G. & Crystal, R. G. (1987) *J. Appl. Physiol.* **63**, 2015–2023.
38. Stratford-Perricaudet, S. D., Makeh, I., Perricaudet, M. & Briand, P. (1992) *J. Clin. Invest.*, in press.