Gene therapy in a xenograft model of cystic fibrosis lung corrects chloride transport more effectively than the sodium defect

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We have developed a model of gene therapy for cystic fibrosis (CF) lung disease, based on growth of human CF bronchial xenografts in *nu/nu* mice. We now report an evaluation of the primary abnormalities in CF lung epithelia — defective Cl secretion and Na hyperabsorption — in xenografts following adenovirus-mediated gene transfer. *In vivo* infection of CF xenografts with a cystic fibrosis transmembrane regulator (CFTR) recombinant adenovirus, at a multiplicity of infection equal to 100, was sufficient to reconstitute near normal levels of cAMP-stimulated Cl transport, despite transducing only 5% of cells in the pseudostratified epithelium. Correction in sodium hyperabsorption was partial and variable. These experiments define aspects of adenovirus-mediated gene therapy relevant to CF protocols based on intrapulmonary genetic reconstitution.

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Cystic fibrosis (CF) is caused by defects in the gene encoding the CF transmembrane conductance regulator (CFTR), which lead to abnormalities in fluid and electrolyte transport across various epithelia[1]. CF epithelial cells are impermeable to chloride and absorb excessive quantities of sodium; both processes potentially affect biochemical properties of mucus and contribute to primary pathogenesis[2]. The end result is inspissation of secretions in ducts of affected organs and pathology in several systems including lung, pancreas and liver[3].

Genetic reconstitution of CFTR expression in lung with recombinant adenoviruses is being evaluated in the treatment of the pulmonary manifestations of CF[4]. One problem in predicting the potential efficacy of this approach is that recombinant adenoviruses, which nonspecifically target genes to surface airway epithelial cells, will not accurately reconstitute the expression of endogenous CFTR in human lung[5,6]. The challenging task of demonstrating clinical efficacy will require longitudinal evaluation of clinical endpoints or the characterization of surrogate endpoints that predict clinical outcome. The most relevant direct functional measures of CFTR correction in CF epithelia are reconstitution of cAMP-regulated chloride (Cl) secretion and blunting of sodium (Na) hyperabsorption[7]. It is hoped that normalization of these surrogate endpoints by gene therapy will help to quell the primary and secondary disease processes that lead to bronchiactesis and ultimately respiratory failure.

Two general strategies for evaluating the safety and biological efficacy of gene therapy to the airway in patients with CF have emerged. One approach evaluates correction of CFTR function in nasal epithelia based on the hypothesis that epithelial structures within the nose are an extension of intrapulmonary respiratory epithelia[8,9]. The advantage of this approach is that the nasal cavity can be accessed noninvasively for virus administration, tissue harvesting and serial measurements of CFTR function *in situ* through measurement of transepithelial potential differences (PD). However, nasal epithelia differ from those of the lung and important issues of safety in the lung are difficult to extrapolate from these studies. The other approach is to instill recombinant adenovirus directly into the lung of CF patients with the obvious advantage that the critical parameters of biological efficacy and safety are studied at the actual site of disease[10–12]. But the relative inaccessibility of intrapulmonary epithelia limits recovery of biological material for direct analysis of gene transfer and complicates an *in situ* assessment of CFTR function using the techniques of transepithelial PD measurements that have been so useful in the nose.

Difficulties in evaluating CFTR gene transfer within the human lung and the potential limitations of the nasal cavity reaffirm the importance of developing animal models of human CF lung disease. Grubb *et al.*[13] recently used the *Cftr*-deficient mouse to evaluate the biology of adenovirus-mediated gene transfer directed to nasal epithelia. The presence of an alternative Cl channel in mouse airway potentially spares the CF mouse from developing pathology and, therefore, has limited its usefulness in evaluating lung-directed gene therapy[14]. In this study, we have used a model of the proximal human intrapulmonary airway to evaluate the potential efficacy of adenovirus-mediated gene therapy directed to human lung. This model, which is based on the growth of human bronchial xenografts from CF patients in *nu/nu* mice, produces an epithelial structure similar in morphology

and function to proximal human CF conducting airway and potentially useful to the study of human adenoviral biology[15,16]. Adenovirus infected xenografts were evaluated for efficiency of genetic reconstitution and functional correction of Cl and Na transport.

Efficient gene transfer in CF bronchial xenografts

Human bronchial xenografts were established from cells of nine CF patients (n=25) and five non CF patients (n=10). All grafts developed a fully differentiated pseudostratified epithelium at the time of study. Undifferentiated, incompletely formed glands were occasionally seen. First-generation recombinant adenoviruses expressing either *lacZ* (H5.010CMV*lacZ*) or CFTR (H5.020CB*CFTR*) were instilled into the lumen of grafts at multiplicity of infections (MOI) approximately equal to 100 (5×10^9 pfu in 100 µl) or 10 (5×10^8 pfu in 100 µl). Transport properties of the grafts were evaluated before and after gene transfer using the technique of transepithelial PD measurements similar to that utilized in the human trials of gene transfer to nasal epithelia[8]. Upon completion of these studies, the grafts were explanted and evaluated for transgene expression using various cytochemical techniques (Fig. 1).

In general, the distribution and frequency of transgene expressing cells did not vary significantly within regions of the xenograft. Analysis of CF grafts by *in situ* hybridization with an antisense probe to the R domain of CFTR demonstrated transgene expression in 6.8% +/– 1.8 (mean +/– 1 S.D., n=4, range 4.8–8.7) and 0.3% +/– 0.4 of cells (n=3, range 0.1–0.8) when grafts were infected with 5×10^9 (Fig. 1*c,d*) and 5×10^8 (Fig. 1*e,f*) pfu total CFTR virus, respectively. No hybridization was detected to the CFTR antisense probe in *lacZ* infected grafts (Fig. 1*a,b*) documenting the specificity of the assay. Xenografts from three independent CF patients, that were infected with 5×10^9 pfu CFTR virus, were analysed for CFTR protein expression by immunofluorescence. These experiments confirmed the estimate of gene transfer based on *in situ* hybridization (that is, 5%, 6.5% and 7.5% of cells expressed recombinant CFTR protein in the three grafts analysed) and demonstrated apical localization of the recombinant protein (Fig. 1*g,h*). Both secretory and ciliated cells expressed recombinant CFTR. *In situ* hybridization and X-gal histochemical analysis of CF grafts infected with *lacZ* virus demonstrated similar levels of transgene (*lacZ*) expression. Grafts infected with 5×10^9 pfu lacZ virus revealed transgene expression in 7.1% +/– 2.3 (n=3) of cells by *in situ* and 5.6% +/– 1.5 (n=3) of cells by X-gal. Grafts infected with 5×10^8 pfu *lacZ* virus had transgene expression in 0.7% +/– 0.3 (n=3) of cells by *in situ* and 0.6% +/– 0.3 (n=3) of cells by X-gal.

Correction of Cl secretory defect

Analysis of the chloride secretory defect was facilitated by the application of the sodium channel blocker, amiloride, to the apical surface which converts the xenograft from a sodium absorbing to chloride secreting epithelium[17]. In non CF tissues, the subsequent replacement of luminal Cl by gluconate creates an electrochemical gradient for Cl efflux that is reflected as an increase in PD, which is further enhanced when the CFTR channel is activated

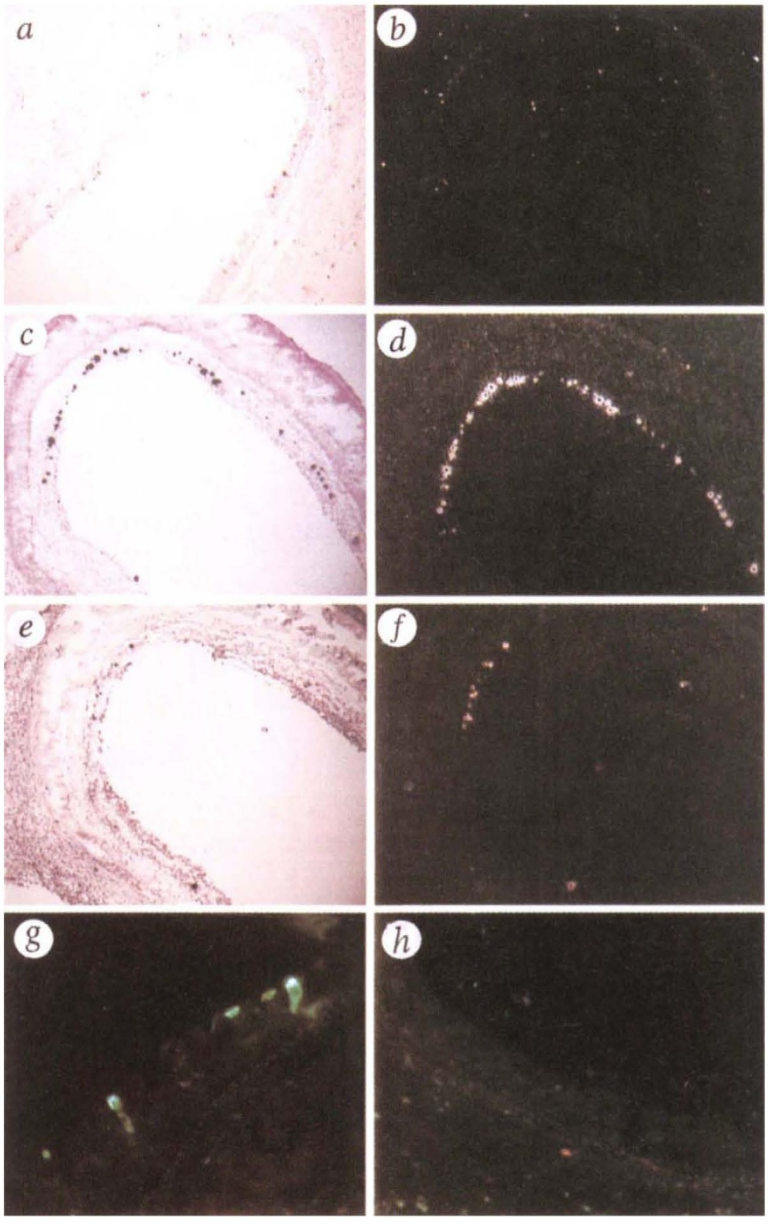

Fig. 1 Analysis of xenografts for expression of CFTR RNA and protein expression. All xenografts were harvested within 14 days of virus instillation for morphological characterization and analysis for recombinant gene expression using techniques of *in situ* hybridization and immunocytochemistry. Representative examples of the *in situ* hybridization experiments are presented (*a*, *c* and *e*, bright fields; *b*, *d*, and *f*, dark fields): H5.010CMV*lacZ* infected CF xenograft (5×10^9 total pfu) hybridized to an antisense CFTR probe (*a* and *b*); H5.020CB*CFTR* infected CF xenograft (5×10^9 total pfu) hybridized to an antisense CFTR probe (*c* and *d*); and H5.020CB*CFTR* infected CF xenograft (5×10^8 total pfu) hybridized to an antisense CFTR probe (*e* and *f*). Serial sections also were hybridized with a sense probe and the antisense probe after treatment of the section with RNase. Specific hybridization was never seen under these conditions confirming the specificity of the assay. Selected grafts were analyzed for CFTR protein expression by immunocytochemistry using a polyclonal antibody to the C terminus of CFTR. Analyses were performed with a CF xenograft infected with 5×10^9 total pfu H5.020CB*CFTR* (*g*) and H5.010CMV*lacZ* (*h*) virus.

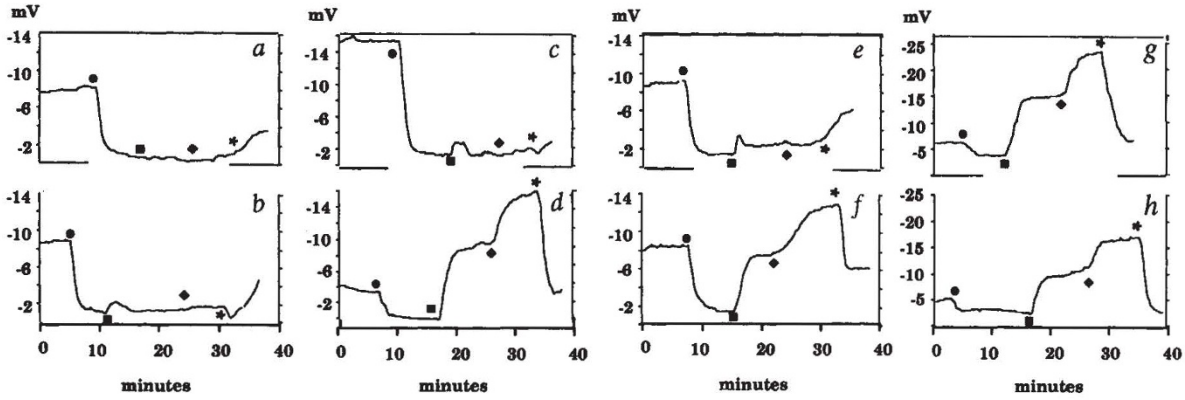

Fig. 2 Recordings of transepithelial potential differences. Bronchial xenografts generated from epithelial cells derived from CF and non CF patients were subsequently instilled with first generation recombinant viruses (H5.010CMV*lacZ* or H5.020CB*CFTR*, 5×10^9 pfu). Xenograft PD responses to perfusion with different solutions measured by modifications of previously described techniques were characterized before and after exposure to the adenovirus[7,22]. Each recording measured voltage (y axis) as a function of time (x axis) in response to the sequential perfusion of: (i) Hepes phosphate buffered ringers solution (HPBR); (ii) HPBR with amiloride (circle); (iii) chloride free HPBR with amiloride (square); (iv) chloride free HPBR with amiloride, 8-cpt cAMP, and forskolin (diamond); and (v) return to HPBR (asterisk). Recordings were performed in the following experimental models: CF xenograft infected with H5.010CMV*lacZ* before (*a*) and after (*b*) gene transfer; CF xenograft infected with H5.020CB*CFTR* before (c) and after (*d*) gene transfer in which baseline PD was shown to correct; CF xenograft infected with H5.020CB*CFTR* before (*e*) and after (*f*) gene transfer in which baseline PD did not correct; non CF xenograft infected with H5.010CMV*lacZ* before (*g*) and after (*h*) gene transfer.

with cAMP. Representative transepithelial PD recordings measured as voltage (mV) are presented in Fig. 2 and summarized in Fig. 3. PD in CF grafts was unresponsive to both Cl free buffer and cAMP mediated activation with forskolin and 8-4-chlorophenylthio-cAMP (8-cpt cAMP) (–1.9+/–0.9 mV (amiloride) to –1.9+/–0.8 mV (Cl free) to –2.0+/–0.9 mV (cAMP)), whereas large and significant increases were seen in non-CF grafts under the same conditions (–2.4+/–1.0 mV (amiloride) to –10.2+/–3.4 mV (Cl free) to –18.7+/–3.3 mV (cAMP)). Activation of CFTR with the β-agonist isoproterenol polarized the epithelium of non-CF grafts (Fig. 4*d*) similar to that observed with forskolin and 8-cpt cAMP (Fig. 4*b*), whereas no change in PD was noted under similar conditions in CF xenografts (Fig. 4*c*). Stimulation of alternate Cl channels with ATP resulted in a return of PD to baseline in CF grafts confirming the bioelectric integrity of the epithelia (Fig. 4*e*).

Infection of CF xenografts with 5×10^9 pfu of H5.020CB*CFTR* significantly improved the basal Cl transport measured in Cl free solution. PD increased from –1.9+/–0.9 mV (uninfected CF xenografts) to –8.8+/–2.2 mV (corrected CF grafts) as compared to –10.2+/–3.4 mV (mock infected non CF grafts); the difference between CF and CF-corrected was highly significant ($P<0.001$) whereas non CF could not be distinguished from CF-corrected ($P=0.26$) indicating that reconstitution of basal Cl secretion was substantial. Correction of the chloride secretory defect was also demonstrated following stimulation of cAMP with forskolin/cpt cAMP. PD increased from –10.2+/–3.4 mV (Cl free) to –18.7+/–3.3 mV (cAMP) in uninfected non-CF grafts, and –8.8+/–2.2 mV (Cl free) to –14.7+/–2.5 mV (cAMP) in CFTR corrected CF grafts; differences between CF and CF-corrected ($P<0.001$) and CF-corrected and non-CF ($P<0.002$) were significant indicating substantial but incomplete correction. Instillation of xenografts with a similar dose of lacZ virus (5×10^9 total pfu) or a 10-fold lower dose of CFTR adenovirus (5×10^8 total pfu, MOI=10) failed to correct baseline or stimulated Cl secretion (Fig. 3).

Variable correction of sodium hyperabsorption

Another significant transport abnormality in CF airway epithelia is hyperabsorption of sodium, which is reflected in an increased voltage under basal conditions of isotonic saline in the luminal buffer (Figs 2 & 3). It was possible to discriminate CF from non-CF in terms of basal PD (–10.8+/–2.8 mV in CF versus –4.8+/–1.2 mV in non-CF; $P<0.001$), however, there was some overlap. The ability of high dose CFTR virus to correct baseline PD was mixed. Average baseline PD decreased in CF grafts from –10.8+/–2.8 mV to –7.1+/–2.4 mV following CFTR gene transfer ($P<0.001$) although it remained significantly higher than non CF (4.8+/–1.2; $P<0.01$). Detailed analyses of these individual recordings indicate there is heterogeneity in CF xenografts infected with high dose CFTR virus with respect to correction of baseline hyperpolarization: 5/9 xenografts showed significant correction whereas 4/9 did not respond to gene transfer (Fig. 5). (Individual recordings of CFTR infected CF grafts which demonstrate this heterogeneity are presented in Fig. 2*c,d* (responder) and Fig. 2*e,f* (nonresponder).) Depolarization found in response to CFTR virus is not a non-specific consequence of adenovirus-mediated damage to the epithelia because there were no detectable morphological changes in the epithelium based on light microscopic analyses (data not shown) and similar doses of *lacZ* adenovirus had no effect on baseline PD (Fig. 3).

Discussion

The human bronchial xenograft system has many features important to its use as a model for CF gene therapy of the lung. The epithelium that develops in this model recapitulates the normal architecture, morphology and

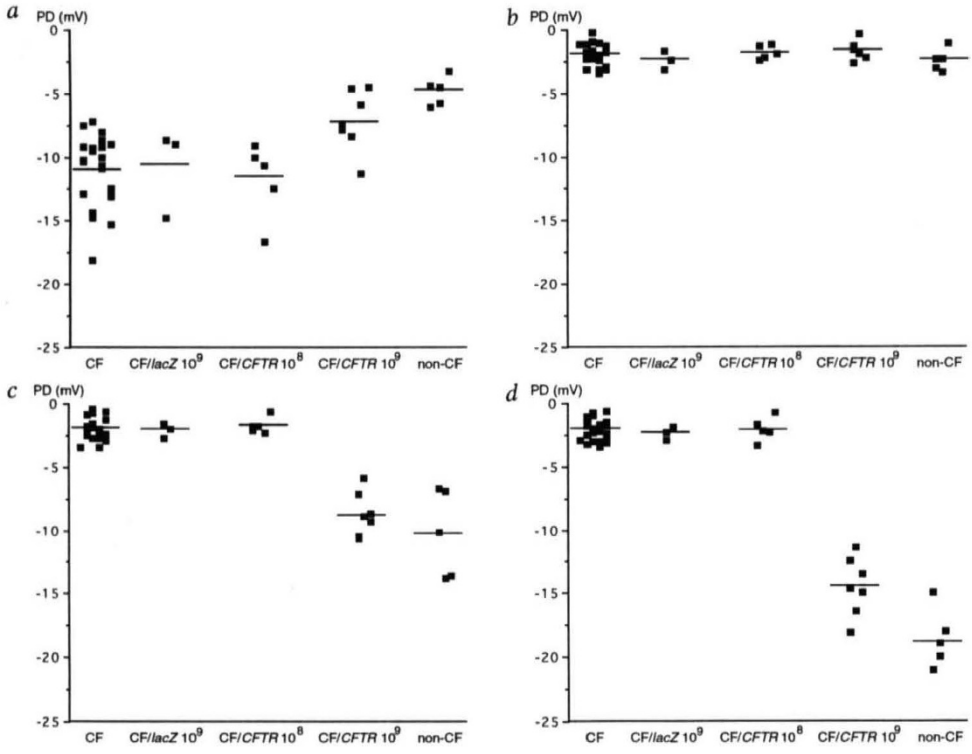

Fig. 3 Summary of transepithelial potential difference measurements made in bronchial xenografts. Samples from nine CF lungs (25 xenografts) and five non-CF lungs (10 xenografts) were not infected or exposed to H5.010CMV*lacZ* or H5.020CB*CFTR*. Each xenograft was characterized with respect to transepithelial cell conductance (see Fig. 2 legend). Steady state voltages (PD (mV)) for uninfected non CF grafts and CF grafts that were uninfected or infected with 5×10^8 and 5×10^9 pfu of CFTR virus or 5×10^9 pfu of lacZ virus are presented. These include voltages at baseline in HPBR buffer (*a*), in HPBR plus amiloride (*b*), amiloride in low chloride buffer (*c*), and amiloride in low chloride buffer in the presence of forskolin and 8-cpt cAMP (*d*). Transepithelial PD recordings from a CF xenograft exposed to 5×10^{10} pfu of CFTR virus was indistinguishable from that obtained with ten-fold lower virus.

bioelectric properties of a human bronchus, including abnormalities of Cl secretion and Na hyperabsorption in grafts reconstituted with CF-derived cells that are the hallmark of this disease. The biology of human adenovirus transduction is well studied in this system because the host cells are of human origin, as opposed to other models such as the mouse where these viruses are not permissive for replication[18]. The fact that the xenografts are grown in athymic animals simplifies the study of gene transfer because the confounding issues of inflammation within the airway due to bacterial infections or immune responses to the genetically modified cells are irrelevant. While this feature of the model simplifies the study of gene transfer and functional correction, it limits an evaluation of the host's immunologic response to the virus or virus-infected cells — aspects of gene therapy critical to its eventual success.

We have used the human xenograft model to address several important aspects relevant to the feasibility of gene therapy to the intrapulmonary airways. What is the efficiency and cellular distribution of gene transfer? Can the chloride secretory defect be fully restored with doses of viruses that are reasonable to use in humans? Is there a relationship between reconstitution of the chloride secretory defect and blunting of the hyperabsorption of sodium? What is the relationship between efficiency of gene transfer and functional correction?

Critical to an evaluation of the potential of gene therapy for CF lung disease is an assessment of the efficiency with which recombinant adenoviruses transfer genes to the superficial epithelia of the conducting airway of the lung. Changes in the biology of the target cell, such as its state of differentiation, can have profound effects on transduction efficiency. Studies in the mouse suggest an anatomical gradient of susceptibility to adenovirus-mediated gene transfer within the conducting airway, with more proximal structures such as nose intrinsically less susceptible to gene transfer than the

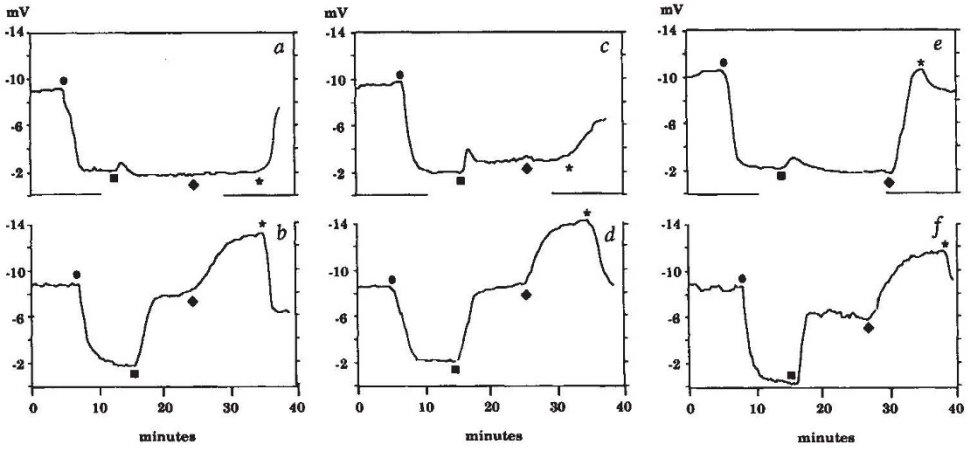

Fig. 4 Transepithelial PD measurements in the presence of different conditions for channel activation. Xenografts from a CF (*a*, *c* and *e*) and non CF (*b*, *d* and *f*) patient were subject to different protocols and transepithelial PD measurements were recorded. The structure of these experiments is as described in the Fig. 2 legend, except for the composition of solution 4 which contains chloride-free HPBR with amiloride in addition to: 8-cpt-cAMP and forskolin (*a* and *b*), 10 μm isoproterenol (*c* and *d*) and 100 μm ATP (*e* and *f*).

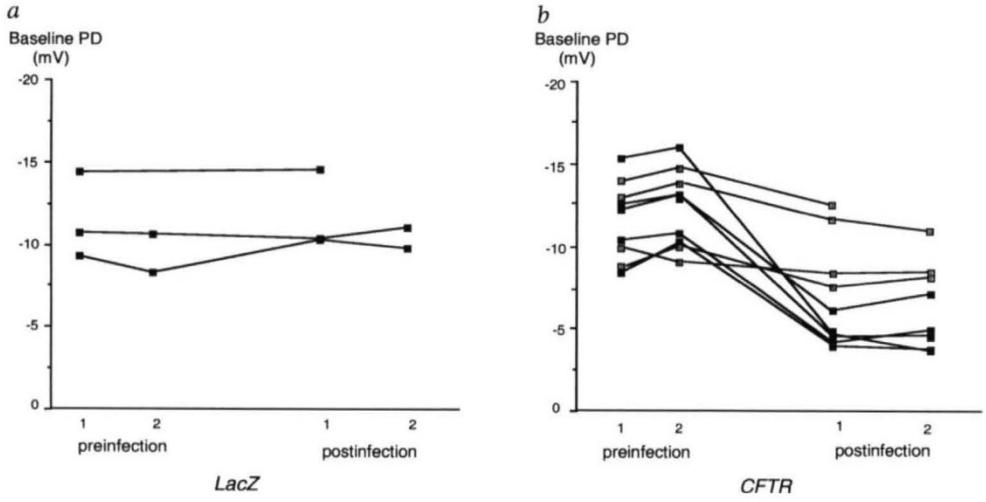

Fig. 5 Baseline PD (mV) in xenografts infected with *lacZ* and CFTR virus. CF xenografts infected with 5×10^9 total pfu of H5.010CMV*lacZ* (*a*) and H5.020CB*CFTR* (*b*). In *b*, closed squares (n=5) represent responders and open squares (n=4) nonresponders. Baseline PD in mV was measured twice over seven day intervals before and after gene transfer.

distal intrapulmonary airway. Grubb *et al.*[13] recently demonstrated gene transfer and functional correction in nasal cavity of the CF mouse, but only at high doses (MOI ~10,000) of repeatedly administered virus. This contrasts with the results obtained following intratracheal instillation of the same viruses into CF mice, where gene transfer can be accomplished in the majority of conducting airway epithelial cells with 500-fold less virus than that required to show an affect in CF mouse nasal epithelia[19].

Less is known about gene transfer in respiratory epithelia of humans. Results in the nasal cavity of CF patients are not consistent, with Zabner *et al.*[9] reporting functional correction with low MOIs (1–25), whereas the clinical trial of Knowles and Boucher suggests MOIs >10,000 will be required for significant correction (unpublished data). Early reports of the human trials of lung-mediated gene transfer have demonstrated detectable, but variable, gene transfer at relatively low doses of virus. Crystal and colleagues[10] detected recombinant CFTR protein in bronchial cells of a patient who received 2×10^6 pfu of virus (equivalent to an MOI <1) while we have documented recombinant CFTR RNA by *in situ* hybridization in bronchial epithelial cells of a patient that received 7×10^6 pfu of virus (data not shown).

Our work in the xenograft model shows a clear relationship between dose of virus and gene transfer with meaningful levels of gene transfer (~5%) achieved with doses of virus equivalent to an MOI of 100. Recombinant CFTR was detected in both ciliated and secretory cells of fully differentiated epithelia. Efficiency of gene transfer increased substantially when the epithelium was not fully developed (data not shown) consistent with previous observations in the mouse[13]. Based on the preliminary human data and those derived from our study, it is difficult to predict what dose and method of administration will be required to obtain gene transfer at therapeutic levels consistently in distal human lung.

Distribution of endogenous CFTR expression within a non-CF bronchus is complex with low levels present in most cells of the superficial epithelium and high levels found in a subset of cells within the submucosal glands[5,6]. A critical question is whether or not the level and distribution of gene transfer achieved with recombinant adenoviruses (in other words, high level correction in a small subset of superficial epithelial cells) will correct the defect in chloride secretion at a microscopic level. Studies in the xenograft model indicate that virtually complete normalization of the chloride secretory defect is possible with as little as 5% genetic correction. There is a clear threshold in this effect as evidenced by the lack of any detectable functional improvement in grafts that contain less than 0.5% corrected cells. This confirms previous studies based on *in vitro* analysis of viral transduced monolayers of polarized epithelial cells[20] and the recent studies in nasal epithelia of mouse nose[13].

It is striking that the Cl secretory defect was consistently and almost completely corrected following exposure to CFTR virus, whereas the defect in Na absorption was not. Mechanisms to explain the apparent uncoupling of Cl secretion and Na reabsorption in some xenografts following gene transfer are difficult to understand in light of our lack of insight into the normal regulation of the amiloride sensitive sodium channel. It is possible that the lack of correction in Na reabsorption in some grafts is simply a threshold effect of genetic reconstitution, however, it did not appear to correlate with either efficiency of gene transfer or quantitative recovery of Cl secretory responses. The CF genotype or heterogeneity at other genetic loci may influence the responsiveness of the epithelia to correction of Na reabsorption following gene transfer. The patients that did not correct Na transport had the unusual genotypes ΔF508/unknown and ΔF508/621+G>T, whereas the responding patients had different genotypes, including ΔF508/1717-1G>A and the more common ones ΔF508/G542X, ΔF508/ΔF508. These findings underscore the importance of establishing multiple functional criteria for assessing CFTR gene transfer *in vivo*.

Our studies suggest that physiologically meaningful gene transfer can be achieved in human CF bronchial epithelia with recombinant adenoviruses. The host's immune response to the virus and virus-infected cells could not be evaluated in the bronchial xenograft model because it is grown in an immune-deficient mouse. A better understanding of the mechanisms of Na hyperabsorption in CF patients and its variable response to partial genetic reconstitution will be important if therapeutic efficacy of gene therapy requires its correction.

Methodology

Recombinant adenoviruses. The structure and production of E1 deleted viruses that express *lacZ* (H5.010CMV*lacZ*; CMV promoter in sub360 backbone) and *CFTR* (H5.020CB*CFTR*; CMV enhanced β-actin promoter in dl7001 backbone) have been described[15,21]. CFTR virus was derived from the actual production lot currently used in our clinical trial[11].

Human bronchial xenografts. Human bronchial tissues were obtained from donor and explanted lungs at the time of lung transplantation and xenografts were prepared as described[15,16]. Surface epithelial cells were removed by incubating the bronchus in protease 14. These cells were plated in primary culture and maintained for 7–10 days prior to release with trypsin. Donor rat tracheas were harvested from CO_2 asphyxiated male Fisher 344 rats (250–350 g), the epithelium was denuded by three rounds of freeze thawing, and the tracheas were ligated to tubing at both ends after seeding of $2–5 \times 10^6$ primary bronchial epithelial cells in 30 µl of hormonally defined medium. Grafts were then implanted subcutaneously in the flanks of male *nu/nu* BALB/c mice and irrigated biweekly to remove accumulations of mucus and secreted proteins. Grafts were maintained for 3–4 weeks *in vivo* to allow cellular maturation to fully differentiated epithelia. Virus was instilled into the graft lumen (5×10^{10} or 5×10^9 pfu/ml, 100 µl) and expelled with air 12 h later.

Samples from nine CF lungs were used to generate 25 functioning xenografts which were exposed to H5.010CMV*lacZ* at 5×10^9 (3 xenografts) and 5×10^8 (3 xenografts) total pfu and H5.020CB*CFTR* at 5×10 (ref. 10) (1 xenograft), 5×10^9 (9 xenografts), and 5×10^8 (5 xenografts) total pfu; the remaining four grafts were studied in the absence of viral infection. CF samples were derived from patients with several genotypes including ΔF508/ΔF508 (four patients), ΔF508/G542X (one patient), ΔF508/1717-1G>A (one patient), ΔF508/621+1G>T (two patients), and ΔF508/unknown (one patient). Xenografts from non-CF tissues were analysed in the absence of virus (5 grafts) or after exposure to 5×10^9 total pfu of H5.010CMV*lacZ* (5 grafts).

Measurement of transepithelial potential differences. Xenografts were analysed for changes in PD in response to perfusion with different solutions before and after gene transfer using the technique developed by Knowles and co-workers[7,22]. Animals were anaesthetized with ketamine/xylazine (100 µl; 10% v/v in PBS) intraperitoneally in preparation for these measurements. Agar bridges were prepared by filling 21 gauge butterfly needles and tubing with 1 M KCl in 4% agar and the reference bridge was implanted subcutaneously in the back of the mouse while the exploring bridge was in contact with a reservoir of buffered solution being delivered at a constant rate of 2 ml min^{-1} at 37 °C by a syringe pump. Each bridge was connected by a calomel half cell to a voltmeter. Measurements were recorded every 5 s by a computer interfaced with the voltmeter. Prior to use, each pair of bridges was placed in a common reservoir of buffered solution. Only agar electrodes differing less than 0.2 mV were used for analysis. Each recording measured voltage (y axis) as a function of time (x axis) in response to the sequential perfusion of: (i) Hepes phosphate buffered ringers solution (HPBR) containing 10 mM Hepes pH 7.4, 140 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM Ca gluconate, 2.4 mM K_2HPO_4, and 0.4 mM KH_2PO_4; (ii) HPBR with 100 µM amiloride; (iii) chloride free HPBR (gluconate replaces chloride) with 100 µM amiloride; (iv) chloride free HPBR with 100 µM amiloride, 200 µM 8-cpt cAMP, and 100 µM forskolin; and (v) return to HPBR. The exploring electrode was advanced to the middle section of graft where there were little, if any, regional differences in PD. Post-infection PD measurements were done at four days, then every 3–4 days until the grafts failed or they were harvested. Most grafts were measured twice prior to viral infection and again twice after infection with virus in four day intervals. Statistical comparisons were performed between CF and non CF grafts using an unpaired student's *t*-test. The significance of adenovirus-mediated gene transfer was assessed using a paired student's t-test comparing within individual grafts Vt measurements before and after gene transfer.

Cytochemical analysis of grafts for transgene expression. Upon completion of the transepithelial PD analyses, the xenografts were explanted, embedded in OCT (Tissue-TCK, Miles Inc.), cryosectioned, and analysed by *in situ* hybridization (*CFTR* and *lacZ*), immunocytochemistry (CFTR), and x-gal histochemistry (*lacZ*). *In situ* studies were performed as described with ^{35}S-labelled cRNA probes to *lacZ* and human *CFTR*[5,15]. CFTR protein was evaluated by immunocytochemistry using a polyclonal rabbit antibody to a C-terminal peptide of *CFTR* while *lacZ* protein expression was detected using x-gal histochemistry[15]. The efficiency of gene transfer was determined by dividing the number of positive signals by the total number of cells present in 20 sections derived from multiple blocks of each xenograft.

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