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***VIRAL TRANSFER TECHNOLOGY*** ***BRIEF COMMUNICATION***

# *Gene transfer to adult human lung tissue ex vivo*

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*The potential of gene therapy for treatment of lung disease remains unrealised. Early model systems often resulted in promising efficiency of gene transfer, only to prove irreproducible in the clinic. While problems such as induction of host immune responses and duration of expression also need to be addressed, it is now widely believed that alternative, relevant models which more accurately reflect gene transfer efficiencies in human lungs are urgently required. We report here on a human lung slice culture system to assess gene transfer to adult lung epithelium. A lacZ-expressing adenovirus (AdCA35lacZ) was used as a reporter vector. A solution of AdCA35lacZ was instilled via bronchioles into resected lung tissue, a route analogous to clinical administration. Following a 1 h incubation, the tissue was inflated with a 0.4% agarose solution, instilled via the same bronchioles. Once solidified, 500 μm slices of the tissue were prepared and cultured for 4 days. β-Galactosidase staining revealed lacZ transgene expression in bronchiolar and alveolar cells of the lung slices throughout the 4 days in culture. This system, which can also be used to study other viral and liposome vectors, could prove to be a useful alternative model for assessing gene delivery to adult human lung epithelium.* Gene Therapy (2000) **7,** 675–678.

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Lung diseases such as cystic fibrosis (CF) and α1-antitrypsin deficiency, as well as neonatal disorders such as respiratory distress syndrome and bronchopulmonary dysplasia, have significant potential for gene therapy strategies. Current treatment regimens do not have high success rates but the identification of genes involved in these diseases has prompted investigations into corrective gene replacement approaches.[1] In comparison to other organs, the lung offers relatively straightforward access for gene delivery via the trachea, or alternatively via the pulmonary artery. In the case of CF, gene delivery vectors such as adenovirus and cationic lipid/liposomes have successfully produced CFTR protein expression in humans *in vivo* with some demonstrable restoration of chloride transport.[2,3] However, vector-related problems such as target cell specificity, duration of gene expression, host cell toxicity, ensuring replication defectiveness and immunogenicity have proved difficult to solve. As a result, despite initial promising results, no gene therapy drug has yet been approved for human use.[4,5]

A recurrent stumbling block has been the inability to reproduce results in the clinic based on the animal models and *in vitro* human systems used to test vectors. Transfer efficiencies achieved in murine and human primary epithelial cell cultures are frequently irreproducible when tested *in vivo* and most animal models do not exactly mimic human respiratory disease.[6,7] Adenovirus-mediated gene transfer to primary cultures of human lung type II cells can be up to 50 times more efficient than that observed *in vivo*[8] possibly due at least in part to altered membrane receptor distribution. Furthermore, uniform expression of adenoviral reporter genes has been observed in fibroblasts and type II cells in monolayer cultures derived from digested human fetal lungs.[9] This contrasts with adenovirus-infected cultured fetal human explants[10] and fetal lambs infected intratracheally *in utero*[11] where epithelial cells expressed reporter genes but little to no expression was found in mesenchymal cells. Therefore, while cultured cell monolayers can address important mechanistic questions, explant culture appears to provide a more accurate assessment of gene transfer efficiency and target cell specificity *in vivo*.

When tested on CF patients, vectors are usually applied to nasal mucosa. However, not only is this not the area clinically affected, but one of the cellular receptors involved in adenoviral entry is absent in nasal mucosa, but present in distal conducting airways.[12] This may account for differences between nasal and pulmonary epithelia in efficiency of gene transfer and demonstrates the need for more appropriate means of testing adenoviral and other gene therapy vectors. Few models of gene delivery using adult human pulmonary tissue have been reported. These have involved subcutaneous transplantation of segments of intact human bronchus, or denuded rodent trachea reseeded with cultured human bronchial cells, into the flanks of immunodeficient mice.[8,13] Vectors are subsequently delivered to the lumen of the xenografts via a surgical incision. While gene transfer and expression in bronchial epithelial cells were successfully demonstrated in these models, they have not been widely used, possibly because they are technically difficult systems.

We have developed a human lung slice culture system

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as an alternative model for assessing gene delivery vectors. With this system, we can closely reproduce clinical procedures by administration of adenoviral vectors via bronchioles to adult human lung tissue *ex vivo*. The tissue is then sliced and maintained in culture. Gene delivery and protein expression can subsequently be analysed *in situ*. Here, we report on the successful infection of human lung tissue with an adenoviral reporter vector (AdCA35lacZ) and subsequent detection of β-galactosidase in the epithelium of cultured lung slices.

Slices were stained for β-galactosidase activity at 24, 48, 72 and 96 h after infection. At each time-point, X-gal incubation of AdCA35lacZ infected lung slices resulted in positive staining of bronchiolar and alveolar epithelial cells (Figure 1a–f). Maximal β-galactosidase gene expression was apparent by 24 h with an approximate equivalent overall number of positive cells present at each time-point. Very occasional staining occurred in some smooth muscle cells (less than 5% of total positive cells). No staining occurred at any stage of the culture in control slices which had been instilled with culture medium without AdCA35lacZ. In order to compare gene transfer and expression in this *ex vivo* slice system with that found in existing *in vivo* models, a similar approach was employed using cultured murine lung slices. Following intratracheal instillation of AdCA35lacZ, β-galactosidase activity was observed in bronchiolar and alveolar epithelial cells within the cultured slices at 24, 48, 72 and 96 h after infection, with little staining of mesenchymal cells. As with the human slices, maximal activity was observed at 24 h with a similar distribution pattern of staining apparent up to 96 h (Figure 1g). No staining occurred in control slices instilled with culture medium without AdCA35lacZ. These findings are consistent with *in vivo* studies in rodents where adenovirus infection using similar doses resulted in transgene expression in the same cell types and at similar frequencies.[14,15] This would indicate that the *ex vivo* slice system is comparable with *in vivo* instillation models.

The benefits of agarose instillation into the tissue are two-fold. First, the collapse of the airways and airspaces during slicing and culturing is prevented, resulting in retention of tissue architecture and corresponding maintenance of cell–cell and cell–matrix contacts. Epithelial cell polarity and associated localisation of cell surface receptors are important factors during adenoviral infection of cells. The lung slice model is, therefore, more representative of *in vivo* epithelia than cultured monolayers, and is a more relevant model for viral infectivity. Second, because the agarose is mixed with culture medium, nutrients are delivered throughout the tissue and cells are not entirely dependent upon nutrient diffusion from the external culture medium. A limiting factor of the system is the relatively short culture period of 4 days. After this time, epithelial cell death is apparent in both control and infected slices. Optimisation of the culture conditions, such as improving the culture medium or altering the agarose solution, should extend the culture period. We have successfully established a culture system in which murine lung slices can be cultured for up to 3 weeks with good retention of tissue architecture and cell viability, by adapting previously published methods.[16,17] Achieving similar lengths of culture for human slices will permit further studies of duration of transgene expression.

This is a unique model system for assessment of gene delivery to distal adult human respiratory epithelium. This region of the lung has not been represented in any other *in vivo*/*ex vivo* human model of gene delivery to date, but it is affected by most of the diseases advocated as suitable for gene therapy. Studies using human fetal lung tissue have demonstrated gene transfer to nonciliated epithelial cells lining the acinar tubules, including type II cells and their glycogen-rich precursors.[10,11] The extrapolation that gene transfer would occur in corresponding cell types in distal adult human lung is confirmed in our model.

While an adenoviral vector was used in these experiments, the system is equally applicable to other viral and liposome vectors. The ability to delivery genes to adult human lung tissue *ex vivo* followed by culture and determination of gene delivery and expression *in situ* should enable us to address some of the outstanding problems hindering progress with gene therapy. Vector-related issues such as target cell specificity, efficacy of gene delivery, appropriate vector solute composition and vector toxicity may be explored using this system, before clinical tests.

The critical problem of adenovirus-associated immunogenicity cannot be assessed in this model under present conditions. However, among those researchers who are currently assessing the impasse at which gene therapy for lung disease now seems to be, many believe that inefficiency of gene transfer is a significant hurdle to be overcome, and that alternative model systems are required for more accurate assessments of vectors.[4,5] The *ex vivo* human lung slice system described here can potentially contribute to our understanding and development of gene therapy vectors.

A replication deficient (E1 deleted), sero type 5 adenovirus containing the *LacZ* reporter gene under the control of the murine CMV promoter (AdCA35LacZ) was used.[18] The virus was propagated and titrated as previously described.[19] Stock virus was aliquoted and stored at –70°C before use.

Macroscopically normal human lung tissue from an uninvolved lobe was obtained following pneumonectomy for lung cancer and stored at 4°C in 1:1 mixture of M-199 medium (Gibco, Life Technologies, Paisley, UK) and Hams F-12 (Gibco) before use. For most of our experiments, including the data presented here, lung tissue was stored for no longer than 4 h at 4°C. While we have also infected tissue following overnight storage at 4°C and observed *lacZ* staining in slices cultured from this tissue, cell death was increased in tissue which had been stored overnight and instillation of solutions was more difficult. A cannula and syringe were used to instil $1 \times 10^9$ p.f.u. of AdCA35lacZ suspended in culture medium (see below), or a control solution (culture medium only), via exposed bronchioles. Tissues were incubated, submerged in culture medium (1:1 M-199 medium and Hams F-12 medium, supplemented with 1 μg/ml insulin, 0.5 μg/ml transferrin, 0.1 μg/ml hydrocortisone, 10 ng/ml EGF, 0.1 μg/ml retinyl acetate, 2 mM L-glutamine and 0.1 mg/ml Pen/Strep) at 37°C for 1 h. A 0.4% agarose solution (1:1 mixture of 0.8% molten agarose/$H_2O$ and 2× culture medium, maintained at 37°C) was then instilled into the tissues via the same bronchioles used for adenovirus instillation. Following incubation at 4°C for 20 min to permit agarose solidification, tissues were cut into strips approximately 0.75 cm

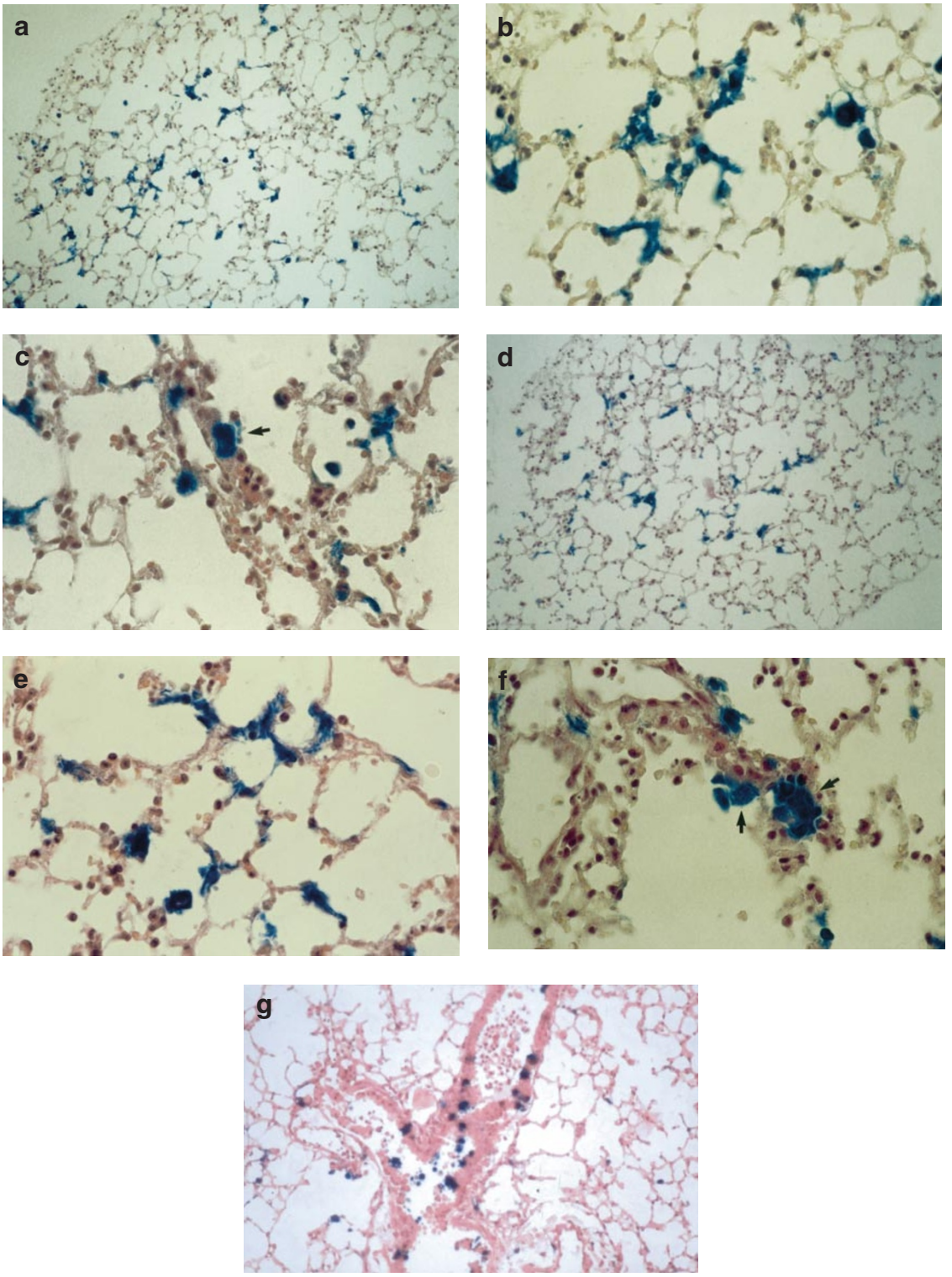


***Figure 1*** *Human lung tissue was infected with AdCA35lacZ ($1 \times 10^9$ p.f.u.) and cultured slices were stained for β-galactosidase expression. At 24 h after infection, staining of (a) and (b) alveolar and (c) bronchiolar (arrow) epithelial cells was observed. Slices stained at 96 h after infection displayed similar staining of (d) and (e) alveolar and (f) bronchiolar (arrows) epithelial cells. Murine lungs were infected intratracheally with AdCA35lacZ ($1 \times 10^9$ p.f.u.). Epithelial cells within the cultured slices were positive for β-galactosidase expression at each time-point, including (g) 96 h shown here. Magnification: (a) and (d): ×100; (b), (c), (e) and (f): ×400; (g): ×200.*

in diameter, embedded in 3% agarose and sliced into 500 μm thick sections using a Krumdieck Tissue Slicer (Alabama R & D Corporation, Munford, AL, USA). The embedding agarose detached easily from the slices once cut. Slices were cultured at the liquid–air interface in six-well plate Netwell inserts (Costar; Corning, High Wycombe, UK) in culture medium and incubated at 37°C in humidified 5% $CO_2$.

Mice approximately 5 weeks old were killed by intraperitoneal injection of pentabarbitone. Lungs were perfused with saline via the pulmonary artery with simultaneous artificial ventilation of the lungs using a syringe and cannula inserted into the trachea. Intact lungs and trachea were then removed and 0.8 ml culture medium (see above) containing $1 \times 10^9$ p.f.u. of AdCA35lacZ was instilled into the lungs via the trachea. Lungs were incubated, submerged in culture medium, at 37°C for 1 h. 0.8 ml 0.4% agarose solution was then instilled into the lungs via the trachea, allowed to solidify and individual lobes were embedded, sliced and cultured as described above.

Slices were rinsed in PBS, fixed for 5 min in 0.05% glutaraldehyde at 4°C, and then rinsed twice in PBS for 10 min. Slices were then incubated in X-gal solution (20 mM $NaH_2PO_4$, 250 mM $Na_2HPO_4$, 1.3 mM $MgCl_2$, 3 mM $K_3Fe(CN)_6$, 3 mM $K_4Fe(CN)_6$, 1 mg/ml X-gal (dissolved in dimethylformamide) in $H_2O$) at 37°C in a humidified chamber. Staining was visible after 1 h and the reaction was stopped after 6 h by removing the staining solution and rinsing the slices with PBS. Tissues were embedded in paraffin and 3 μm sections were cut and stained with haematoxylin and eosin.

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