3

NON-VIRAL GENE THERAPY FOR PULMONARY DISEASE

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INTRODUCTION

Non-viral gene therapy now includes a number of different strategies ranging from direct intramuscular injection of naked DNA to the systemic or local administration of formulations comprising DNA and lipid, proteins, peptides or polymers. While the main limitation of non-viral gene transfer methods is their relatively low efficiency *in vivo*, both preclinical and clinical studies indicate that these methods exhibit safety profiles similar to conventional pharmaceutical and biological products. Cationic liposome-based gene therapy has been extensively studied for the treatment of the genetic disease cystic fibrosis, where research has reached the stage of clinical trials. Non-viral gene delivery has also been studied with a view to developing treatment for the acute lung injury and pulmonary oedema associated with the adult respiratory distress syndrome (ARDS). In this chapter, following a brief overview of non-viral gene transfer, the progress of both clinical studies for CF gene therapy and preclinical studies for pulmonary oedema are described.

NON-VIRAL VECTORS

Non-viral gene delivery systems currently in use for the development of pulmonary gene therapy have focused on cationic liposomes, with or without DNA condensing agents or attached molecules intended to bind to receptors or to assist escape from endosomes. More recently, cationic polymers such as polyethylenimine (PEI) (1) and polyamidoamine dendrimers (2) have been used for gene transfer. Lipids and polymers are somewhat less efficient at gene transfer than recombinant viruses but lack their immunological and proinflammatory disadvantages and pose no risk of insertional mutagenesis.

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They can, moreover, accommodate large DNA plasmids. Current non-viral research aims to modify liposomes to incorporate advantageous viral features without introducing the inherent penalties of these vectors.

Cationic lipids vary in structure but are invariably composed of a hypdrophobic lipid anchor (an aliphatic chain or cholestrol ring), a positively charged head group that interacts with and condenses the DNA, and a linker that bridges these two components. The positively charged lipid forms a particle by condensing negatively charged DNA through ionic interactions. Particulate complexes subsequently form through further hydrophobic interactions amongst the bound lipids, and these may contain several plasmids, Within the complexes, the DNA is not simply encapsulated by lamellar lipid structures. Rather, freeze fracture electronmicroscopy reveals that the structure of lipid/DNA complexes is heterogneous (3). Aggregated and fused liposomes together with tubular structures (composed of condensed DNA) give rise to the term "spagetti and meatballs" and it has been suggested that the spagetti could give rise to transfection due to its diameter (~10 nm) which is close to the diameter of nuclear pore complexes (~7 nm).

DOTMA was the first synthetic cationic lipid used for gene transfer (4). Since then, a large number of cationic lipids have been assessed as transfer agents for lung gene delivery, and considerable progress has been achieved in improving efficiency. Some of those tested include the so-called first generation vectors with structures analogous to DOTMA such as DMRIE (5) and DOTAP (6), cholestrol-containing cationic lipids such as DC-Chol/DOPE (7) and second generation vectors which bear a polycationic headgroup (eg. spermidine) like GL-67/DOPE/DMPE-PEG_{500} (8). The basic mechanism of lipid-mediated transfection, though poorly understood, involves an initial association between cationic lipids, usually in the form of liposomes and plasmid DNA as described above. The resulting complexes are then thought to bind to the cell by an electrostatic interaction between the cationic lipid and negatively charged components of the plasma membrane such as sialylated glycoproteins or possibly by coupling with sulphated membrane-associated proteoglycans (9). Following membrane association, entry of cationic lipid/DNA complexes into the cell is thought to occur via an endocytic process which differs from either receptor-mediated uptake or pinocytosis (10). Escape from the endosome is a limiting factor for non-viral gene delivery and is thought to occur via destablisation of the endosomal membrane. It has been postulated that destabilisation results in the flip-flop of anionic lipids located principally in the cytoplasmic monolayer. These subsequently diffuse laterally into the complex where they form ion pairs

with the cationic lipids, resulting in charge neutralisation and release of DNA into the cytoplasm (11).

Intracellular trafficking and nuclear entry of the DNA is not, as yet, fully elucidated, although recent studies point to important roles for cytoskeletal elements, a number of nucleocytoplasmic transport factors and the nuclear membrane. Thus, escape from the endosomal pathway is followed by important regulation by cytoskeletal elements of subsequent transfer of the DNA to the nucleus. The finding *in vitro*, that inhibition of microfilament polymerisation significantly enhances gene expression, irrespective of the magnitude of endosomal entrapment (10) supports a critical role for these elements. Nucleo-cytoplasmic transport factors (12) which include the nuclear-localising signal (NLS) and their cognate receptors - importin-α, importin-β and related proteins - are known to play an important role in the succesful entry of exogenous DNA into the nucleus. Finally, synchronisation of the cell into the G1 phase, likely to mimic conditions found in the terminally differentiated airway epithelium, has been shown to significantly attenuate gene expression (10), while mitotic nuclear disassembly has the opposite effect.

GENE THERAPY FOR CYSTIC FIBROSIS

Cystic fibrosis is an autosomal recessive disease affecting more than 50,000 individuals worldwide, primarily although not exclusively, caucasians. The disease affects epithelial-lined organs, such as the respiratory and intestinal tracts, of which the former are the site of major morbidity and mortality. Recurrent chest infections and colonisation of the lower airways with organisms such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* progress to subsequent bronchiectasis and finally respiratory failure, the most common cause of death.

Current treatment includes daily physiotherapy, vigorous antibiotic treatment of pulmonary infections, pancreatic enzyme supplementation for pancreatic insufficiency and intensive dietary support. This combination has resulted in great improvements in life expectancy, but the treatment remains time-consuming and intrusive for patients and is essentially symptomatic. In 1989, the gene mutated in CF was identified and the protein for which it codes - the cystic fibrosis transmembrane conductance regulator (CFTR) - was characterised (13). This opened the way for the development of gene therapy for the disease, offering the long-term possibility of a promising new and more effective approach to treatment.

The proposed strategies for gene therapy in CF are based on what is currently known about the molecular and biochemical pathology of the disease. CFTR functions as a cAMP-regulated chloride channel in the apical membrane of epithelial cells (14). Increases in intracellular cAMP result in increased chloride transport through CFTR followed by osmotic movement of water, and this probably contributes to maintenance of mucosal surface hydration. Mutations in the CF gene, located on the long arm of chromosome 7, result in a CFTR that is either mislocalised or dysfunctional. This in turn results in disturbance of chloride transport across the epithelial cells of the airways, gut, pancreas, biliary tract and sperm ducts. Secondary to the chloride transport defect there is also an increase in sodium absorption in the airways (15), although how this occurs is presently unclear.

The relationship between these defects and the clinical manifestations of cystic fibrosis still remains speculative. However, a reasonable hypothesis is that the ion transport defects may lead to suboptimal volumes of airways surface liquid resulting in impaired mucociliary clearance, bacterial colonisation and repeated infection. The idea of gene therapy was simply to administer the normal CF gene or protein as if it were a drug, to the organ most affected — the lungs. In theory this should result in restoration of normal cellular function and so prevent or treat the disease. A vigorous research effort has therefore followed identification of the gene. The first reports of *in vitro* CFTR gene transfer appeared in 1990 (16), only one year after identification of the gene. Further *in vitro* studies (17), were followed by *in vivo* gene transfer to the airway epithelial cells of transgenic CF mice (18-19), confirming that it was possible to achieve some functional restoration of the ion transport defects in these cells following transgene expression. Within four years, at least four clinical studies of gene therapy in CF patients had been reported (20-23) and since then, the number has risen to over 20, all Phase I safety studies. Liposome/plasmid complexes have been tested in at least eight clinical trials (Table 1)(23-30). Of these, five were conducted in the U.K. and were all double blind studies. Three trials with similar design showed partial correction of the chloride transport defect in the nose (23-25). In the first, Caplen *et al* (23) tested a pSV-CFTR plasmid complexed with DC-Chol:DOPE in the nasal epithelium of ΔF508 homozygous CF patients. Three DNA doses were tested (10, 100 and 300 μg) in patient groups of three each, while a further 6 patients received only the equivalent lipid dose as control. No safety problems were encountered and of eight biopsies taken from the treated patients evidence for the transgene was seen in all but one sample. In 5 of these 7 positive samples, transgene expression was detected. The CF chloride secretory defect, as assessed by measurement of *in vivo* transepthelial potential difference (PD) (31), showed a significant 20% increase towards normal values, with two of the patients reaching the normal

range for non-CF subjects. This change was maximal 3 days after gene administration and had reverted to pretreatment values after 7 days.

DC-Chol:DOPE was tested in a second trial of CFTR gene transfer to the nasal epithlium by Gill *et al.* (24). Again, DC-Chol:DOPE was used to deliver a CFTR-carrying plasmid driven by a RSV-LTR promoter to the nasal epithelium of 12 CF patients (mixed genotypes) by direct instillation. Two DNA doses were studied: 40 μg (4 patients), 400 μg (4 patients), and placebo (either buffer or lipid:plasmid encoding non-functional CFTR). No safety problems were encountered. Both *in vivo* nasal PD measurements and *ex vivo* SPQ analysis (32) of nasal epithelial cells obtained by brushings, were used as end-points to assess the effect of gene transfer on ion transport. The combined results showed evidence of functional CFTR gene transfer in 6 out of 8 treated patients. Correction of the CF ion transport abnormalities were more sustained than those reported in the first trial above (23), lasting for 7 days in one patient and fifteen days in another. No change was observed in sodium-related measurements.

In the third of these nasal trials (25), a single dose of 400 μg pCMV-CFTR complexed with 2.4 mg of DOTAP was administered to the nasal epithelium of CF patients (mixed genotype). Again, the trial was double-blind and placebo controlled, with 8 patients receiving treatment and a further 8 receiving buffer only. No safety problems were encountered. Vector-specific DNA was detected at 3 days and 7 days in seven out of eight treated patients

***Table 1*: Clinical Trials of Liposome-mediated Gene Therapy for CF**

Research Center	Delivery System	Target Organ(s)	**Subject #**
Royal Brompton Hospital, National Heart and Lung Institute, London, UK (23)	DC-Chol:DOPE	Nose	18
University of Oxford/University of Cambridge, UK (24)	DC-Chol:DOPE	Nose	19
MRC Human Genetics Unit, Western General Hospital, Royal Infirmary, University of Edinburgh, Edinburgh, Scotland (25)	DOTAP	Nose	20
University of Iowa College of Medicine, Iowa, USA (26)	GL-67	Nose	21
Royal Brompton Hospital, National Heart and Lung Institute, London, UK (27)	GL-67	Nose and Lungs	22
University of Oxford/University of Cambridge, UK (28)	DC-Chol:DOPE	Nose (repeat dose)	12
University of Alabama, Birmingham, AL, USA (29)	DMRIE:DOPE	Lungs	Still to be reported
University of North Carolina at Chapel Hill, Chapel Hill, NC, USA (30)	GR213487B	Nose	23

and at day 28 in two of these seven. Vector-derived mRNA was detected in 2 patients at days 3 and 7. Two treated patients demonstrated changes in their nasal PD following treatment consistent with partial correction of the chloride transport defect, with a mean change towards non-CF values of 20%.

The following two clinical trials (26-27) made use of a second generation cationic lipid GL-67/DOPE/DMPE-PEG_{500}, shown to be non-toxic when administered by nebulisation to the lungs of normal volunteers (33). Zabner *et al* (26) administered naked DNA (1.25 mg of pCF1-CFTR driven by a CMV promoter) to the epithelial surface of one nostril and the same dose of DNA but complexed with lipid Gl-67 to the other nostril in a randomised, double-blind fashion. Nine subjects were studied. Functional gene transfer was demonstrable by RT-PCR and *in vivo* nasal potential difference measurements, with little difference observed between the two vector systems studied. In a further double-blind, placebo-controlled trial using GL-67, the complex was delivered to both nose and lungs of 16 CF patients by nebulization (27). 4.2 mg of pCF1- CFTR complexed with 229 mg of the lipid was administered to the lungs of 8 patients while a further 8 received an equivalent dose of lipid alone. A lower dose (11.8 mg DNA) was nebulised into the nose. Using *in vivo* PD measurements and *ex vivo* SPQ fluorescence analysis, partial, but significant correction of chloride transport was seen at both sites — approximately 25% correction towards normal values in the lungs and 20% in the nose. Furthermore, for the first time, reduced binding of *Pseudomonas aeruginosa* to airway epithelial cells (34) was observed in both nose and lungs. Administration of the gene-lipid complexes to the lungs in this trial was associated with a transient febrile reaction which did not occur in the control group who received the lipid on its own. This side effect may be attributable to the bacterial origin of the DNA (35). None of these studies showed any correction of the sodium transport defect and vector-specific mRNA was only inconsistently found.

More recently, a double-blind study to evaluate the safety and efficacy of multiple doses of CFTR-cDNA complexed with DC-Chol:DOPE to the nasal epithelium of CF patients has been reported (28). Ten subjects received the complexed DNA while a further 2 patients received placebo. Each subject received three doses, administered respectively at 4 weekly intervals. There was no evidence of inflammation, toxicity or immunological response to either the complexes or to the expressed transgene. End-point assays included quantification of vector-specific DNA and mRNA, immunocytochemistry of CFTR protein, bacterial adherence to epithelial cells, *in vivo* nasal potential difference and *ex vivo* SPQ analysis. On average, 6 of the treated subjects were positive for CFTR gene transfer after each

dose. Of these, all subjects demonstrating some correction of the CF ion transport defect were also positive for plasmid-derived DNA, mRNA and CFTR protein by immunocytochemistry. This study suggested, that unlike high-dose recombinant adenoviral gene therapy, cationic lipid/DNA complexes could be succesfully re-administered without apparent loss of efficacy.

Finally, a recently reported study using a novel second generation cationic lipid, [p-ethyl-dimyristoylphosphatidylcholine(EDMPC) cholestrol] (30) reported that the lipid-DNA complex were safe but did not produce consistent evidence of gene transfer to the nasal epithelium.

FUTURE CONSIDERATIONS

While clinically applicable treatment using cationic lipid-mediated gene therapy has not yet been realised, a wealth of data has emerged from all of the above studies. These reflect both enormous progress but also bring to light numerous difficulties that were not anticipated. Gene transfer efficiency currently remains suboptimal, not only for cationic lipids, but also for the two viral systems tested clinically (recombinant adenovirus and AAV). Further progress will need resolution of a number of issues:

The Target

CF affects the conducting airways, rather than the alveoli. These include both the larger bronchial regions lined by a pseudostratified columnar epithelium and containing numerous submucosal glands, and the small bronchioar regions, lined by a simple columnar epithelium devoid of glands. A central question for CF gene therapy is which cell type and which region (large or small) to target. Although ciliated superficial epithelium is abundant and displays the ion transport defects in patients with CF, the submucosal glands are the highest expressing CFTR cells in the lung and might well need to be targeted for clinical benefit. This raises considerations of delivery, since topical delivery will only reach these cells with difficulty and may therefore require systemic delivery of CFTR-vectors. Further, most data suggest that small airways are both the initial and major site of disease in CF. Effective cDNA delivery to these areas is not inevitable using current nebuliser technology and remains an important strategic issue.

The Barriers

An intrinsic function of the lining epithelium of the airways is to prevent penetration of the airways and interstitium by foreign material and invading organisms. Thus, a complex series of extracellular barriers, including a normal mucus layer which inhibits gene transfer (10), a glycocalyx, an apical

cell membrane, and tight junctions between the cells, conspire to keep out intraluminally delivered materials including both viral and non-viral vectors. This problem is compounded in CF, by the presence of thick, infected sputum, also known to inhibit gene transfer (36), and mucus plugging of the small airways. Removal of the mucus layer has been shown to increase transgene expression 25-fold in an *ex vivo* model of airway epithelium using native sheep trachea (10). Thus, the use of adjunctive mucolytic agents or abrogation of tight junction barrier function using either detergents or antibodies to intrinsic tight junction components are just two novel strategies being investigated to overcome these barriers.

The apical cell membrane presents another significant barrier, serving, *in vivo,* an important protective function by preventing entry of macromolecules into the cell. The extent of this barrier is however variable. Studies using airway epithelial cell cultures have shown that differentiated cells differ both in their surface charge and their ability to endocytose DNA-lipid complexes in comparison with dedifferentiated cells at the edge of the culture (37). These differences are paralleled by significantly more efficient gene transfer in the latter.

Once inside the cell, plasmid DNA, is then subject to a further series of potential barriers, and unlike viruses, non-viral vectors lack any strategy to overcome intracellular barriers including the endosome and nuclear membrane. It is estimated that only 1 in 1000 liposome-delivered plasmids reach the nucleus and are expressed. It is difficult to quantify the relative importance of the different barriers facing lipid-mediated gene transfer. Further strategies to overcome the cell membrane and intracellular barriers might include (i) optimisation of receptor mediated endocytosis using polycations such as poly-L-lysine or protamine to condense the DNA together with receptor binding chemicals such as transferrin, antibodies to cell surface antigens like the polymeric IgA receptor and viral entry proteins (38,39); (ii) pH sensitive ligands to encourage more efficient endosomal escape; (iii) the use of nuclear proteins (e.g. HMG-1), to enhance trafficking of the DNA into the nucleus, and (iv) the addition of relevant nuclear localisation signals so that the DNA can enter the nucleus more efficiently.

The Vectors

With respect to non-viral gene transfer, strategies aimed at improving gene transfer efficiency include the ongoing modification of lipid vectors currently in use. Third generation synthetic vectors have been designed, and include:

i) dendrimers (2)

ii) ligand-polylysine-DNA complexes for receptor-mediated gene transfer, for example lactosylated poly-L-lysine has been shown to

efficiently transfer CFTR cDNA to CF airway epithelial cells *in vitro* (40)

iii) DNA-gelatin nanospheres (41)

iv) polyethylenimine (PEI) (1) which has been shown to transfer genes both *in vitro* and *in vivo*. It has a number of chemical/structural properties which are quite different to cationic liposomes and may be advantageous for gene delivery. The size of PEI/DNA complexes is very small (<100 nm), potentially allowing for better distribution. It also appears to be efficient at endosomal escape, thus reducing DNA degradation.

v) cationic detergents able to condense plasmid DNA into small uniform complexes (42)

vi) virosomes — which comprise liposomes with viral proteins such as Sendai virus haemaglutinin (HVJ liposomes). These complexes are thought to to introduce DNA into the cytoplasm of the cell following haemaglutinin-mediated fusion of the liposome with the target cell membrane (43)

How much is enough?

The vexing question of the level of gene expression needed to achieve clinical benefit remains unresolved, but it is likely that the level of transduced CFTR per cell required will be low. The percentage of CF cells which need to be corrected has been suggested by a study (44) demonstrating *in vitro* that 6-10% of cells within a monolayer must consist of 'corrected CF cells' in order to restore normal chloride transport. The percentage required to correct the sodium transport defect is however, much higher than this (45). The optimal strategy for functional correction would be to mimic normal expression ie. to correct as close to 100% of cells as possible at low levels of expression per cell. This remains a challenging target, with a realistic aim of achieving somewhere between 10 and 100% of cells.

Thus, while CFTR gene therapy using first and second generation non-viral vectors has been shown to be safe and associated with some, albeit small, detectable functional correction of airway epithelial cells, it still remains elusive as a practical treatment for CF lung disease. However, the unresolved questions that remain to be answered for successful CF gene therapy, have now been clearly defined — a prerequisite for overcoming the technical problems of efficient gene transfer in the right target cells. Gene therapy for CF remains the most promising possibility for curative rather than symptomatic therapy. It will likely prove most beneficial if given very early - prior to the onset of established infection/inflammation in the lungs. While ongoing research worldwide aims to resolve the problems delineated above, questions about the execution and design of trials in the paediatric

population, not studied thus far, will become the focus of new efforts. Rigorous measurement of gene transfer efficiency *in vivo* and the development of markers - both real and surrogate - of clinical benefit remain important challenges. One can still be optimistic that once gene transfer efficiency reaches the 10 to 100% target, clinical benefit when judged from an early stage of the disease, will be easily apparent.

GENE THERAPY TO ENHANCE RESOLUTION OF PULMONARY OEDEMA

Resolution of pulmonary oedema is now known to be mediated by active absorption of liquid across the alveolar epithelium (46-49). While the mechanisms remain only partially understood, most data suggest that the driving force for this process is active sodium transport via apically located amiloride-sensitive channels and basolaterally located, ouabain-sensitive sodium-potassium ATPase (Na,K-ATPase) pumps (50). This process generates a transepithelial osmotic gradient that causes water to move out of the alveolus as a secondary phenomenon. Thus, the alveolar epithelium functions not only as an important barrier to alveolar flooding but also as an active organ in the reabsorption process. Maintenance of its structural and functional integrity has been shown to be an important determinant of the outcome of lung injury (51).

Na,K-ATPases are complex transmembrane multimeric proteins containing at least three subunits (α, β, and γ) each of which exists as a number of isoforms (52-54). The larger (112 kD) α subunit contains the domains directly involved in ion translocation and ATP hydrolysis as well as binding sites for substrates and inhibitors of enzyme function. The smaller (55 kD) β subunit is a glycoprotein thought to control heterodimer assembly and membrane insertion (55). Both the α and β subunits are required for normal Na,K-ATPase activity (56-57). In the lung, the protein is expressed in alveolar type I and type II cells and both expression and function of the enzyme appears to be developmentally regulated. Thus, in the foetal rat lung, for example, Na,K-ATPase mRNA, protein and function increase just before birth (55,58) while in the developing mouse lung, a dramatic increase in α and β_1-mRNA has been documented just before birth (59). Further, in the rabbit lung, alveolar type II cells have been shown to raise their Na,K-ATPase activity perinatally (60). All of these observations point to a central role for Na,K-ATPase in the generation and maintenance of dry alveoli at a crucial time when the lungs need to clear liquid as they adapt to air breathing.

A large body of evidence now also suggests that in the developed lung, clearance of lung liquid remains related to active sodium transport and hence

to Na,K-ATPase activity. Upregulation of alveolar fluid transport secondary to increased sodium transport by alveolar epithelial cells has been demonstrated in isolated type II alveolar cells (61-62), isolated lungs *ex vivo* (63-64) and in a number of alveolar flooding models induced either by liquid instillation (46-48) or by acute lung injury using hyperoxia (65), endotoxin or bacterial pneumonia (66-67). Further, ouabain, a specific inhibitor of Na,K-ATPase, depresses sodium (63-64) and liquid (49,64,68) transport in isolated lungs. The most compelling evidence for the role of Na,K-ATPase in the resolution of pulmonary oedema derives from an *in vivo* model of high permeability pulmonary oedema induced in rats by intraperitoneal thiourea (69). This study demonstrated that lung Na,K-ATPase activity was increased, particularly in alveolar type II cells, during recovery from oedema. This, in part, related to an increased Na,K-ATPase protein production in the lung. Enzyme activity began to increase four hours after the induction of oedema, approximately two hours after maximal pulmonary oedema. Activity reached maximal levels at twelve hours and was associated with marked resolution of the oedema, providing evidence that this active sodium transport mechanism is endogenously upregulated during this resolution process.

On the basis of the above evidence, two independent groups of researchers hypothesised that lung liquid clearance and hence oedema resolution could be further upregulated by transfer and expression of exogenous Na,K-ATPase cDNA. This may be particularly important in refractory oedema where maximal endogenous Na,K-ATPase upregulation remains inadequate either because of injury to the epithelium or because the rate of oedema formation overwhelms the epithelial capacity for reabsorption. This hypothesis was tested using both adenoviral-mediated and liposome-mediated gene transfer. In the first study, Factor *et al* (70) used replication-deficient human type 5 adenoviruses encoding, respectively, the rat α_1 and β_1 Na,K-ATPase subunits which were tested in isolated rat airway type II cells, airway epithelial cell monolayers and in isolated rat lungs. The data from this study demonstrated for the first time that transfer of the β_1 Na,K-ATPase subunit could augment Na,K-ATPase function and increase the rate of lung liquid clearance. These observations were supported by a study in which the same hypothesis was tested in a murine disease model of pulmonary oedema (71). Here, the second generation cationic liposome GL-67 was used to transfer cDNA encoding both α and β subunits of Na,K-ATPase (pCINaK) to the lungs of a mouse model of pulmonary oedema induced by thiourea. The study demonstrated that *in vivo* resolution of pulmonary oedema may be significantly enhanced by pretreatment of the lungs with Na,K-ATPase gene transfer. This was associated with increased lung Na,K-ATPase activity and detection of vector-specific mRNA confirming successful gene transfer.

These observations supported the hypothesis that exogenous upregulation of Na,K-ATPase, over and above the inherent capacity of the endogenous enzyme to upregulate acutely in response to oedema formation, can augment lung liquid clearance. Despite prior optimisation of gene transfer efficiency (72) however, resolution of pulmonary oedema following pCINaK transfer in these studies was not complete. Although, simply improving transfection efficiency may enhance this process of liquid clearance, it is possible that other components of the complex machinery of lung water clearance may also need to be considered. Thus, while Na,K-ATPase is thought to be the principal driving force for vectorial sodium and water transport across the alveolar epithelium, the epithelial sodium channel (EnaC)(73), is also involved in lung liquid clearance. Increased Na,K-ATPase activity must be associated with increased sodium ion entry at the apical surface of epithelial cells and since increased ENaC expression may also be required, co-transfection of cDNA for this components may ultimately be needed to achieve further augmentation of lung liquid clearance.

Although these studies provide only proof-of-principle in a mouse model in which gene transfer was applied as pretreatment, prior to the onset of lung injury, these data suggest this approach may be applied clinically. The acute, and inevitable, pulmonary oedema associated with the reperfusion syndrome following lung transplantation (74) may, for example, be an important clinical target where pretreatment of donor organs may be sufficient to prevent the development of pulmonary oedema. Adult respiratory distress syndrome remains a disease with a high mortality, and while this relates principally to multiorgan failure, an important study has shown that patients who were able to reabsorb some of their oedema fluid within 12 hours of intubation after acute lung injury, recovered more rapidly from respiratory failure and had a lower mortality (51). This study also showed that the ability of the alveolar epithelial barrier to reabsorb fluid following acute lung injury is preserved in 30% to 40% of patients. Thus, pre-selection of patients known to be at high risk of developing ARDS and manipulation of the epithelial barrier with Na,K-ATPase gene therapy, may accelerate the resolution process in zones of the lung where the epithelium remains intact. Efficient delivery to the alveolar regions of the lung, and optimisation of gene transfer remain important challenges.

CONCLUSION

Non-viral mediated gene therapy for lung disease remains an attractive therapeutic strategy in view of its low toxicity and lack of immunogenicity. A great deal of progress has been made within the field and non-viral vectors have provided important proof-of-priciple data, particularly for cystic

fibrosis. However, gene transfer efficiency using non-viral vectors remains currently inadequate for clinical benefit and ongoing research will focus on achieving optimum levels of transgene expression for therapeutic efficacy.

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