The pathogenesis of cystic fibrosis and progress towards gene therapy

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Cystic fibrosis (CF) is the most common autosomal recessive disease of Caucasians, affecting more than 50,000 individuals worldwide. Extensive research over the past decade has resulted in a number of important advances:

1. Life expectancy has been substantially improved to the current median of about 30 years by conventional therapy consisting of physiotherapy and aggressive antibiotic treatment for pulmonary infections, as well as pancreatic enzyme supplementation and nutritional support.

2. Considerable advances have been made in elucidating the molecular defects in CF cells, including the cloning of the CF gene and the identification of the CF transmembrane conductance regulator (CFTR) protein encoded by this gene, raising the possibility of gene therapy as a curative treatment.

The disease is, however, still associated with significant morbidity and mortality, and the relationship between the genetic defect and the clinical manifestations that lead to chronic respiratory infections and ultimate respiratory failure remains incompletely understood.

This article will review the molecular and biochemical pathology of CF, and present the current (and controversial) hypotheses about pathogenic mechanisms. It will also summarise the progress of gene therapy from the earliest in vitro experiments to the recent clinical trials, and identify some of the difficulties that remain to be resolved.

Clinical pathology

The organs affected in CF include the gut, pancreas, liver and reproductive tract, but the most important clinical consequences of CF are those affecting the lungs (Fig 1). Although the lungs of patients with CF are thought to be normal at birth, they show microscopic evidence of small airway inflammatory changes early in infancy. It remains unclear whether infection precedes inflammation or intrinsic defects in inflammatory regulation facilitate infection. However, detectable endobronchial colonisation with organisms such as Staphylococcus aureus and Haemophilus influenza commonly occurs within the first 1–2 years of life, and eventually nearly all patients become infected with Pseudomonas aeruginosa. This organism is innocuous in normal individuals but in CF is associated with progressive pulmonary deterioration, although the rate and severity are highly variable and multifactorial. Chronic inflammation contributes to obstructive lung disease and tissue destruction, most severe in the upper lobes and involving a huge infiltration of neutrophils. The subsequent development of bronchiectasis ultimately leads to respiratory failure and death.

Fig 1. Typical appearance of a cystic fibrosis (CF) lung showing thick sticky secretions (arrow). (Reproduced courtesy of Dr A G Nicholson, Royal Brompton Hospital.)
Molecular pathology

Studies in the early 1980s identified defects in the chloride conductance properties of CF epithelial cells. This was followed by the cloning of the CF gene in 1989 and the identification of CFTR, the gene product. CFTR, a complex transmembrane protein (Fig 2), is a member of the adenosine triphosphate (ATP) binding cassette (ABC) family, and is now known to have at least two of the diverse functions displayed by this family of molecules:

- It functions as a cAMP-regulated chloride channel located in the apical membrane of epithelial cells. Mutations in the gene, located on the long arm of chromosome 7, result in CFTR that is either mislocalised or dysfunctional, with consequent impairment of chloride transport across the epithelial cells of the airways, gut, pancreas, biliary tract and sperm ducts.
- CFTR can also regulate other membrane proteins, its negative regulation of the amiloride-sensitive epithelial sodium channel (ENaC) probably being the most well-documented. Again, mutations in the CFTR disrupt this function, leading to the characteristically increased sodium absorption in CF.

CFTR therefore probably plays multiple roles in epithelial cell ion transport, and this is emphasised in most current hypotheses of CF disease.

The link between clinical and molecular pathology

Two hypotheses attempt to link defects in CFTR-mediated ion transport to CF lung disease:

- The 'low volume' hypothesis (Fig 3(a)) postulates that, compared to normal airways surface liquid (ASL), which has salt levels approximately equal to those in plasma, CF ASL is salt-depleted because faulty CFTR is unable to inhibit ENaC. Thus, abnormally increased isotonic fluid absorption depletes the ASL volume, leading to impaired mucociliary clearance, a failure to clear thickened mucus from the airway surface and the initiation of chronic infection.
- The 'high salt/defensin' hypothesis (Fig 3(b)) postulates that defective or missing CFTR results in reduced transepithelial chloride conductance, allowing ASL salt levels to remain similar to plasma levels. The high salt concentrations (>100 mM) interfere with the function of salt-sensitive epithelial cell-derived natural antibiotics such as defensins, lysozyme and lactoferrin, rendering the airways susceptible to bacterial colonisation and infection.

The controversy surrounding these two hypotheses has not yet been resolved. Technical difficulties are associated with in vivo measurements of ASL ionic composition and volume because the volume of fluid to be sampled is tiny and rapidly altered when disturbed. Despite a number of studies, including two in vivo studies, showing no
difference between CF and non-CF subjects, to date there is no consensus on the ionic composition of ASL in normal and CF airways. Clearly, each theory has implications for proposed therapy for CF: the first hypothesis implies that the disease will best be managed by restoring the volume of ASL, while the second suggests that therapy should rather be directed at modulating ionic composition.

Intrinsic abnormalities of CF epithelia have also been implicated in the pathogenesis of CF lung disease. Here, at least two further hypotheses have sought to explain the chronic bacterial colonisation:

- An increased density of the *P. aeruginosa* receptor, asialo-ganglioside-GM1, mediates increased binding of *P. aeruginosa*.
- CFTR itself serves as a receptor for *P. aeruginosa*, which is then cleared by epithelial cell ingestion. Decreased levels or abnormal function of CFTR would thus result in defective clearance of the bacteria.

It is likely that the clinical pathology of CF lung disease represents the outcome of a combination of all these factors, although the relative contribution of each mechanism remains unclear. Gene therapy for CF – that is transfer of normal copies of cDNA encoding CFTR to CF airway epithelial cells – would therefore be a logical approach to treatment, encompassing all possible CFTR-mediated mechanisms rather than attempting to focus on each separately.

### Gene therapy for cystic fibrosis

Given the cloning of the gene and the accessibility of the lungs, CF appeared to provide an excellent pathfinder for the development of gene therapy. Theoretically, transfer of normal CFTR-cDNA into sufficient numbers of affected CF cells early in life should result in the production of enough normal CFTR to prevent the major clinical manifestations of CF. To this end, a vigorous research effort followed identification of the gene in 1989, with first reports of *in vitro* CFTR gene transfer appearing only one year later. These studies were followed by *in vivo* gene transfer to the airway epithelium of transgenic CF mice, confirming that it was possible to achieve some functional restoration of the chloride defect in these cells following transgene expression. Within four years, four clinical studies of gene therapy in CF patients had been reported, and since then, many more. These have all been Phase I safety studies using either viral (recombinant adenovirus or adeno-associated virus) or non-viral (cationic liposomes) vectors to deliver CFTR cDNA topically to the nose or lungs of patients by direct liquid instillation or aerosol.

A wealth of data has emerged from these studies, reflecting enormous progress, but also bringing to light numerous unanticipated difficulties. Thus, while CFTR gene therapy using first- and second-generation vectors has been shown to be quite safe and associated with some, albeit small, functional correction of airway epithelial cells, it remains elusive as a practical treatment for CF lung disease. Some of the problems relate to logistic
considerations of vector design and delivery that are required to optimise gene transfer efficiency. Many problems, however, may be resolved only when the pathogenesis of the disease and the role of CFTR have been fully elucidated.

The issues to be resolved

The target

CF affects the conducting airways rather than the alveoli. The former include both the larger bronchial regions, lined by a pseudostratified columnar epithelium containing numerous submucosal glands, and the small bronchiolar regions, lined by a simple columnar epithelium devoid of glands. Numerous other cell types are present within both regions. A central question for CF gene therapy is which cell type and which region (large or small airways) 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 may need to be targeted for clinical benefit. If so, this would cause considerable difficulties. Topical delivery would not reach these cells, and systemic delivery of CFTR vectors might be required. Importantly, most data suggest that small airways are both the initial and the major site of disease in CF, and an as yet unidentified population of cells here also expresses quite high levels of CFTR. Again, effective cDNA delivery to these areas would be difficult both because of current nebuliser technology and because of the presence within this region of abnormal secretions from early in life. We suspect that these small airways may be the key target and that delivery is an important 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 epithelial barriers, including a mucus layer which inhibits gene transfer, 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, which is also known to inhibit gene transfer and mucus plugging of the small airways. Two novel strategies being investigated to overcome these barriers are:

- the use of adjunctive mucolytic agents
- the abrogation of tight junction barrier function using either detergents or antibodies to intrinsic tight junction components like occludin.

Vectors

An alternative strategy aimed at improving gene transfer efficiency is the ongoing modification of currently used vectors. Current gene transfer efficiency is probably too low to obtain clinical benefit, although this has never been tested. In the case of viral vectors, the appropriate receptors for both adenovirus and adeno-associated virus have been identified on the inaccessible basolateral cell membrane. Attempts are now being made to adapt viral entry into cells using other receptors such as the apically sited ATP/uridine triphosphate receptor known as P2Y2. Adenoviral vectors have two additional problems which make repeated dosing difficult:

- a cytotoxic T-lymphocyte response which limits the dose-dependent titre that can be applied
- the production of humoral antibodies.

Recombinant viral technology continues to adapt the viral structures to reduce these problems. Cationic liposomes have been used to achieve an approximately 25% functional correction of lower airway epithelium following CFTR gene transfer to patients’ lungs by nebulisation. Overall, they are probably less inflammatory and immunogenic than viral vectors. However, reactions to the cDNA were identified in this trial which are likely to be due to its bacterial origin. Gene transfer efficiency using this vector remains low and efforts continue to adapt the liposome particle to address this problem.

The end-points

Molecular and functional assays to determine gene transfer efficiency are now established. These include:

- reverse transcription polymerase chain reaction for the detection of vector-specific mRNA
- immunohistochemistry to detect CFTR protein
- measurement of in vivo transepithelial potential difference to detect restoration of ion transport.

Adherence of P. aeruginosa to CF epithelial cells in vitro reduces following liposome-mediated CFTR-gene transfer.
Quantification of bacterial adherence has subsequently been used as a potentially relevant clinical end-point in clinical trials of CF gene therapy. Further clinical outcome measures, for example airway inflammation, pulmonary function and the number of infective exacerbations, have not yet been tested in clinical trials. The development of clinical, non-invasive and accurate end-points applicable to large numbers of patients in Phase III studies remains an important challenge.

How much is enough?

The key question of how much effective gene delivery and expression will be required to achieve clinical benefit remains unresolved. There are two ways to consider this:

- If the gene gets into every cell, what level of normal CFTR needs to be achieved? Studies in CF mice suggest that low levels of normal CFTR mRNA (5%) within every cell are sufficient both to correct the chloride defect and to prevent intestinal disease in these animals.

- If the gene is delivered to only a few cells, how many need to be transfected? One in vitro study has shown that 6–10% of non-CF cells mixed into a monolayer of CF cells is sufficient to restore normal chloride transport. The percentage required to correct the sodium transport defect is, however, much higher.

The most likely strategy to ensure functional correction would be to attempt to mimic normal expression by correcting as close as possible to 100% of cells at low levels of expression per cell. This remains a challenging target, for which a realistic aim is to achieve somewhere between 5% and 50% of cells.

The future

Gene therapy for CF is likely to prove most beneficial if given early, before the onset of established infection/inflammation in the lungs. Thus, while ongoing research aims to resolve the problems delineated above, questions regarding trials in the paediatric population, not studied thus far, have 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. Gene therapy for CF is progressing steadily and is likely to become an important therapeutic option for this disease.

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Adult obstructive sleep apnoea syndrome

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Some degree of obstructive sleep apnoea (OSA) is a common finding in 5–20% of adult men, although only about one in five of these individuals will have associated daytime sleepiness (obstructive sleep apnoea syndrome (OSAS)). Much has been discovered about this condition since the publication of the first widely-read account of the syndrome in 1976. Improvements in our understanding of the pathophysiology of OSAS, and the effects of even minor upper airway obstruction on sleep architecture, have refined our approach to treatment which should now be based on the severity of daytime symptoms rather than on nocturnal respiratory disturbance. This article outlines current approaches to diagnosing and managing OSAS, including issues related to driving and cardiovascular disease. The evidence for the efficacy of nasal continuous positive airway pressure (CPAP) is presented, and other management approaches discussed, including dental appliances and surgery.

Definition

The original definitions of OSAS were specific, requiring an arbitrary number of respiratory events (apnoeas or hypopnoeas) per hour of sleep (apnoea index or apnoea/hypopnoea index) to confirm a diagnosis. It is now recognised that increases in upper airway resistance alone, without an apnoea, hypopnoea or even hypoxic event can cause recurrent arousal from sleep, so this index is no longer considered ideal either for defining sleep apnoea or for grading its severity. A clinical definition of OSAS is more appropriate: for example, significant daytime symptoms (e.g. sleepiness) in conjunction with evidence of sleep-related upper airway obstruction and sleep disturbance.

Diagnosis by sleep study

The aims of a sleep study are to:
- recognise upper airway obstruction
- identify whether this is responsible for substantial sleep disturbance
- exclude other conditions that may also cause daytime hypersomnolence.

Many different physiological signals can directly or indirectly provide this information, and these will not be discussed in detail in this article. No single signal can reliably produce all the necessary information and a 'montage'

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