CYSTIC FIBROSIS

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INTRODUCTION

Cystic fibrosis, an autosomal recessive disease, is the most common genetic disorder in Caucasians. Within this group, 1 in 25 individuals is a carrier of the defective gene, and about 1 in 2500 births results in an affected child. The disease is infrequent in African-Americans (1:17,000) and is rare in native African and oriental populations (< 1:100,000). Although reports of clinical symptom constellations consistent with cystic fibrosis range to the 1650's, it was not until 1938 that Anderson assimilated the array of clinical features into the formulation of cystic fibrosis, with the name being derived from characteristic pathologic findings within the pancreas of affected individuals.

From a clinical standpoint, cystic fibrosis is a disease of multi-organ failure in which the predominant clinical problems include pulmonary and pancreatic disease (Table I). The mode of presentation is in part a function of the patient's age. In pediatric patients, 12% present with meconium ileus at birth. Later childhood manifestations of the disease include recurrent bronchitis or pneumonia, or persistent, unexplained cough. Although internists generally encounter patients who have carried the diagnosis of cystic fibrosis for years, it is becoming apparent that very subtle forms of the disease (male infertility) exist, and that genotypic variability has a radical effect on the extent of disease.

Cystic fibrosis (CF) is thus no longer a disease which is confined to children. During the past 10 years, there has been a marked increase in the survival rate of those with CF. In 1977, adults (defined as individuals over the age of 18) constituted about 23% of all CF patients. In 1990, this number had risen to 31% (Figure 1) and will continue to rise, thus assuring that the disease will increasingly fall within the domain of the internist. Despite dramatic advances in our understanding of the molecular pathogenesis of this disease, CF remains a disease of limited treatment options, which is ultimately fatal, requiring prolonged periods of hospitalization in its later stages.

This review focuses upon the definition of the molecular basis of the disease, and the impact that this knowledge has had upon our understanding of the physiology and pathophysiology of the secretory processes involved. In addition, new treatment strategies, including approaches to gene therapy, are discussed.

TABLE I

CLINICAL FEATURES OF CYSTIC FIBROSIS

ORGAN SYSTEM INVOLVED	PATIENTS AFFECTED %
Respiratory Tract	
Upper	
Nasal polyps	6-20
Pansinusitis	90-100
Lower	
Bronchiolitis	Eventually 100
Bronchitis	
Bronchiectasis	
Gastrointestinal tract	
Pancreatic insufficiency	85
Meconium ileus	10
Meconium ileus equivalent	10-30
Rectal Prolapse	20
Intussusception	1
Pancreatitis	5
Cholelithiasis	?
Liver	
Fatty	20
Focal biliary fibrosis	20
Multilobar cirrhosis	5
Endocrine	
Diabetes mellitus	15
Delayed puberty	? 85
Reproductive	
Men, obstructive azoospermia	98
Women: thick cervical mucus	Most
Sweat	
Chloride >60 mEq/L	98
Heat illness	-

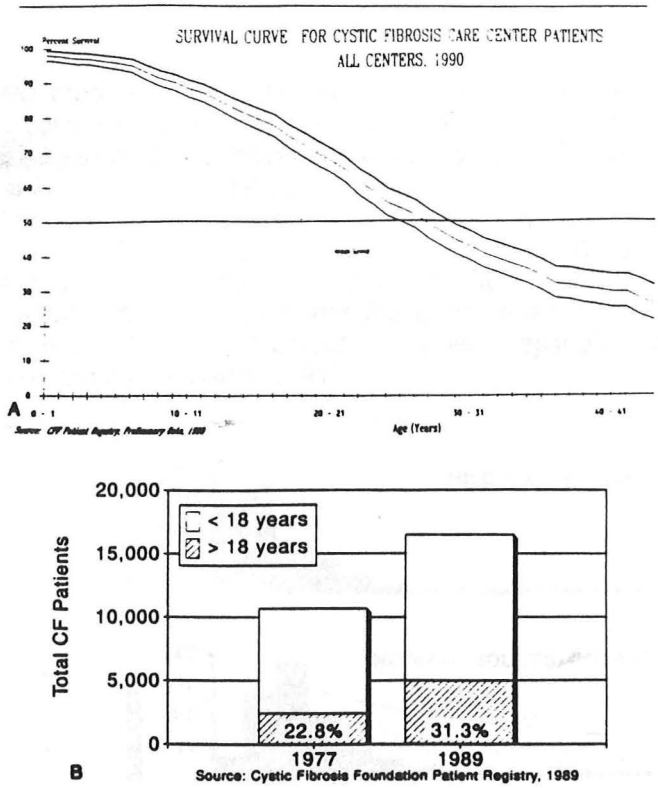

Figure 1. CF Foundation patient survival data. (A) The medial survival of CF patients in 1990 was 28 years. (B) The percentage of adult patients has been increasing from 22.8% in 1977 to 31.3% in 1989. If this trend continues, it is estimated that 50% of CF patients will be adults in 1995.

MOLECULAR BASIS OF CYSTIC FIBROSIS

The history of the investigation of the basis of CF is a revealing picture of the very nature of biomedical research, and the important impact that advanced molecular techniques has had on the process. Accepting 1938 as the date at which CF was recognized as a disease entity, there evolved some 50 years of clinical and laboratory investigations aimed at defining the common denominator of the clinical signs and symptoms of the disease. This resulted in numerous articles and symposium presentations claiming identification of 'the factor' responsible for the development of CF. (All have since gone the way of transfer factor.) This attempt to elucidate causal mechanisms based on clinical clues has

been designated by some as the 'scenic route'. Practically, this entails the biochemical elucidation of the factor responsible for disease, and probably still represents the mainstay of clinical research. It is an approach which has had its obvious successes and has had numerous spinoff (side route) benefits, but one that has too often failed to elucidate the cause of genetic-based diseases. Credit goes to this approach for linking the defect in cystic fibrosis to abnormal epithelial chloride transport. Building upon the demonstration that individuals with CF have an elevated sweat chloride concentration (Figure 2), electrophysiologists determined that the gene defect somehow resulted in defective function of a cAMP-activated chloride channel in epithelial cells of sweat glands, and bronchial mucosa (Anderson, 1991).

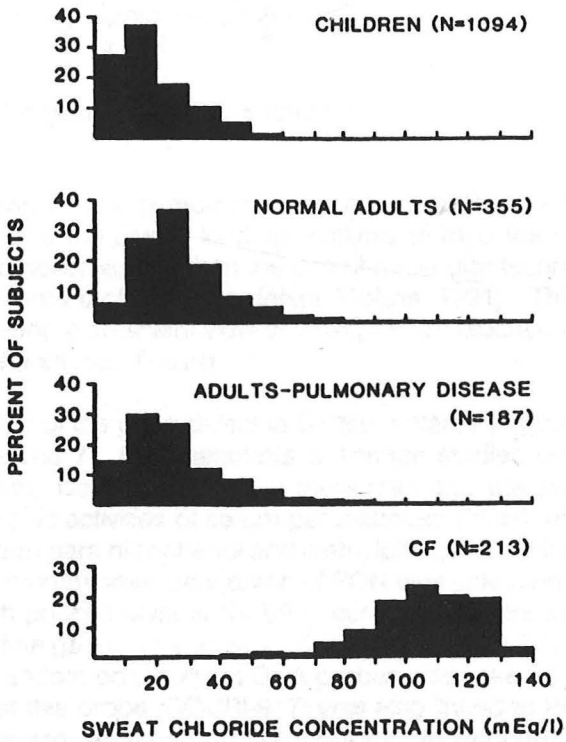

Figure 2. Distribution of sweat chloride concentrations in 1094 children without CF (top panel), 355 healthy normal adults (second panel), 187 adults with pulmonary disease (third panel), and 213 patients with CF (bottom panel).

Shown in Figure 3 is a model of this chloride channel in a cellular context.

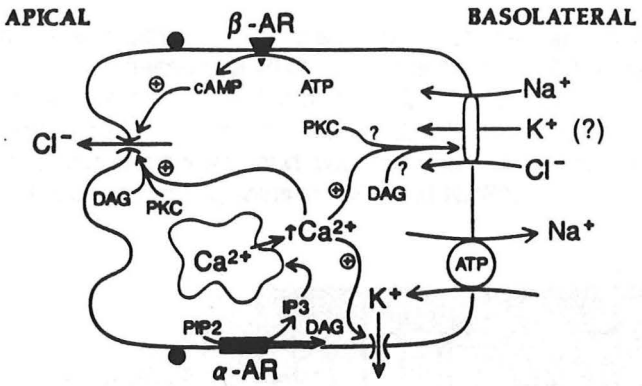

Figure 3. Model of cAMP-regulated chloride secretion.

In contrast, there has been a new approach to genetic disease, the so called 'direct route'. This paradigm entails the use of kindred analysis to map the culprit gene to a general position on a chromosome, and then the use of molecular techniques to identify the disease locus and the nature of the gene defect (Collins, 1991). This approach has the advantage of not requiring a prescient view of what primary biochemical defect might lead to a given clinical (phenotypic) finding.

The direct route to definition of the gene defect in CF first entailed mapping the defect to the long arm of chromosome 7. Initial attempts at linkage studies used polymorphic protein markers (Goodchild). Despite the rarity of these markers, the gene encoding CF was linked to the polymorphic activities of serum paraoxonase (PON), an enzyme which hydrolyses paraoxon to form para nitrophenol and diethylphosphate (Eibert). Initially this was of little utility, as the chromosomal localization of PON was unknown. Subsequently, restriction fragment length polymorphisms (RFLPs) were followed through hundreds of CF kindreds, using candidate genes, or anonymous DNA, as probes. The chance finding that one of two hundred random polymorphic DNA probes was linked to the CF gene at a distance 15 cM, and that this probe (DOCRI-917) was also linked to PON, led the way to localization of the CF gene to chromosome 7 (Knowlton). Subsequently, markers more tightly linked to the CF locus were identified, allowing for the mapping of the CF locus to the middle of the long arm of the chromosome (Tsui, 1985; Wainwright). Direct molecular techniques were then employed, and through the use of walking and jumping clones, Riordan, Tsui, and Collins succeeded in identifying the gene responsible for cystic fibrosis. Comparison of clones from normal individuals, and those from individuals afflicted with CF, revealed that the latter differed by a three base pair deletion. This mutation resulted in the deletion of a phenylalanine residue at position 508 (ΔF508). Further testing

revealed that only individuals with CF were homozygous for this genotype, and that 68% of all CF patients tested carried this mutation.

The gene responsible for CF spans 250 kilobases and contains 27 exons, which are spliced to form a messenger RNA of approximately 6500 nucleotides. The mature protein is a single polypeptide of 1480 amino acids. The structure of the protein, shown in Figure 4, is predicted to contain two hydrophobic regions, each composed of six transmembranous sectors, and two regions with nucleotide (ATP) binding domains termed nucleotide binding folds (NBF1 and NBF2).

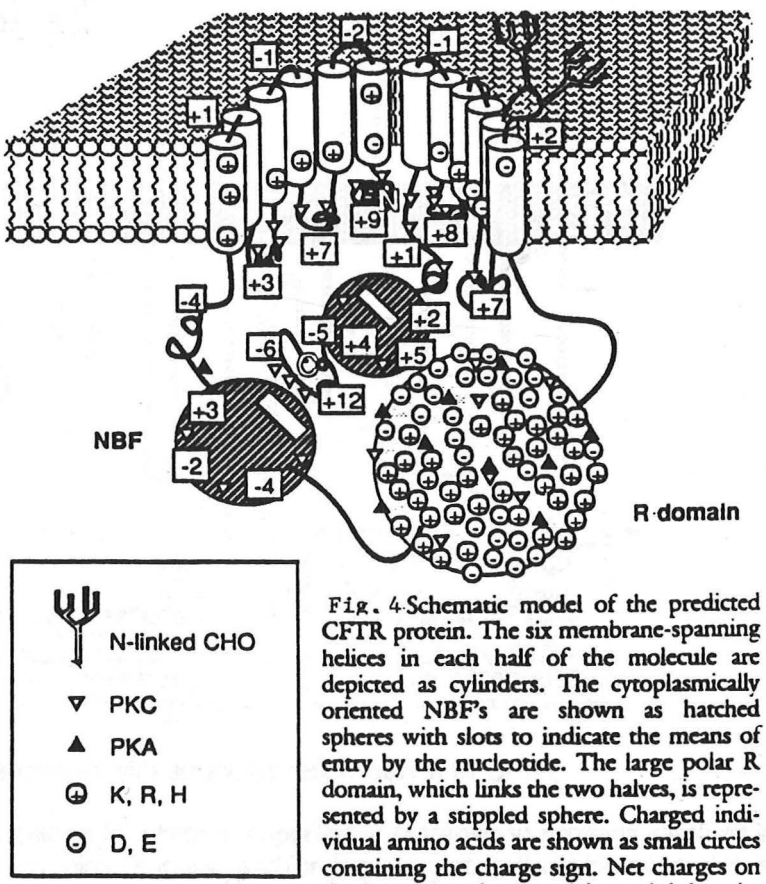

Fig. 4 Schematic model of the predicted CFTR protein. The six membrane-spanning helices in each half of the molecule are depicted as cylinders. The cytoplasmically oriented NBF's are shown as hatched spheres with slots to indicate the means of entry by the nucleotide. The large polar R domain, which links the two halves, is represented by a stippled sphere. Charged individual amino acids are shown as small circles containing the charge sign. Net charges on the internal and external loops joining the membrane cylinders and on regions of the NBF's are contained in open squares. Potential sites for phosphorylation by protein kinases A or C (PKA or PKC) and N-glycosylation (N-linked CHO) are as indicated. K, Lys; R, Arg; H, His; D, Asp; and E, Glu.

The predicted protein also has a highly polarized segment, name the R-domain, which is believed to have a regulatory role as the site of phosphorylation catalyzed by protein kinase A (PKA). Because the predicted structure of this protein did not resemble any known chloride channel, the gene product responsible for CF was termed cystic fibrosis transmembrane regulator, or CFTR.

The general structure of CFTR resembles that of the multiple drug resistance (MDR) proteins (Figure 5) and STE6, a yeast mating factor protein.

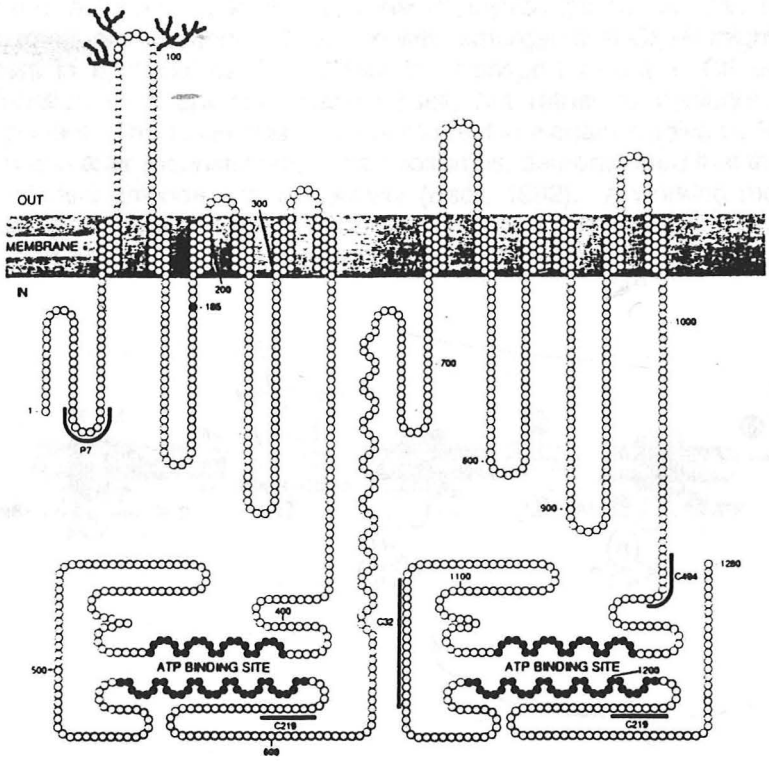

Figure 5. MDR-type protein with structural resemblance to CFTR.

All of these proteins belong to a broad, superfamily of transport proteins involved in the trafficking of drugs, proteins, sugars, and/or ions across cell membranes. In some instances, these transport proteins utilize the hydrolysis of ATP to energize the uphill movement of a molecule. Members of this group, in addition to containing the two sets of transmembranous sectors, also have conserved NBF domains. Distinguishing CFTR from other members of this superfamily is the R, or regulatory domain, which is the potential phosphorylation site.

Following the identification of the gene responsible for CF in 1989, there came a three year period of debate as to the physiological role of the protein. As noted previously, this protein was designated CFTR or cystic fibrosis transmembrane conductance regulator because the structural evidence available was not sufficient to claim that the protein was a channel. In this respect, the identification of CFTR as being a member of the general class of MDR-type transporters was perhaps most contributory to the confusion over function. In essence, no MDR-type transporter has been shown to have channel like activity; rather MDR-type transporters generally hydrolyze ATP and thereby pump substrates (eg. chemotherapeutic agents) across cell membranes. Such ATP-driven transporters seldom, if ever, move greater than 500 molecules /transporter/second, whereas a 'channel', in the true electrophysiologic sense, can typically move $>10^6$ ions/transporter/second. Thus the view emerged that CFTR might regulate a channel present in epithelial cells, and that the transport defect in CF owed not to intrinsic dysfunction of a chloride channel itself, but rather to dysfunction of its regulatory component. This issue was finally put to rest in elegant studies by Riordan, who purified CFTR and after reconstituting it into liposomes, demonstrated that the CFTR protein itself has intrinsic chloride channel activity (Bear, 1992). A working model of the channel, based upon these findings, is shown in Figure 6. The role of the ATP-binding motif in transport function is currently under investigation (Bear, 1993).

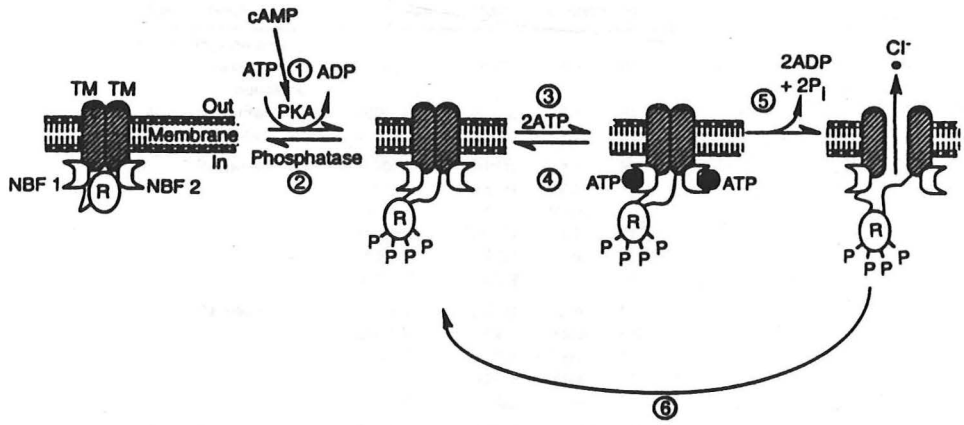

Figure 6. Hypothesis for the dual control of CFTR by PKA and ATP. In the absence of R-domain phosphorylation, the channel is closed. After cyclic AMP stimulates PKA to phosphorylate one or more serine residues in the R domain (step 1), the CFTR channel is poised to bind ATP (step 3), which is cleaved to induce a conformational charge (step 5), opening the chloride channel. This is envisioned to decay back (step 6) to a closed state. The separation of ATP binding, hydrolysis, and channel opening (steps 3 and 5) is speculative. P_1, inorganic phosphate; cAMP, cyclic AMP, and TM, transmembrane domain.

MOLECULAR MECHANISMS OF CFTR DYSFUNCTION

Although the Δ F508 mutation described previously was of landmark importance in the original identification of the gene defect responsible for CF, it is now apparent that the phenotype of CF arises from a wide array of mutations which are scattered through the CFTR chloride channel. Shown in Table II is a partial list of over 300 mutations which have been identified to cause CF. As indicated, about 67% of the instances of CF owe to the Δ F 508 mutation. Considerable work is underway to characterize these mutants with respect to their particular defect in function. Essentially, four classes of mutations have been identified to date: deficiencies in protein production; processing mutants; mutants with altered or deficient regulation; and mutations which an isolated defect in (presumably) the chloride channel domain of the CFTR. These types of mutants are shown schematically in Figure 7.

TABLE II

CLASSES OF CFTR MUTATIONS THAT CAUSE CF

Class	Defect	Examples	Do-main	Fre-quency	Clin-ical
I	Protein production				
	Nonsense mutations	G542X	NBD1	3.4	PI
	Frameshift	3905 insT	NBD2	2.1	PI
	Splice	621 + G→T	MSD1	1.3	PI
II	Processing	Δ1507	NBD1	0.5	PI
		ΔF508	NBD1	67.2	PI
		S549I	NBD1	Rare	
		S549R	NBD1	0.3	PI
		A559T	NBD1	Rare	
		N1303K	NBD2	1.8	PI
III	Regulation	G551D	NBD1	2.4	PI
		G551S	NBD1	Rare	PS
		G1244E	NBD2	Rare	PI
		S1255P	NBD2	Rare	PI
		G1349D	NBD2	Rare	PI
IV	Conduction	R117H	MSD1	0.8	PS
		R334W	MSD1	0.4	PS
		R347P	MSD1	0.5	PS

NBD, nucleotide-binding domain; MSD, membrane-spanning domain; PI, pancreatic insufficiency; PS, pancreatic sufficiency. Frequency of mutations is expressed as the percentage of all CF mutations (from

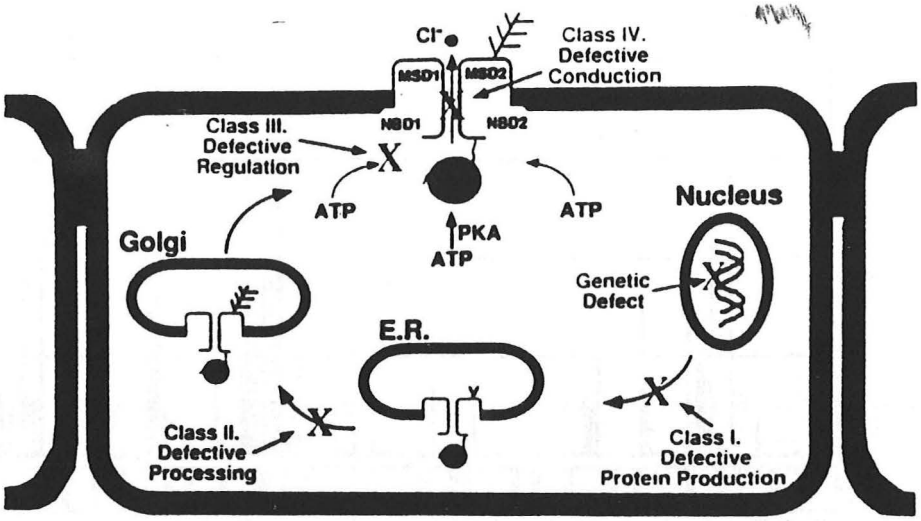

Figure 7. Biosynthesis and Function of CFTR in an Epithelial Cell.

The Class I mutants are scattered through the CFTR gene (Figure 8) and cause defective protein production because of premature termination signals, frameshifts or nonsense mutations. These mutations result in either unstable mRNA (and no detectable protein), or a severely truncated CFTR protein which has an abbreviated half life.

Class II mutations primarily result in defective trafficking of CFTR. The most common mutant, Δ F508, falls within this class, and it is now clear that the central defect of this mutation is one of localization. That is, when cells bearing this mutated form of CFTR are grown at 37°C, the chloride channel does not escape from the endoplasmic reticulum. Interestingly, if the same cells are grown at a lower temperature (23-30°C), some of the mutant protein leaks through the endoplasmic reticulum, is properly processed within the Golgi, and is inserted in a fully functional form in the plasma membrane. It is thus suspected that the Δ F508 mutation results in misfolding of CFTR at 37°C, and that the heat-labile, denatured protein is screened at the endoplasmic reticulum level.

Class III mutants, which have been observed only rarely, are correctly localized to the plasma membrane, but carry mutations in the nucleotide-binding domains. As it is likely that cellular ATP regulates the opening of CFTR through interactions with these domains, these channels have a primary regulatory defect, which ranges from very little function to a blunted response to agonists in vitro.

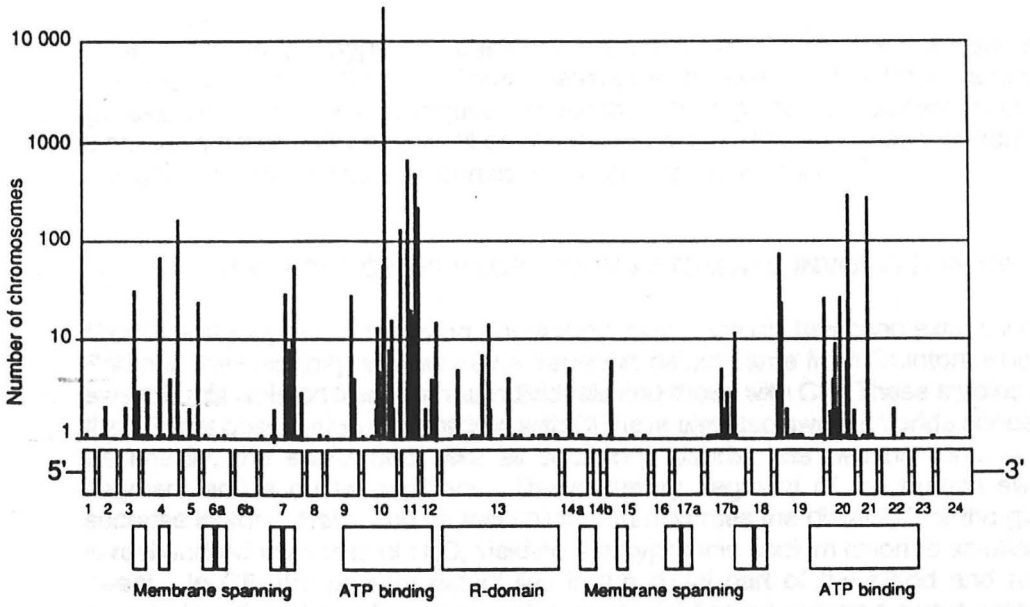

Figure 8. Localization and frequency of mutations within CFTR.

Finally, Class IV mutations are characterized by normal regulation, as judged by in vitro agonist addition studies, but have reduced current flow in response to these agents. The simplest explanation of this observation is that the defect lies within the channel portion of the protein itself.

Categorization of these defects is not an idle exercise in electrophysiology. Rather, it is likely that these different classes of mutations have differences in their phenotype, and may require different approaches to therapy. Although it has been difficult to distinguish genotype based upon the phenotype of the type or degree of pulmonic lesions, there does appear to be a rough correlation between genotype and the extent of pancreatic dysfunction. It is well appreciated that significant differences in degrees of pancreatic insufficiency occur in patients with CF; whereas most patients have total pancreatic failure, others retain function sufficient to obviate the need for protease replacement therapy. To date, Class I and II mutations, which would be predicted to result in the most severely dysfunctional CFTR, have been associated with a severe pancreatic-insufficiency phenotype. In contrast, Class III and IV mutations are often associated with a less severe phenotype - a finding which might be expected since CFTR function is diminished, rather than absent in these instances (Kristidis). Most dramatically, some individuals with Class III mutations do not have clinically recognizable CF, but simply have isolated male infertility owing to absence of the vas deferens (Anguiano).

In addition, the genotype of a particular individual with CF has the potential of dictating the most appropriate therapy. Thus a patient with a Class I or II mutation (and total CFTR deficiency) would seem to require genetic therapy directed at replacement of the gene, whereas a patient with a Class III or IV mutation could conceivably benefit from a therapy designed to open existing channels which are poorly functional.

PHYSIOLOGY OF SECRETORY PATHWAYS INVOLVED IN CF

From the standpoint of transport physiology, cystic fibrosis has been extensively studied. Perhaps the first unifying view of the transport defect came from Quinton, who perfused sweat ducts isolated from normal individuals and those with CF. These studies built upon the original observation that children with CF have elevated sweat chloride concentrations. Essentially, the sweat duct, like all secretory glands, has two portions: a secretory segment and a ductal segment. The secretory segment of the human sweat gland secretes isotonic NaCl, and as this solution transverses the distal 2/3 of the gland, NaCl is reabsorbed in excess of H_2O, yielding the hypotonic sodium chloride solution which is sweat. In CF, the primary defect lies in the distal part of the gland and as a result, secreted sodium chloride is not reabsorbed, yielding an elevated sweat chloride. This is generally of trivial clinical consequence, although this mechanism may contribute to volume depletion of children with CF who are exposed to heat for extended periods.

More significant are the consequences of a defective chloride transporter in the pancreas. Shown in Figure 9 is a model of solute transport in the pancreatic duct. Like the sweat gland, the exocrine pancreas is composed of two portions: a proximal acinar device, and a distal ductal conduit. The former secretes NaCl, just as does the sweat gland. The trafficking of ions in the pancreatic duct is however more complex. Essentially, chloride is secreted by the ductal cells, yet fully processed pancreatic secretions are low in chloride. The explanation for this phenomenon entails a futile cycling of chloride across the apical membrane. Thus chloride, secreted through CFTR, is reabsorbed by a chloride/bicarbonate exchanger, with the net effect of bicarbonate secretion. Secretion of bicarbonate, in turn, obligates paracellular sodium movement and thus $NaHCO_3$ appears in the ductal lumen. Importantly, net secretion of $NaHCO_3$ causes H_2O movement across the epithelium of the duct, resulting in isotonic pancreatic secretions. Inhibition of chloride secretion through CFTR (as in CF) results ultimately in decreased $NaHCO_3$ and water secretion. The latter is of particular consequence, and ductal dehydration leads to concentration and inspissation of pancreatic secretions, culminating in chronic pancreatitis and the pancreatic insufficiency which is characteristic of CF.

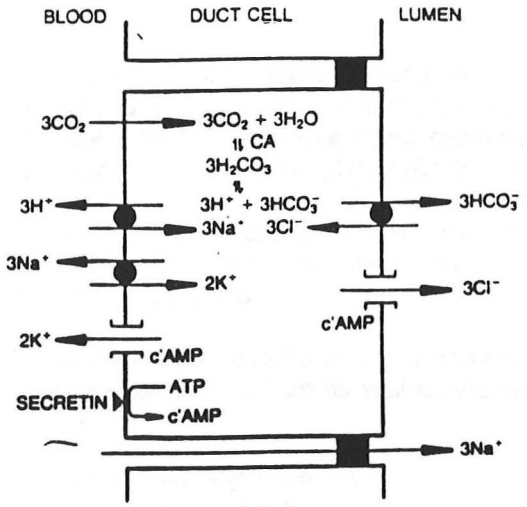

Figure 9. Cellular model of bicarbonate secretion by pancreatic duct cells. CA = carbonic anhydrase.

The trafficking and net flow of NaCl and water through the pulmonary bed, extending from alveolus to trachea is less well understood. However, transport within the larger bronchi and trachea is well characterized, and the essential feature of this process is one of chloride secretion, and thus net water movement (Liedtke). In simplest terms, inhibition of the chloride current through the defect of CF results in dehydration (and hypertonicity) of the mucosal surface of the airway epithelia, which sets the stage for numerous pathophysiologic sequences, as discussed below.

PATHOPHYSIOLOGY OF PULMONARY DISEASE IN CF

As noted previously, the most devastating clinical manifestations of cystic fibrosis involve the pulmonary bed. Although the direct effect of the mutant gene is to increase the viscosity of pulmonary secretions, definition of ensuing steps leading to end stage pulmonary disease is of considerable pathophysiologic and therapeutic importance. Several theories are under investigation at present, and there is a converging focus on the role of mucins in the pathogenesis of airway disease and in the propensity of Pseudomonas to colonize patients with CF (Al-Awqati).

Many investigators have found that mucins from the respiratory and gastro-intestinal tracts of CF patients have alterations in glycoprotein structure, as compared to mucins isolated from normal individuals or patients with other types of chronic obstructive pulmonary disease. A phenotype found by many investigators includes a marked increase in sulfate content, a higher fucose content, and less sialic acid in CF mucins, and other glycoproteins of cells of CF patients (Cheng). This finding has not, however, been

uniform, and there is considerable individual variability in the structure of mucins among CF patients (Chernick, Lamblin, Rose). In part, this variation may owe to secondary pathologic factors, such as neutraphil-mediated carbohydrate modification (Houdret). Clear evidence for malprocessing during glycosylation in CF cells comes from studies of cultured cells, where it has been noted that the secretions from explants show consistent variation in the terminal (capping) reaction which is carried out in the trans-Golgi network (Rose). Thus speculation has arisen that alterations in Golgi function might account for the mucin defects seen in CF patients.

Glycosylation of proteins within the cell involves a complex series of reactions which are carried out in the endoplasmic reticulum as well as the Golgi apparatus (Figure 10).

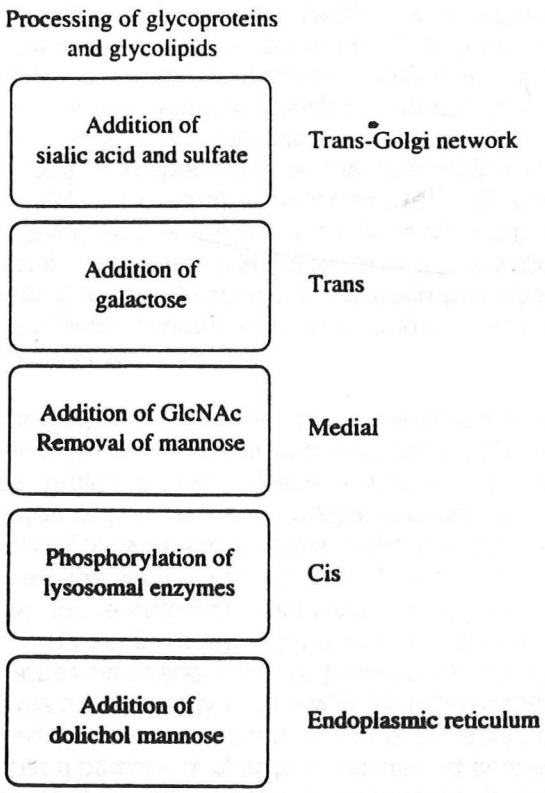

Figure 10. Compartmentalization of Golgi functions in different stacks. Only the trans-Golgi network and in some cell the trans Golgi are acid.

Nascent proteins begin to become glycosylated in the endoplasmic reticulum with transfer of carbohydrates from a dolichol donor. Subsequently, post-translational modification is carried out sequentially within the Golgi as the maturing protein trafficks from the cis to medial to trans stacks of the network. During this migration, the carbohydrate moieties are added, modified, removed, and readded to yield a mature glycoprotein, which then exits the trans-Golgi network to its final cellular, or extracellular, destination. Thus there are multiple enzymatic steps which are potentially important in the malprocessing of mucins in patients with CF. However, characterization of the particular defects seen in the secretions of CF patients strongly implicates the trans stack of the Golgi as the site of improper glycosylation. In addition, it has been further proposed that the glycosylation defect in the terminal stack of the Golgi owes not to a specific enzyme defect, but to a loss of acidity in this organelle (Al-Awqati).

Within all nucleated cells there exists an extensive network of acid organelles. Vesicles of the constituitive sorting pathway, including clathrin-coated vesicles, endosomes, lysosomes, and the Golgi membrane are acidic, as are the organelles of specific secretory pathways, including chromaffin granules, synaptic vesicles, mast cell granules and insulin processing vesicles. In each instance, this acidity carries out a specialized function - in endosomes, this acidity (pH 5.5) is responsible for the processing of endocytosed receptor-ligand complexes (eg, Fe^{2+}-transferrin); in lysosomes (pH 4.5), acidity is required for the activity of acid hydrolases, and in the trans stack (Anderson, 1985) of Golgi membranes (pH ~ 6.5), acidification plays a role in the terminal glycosylation steps which are carried out in the organelle (Stone). Recently, it has been proposed that specific defects in this acidification pathway contribute to the pathogenesis of the altered secretions in the airways of patients with CF.

In order to explain this hypothesis, it is necessary to review essential features of the acidification process of cellular organelles. The driving force for acidification of all these organelles is a vacuolar-type proton pump. These enzymes are complex hetero-oligomers with molecular masses of greater than 700 kDa, and are composed of 8-10 different subunits. As depicted in Figure 11, this enzyme hydrolyses ATP, and the energy captured from this reaction fuels the vectorial movement of protons into the interior of organelles (Stone). Importantly, this acidification is an electrogenic process. That is, the vacuolar proton translocating ATPase, in catalyzing the movement of a proton into the interior of the organelle, generates an interior positive potential in the organelle lumen. This generation of charge serves as an energy barrier which blocks further movement of protons, and it must be relieved by either the co-movement of an anion, or the counter-movement of a cation, such that a balance of charge is maintained across the organelle membrane. In all organelles studied, this charge compensation is achieved by the movement of chloride into the organelle interior (Xie, 1983) This movement of chloride occurs through a second transport protein, a chloride channel, which is structurally independent of the proton pump, and has its own regulatory devices (Xie, 1989). Thus organelle acidification occurs by the primary active movement of protons, and the secondary movement of chloride into the organelle; with HCL movement, the interior pH

falls. This model is shown in Figure 11.

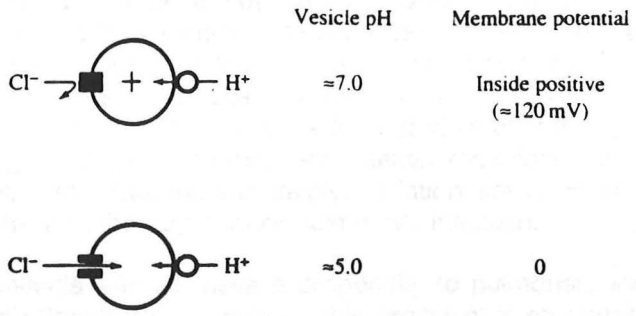

Figure 11. A model for the generation of pH differences and membrane potential by intracellular organelles.

Predictably, regulation of acidification in these organelles might occur (Xie, 1993) by a variety of mechanisms including modulation of either the proton pump or chloride channel (Xie, 1989). In investigation of this point, it has been reported that Golgi containing membrane fractions harvested from CF patients are defective in acidification as compared with control vesicles prepared from normal respiratory tissue explants. Moreover, evidence suggests that the defect lies at the level of the chloride channel, rather than the proton pump, and thus acidification (in the terminal stack of the Golgi) is decreased because of reduced charge compensation (Barasch). This in turn, might effect processing of glycoproteins and mucins because of the pH dependency of the activation of these enzymes participating in the glycosylation pathway. Enzymes responsible for glycosylation vary considerably in their pH dependence. Those responsible for the glycosylation steps in the terminal stack of the Golgi exhibit maximal activity in an acid (pH 5.5-6.5) range and would be expected to have decreased activity in the setting of an elevated organelle pH.

How does the mutation in CFTR result in this reduced capacity for organelle acidification? Certainly a unifying hypothesis is not intuitive, in that CFTR is expressed in the cell surface and the defect in acidification occurs rather deeply in the exocytic pathway. One hypothesis is that CFTR, by virtue of its maldistribution, is responsible for the defect. This, in light of the recent demonstration that CFTR is itself a chloride channel (which differs from the chloride channel native to cellular organelles), seems untenable. It seems most likely that the dysfunctional acidification activity and resultant mucin malprocessing owe to a 'sick cell' phenotype' of explanted CF cells and probably represents one of a number of nonspecific defects in constitutive cell processes. This does not, however, detract from the potential importance of the observation, or from the potential role of altered glycosylation in the generation of an illicit Pseudomonas receptor.

As a first step, colonization of an epithelial surface with bacteria would seem to require adherence, perhaps to a cell surface receptor. The presence of such a receptor for Pseudomonas in CF airways is suggested by the observation of a seven-fold increase in the numbers of Pseudomonas cells adhering to buccal cells of CF patents, as compared with controls. In these studies, adherence was abolished by treatment with neuraminidase, suggesting that the buccal cell receptor is an asialo glycoprotein or glycolipid. Further support of this notion comes from studies attempting to define the nature of the Pseudomonas receptor, in which two potential receptors were shown to be an asialo-GM_1, and asialo-GM_2. Thus incomplete glycosylation may uncover (or generate) a latent bacterial receptor and thereby nurture pulmonary infection.

As noted previously, patients with CF have a propensity to pulmonary infiltration with Pseudomonas, and, with Staphylcoccus aureus, this organism is responsible for much of the 'secondary' damage to the pulmonary bed. Perhaps because of this illicit Pseudomonas receptor, patients with CF become colonized with Pseudomonas by the age of 10. Most commonly, this colonization is with the 'rough' form of the organism. Within the next 10 years this non-mucous producing form transforms to a mucoid phenotype, which is of considerable consequence. This 'mucoid' phenotype results from the synthesis of alginates by the transformed strain. Alginates are polymers of mannuronic and guluronic acids which have become acetyleted. This alginate, which gives colonies of Pseudomonas a 'slime'-like appearance, actually serves as an adhesion for respiratory epithelia and thus promotes the apposition of Pseudomonas with the host. Moreover, alginate itself is an important component in the formation of bronchial bacterial micro-colonies and plugs. This in turn shields these colonies from phagocytic attack by neutrophils, and anti-microbial agents.

The nature of the transition from rough to mucoid phenotype has been the subject of intense investigation because of its pathological consequence. It appears that the 'mucoid' transition is controlled by at least three different loci in the bacterial chromosome (Govan). Evidently, early infections with Pseudomonas are characterized by considerably heterogeneity in the genotype of the organisms, and with time there is an adaptation to the host environment leading to a preponderance of the mucoid form. In this regard, it is notable that increased medium tonicity leads to increased alginate production by Pseudomonas bearing the muc mutations, thus suggesting that the hypertonic environment of the bronchial tree in CF patients favors increased alginate production, in a vicious pathophysiologic cycle.

The issue of Pseudomonas infection in CF patients has recently been further complicated by the emergence of a second pathogenic strain, Pseudomonas cepacia, in infections complicating CF. Pseudomonas cepacia was recognized for many years as a plant pathogen. Increasingly, however, it is now being identified as the causative organism in CF patients who develop a severe necrotizing pneumonia. In other instances, CF patients harboring this organism have a slower course of pulmonary destruction caused by the organism. The organism is less invasive than Pseudomonas aeruginosa, but is extremely

refractory to therapy. Unlike Pseudomonas aeruginosa, Pseudomonas cepacia is spread by direct patient contact, and fraternization among CF patients outside of the hospital environment in support groups is discouraged, in order to curtail spread of the organism (Smith).

THERAPY

At present, the mainstay of therapy for the complications of CF remain conventional: antibiotics and chest physical therapy for pulmonary disease, and replacement enzymes for pancreatic insufficiency. Despite this, new approaches to treatment of the pulmonic complications are emerging, based upon knowledge of the basic defect of the disease. These include the use of aerosolized amiloride and nucleotides to increase hydration of the airway secretions. In addition recombinant DNase is undergoing clinical trial as a means of reducing the viscosity of pulmonary secretions. As such, these therapies can potentially ameliorate the course of CF. Beyond this, "replacement" therapies carry the potential for cure of the disease. As an extreme, combined heart-lung transplantation is an heroic solution, and more precisely, gene therapy for cystic fibrosis is under development. These approaches are discussed below.

As noted previously, the likely instigating factor in the cascade of pathogenic events culminating in COPD is hypertonicity in the airways owing to decreased chloride secretion, increased sodium reabsorption, and decreased hydration of the mucosal surface. Short of direct gene therapy, attempts have been made to influence the nature of the secretory product of airway epithelia by pharmacologic manipulation with diuretics (amiloride) and nucleotides (ATP, UTP).

Several electrophysiological studies have documented that the apical sodium channel of respiratory epithelial cells, like that of the renal cortical collecting duct, is inhibited by amiloride in micromolar concentrations. Blocking this channel would inhibit apical sodium reabsorption, and thereby increase the water content of mucosal secretions. To determine whether the inhibition of excessive absorption of sodium might affect the course of pulmonary disease in CF, a doubled-blinded, crossover trial comparing aerosolized amiloride and placebo was performed. In this study, 14 patients with CF were followed for a one year period in which they were treated with nebulized amiloride in concentrations of 100 μm (about 100 times the dose necessary to block the sodium channel of airway epithelial cells). Amiloride effects were studied by measurements of FVC and FEV, as well as measurement of the viscosity of airway secretions. The latter was shown to decrease in patients receiving amiloride, and importantly, the rate of loss of FVC was reduced from 3.00 ml to per day to 1.44 ml per day. No toxic effects were observed, and specifically, no effect on systemic electrolyte parameters were noted (Knowles, 1990). Currently, expanded, long-term investigations of the treatment of CF patients with established lung disease with amiloride are underway.

A second mechanism by which the primary defect in CF might be alleviated is through stimulation of the defective chloride channel (CFTR) itself. Whereas chloride secretion in normal individuals can be stimulated by beta-adrenergic agents (which include cellular cAMP levels), such agents have no such effect on CF patients owing to the non-responsiveness of mutated CFTR to cAMP. However, it has been noted that ATP can directly effect an increase in airway epithelial cells when applied to the mucosal surface. Studies testing the effect of nucleotides (ATP and UTP) were conducted with nasal epithelial cells isolated form CF patients and normal controls. Whereas these agents had no effect by themselves, they significantly potentiated the amiloride provoked changes in salt transport and specifically restored the chloride current to normal levels. At present, it is not known how extracellular ATP exerts its effect. Possibilities include stimulation of CFTR (or other chloride channels) through P_2-type purinergic receptors. Alternatively, ATP has recently been shown to have a direct gating effect on CFTR, perhaps accounting for its effect (Knowles, 1991). At present, this potential therapeutic modality has not been extended to in vivo testing.

As noted previously, the viscosity of purulent sputum itself is highly contributory to the pathogenesis of chronic obstructive pulmonary disease in patients with CF. The cause of this hyperviscosity is multifactorial, and owes to decreased water content (secondary to the basic defect of CF itself), as well as alginate production by the mucoid form of Pseudomonas aeruginosa. In addition, a third factor, DNA, contributes significantly to the viscosity.

Experiments in the 1950's revealed that DNA is present in high concentrations (3-14 mg/ml) in purulent, but not normal, sputum (Armstrong). DNA, as a viscous polyamine, contributes to the viscosity of lung secretion. Early experiments demonstrated that in vitro treatment of lung secretion with bovine pancreatic DNaseI greatly reduced viscosity (Lieberman) and because of this, bovine pancreatic DNaseI was approved for human use in 1958. Subsequently, numerous studies supported the utility of this enzyme in the treatment of patients with pneumonia, as well as CF. However, the course of treatment was occasionally compromised by severe allergic reactions which were triggered by the bovine DNaseI, or contaminating enzymes from the pancreatic extract.

Several groups have now launched experimental and clinical studies to determine the utility of highly purified DNaseI in the management of pulmonary complications of CF. Rather than isolating bovine pancreatic DNaseI, the focus is now upon the use of human recombinant DNaseI. In vitro studies have documented that DNaseI markedly reduces the viscosity of sputum harvested from CF patients (Shak), and have shown that this decrease in viscosity is accompanied by a decrease in DNA content of the sputum. Subsequently, a preliminary clinical trail in patients with CF has indicated that this treatment modality may be of significant utility. To date, no adverse effects were noted in this study, likely owing to the purity of the DNaseI preparation. Subjective improvement was reported by 11/16 patients and importantly, FCV and FEV, were shown to increase in treated patients documenting at least short term benefits of the protocol (Hubbard).

Further studies are underway to determine the long term benefits of this treatment protocol.

Whereas these therapies offer immediate benefit to patients with manifest CF, the ultimate approach to this disease is prevention through genetic screening and gene therapy (Collins, 1992 & Crystal).

GENETIC SCREENING

Identification of the gene responsible for CF bears the potential for precise prenatal diagnosis of homozygotes, and identification of heterozygotes (Ryley). In addition, screening for carriers of CFTR mutants can be of use in the diagnosis of individuals with clinical symptoms of CF when classical methods are inconclusive for carrier testing for genetic disorders. In practice, this screening is complicated by the fact that over 300 mutations resulting in CF have been identified to date. Because of this, the American Society of Human Genetics has officially recommended that screening be conducted only in instances where individuals have a family history of CF (Nance). In fact, more modern screening strategies have recently been developed and are based upon the use of probes representing the most frequent mutations observed in the test population. Practically, this entails screening with a 'cocktail' of 32 different probes including ΔF508. By using this mixture, the CF carrier state can be reliably identified in 90% of Western European and American Caucasians. For Ashkenazi Jewish populations, analysis of only five mutations achieves 97% carried detection rate. However, in African Americans, the most commonly used probe 'cocktail' identifies only 45% of carriers, and is not recommended for screenings in settings without a family history.

GENE THERAPY

Perhaps the most successful mode of gene therapy at present, and one which produces a genuine cure, employs a retroviral approach. This has been utilized for the management of severe combined immune deficiency syndrome, and children who have received gene replacement therapy for this disease now are able to live a nearly normal life with a functioning immune system and with a reduced risk of life-threatening infection. The basis of retroviral therapy entails the use of a modified RNA virus which is incapable of directing the synthesis of new virus and contains the replacement gene of interest. Through reverse transcription, the viral RNA is converted into DNA and double stranded DNA is then inserted (stably) into the host genome. The new gene can then be passed to the progeny of the (infected) dividing cell. This, however, points to a potential problem to this approach, in that a randomly-inserted gene might be introduced by chance into a crucial site within the host cell genome, and could, for example, activate an oncogene, or deactivate an anti-oncogene. A further disadvantage of the retro-viral approach with respect to CF is the necessity for purification of the target cell. Therefore, the recombinant retroviral approach to gene therapy has been largely confined to ex vivo strategies, in which host peripheral blood cells are removed, transfected, and transfused

back into the donor.

A second approach, that of adenovirus-mediated gene transfer, has recently been given high visibility (Siegfried). This virus, a double stranded DNA virus, has as its native target the upper and lower respiratory tract of humans. It enters a cell through an endocytic process and then gains entry to the cytosol via a pH-dependent conformational change in the endosome. Unlike the retrovirus, which directly integrates into the host genome, the adenovirus DNA functions in an extrachromosomal fashion. In attempts at gene therapy using this system, the virus is modified to include the recombinant gene and to render it replication deficient. Thus this system overcomes the problem of the insertional mutation potential of the retroviral approach, but has the inherent disadvantage of not being passed to the progeny of the modified cell. As a result, adenovirus-mediated gene therapy would need to be given periodically. Since the target cells of pulmonary epithelium proliferate slowly, this approach would seem to allow for a transfection which would be site-directed and semi-stable. The need for repeated treatments, however, has raised the concern of immunization with neutralizing antibodies that might prevent continued, successful treatment.

Preliminary studies, however, suggest that significant immunization provoked by an adenovirus-based transfection system may be unimportant (Zabner). The safety and efficacy of repetitive adenovirus mediated transfer of CFTR cDNA to airway epithelia was tested in primates (Rhesus monkeys) and cotton rats. In both species, CFTR mRNA and protein were detected after administration, and in monkeys, protein was detected six week after repeat administration. The vector did not replicate and was rapidly cleared. All monkeys tested developed circulatory antibodies against the adenovirus, and typically initial rises were observed within two week of therapy. Neutralizing antibodies developed late in the course of the study and only after a second administration of adenovirus. Despite this, repeated administration of recombinant adenovirus neither provoked local inflammation nor altered the efficacy of the treatments, perhaps because neutralizing antibodies could not be detected in airways. At present, it is not known whether secretory immunoglobulins (IgA) were raised against the adenovirus, or if an aggravated immune response might develop with a longer treatment course. In addition, it is important to note that these studies were conducted in animals with functional and immunologically normal CFTR.

Most recently, liposome-mediated gene delivery has emerged as a potential method of gene therapy in cystic fibrosis. In this delivery system, small, single lamellar liposomes are prepared by the sonication of cationic lipids. Subsequently, a plasmid containing the cDNA encoding human CFTR is mixed with the liposomes and because of charge differences, the two become adherent. Subsequently, the mixture is presented to the pulmonary bed by aerosolization. The liposomes are essential, in that plasmids do not enter cells easily by themselves, and if they do, they are frequently shunted to the lysosome pathway, where they are rapidly degraded. This modality thus has the advantage of avoiding the potentially immunogenic complication of the adenovirus

transfection system. In fact, this lipofection method has been successfully employed in two transgenic mice models of CF (Alton; Hyde). In both of these models, the CFTR gene of mice was knocked out by an insertional mutagenesis approach. Human cDNA-liposome mixtures were then infiltrated into the lungs of mice. Subsequently, expression of human CFTR was demonstrated not only in the trachea of the mice, but also within deep alveoli of the lung. In one study, the liposome therapy was extended to include the gastrointestinal tract.

Finally, future directions in the management of cystic fibrosis are likely to represent a tailoring of treatment to the particular genotype of individuals. In cases of severe CFTR dysfunction, gene replacement therapy holds the greatest promise, whereas more accessible treatments are becoming available to individuals with milder disease forms. As a result of increasingly successful management of the pulmonary complications of cystic fibrosis, we can expect the pancreatic complications of the disease to become dominant in dictating the clinical course. Thus ultimately, systemic gene therapy remains the final goal for management of the disease.

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