

## Cystic Fibrosis

Cystic fibrosis (CF) is a monogenetic disorder that presents as a multisystem disease. The first signs and symptoms typically occur in childhood, but nearly 4 percent of patients are diagnosed as adults. Due to improvements in therapy, approximately 34 percent of patients reach adulthood and nearly 10 percent live past the age of 30. The average life span for both male and female CF patients is similar, ~28 years. Thus, CF is no longer only a pediatric disease, and internists must be prepared to recognize and treat its many complications. This disease is characterized by chronic airway infection that ultimately leads to bronchiectasis and bronchiolectasis, exocrine pancreatic insufficiency and intestinal dysfunction, abnormal sweat gland function, and urogenital dysfunction.

### Pathogenesis

### Genetic Basis

CF is an autosomal recessive disease resulting from mutations in a gene located on chromosome 7. The prevalence of CF varies with the ethnic origin of a population. CF is detected in approximately 1 in 3000 live births in the Caucasian population of North America and northern Europe. It is being deduced that, 1 in 20 Caucasians harbors one copy of the mutated CF gene. The most common mutation in the CF gene (~70 percent of CF chromosomes) is a 3-base-pair deletion that results in an absence of phenylalanine at amino acid position 508 ( $\Delta F_{508}$ ) of the CF gene protein product, known as the CF transmembrane regulator (CFTR). The large number (>400) of relatively uncommon (<2 percent) mutations identified in the CF gene makes it difficult to use DNA diagnostic technologies for identifying heterozygotes in populations at large, and no simple physiologic measurements allow heterozygote detection.

The reason for this relatively high incidence of heterozygosity is due to the fact that CF heterozygotes have a selective advantage against cholera-induced secretory diarrhea (*issue for discussion*).

### CFTR Protein

The CFTR protein is a single polypeptide chain containing 1480 amino acids that appears to function both as a cyclic AMP-regulated  $Cl^-$  channel and, as its name implies, as a regulator of other ion channels. The fully processed form of CFTR is found in the plasma membrane in normal epithelia (Fig. 1). Biochemical studies indicate that the  $\Delta F_{508}$  mutation leads to improper processing and intracellular degradation of the CFTR protein. Thus, absence of CFTR at appropriate cellular sites is often part of the pathophysiology of CF. However, other mutations in the CF gene produce CFTR proteins that are fully processed but are nonfunctional or only partially functional at the appropriate cellular sites.

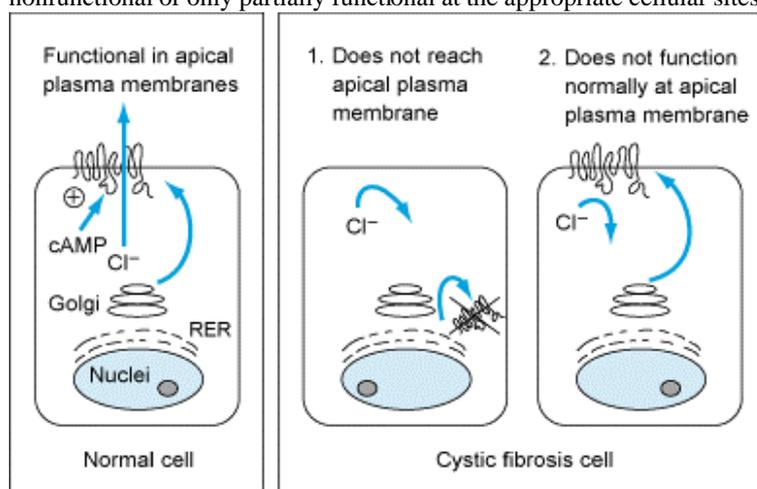


FIGURE 1: Cellular metabolism of the CFTR protein.

In a normal cell (*left*), CFTR is synthesized in the rough endoplasmic reticulum (RER), glycosylated in the Golgi apparatus, and functions as a  $Cl^-$  channel and regulator of other ion channels when located in the plasma membrane. Two possible outcomes of mutations in the CF gene are shown (*right*). (1) If a mutation disturbs protein folding, e.g., the  $\Delta F_{508}$  mutation, CFTR is degraded intracellularly so that no protein is transported to the plasma membrane. (2) With other mutations, the abnormal protein is processed and traffics to the plasma membrane but functions abnormally at that site.

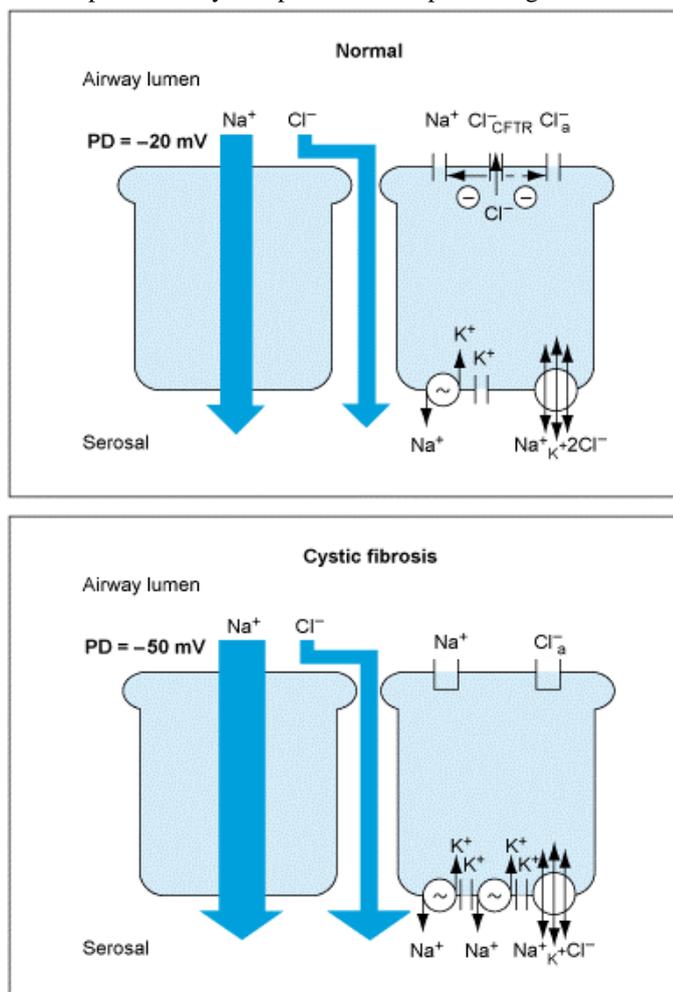
## Epithelial Dysfunction

The epithelia affected by CF exhibit different functions in their native state; i.e., some are volume-absorbing (airways and intestinal epithelia), some are salt-absorbing but not volume-absorbing (sweat duct), whereas others are volume-secretory (pancreas). Given this diverse array of native activities, it should not be surprising that CF produces very different effects on patterns of electrolyte and water transport. However, the unifying concept is that all affected tissues express abnormal ion transport function.

## Organ-Specific Pathophysiology

### Lung

The diagnostic biophysical hallmark of CF is the raised transepithelial electric potential difference (PD) detected in airway epithelia. The transepithelial PD reflects components of both the rate of active ion transport and the resistance to ion flow of the superficial epithelium. CF airway epithelia exhibit both raised transport rates ( $\text{Na}^+$ ) and decreased ion permeability ( $\text{Cl}^-$ ) (Fig.2). The  $\text{Cl}^-$  transport defect reflects at least in part the absence of cyclic AMP-dependent kinase and protein kinase C-regulated  $\text{Cl}^-$  transport that is mediated by the  $\text{Cl}^-$  channel functions of CFTR. An important observation is that there is an alternative  $\text{Cl}^-$  channel expressed in airway epithelia. This "alternative"  $\text{Cl}^-$  channel ( $\text{Cl}_a^-$ ) is different from CFTR and is regulated by intracellular  $\text{Ca}^{2+}$  levels or by extracellular triphosphate nucleotides, e.g., uridine - adenine triphosphate (UTP), and perhaps by CFTR itself. This channel can substitute for CFTR with regard to net  $\text{Cl}^-$  transport and may be a potential therapeutic target.



**FIGURE 2: Comparison of ion transport properties of normal (*top*) and CF (*bottom*) airway epithelia.**

The vectors describe routes and magnitudes of  $\text{Na}^+$  and  $\text{Cl}^-$  transport. The normal basal pattern for ion transport is absorption of  $\text{Na}^+$  from the lumen via an amiloride-sensitive  $\text{Na}^+$  channel. This process is accelerated in CF. The capacity to initiate cyclic cAMP-mediated  $\text{Cl}^-$  secretion is diminished in CF airway epithelia due to absence/dysfunction of the CFTR  $\text{Cl}^-$  channel. The accelerated  $\text{Na}^+$  absorption in CF reflects the absence of CFTR inhibitory effects on  $\text{Na}^+$  channels.  $\text{Cl}_a^-$ , alternative  $\text{Cl}^-$  channel; PD, potential difference; CFTR, cystic fibrosis transmembrane regulator.

Raised  $\text{Na}^+$  absorption is a feature of CF airway epithelia.  $\text{Na}^+$  transport abnormalities are not a widespread feature of the CF epithelial phenotype and appear confined to volume-absorbing epithelia. Recent studies demonstrate that the increased  $\text{Na}^+$  transport reflects the absence of CFTR's tonic inhibitory regulatory function on  $\text{Na}^+$  channel activity. It appears that CFTR inhibits  $\text{Na}^+$  channel activity as a part of its general function to act as a "switch" that coordinates the balance between  $\text{Na}^+$  absorption and  $\text{Cl}^-$  secretion. The central hypothesis of CF airways pathophysiology has been that the abnormal  $\text{Na}^+$  and  $\text{Cl}^-$  transport rates produce secretions that are dehydrated and poorly cleared. The unique predisposition of CF airways to chronic infection by *Staphylococcus aureus* and *Pseudomonas aeruginosa* raises the issue that other as yet undefined abnormalities in airway surface liquid ionic composition also may contribute to the failure of lung defense.

### Gastrointestinal Tract

The gastrointestinal effects of CF are diverse. In the exocrine pancreas, the absence of the CFTR  $\text{Cl}^-$  channel in the apical membrane of pancreatic ductal epithelia limits the function of an apical membrane  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchanger to secrete bicarbonate and  $\text{Na}^+$  (by a passive process) into the duct. The failure to secrete  $\text{NaHCO}_3$  and water leads to retention of enzymes in the pancreas and ultimately destruction of virtually all pancreatic tissue. The CF intestinal epithelium, because of the lack of  $\text{Cl}^-$  and water secretion, fails to flush the secreted mucins and other macromolecules from intestinal crypts. The diminished CFTR-mediated secretion of liquid may be exacerbated by excessive absorption of liquid, reflecting abnormalities of CFTR-mediated regulation of  $\text{Na}^+$  absorption (both mediated by  $\text{Na}^+$  channels and possibly other  $\text{Na}^+$  transporters, e.g.,  $\text{Na}^+$ - $\text{H}^+$  exchangers). Both dysfunctions lead to desiccated intraluminal contents and obstruction of both the small and large intestines. In the hepatobiliary system, defective hepatic ductal salt ( $\text{Cl}^-$ ) and water secretion causes retention of biliary secretions and focal biliary cirrhosis and bile duct proliferation in approximately 25 to 30 percent of CF patients. The inability of the CF gallbladder epithelium to secrete salt and water can lead to both chronic cholecystitis and cholelithiasis.

### Sweat Gland

CF patients secrete nearly normal volumes of sweat in the sweat acinus. However, CF patients are not able to absorb  $\text{NaCl}$  from sweat as it moves through the sweat duct due to the inability to absorb  $\text{Cl}^-$  across the ductal epithelial cells.

### Diagnosis

Because of the large number of CF mutations, DNA analysis is not used for primary diagnosis. The diagnosis of CF rests on a combination of clinical criteria and analyses of sweat  $\text{Cl}^-$  values. The values for the  $\text{Na}^+$  and  $\text{Cl}^-$  concentration in sweat vary with age, but typically in adults a  $\text{Cl}^-$  concentration of  $>70$  mmol/L discriminates between CF patients and patients with other lung disease.

A reason for the

### Screening for cystic fibrosis

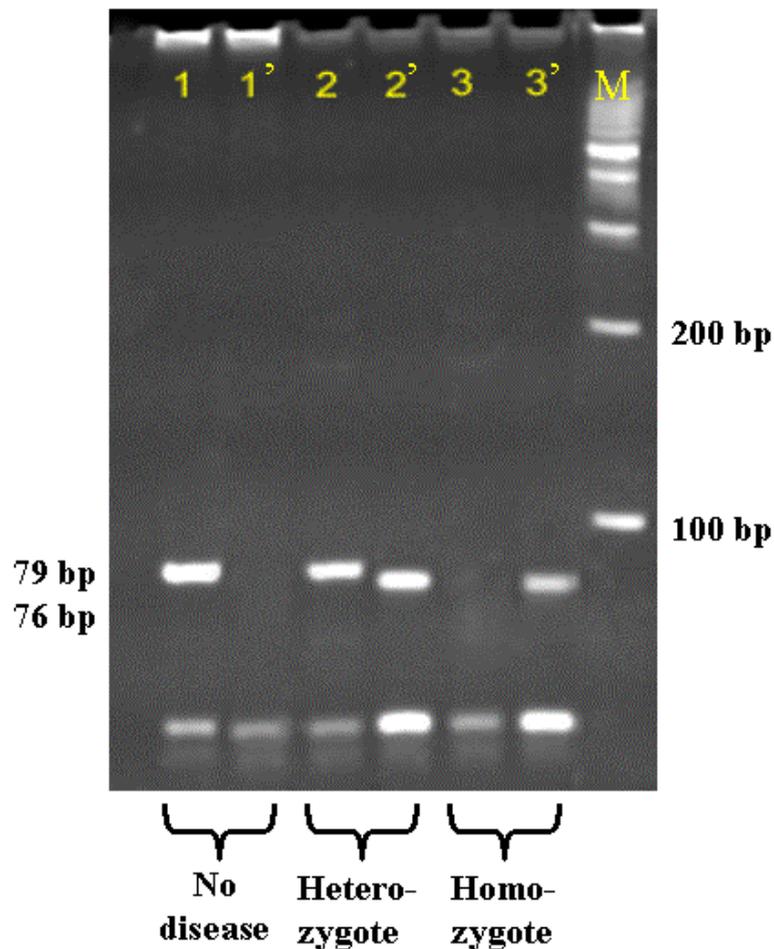
A number of different screening strategies have been suggested, including prenatal, preconceptional, school and neonatal carrier screening, as well as screening of newborns to identify affected infants. Currently, screening to identify carriers during the newborn period or among school age children is inadvisable, mainly on psychosocial and cost-effective grounds. Although early diagnosis of CF may improve prognosis, current scientific evidence is not sufficient to support screening newborns to identify affected

infants. Of the remaining two options, prenatal screening has a practical advantage because of existing facilities, while with screening before conception all reproductive options are, in principle, open to detected carrier couples.

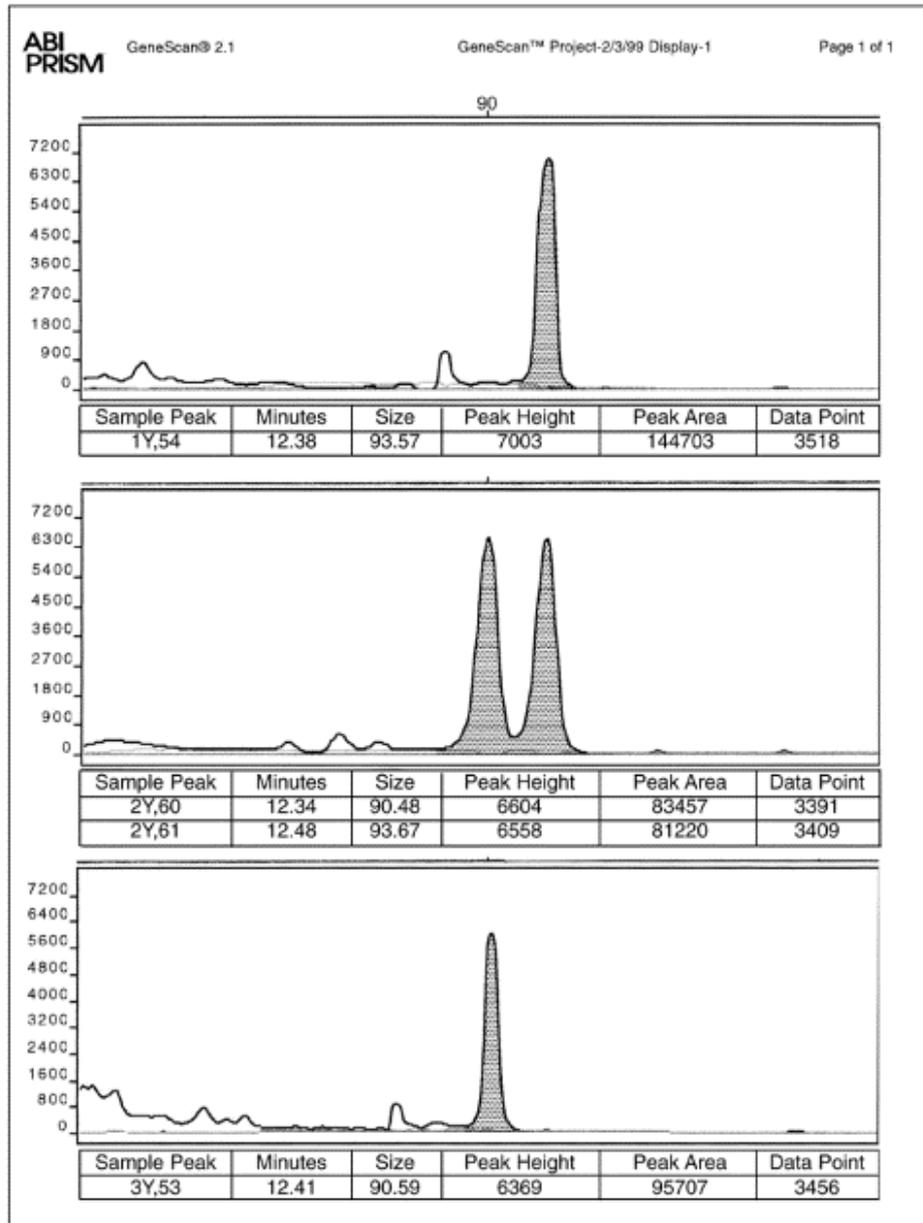
Prenatal diagnosis is primarily performed by a PCR analysis of samples obtained by CVS (Chorionic Villus Sampling), amniocentesis or percutaneous villus sampling (PUBS), (although for the latter there is no any practical indication).

This method is being currently applied in the Semmelweis University, 1st Department of Obstetrics and Gynecology,

Genetic Laboratory, by Dr. Bán Zoltán. In their setting, the  $\Delta F 508$  mutation is being detected by fluorescence PCR [F-PCR] (see below).



### The three typical patterns of F-PCR in the detection of DF 508 mutation



**Amniocentesis:** This involves withdrawing 10 to 20 ml of amniotic fluid from the amniotic cavity between the 14<sup>th</sup> and 16<sup>th</sup> week of gestation, using the transabdominal approach. Amniotic fluid cells are mainly fetal in origin and can easily grow in culture.

**CVS:** A sample of Chorionic villi can be obtained by inserting a flexible catheter through the vagina and cervix, and advancing it to the site of fetal implantation under direct ultrasound guidance or by transabdominal needle (also with ultrasound guidance). About 10 to 30 mg of villi are then aspirated into a syringe; any contaminating maternal tissue is removed under a dissecting microscope. Karyotypes are obtained after short-term (3 to 5 days) and long-term (10 days to 2 weeks) culture. The latter is now believed to reflect more accurately the karyotype of the fetus. Important advantages of CVS are these: It is done at 8 to weeks of gestation, which is 6 to 8 weeks earlier than amniocentesis, and results are available more quickly. Thus, if as couple elects termination of pregnancy because of an abnormal result, a 1<sup>st</sup>-trimester abortion is easier and safer than one at the 20<sup>th</sup> week.

**PUBS:** A sample of fetal blood can be obtained by a needle inserted through the maternal abdomen into a placental blood vessel at the site of the attachment of the umbilical cord. Previously requiring fetoscopy, it is now done under ultrasound guidance from as early as 12<sup>th</sup> week of gestation until term. PUBS is useful for obtaining a rapid karyotype (2 to 3 days) after ultrasound detection of congenital anomalies or for WBC, serum, or Hb disorders not yet detectable by enzyme assay or molecular techniques. The risk of fetal loss ranges from 1 to 5%.

- Preconceptional screening is made by detection through PCR analysis of material obtained by a mouthwash or bloodspot. With the mouthwash procedure there is no need for medical supervision or sample collection. The mouthwash procedure has, theoretically, an almost perfect sensitivity and specificity, apart from laboratory errors.

### **Clinical Features**

Most CF patients present with signs and symptoms of the disease in childhood. Approximately 15 percent of patients present within the first 24 h of life with gastrointestinal obstruction, termed **meconium ileus**. Other common presentations within the first year or two of life include respiratory tract symptoms, most prominently cough and/or recurrent pulmonary infiltrates, and failure to thrive. A significant proportion of patients (~4 percent), however, are diagnosed after age 18.

### **Respiratory Tract**

Upper respiratory tract disease is almost universal in CF patients. Chronic sinusitis is common in childhood and leads to nasal obstruction and rhinorrhea. The occurrence of nasal polyps approaches 25 percent and often requires surgery.

In the lower respiratory tract, the first symptom of CF is cough. With time, the cough becomes persistent and produces viscous, purulent, often greenish-colored sputum. Inevitably, periods of clinical stability are interrupted by "exacerbations," defined by increased cough, weight loss, increased sputum volume, and decrements in pulmonary function. These exacerbations require aggressive therapy, including frequent postural drainage and oral antibiotics, and often intravenous antibiotics (see below), with the goal being recovery of lost lung function. Over the course of years, the exacerbations become more frequent and the recovery of lost lung function less complete, leading to respiratory failure.

CF patients exhibit characteristic sputum microbiology. *Haemophilus influenzae* and *S. aureus* are often the first organisms recovered from samples of lung secretions in newly diagnosed CF patients. *P. aeruginosa* is typically cultured from lower respiratory tract secretions thereafter.

The earliest chest x-ray change in CF lungs is hyperinflation, reflecting small airways obstruction. Later, signs of luminal mucus impaction, bronchial cuffing, and finally, bronchiectasis, e.g., ring shadows, are noted. For reasons that are still unknown, the right upper lobe displays the earliest and most severe changes. Neither CT nor MRI scanning is routinely performed on CF patients.

CF pulmonary disease is associated with many intermittent complications. Pneumothorax is common (>10 percent of patients). The production of small amounts of blood in sputum is common in CF patients with advanced pulmonary disease and appears to be associated with lung infection. Massive hemoptysis is life threatening and difficult to localize bronchoscopically. With advanced lung disease, digital clubbing becomes evident in virtually all patients with CF. As late events, respiratory failure and cor pulmonale are prominent features of CF.

### **Gastrointestinal Tract**

The syndrome of meconium ileus in infants presents with abdominal distention, failure to pass stool, and emesis. The abdominal flat plate can be diagnostic, with small intestinal air fluid levels, a granular appearance representing meconium, and a small colon. The characteristic intestinal abnormalities are complicated by exocrine pancreatic insufficiency in more than 90 percent of CF patients. Insufficient pancreatic enzyme release yields the typical pattern of protein and fat malabsorption, with frequent, bulky, foul-smelling stools. Signs and symptoms of malabsorption of fat-soluble vitamins, including vitamins E and K, are also noted. Because pancreatic beta cells are typically spared, the appearance of hyperglycemia and a requirement for insulin is a late finding in CF and occurs in about 5 percent of patients.

## Genitourinary System

Late onset of puberty is common in both males and females with CF. The delayed maturational pattern is likely secondary to the effects of chronic lung disease and inadequate nutrition on reproductive endocrine function. More than 95 percent of male patients with CF are azoospermic, reflecting obliteration of the vas deferens that probably reflects defective liquid secretion. Twenty percent of CF women are infertile due to effects of chronic lung disease on the menstrual cycle and thick, tenacious cervical mucus that blocks sperm migration. More than 90 percent of completed pregnancies produce viable infants, and CF women are generally able to breast-feed infants normally.

## Treatment

The major objectives of therapy for CF are to promote clearance of secretions and control infection in the lung, provide adequate nutrition, and prevent intestinal obstruction. Ultimately, gene therapy may be the treatment of choice.

The goal of gene therapy is correction of the mutant CFTR gene with wild-type (WT) DNA sequences to restore normal CFTR protein and function. Experiments with wtCFTR cDNA expression vectors have shown that Cl<sup>-</sup> ion transport phenotype associated with CF can be corrected to resemble that in normal cells. Gene targeting is done mostly with the use of oligonucleotides or adenoviral vectors.

Cells of the respiratory epithelium are the logical targets for gene therapy of CF. Lung cells cannot be excised, cultured and transfected with CFTR gene in the laboratory and transplanted back to the patients. The normal approach is limited to *in vivo* delivery of the normal version of CFTR gene to the correct cell type. Regenerating poorly differentiated cells of the human airway epithelium represent preferential cell targets for the recombinant adenoviral gene vectors. None of the methods are 100% efficient but correction of less than 10% cells with normal CFTR gene would produce a lining epithelium with normal functioning of the chloride channel.

In a study of *in vivo* adenovirus-mediated transfer of human CFTR cDNA to Rhesus monkeys, mild to moderate perivascular and peribronchial lymphocytic infiltrates were seen only after high doses of recombinant adenovirus. Rechallenge of these animals with further doses of recombinant adenovirus resulted in gene transfer at all levels of lungs and airways without additional histopathological changes. Circulating adenovirus antibodies were detected. These studies show that adenovirus vectors can be used for CFTR gene delivery to the respiratory epithelium without undue toxicity.

### **- Clinical Protocol (issue for discussion).**

Links on Cystic Fibrosis to the Web: <http://www.genet.sickkids.on.ca/cfr>

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Pictures of F-PCR kindly provided by Dr. Bán Zoltán, Genetic Laboratory, 1st Department of Obstetrics and Gynecology, Semmelweis University.