than in sinusoidal membrane vesicles, suggesting that ATP-dependent transport is localized to this domain. Using a mixture of canalicular and sinusoidal membrane vesicles, ATP-stimulated uptake of radiolabeled LTC_4, LTD_4, LTE_4 and *N*-acetyl-LTE_4 was observed in membrane vesicles from normal rat liver but not from the mutant Wistar rats. Significantly, LTB_4 uptake was not stimulated by ATP, in contrast to the cysteinyl leukotrienes, suggesting that the cysteine moiety is crucial for ATP-dependent transport. Although the ATP-dependent component of transport was present only in vesicles isolated from control rats, a significant uptake of all leukotrienes was seen in the absence of ATP in vesicles from both normal and mutant animals. The time course of LTC_4 uptake in the absence of ATP showed a transient accumulation of fivefold over equilibrium values (overshoot). This transient accumulation was attributed to the binding of LTC_4 to the surface of plasma membrane vesicles and not to the transmembrane movement of LTC_4 into vesicles. However, this interpretation is tenuous because other criteria of mediated transport were not excluded. The preliminary work of Nishida et al. (11) suggests that both ATP-dependent and ATP-independent mechanisms function to transport certain organic anions across the canalicular membrane into bile.

ATP-stimulated cysteinyl leukotriene uptake in mixed liver plasma membrane vesicles was specific for ATP because ADP, AMP and ATP analogs had no effect; it was inhibited by vanadate but not by ouabain, EGTA, acivicin or D-penicillamine. Because ATP-dependent transport of [^{3}H]LTC_4 was not inhibited by doxorubicin, daunorubicin, verapamil or a monoclonal antibody to P-glycoprotein (C219), this transport pathway is probably distinct from the ATP-dependent primary active pump for cytotoxic drugs.

Initial rates of ATP-stimulated uptake of LTC_4, LTD_4 and *N*-acetyl-LTE_4 were saturable with increasing concentrations, with apparent K_m values of 0.25, 1.5 and 5.2 μmol/L, respectively. Uptake of ^{3}H-labeled cysteinyl leukotrienes was mutually inhibited by unlabeled cysteinyl leukotrienes (except for the lack of inhibition of [^{3}H]LTC_4 uptake by LTE_4) and unlabeled DNP-SG. From these observations the authors concluded that cysteinyl leukotrienes and DNP-SG are transported by way of a common transport system and that the carrier has a high affinity toward LTC_4 as compared with DNP-SG. However, this conclusion is not warranted because no evidence was presented that cysteinyl leukotrienes actually inhibit the transport of DNP-SG across liver membranes and, if so, whether it is a competitive inhibition.

Nevertheless, these findings provide the first direct evidence that the four major cysteinyl leukotrienes are transported across the canalicular membrane by a common ATP-dependent mechanism that is distinct from P-glycoprotein. Furthermore, because the ATP-stimulated transport system was localized to the canalicular membrane, transport of cysteinyl leukotrienes across the sinusoidal membrane is presumably mediated by a different ATP-independent mechanism. Additional studies are needed to define the physiological significance of the ATP-independent components of cysteinyl leukotriene transport on both the canalicular and sinusoidal membranes and the relation between the ATP-dependent transport of cysteinyl leukotrienes and that which mediates ATP-dependent DNP-SG transport.

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CYSTIC FIBROSIS – CORRECT CHLORIDE CONDUCTANCE CAN CURE CELLS

Drumm ML, Pope HA, Cliff WH, Rommens JM, Marvin SA, Tsui LC, Collins FS, et al. Correction of cystic fibrosis defect *in vitro* by retrovirus-mediated gene transfer. Cell 1990;62:1227-1233.

ABSTRACT

We have used retrovirus-mediated gene transfer to demonstrate complementation of the cystic fibrosis (CF) defect in vitro. Amphotropic retroviruses were used to transduce a functional cystic fibrosis transmembrane conductance regulator (CFTR) cDNA into

CFPAC-1, a pancreatic adenocarcinoma cell line derived from a patient with CF that stably expresses the chloride transport abnormalities characteristic of CF. CFPAC-1 cells were exposed to control virus (PLJ) and CFTR-expressing virus (PLJ-CFTR); viral-transduced clones were isolated and subjected to molecular and physiologic analysis. RNA analysis detected a viral-derived CFTR transcript in all of the PLJ-CFTR clones that contained unrearranged proviral sequences. Agents that increase intracellular cAMP stimulated ^{125}I efflux in PLJ-CFTR clones but not PLJ clones. Whole-cell patch-clamp performed on three responding clones showed that the anion efflux responses were due to cAMP stimulation of Cl conductance. Our findings indicate the expression of the normal CFTR gene confers cAMP-dependent Cl channel regulation on CF epithelial cells.

Rich DP, Anderson MP, Gregory RJ, Cheng SH, Paul S, Jefferson DM, McCann JD, et al. Expression of cystic fibrosis transmembrane conductance regulator corrects defective chloride channel regulation in cystic fibrosis airway epithelial cells. Nature 1990;347:358-363.

ABSTRACT

The cystic fibrosis transmembrane conductance regulator (CFTR) was expressed in cultured cystic fibrosis airway epithelial cells and Cl^- channel activation assessed in single cells using a fluorescence microscopic assay and the patch-clamp technique. Expression of CFTR, but not of a mutant form of CFTR (ΔF508), corrected the Cl channel defect. Correction of the phenotypic defect demonstrates a causal relationship between mutations in the CFTR gene and defective Cl transport which is the hallmark of the disease.

COMMENTS

Cystic fibrosis (CF), one of the most common and often devastating genetic diseases in white populations, has long fascinated clinical investigators and physiologists, in part because of its effects on a wide variety of epithelial tissues, including the airway epithelium, liver, pancreas and intestine. Recently, work on CF has shifted into a higher gear as the identification of the presumed CF gene (1) has allowed investigators to combine molecular biology with physiology to better understand the underlying pathophysiology of this scientifically appealing and clinically destructive disease. The two papers reviewed here constitute a landmark step forward in CF research because they both provide strong evidence that the previously described "CF" gene is the one that is defective in CF tissues and, importantly, show that the restitution of a normal gene corrects the function defects in cultured cell lines from CF airway and pancreatic epithelium. The latter finding sets the stage for the development of effective gene therapy for CF.

CF is emerging as a disorder in the regulation of ion, specifically chloride, transport in a variety of epithelial tissues (2, 3). Many of the well-known clinical features of CF, such as inspissated and thickened airway mucus, plugging of biliary and pancreatic ducts, inspissated intestinal meconium and high sweat chloride, may reflect defective secretion of chloride, sodium and water by airway and gastrointestinal epithelia or defective reabsorption of secreted salts in sweat glands. Indeed, the reason for the persistence of the CF gene may be a heterozygote advantage—heterozygotes may develop much less secretory diarrhea during bacterial, viral or parasitic gastrointestinal infections, leading to decreased childhood mortality (4-6). The currently accepted model for epithelial salt secretion and/or reabsorption includes transcellular movement of chloride across epithelial cells through specific membrane transport proteins on apical and basolateral cell membranes. In most epithelia studied to date, one of these membrane transporters is a chloride channel (or permeability) located on the apical cell membrane that allows rapid movement of negatively charged Cl^- ions. Cl^- ions accumulate on one side of the epithelial cell layer, and cations (Na^+) and water follow the chloride ions by electrical and osmotic forces to constitute fluid secretion (if Cl^- is transported to the apical or luminal side) or fluid absorption (if Cl^- is transported to the basolateral or blood side). Epithelial electrolyte and fluid transport is regulated by a variety of agents, including hormones and neurotransmitters, and may be strikingly altered by drugs or bacterial products. Indeed, for gastroenterologists, cholera is an excellent example of unrestrained intestinal chloride secretion that is induced by a bacterial toxin.

In light of this model, many studies of CF tissues demonstrate a defect in epithelial chloride transport, specifically a defect in chloride channel function. In early studies, decreased permeability to chloride was demonstrated in CF sweat glands and airway tissue (2). More recent studies have directly examined cell membrane chloride transport. Chloride secretion in many epithelial tissues is stimulated by agents that increase either cyclic AMP (cAMP) or intracellular Ca^{+2} or both by way of the opening of apical chloride channels and basolateral potassium channels. In contrast, chloride secretion by CF airway epithelia, intestinal mucosa and pancreatic duct cells can be elicited only by Ca^{+2}—these tissues display little or no response to cAMP or any of its functional analogues (3, 7). The CF defect is not, however, in the cAMP pathway *per se* because these cells generate adequate cAMP if given appropriate stimuli and contain active cAMP-dependent protein kinase. Furthermore, CF cells do contain functional chloride channels. Patch-clamp studies, in which bits of membrane containing ion channels are removed from cells and studied in isolation, show that CF cells have chloride channels that can be opened by nonphysiological maneuvers such as the imposition of a large transmembrane voltage (8, 9). Rather, the defect in CF seems to be one of regulation. Chloride channels in normal cells are opened when exposed to activated protein kinase A, presumably through phosphorylation of the channel itself or of an associated regulatory protein, whereas the chloride channels in CF cells are not opened by protein

kinase A. It is suggested that the chloride channels in CF cells, or an associated regulatory protein, may exhibit defects in a phosphorylation site. Because it is likely that most epithelial tissues are constantly bombarded by a variety of regulatory signals, this defect could explain the observed abnormal regulation of Cl^- secretion and of fluid secretion that is seen in CF cells and organs. This defect in chloride transport can be identified in cultured cells by measuring cAMP-stimulated Cl^- (or I^-) transport using radioisotopes, Cl^- sensitive fluorescent dyes or patch-clamp electrophysiological methods that assess movement of Cl through membrane channels (Cl^- currents).

Progress in the genetics of CF has been equally impressive. In 1989, a group of collaborators from the Hospital for Sick Children in Toronto and the University of Michigan localized a putative gene for CF using a combination of linkage analysis based on DNA polymorphisms in a group of affected families and a technique called chromosome jumping (1, 10, 11). These investigators used genetic, not functional, criteria to implicate the gene they found to be the CF gene. Mutations of this gene are found in most CF patients and in carrier parents but not in normal subjects. One mutation (ΔF508), in which a phenylalanine residue is omitted, apparently accounts for about 70% of white CF patients, but a wide variety of other mutations are now being discovered. This gene encodes for a large protein of 1,480 amino acids with a molecular weight of about 170 kD. The encoded protein contains two very similar halves with areas that are likely to represent membrane-spanning domains, ATP-binding sites and phosphorylation sites. The function of this protein is unknown, although it resembles the multidrug resistance P-glycoprotein, an ATP-dependent drug pump. The CF gene product has been termed the CF transmembrane conductance regulator (CFTR) because based on the physiological data already reviewed it is likely to function as a membrane-associated regulatory protein of a chloride channel.

With the identification of a putative CF gene, it became possible to address many questions, including the following:

(a) Does the CFTR gene code for the defective protein in CF or is it simply a closely linked gene?

(b) What is the function of the CFTR protein?

(c) Can CF be treated by gene therapy?

The two papers reviewed here examine these issues, using the skills of ion transport physiologists and molecular biologists.

The strategy used by both groups was straightforward—to obtain a full-length complementary DNA (cDNA) for the CFTR gene, transfect it into defective CF cells and look for changes in cAMP-stimulated halide transport. An initial stumbling block for both was the inability to obtain a full-length cDNA in bacteria. Apparently the CFTR gene contains a bacterial promoter that facilitates transcription of a toxic protein in bacteria. The group headed by Welsh (the Rich et al. article) at the Genzyme Corporation solved the problem by using a bacterial strain that produced a low number of transcripts. They then transfected copies of the normal gene into CF airway cells in primary culture using a vaccinia virus vector. Because this vector halts cell protein synthesis, the airway cells lived only a short time after transfection, and all experiments were performed as short-term assays. The group headed by Frizzell and Wilson (the Drumm et al. article) inserted several silent mutations into the normal CFTR gene that disrupted the bacterial promoter, inserted the CFTR cDNA into a retroviral vector and transfected a cell line of pancreatic adenocarcinoma cells (CFPANC) derived from a patient with CF. The transfected pancreatic cells constitute a stable system that continuously expressed the transfected gene. Both groups successfully transfected CF cells with the normal CFTR gene and demonstrated the presence of the appropriate messenger RNA, protein or both. Cells transfected with the viral vector alone (Drumm et al.) or with the CF ΔF508 (defective) CFTR gene (Rich et al.) served as controls. Both groups showed convincingly that in cells transfected with the normal CFTR gene, but not in control cells, cAMP-dependent halide transport was restored. Drumm et al. measured cAMP-stimulated ^{125}I efflux in 10 clones of CF pancreatic cells transfected with the normal CFTR gene and 10 clones transfected with the viral vector. All nine clones that expressed the complete CFTR gene exhibited cAMP-stimulated ^{125}I transport in contrast to none of the control clones. Interestingly, however, these investigators noted a poor correlation between the amount of CFTR messenger RNA in transfected cells and the extent of cAMP stimulation of halide transport. This is consistent with a role for CFTR as a regulatory agent, not as the Cl^- channel *per se*.

Further, Drumm et al. performed whole cell patch-clamp recordings of transfected cells. Those cells that expressed the normal CFTR gene showed cAMP-stimulated Cl^- currents that were not seen in vector-transfected control cells. Similarly, Rich et al. used a halide-sensitive fluorescent dye, SPQ, and fluorescence microscopy to demonstrate cAMP-dependent halide transport in CF airway cells transfected with the normal but not the defective (CF) CFTR gene. Rich et al. also demonstrated cAMP-dependent anion currents in whole cell patch-clamp recordings on transfected cells given the normal but not the CF CFTR gene.

Collectively, these studies, which represent a technical *tour de force*, conclusively show that the normal CFTR gene corrects functional abnormalities in halide transport in CF cells that are related to cAMP-dependent regulation of ion transport. These studies suggest that CFTR performs a regulatory function, the precise nature of which remains unknown. Finally, the work clearly shows that the expression of only one copy of the normal CFTR gene can correct CF cells, whereas expression of many copies of the defective CF gene cannot, suggesting that the defective gene, although nonfunctional, is not in and of itself toxic to cells. Thus gene therapy, if successful in incorporating a normal gene into epithelial cells, may cure CF.

Where will this work go from here? Clearly, many important questions remain to be answered, but the tools now seem to be present to proceed at a rapid pace to address many issues, including: (a) At a molecular level, what is CFTR, how does it interact with chloride channels and does it have other functions? The CFTR protein is unlike any other known regulatory protein and may represent a whole new level of cell regulation. (b) Does a defect in CFTR alone completely reproduce the clinical picture of CF? A murine model of CF should be possible using the analogous mouse gene and homologous recombination in embryonic stem cells. (c) Can a defect in Cl^- transport cause the abnormalities in mucin sulfation or other protein posttranslational modifications that are seen in CF patients? (d) Is the CFTR gene expressed in those cells and tissues known to be affected in CF? (e) Can different defects in CFTR account for the wide phenotypic range of CF? Work is currently underway to try to correlate different CFTR mutations to different phenotypic expression of CF (12).

Finally, how does this work relate to readers of HEPATOLOGY? Hepatobiliary disease is common in patients with CF, including patchy biliary cirrhosis, inspissated bile, abnormal bile ductules, extrahepatic biliary strictures and gallstones (13, 14). The origin of these abnormalities is unclear but could relate to decreased hepatic bile flow, decreased bile acid pools, decreased bile acid transport and decreased ductular bile formation. It is not known currently whether Cl^- channels are important for bile formation by hepatocytes or bile ductular cells although Cl^- channels have been identified in the canalicular membrane of rat hepatocytes. Interestingly, recent studies have shown that cAMP stimulates bile formation (both bile acid–dependent and bile acid–independent) and bile acid transport by rat hepatocytes (15). Although not conclusive, these findings suggest that hepatocytes and/or bile duct cells may exhibit a similar cAMP–CFTR–ion transport-fluid secretion motif. Clearly, the next few years will be interesting and exciting ones for those interested in ion transport and disorders thereof.

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β-BLOCKER THERAPY FOR PROPHYLAXIS OF INITIAL VARICEAL HEMORRHAGE: HAS ITS TIME COME?

Roberto J. Groszmann, Jaime Bosch, Norman D. Grace, Harold O. Conn, Guadalupe Garcia-Tsao, Miguel Navasa, Jeannie Alberts, et al. Hemodynamic events in a prospective randomized trial of propranolol versus placebo in the prevention of a first variceal hemorrhage. Gastroenterology 1990;99:1401-1407.

ABSTRACT

In a double-blind randomized trial, the hemodynamic events following the administration of propranolol (n = 51) or a placebo (n = 51) were prospectively studied in cirrhotic patients with esophageal varices. The hepatic venous pressure gradient, heart rate, and variceal size were determined at the baseline and 3, 12, and 24 months after the beginning of therapy. Baseline values were similar in both groups. At 3 months, the hepatic venous pressure gradient decreased significantly in propranolol-treated patients (from 18.1 ± 4.2 to 15.7 ± 3.4 mm Hg; $P < 0.05$) but not in patients receiving the placebo (19.6 ± 6.8 to 17.5 ± 5.3 mm Hg; NS). At subsequent time intervals this gradient decreased significantly from the baseline value in both groups. Heart rate decreased significantly in the propranolol-treated group at all times ($P < 0.001$). Variceal hemorrhage occurred in 13 patients (11 placebo-, 2 propranolol-treated; $P < 0.01$), all of whom had a hepatic venous pressure gradient >12 mm Hg. In 21 patients (14 propranolol-, 7 placebo-