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CFTR in cystic fibrosis and cholera: from membrane transport to clinical practice

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Goodman, Barbara E., and William H. Percy. CFTR in cystic fibrosis and cholera: from membrane transport to clinical practice. Adv Physiol Educ 29: 75–82, 2005; doi:10.1152/advan.00035.2004.—We have used a brief analysis of transport via cystic fibrosis (CF) transmembrane conductance regulators (CFTRs) in various organ systems to highlight the importance of basic membrane transport processes across epithelial cells for first-year medical students in physiology. Because CFTRs are involved in transport both physiologically and pathologically in various systems, we have used this clinical correlation to analyze how a defective gene leading to defective transport proteins can be directly involved in the symptoms of cholera and CF. This article is a “Staying Current” approach to transport via CFTRs including numerous helpful references with further information for a teaching faculty member. The article follows our normal presentation which begins with a discussion of the involvement of CFTR transport in the intestine and how cholera affects intestinal transport, extends to CFTR transport in various organ systems in CF, and concludes with the logic behind many of the treatments that improve CF. Student learning objectives are included to assist in assessment of student understanding of the basic concepts.

salty sweat; enterocyte; airway epithelia; mucus

BACKGROUND

This information has been used in a 90-min lecture-style presentation using PowerPoint slides for first-year medical students and has been team taught by the membrane transport and gastrointestinal physiologists. The presentation occurs either immediately following the membrane transport portion of the physiology course or at the end of the gastrointestinal physiology portion of the course, which follows the general physiology portion (membrane transport and nerve, muscle, and autonomic physiology). The presentation begins with a discussion of the involvement of CFTR transport in the intestine and how cholera affects intestinal transport, extends to CFTR transport in various organ systems in CF, and then extended to CFTR transport in various organ systems in CF. Handouts are given to the students with the major points about the effects of transport changes in each disease state. Basic information explaining how various treatments work is presented as a logical extension of the membrane transport processes. Student learning objectives for cholera are 1) describe the transport implications of cholera for both secretory and absorptive epithelia in the gastrointestinal tract. Differentiate between which transporters are stimulated and which transporters are inhibited; 2) describe how CFTR is involved in cholera; and 3) explain how cholera is treated and why. Student learning objectives for CF are 1) describe how CFTR is involved in the formation of salty sweat in CF patients; 2) explain how CFTR is involved in increased thickness of mucus in the upper airways of the lungs in CF patients; 3) identify the clinical problems that commonly lead to death in CF patients; 4) explain why CF patients might be reproductively sterile; 5) understand what causes meconium ileus in newborns and CF-related pancreatic damage in adults; and 6) explain how cholera and CF might be related.

HISTORY OF CHOLERA

According to the World Health Organization, both Hippocrates (466–377 BCE) and Galen (129–216 CE) described an illness that appeared to be cholera (37). In addition, a cholera-like malady has been known throughout the Ganges River area since antiquity. However, modern understanding of cholera began in the early 19th century during which researchers into the first pandemic (worldwide epidemic which started in 1817) made progress toward understanding the causes and treatment of the disease. In the 1850s, John Snow made the initial link between transmission of cholera and the consumption of fecally contaminated water by deducing that the spread of cholera on Broad Street in London was due to raw sewage in the drinking water (21). When the handle was removed from the offending water pump, the epidemic ended. Subsequently in 1883, Robert Koch was the first researcher to identify the causative organism as Vibrio cholerae 01 (14). Koch described cholera in the Egyptian city of El Tor where he found ulcerations of the intestinal mucosa of affected individuals and postulated that an increased leakage of plasma into the intestinal lumen caused their diarrhea (1). Cholera is now defined as any infection of the gastrointestinal tract by the bacterium V. cholerae. The organism responsible for the seventh pandemic, now in progress on earth, is known as V. cholerae 01, biotype El Tor (37).

PREVALENCE OF CHOLERA

According to data from the World Health Organization, the current seventh pandemic began in 1961 when epidemic cholera first appeared in Celebes, Indonesia (37). The disease spread to other countries in eastern Asia including Bangladesh, India, and the USSR, Iran, and Iraq by 1965–1966. In 1970, cholera reached West Africa, where it had not been seen for more than 100 years. In 1991, cholera appeared in Latin America for the first time in more than a century. Until 1992, only one serogroup (V. cholerae serogroup 01) had been implicated in causing epidemic cholera. Other serogroups had caused sporadic cases of diarrhea, but not epidemic cholera. Later in 1992, additional outbreaks of cholera began in India and Bangladesh caused by a previously unrecognized serogroup of V. cholerae, designated 0139. It is unclear whether this new serogroup from Southeast Asia will spread to other regions of the world. It is estimated that 120,000 people die from cholera worldwide each year (37).
CHOLERA: THE DISEASE

What causes cholera? Vibrios are one of the most common organisms in surface waters of the world (both marine and freshwater) (37). Vibrios are acquired by eating contaminated food or drinking contaminated water and are transmitted from person-to-person by the fecal-oral route. V. cholerae is endemic to areas including India, Asia, Africa, the Mediterranean, and more recently, South and Central America, and the United States. In the Asian countries like India, cholera outbreaks are more prevalent in the late summer and during the monsoon season (June through August). A type of Vibrio is associated with shellfish, especially raw oysters. No major outbreaks of cholera have occurred in the United States since 1911; however, sporadic cases have occurred between 1973 and 1991 and have been mostly associated with the consumption of raw shellfish or shellfish either improperly cooked or recontaminated after proper cooking. The illness is caused by ingestion of viable bacteria, which attach to the wall of the gastrointestinal tract and there produce cholera toxin (37). Recently, investigators found that the infectivity of cholera may be enhanced with the organisms more likely to cause epidemics after various genes that increase survivability are turned on while passing through the human gut (24).

What are the symptoms (and prognosis) of cholera? Cholera is an acute illness characterized by sudden onset of watery diarrhea with a rice water appearance (flakes of mucus and intracellular cAMP causing osmotic diarrhea drawing water into the lumen and leading to the characteristic large volumes of watery diarrhea.

How is cholera prevented? The major prevention against epidemic cholera is a safe water supply, good sanitation and disposal of excreta, and safe food (37). Thus effective control measures include proper case management, surveillance, reporting, sufficient emergency supplies, and access to health facilities, whereas ineffective measures include massive efforts to give antibiotics to entire communities, travel restrictions, and mass vaccinations. Two new oral vaccines have been developed that may provide protection for up to 50% of individuals >5 yr of age for as long as 3 yr after immunization (38). However, these vaccines only protect against V. cholerae 01, are not effective for sustained protection in children <2 years of age, and may lead to travelers becoming overconfident and not following simple safety rules.

How is cholera treated?

Attempts to stop the spread of cholera have been variably successful; however, a major success story has been treatment efforts that have drastically decreased mortality during the current pandemic. While cholera used to have a mortality rate >20%, with the development of oral rehydration therapy (ORT), the fatality rate for cholera has dropped to about 1% (14). “ORT is, in fact, the quintessential example of a primary care intervention: it is a simple technology; it can be made available in the home, encouraging the involvement of the mother and other family members to provide effective care; it is inexpensive; and it effectively treats a common problem.” (7).

In about 1,000 BCE, an Indian physician named Sushruta recommended that his patients with diarrhea drink large amounts of tepid water with dissolved rock salt and molasses (7). Effective treatment of cholera involves rapid replacement of body fluid every hour for several days (if they are adequately rehydrated) (37).

What specific transport processes are affected by cholera toxin in the small intestine? Intestinal crypt cells, the primary secretory cells found in the small intestinal mucosa, respond to numerous secretagogues including acetylcholine, prostaglandins, and vasoactive intestinal peptide and the second messengers Ca$^{2+}$ and cAMP to lead to chloride secretion through CFTRs located on the apical (luminal) side of the cells (see Fig. 1). In the presence of cholera toxin, chloride transport into the intestinal lumen through CFTRs located on the luminal surface of intestinal crypt cells is continuously activated by intracellular cAMP causing osmotic diarrhea drawing water into the lumen and leading to the characteristic large volumes of watery diarrhea.
The approved recipe for ORS is (in mM) 111 sodium, 20 potassium, 90 glucose, 80 chloride, and 30 citrate. Recently, there has been controversy about whether reduced osmolarity ORS that still has approximately equal amounts of sodium and glucose but lower total osmolarity might be better for children with cholera in addition to other diarrheal diseases. Scientific research set the stage for the use of oral rehydration therapy instead of more costly intravenous and sterile rehydration of cholera patients. Enterocytes (intestinal epithelial cells that participate in the absorption of the breakdown products of ingested nutrients) have several different types of transport proteins that facilitate the absorption of sodium and other organic molecules (see Fig. 2). Sodium is known to enter epithelial cells from the lumen via epithelial sodium channels (ENaCs--facilitated diffusion), sodium-dependent substrate cotransporters (secondary active transporters), electroneutral sodium-chloride cotransporters (secondary active transporters), and coupled sodium/proton exchangers and chloride/HCO₃⁻ exchangers (secondary active transporters). Intracellular cAMP is known to inhibit both the electroneutral sodium-chloride cotransporters and the sodium/proton exchangers leading to decreased cellular uptake of sodium and its transport all the way across enterocytes. Thus in addition to enhancing chloride secretion into the intestinal lumen via activating cAMP in the secretory crypt cells, cholera toxin also inhibits absorption via electroneutral sodium-chloride cotransport and sodium/proton exchange out of the intestinal lumen across the absorptive enterocytes, both of which lead to more osmotically driven water in the lumen and the resultant watery diarrhea. However, the sodium-organic substrate (L-amino acids, D-sugars, oligopeptides, etc.) cotransporters are unaffected by cAMP. Thus the approved recipe for ORS is specifically designed to activate sodium and substrate absorption out of the intestinal lumen and to lead to absorption of water molecules osmotically following these solutes. Therefore, simply drinking ORS can be a viable and vital way to quickly rehydrate an individual suffering from infection with cholera.

**HISTORY OF CF**

The disease now known as CF has long been identified by health workers as indicated by the old adage from Northern European folklore “Woe to that child which when kissed on the forehead tastes salty. He is bewitched and soon must die” (35). The first major contribution to determining the cause of CF occurred in 1938 when Dorothy Andersen (after performing autopsies on infants and children with the disease and reviewing their case histories) provided a comprehensive description of their symptoms and the changes produced by the disease in various organs. Andersen noted that there was almost always destruction of the pancreas accompanied by infection of and damage to airways in the lungs. Andersen named the disease “cystic fibrosis of the pancreas.” Subsequently in 1946, researchers deduced that CF was inherited and results from an autosomal recessive mutation. In 1948, there was a devastating heat wave in New York City and hospitals saw a disproportionate number of children with CF who had become dehydrated from losing excessive salt in their sweat (26). This observation by P. A. di Sant’Agnese at Columbia Hospital led to his presentation of his findings to the American Pediatrics Society in 1953 and the development of the cornerstone diagnosis for CF, the sweat chloride test.

Fig. 2. Schematic of the absorptive cells in the small intestine (enterocytes) and the transport proteins involved in net solute transport from the apical side to the basolateral side of the epithelium. A: types of pathways involved in the uptake of sodium by the cell. Electrogenic transport pathways (1 and 2) lead to a net movement of charge across the membrane, and electroneutral transport pathways (3 and 4) do not contribute to a charge difference. B: Na⁺-substrate cotransporters are available for the absorption of nutrients from the intestinal lumen. (Modeled after figures in Ref. 15.)
CF: THE DISEASE

According to data from the Cystic Fibrosis Foundation, more than 10 million white Americans are unknowing, asymptomatic carriers of a defective gene for CF and one of every 3,200 live Caucasian births in the United States has CF (6). One thousand new cases are diagnosed annually with 30,000 children and adults in the United States having CF. “People with CF have a variety of symptoms including: very salty-tasting skin; persistent coughing, at times with phlegm; wheezing or shortness of breath; an excessive appetite but poor weight gain; and greasy, bulky stools. Symptoms vary from person to person due, in part, to the more than 1,300 mutations of the CF gene.” (6)

What are the major common clinical manifestations of CF? One of the major problems with CF for many individuals is blockage of the outflow of digestive enzymes from the exocrine pancreas into the small intestine and the resultant pancreatitis that can lead to the cystic changes in the pancreas previously noted by Andersen (2). A second major difficulty in CF (and probably the most devastating for the long-term health of the individual) is the accumulation of heavy, dehydrated mucus in airways and the resultant changes in the capability of the lungs to fight infections. A third major change that leads to the ability to use the sweat chloride test as the standard diagnostic test for the disease is the abnormally salty sweat of CF patients. A fourth clinical manifestation is sterility in both males and females that is particularly prevalent and may be irreversible in males. A fifth manifestation that may be recognized less frequently than some of the others is abnormal secretion in the small intestine. This aspect has been previously described in detail in the sections on cholera. These common pathophysiological problems lead to the several major symptoms of CF described previously.

How do the various CF mutations affect the function of CFTR proteins? The various mutations (>1,300) that have been shown to cause CF have been categorized into four classes (36). Class I mutations cause defective protein production with a total loss of functional CFTRs. One defect produces a truncated CFTR due to premature stop mutants. This defect may be corrected by certain antibiotics that bypass the shortened protein to make full-length functional CFTR. These antibiotics have restored 25–35% CFTR protein function when only 10% correction appears to be enough for noticeable improvement. Class II mutations cause defective protein processing leading to CFTR that is not in its correct location in the cell or that is different from CFTRs in normal individuals (less glycoproteins and gangliosides on the cell surface in CF cells). The most common mutation found in 70% of CF patients (the ∆F508 deletion) is one of the Class II mutations. Class III mutations cause defective regulation of channel opening of CFTR by changes in the nucleotide binding fold or regulatory (R) domain of CFTR (see Fig. 3) (30). Class IV mutations cause defective ion conduction through CFTRs. These mutations are in membrane-spanning domains of the protein and thus affect the pore that normally allows ion fluxes. Class I and II mutations generally lead to the more serious phenotype of the disease and have accompanying pancreatic insufficiency. Class III and IV mutations generally lead to a less serious phenotype with normal pancreatic function.

How are CFTRs in normal, healthy people regulated? Figure 3 shows a schematic of the CFTR and its regulatory sites. Various studies of CFTR protein function have shown that in the absence of phosphorylation of the R domain, the channel is closed and chloride transport ceases. Sequential phosphorylation of the R domain and subsequent binding of ATP to sites on the nucleotide binding fold increases the open probability of the channel. (Modeled after Figure 1 in Ref. 30.)

At the cellular level, how do mutations leading to CF cause the transport problems in the various organs/systems that are recognized as symptoms of CF? Briefly, in the lung upper airways, there is decreased Cl⁻ (and HCO₃⁻) secretion into the lumen, increased Na⁺ absorption through ENaCs out of the lumen, possibly due to loss of an inhibitory influence of
functional CFTRs and/or ENaCs stimulating the activity of CFTR up to sixfold (18), and normal, but not CF, CFTR may activate water permeability through aquaporins there (28). In sweat glands, sweat is normally produced at the base of the glands and then passes through a narrow duct in which reabsorption of salt occurs [in CF, abnormal Cl− absorption out of the duct via defective CFTRs leads to excessive Na+ and Cl− (3–5 times normal concentration) in sweat] (Fig. 4 and Ref. 27). In the liver of CF patients, plugging of small bile ducts based on lack of Cl− secretion impedes digestion and disrupts liver function; however, liver involvement may be asymptomatic and may be slowly or not at all progressive. In the pancreas, occlusion of ducts (and lack of HCO3−) prevents pancreatic enzymes from reaching the lumen of the intestine and may lead to premature activation of digestive enzymes inside the pancreas causing pancreatitis that may also cause diabetes mellitus. Lack of digestive enzymes in the intestinal lumen can cause symptoms including frequent, loose, oily, and malodorous stools caused by steatorrhea (fat in stool due to lack of active pancreatic lipases). Approximately 80–85% of CF patients have pancreatic insufficiency, which leads to decreased availability of both digestive enzymes and HCO3− in the intestinal lumen and clinical malabsorption based on lack of Cl− secretion. Fortunately, oral pancreatic enzyme supplementation after meals appears to override the digestive problems. In the intestine, obstruction of the gut by a “thick, dehydrated, rubbery, tarry, tenacious, mucoid plug” (26) leads to a condition known as meconium ileus that necessitates surgery in 10% of newborns with CF. In addition, knockout mice with no CFTRs (model developed in 1992) will die from intestinal obstruction by 5 wk of age, and their intestines exhibit an inflammatory state leading to an innate immune response, which may cause additional tissue destruction and pathogenesis. Constipation and distal intestinal obstruction syndrome also occur in older CF patients (CF patients have also been shown to have loss of CFTR function in their colon) with rectal prolapse being common (usually before pancreatic enzyme therapy). All of these situations are based on lack of Cl− secretion. These intestinal CFTRs are the same ones involved in excessive chloride secretion under the effects of cholera toxin. Recently, investigators (4) have shown that while prostaglandin-stimulated electrolyte secretion in the intestine is decreased in CF patients, prostaglandin inhibition of neutral sodium absorption is unaffected. Thus this antabsorptive response may contribute to the low level of intestinal disease in CF patients. In the reproductive tract, absence of fine ducts (i.e., vas deferens) developmentally renders 95% of CF males infertile. The speculation is that the faulty development is due to blockage of the ductules in utero so that most CF males are aspermic or hypospermic. CF women show a significantly lower fertility rate due to physical mucus plugs in the Fallopian tubes which block sperm from fertilizing ova. These transport problems are based on the lack of Cl− secretion. Figure 5 summarizes the important transport implications of CFTR in various organs. Thus in multiple organs, ion and water imbalance contributes to the pathophysiology by failing to dilute the native mucus sufficiently to maintain its normal fluidity. This dehydrated mucus becomes very viscous and relatively immovable.

Therefore, what are the basic goals of clinical studies to help treat patients with CF? The major problems that need to be addressed to assist CF patients to live longer are to 1) fight the chronic lung infections that inevitably lead to lung damage and eventually death, 2) thin the dangerously thick CF mucus particularly in the upper airways of the lungs, 3) reduce the inflammatory response particularly in the lungs which leads to tissue destruction, and ultimately to be able to 4) correct the basic cellular defect (i.e., gene therapy or selective drugs). Thus various efforts are occurring to use or develop therapies for treating CF that 1) correct the abnormal gene via genetic manipulation, 2) correct the abnormal protein via protein rescue/activation, 3) alter ion transport and abnormal mucus secretion via selective drugs, 4) decrease infection and inflammation leading to tissue destruction via anti-inflammatory and anti-infective agents, or 5) overcome organ destruction leading to respiratory failure via lung transplantation. In summary, the various stages of the lung pathogenesis seen in CF are developed from the abnormal gene that translates the abnormal protein that exhibits abnormal transport of ions, which leads to changes in the luminal fluid, causing infection followed by inflammation and finally destruction of lung tissue. The only recourse that one might have may be lung transplantation.

Cells in the upper airways of the lungs of CF patients secrete a mucus layer that is dehydrated, thicker, stickier, and more difficult to clear than in healthy individuals. This layer provides a hospitable environment for pathogens (particularly Pseudomonas aeruginosa and Staphylococcus aureus), which leads to the recurrent infections and progressive loss of respiratory function due to bronchiectasis (destruction of muscular and elastic components of airway walls due to chronic inflammation). Now that digestive problems in CF patients are relatively easily managed if treated before induction of damage to the pancreas, lung impairment in CF patients causes >90% of the disability and death due to the disease. How can ion

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**Fig. 4.** This model of NaCl transport across ductal cells in sweat glands of normal and CF individuals demonstrates the differences in salt reabsorption there that leads to the elevated sweat NaCl in CF patients. Na+ ions utilize ENaCs to enter the cells from the luminal side and Na+-K+-ATPases to leave the cells on the blood side. Cl− ions predominantly utilize CFTRs to enter the cells from the luminal side and other Cl− transporters to leave the cells on the blood side. In CF, the lack of functional CFTR on the luminal side of the cell compromises the movement of cationic Na+ ions and its accompanying anionic Cl− ions leading to less reabsorption of salt out of the lumen. Salt concentrations in CF sweat can be up to 3–5 times normal. (Modeled after Figure 1 in Ref. 27.)
transport and fluid balance across the upper airway epithelium be improved in CF patients? Better dilution of the thick, sticky mucus lining the upper airways will help lower the chances of various bacteria colonizing the upper airways and leading to destruction of lung tissue. This enhanced dilution can be facilitated by simply manipulating concentrations of salts and water across airway epithelia. In other words, net solute movement is accompanied by net water movement by osmosis (see Fig. 6). There are several possible ways to modify salt (and water) movement across the airway epithelial cells. One way would be to use aerosolized amiloride to inhibit ENaCs and thereby decrease sodium reabsorption out of the lumen of the upper airways (13). Less sodium leaving means less water leaving and leads to a larger airway liquid volume. Another way would be to increase chloride secretion either through the CFTR (using various pharmacological agents to rescue the function of mutant CFTR) (13); through other chloride channels (ATP, UTP, and UDP may work via various purinergic receptors to open other Ca\textsuperscript{2+}-activated chloride channels) (19); or by facilitating K\textsuperscript{+} secretion (activation of basolateral Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels by benzimidazolone may promote a sustained Cl\textsuperscript{-} secretory response by providing the accompanying cations needed for net transport) (9). All of these methods lead to more ion secretion into the lumen and more airway liquid volume. Another way might be to increase HCO\textsubscript{3}\textsuperscript{-} secretion because functional CFTRs may facilitate Cl\textsuperscript{-}-coupled HCO\textsubscript{3}\textsuperscript{-} transport, causing abnormally acid luminal fluid in CF patients, which causes precipitation of mucins, plugs various ducts, and facilitates bacterial infections (3).

Although the manipulations described above would likely lead to changes in water levels in airway liquid and may assist in diluting the airway liquid so that it is less thick and tenacious, are there other more indirect methods that can change the characteristics of the airway liquid? The traditional treatment for CF patients has been postural drainage and chest percussion (chest physical therapy) where the patients lie with their heads tilted downward and someone pounds gently and rapidly on their back and chest to physically loosen and clear the secretions in the lung airways. More recently, the purulent airway secretions that have become viscoelastic due to polymerized DNA from the degenerating leukocytes in the lung airways have been treated with recombinant human deoxyribonuclease, thereby reducing the viscosity of the sputum (8). Recurrent infections in the lungs lead to a chronic burden of neutrophils and their resultant neutrophil elastase in upper airways. Thus secretory leukoprotease inhibitor (produced by cells of the mucosal surface) can be used to protect against the assault of neutrophil elastase on airway epithelial cells (23). Alternatively, excess neutrophil elastase can be diminished by high-dose ibuprofen (which decreases neutrophil aggregation and adherence) taken by patients with mild lung disease for a long time (34). Neutralizing the destructive neutrophil elastase by any of these methods may slow the progression of the lung epithelial damage in CF patients. Similarly, it has been shown that CF sputum samples contain filamentous actin; thus human
plasma gelsolin (protein that severs actin filaments) has also been used to reduce the viscosity of sputum in CF patients (32). Finally, since the thick, tenacious sputum is ultimately due to a lack of water in the airway liquid, some relief has been shown with adding ultrasonically nebulized hypertonic saline (6%) to create an osmotic draw for water into the upper airways of CF patients (34).

The ultimate goal for CF patients would be a cure by correcting or replacing the genetic or protein defect (16, 33). What about gene therapy for CF patients? Human gene therapy studies for CF began in 1993 with investigations into their safety and with the first aerosolized CF gene therapy protocol used in 1995. Recent evidence shows that only 6–10% of cells with functional CFTRs will correct the Cl⁻ transport defect but 100% of cells need functional CFTRs to correct the Na⁺ transport defect. Insertion of normal CFTRs into airway cells would be an ideal way to cure the disease; however, finding a good vector for insertion of the gene, which will lead to long-term improvement, has been difficult. Viral vectors (adenovirus, modified adenoviruses, retrovirus) that can be genetically modified to be replication deficient with CFTR cDNA plus a promoter are highly efficient but immunogenic and therefore only effective in the short term. Nonviral vectors (cationic liposome-DNA complexes or molecular conjugates) have greater safety and ease of preparation but low transduction efficiency. Molecular conjugates can be constructed so they are targeted to airway epithelial cells via specific receptor-mediated endocytosis. Since most cells in epithelial tissue are replaced every few months, gene therapy would still have to be administered multiple times a year.

Why is the disease CF so prevalent in humans? There have been several theories about the high incidence of mutations leading to CF in Northern Europeans and their descendants. One theory ties together the relationships between CF and cholera. This theory states that heterozygotes in the population have ~50% functional CFTRs compared with healthy individuals and have a selective advantage in resisting death by cholera because of lower enhanced chloride secretory diarrhea during infection with the disease. Thus these individuals had heterozygote advantage and were more likely to pass on the gene for CF to their offspring. This theory, while reasonable and interesting, does not totally explain the inheritance of CF, because cholera epidemics did not strike Northern Europe until the 19th century. However, diarrheal diseases caused by other organisms, such as Escherichia coli, have affected European humans for centuries and could have led to similar selective pressures (26). However, Cuthbert et al. (5) saw no evidence of genetic advantage in ileal or colonic epithelia of the null CF mouse for acute responses to secretory diarrhea (5). Another theory is that a single copy of the most common ΔF508 mutation causing CF may protect an individual against bronchial asthma (29). These results came about when researchers were searching for evidence about other adult lung diseases in CF heterozygotes and found that only a handful had asthma and even fewer with one allele of the ΔF508 mutation had asthma. Thus there may be some as yet unknown link between defective chloride transport and protection against asthma. A third theory is related to increased resistance to infectious diseases that helps to maintain the mutant CFTR alleles at high levels. It has been shown that Salmonella typhi uses CFTR to enter and infect cells and that when there are fewer functional CFTRs in the cell membrane (as in the ΔF508 mutation) there is less infection (25). Thus diminished numbers of functional CFTRs in CF heterozygotes may have decreased their susceptibility to typhoid fever and preserved the gene at high numbers in the population.

CFTR AND CHOLERA REVISITED

Secretory diarrhea is the leading cause of infant death and has devastating effects on adults in developing countries. It is now known that CFTR is the transporter required for chloride (and accompanying water) secretion both in the intestines and in the upper airways of the lungs. Mutations in CFTR lead to various expressions of CF which is still a lethal genetic disease. Ma et al. (22) have utilized high throughput screening techniques to identify high-affinity blockers of cholera toxin-induced intestinal fluid secretion via CFTRs. In fact, recently a thiazolidinone-type CFTR blocker has been shown to reduce cholera toxin-induced ion and fluid secretion in mouse intestine (31). Thus CFTR inhibitors are being developed as drugs to block intestinal fluid secretion in cholera and other secretory diarrheas. In addition, these compounds might be useful to establish the CF phenotype in cells and animal models to investigate more specifically the mechanisms of lung tissue destruction in patients with CF.

Thus a brief investigation into how CFTRs are involved in transport aberrations in both infections by V. cholerae and the genetic disease CF can help teach students about direct relevance of membrane transport physiological principles to human disease. In addition, the transport principles represented in these two examples are amazingly logical and relevant to basic concepts of diffusion and osmosis already understood by the students.

REFERENCES


