Lung Infections Associated with Cystic Fibrosis

Jeffrey B. Lyczak,[1,2] Carolyn L. Cannon,[1,2,3] and Gerald B. Pier[1,2]*

Channing Laboratory, Brigham and Women's Hospital,[1] Harvard Medical School,[2]
Children's Hospital,[3] Boston, MA 02115

INTRODUCTION

Overview of CF and Bacterial Infection

Cystic fibrosis (CF) manifests as a clinical syndrome characterized by chronic sinopulmonary infection as well as by gastrointestinal, nutritional, and other abnormalities. The genetic basis for CF is a well-characterized, severe monogenic recessive disorder, found predominantly in Caucasian populations of European ancestry, that arises from mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. While the gene defect results in a myriad of medical problems for the patient, the most meddlesome clinical feature, chronic pulmonary infection with *Pseudomonas aeruginosa*, allows the basic pathologic process in CF to be designated an infectious disease. Ultimately, 80 to 95% of patients with CF succumb to respiratory failure brought on by chronic bacterial infection and concomitant airway inflammation. The discovery in 1989 of the genetic defect causing CF sparked an explosion of research efforts, which have led to a greater understanding of the molecular mechanisms underlying the various phenotypic manifestations of the disease. Yet the breadth of the link between mutant forms of the CF gene product, CFTR, and chronic bacterial respiratory infections, particularly by *P. aeruginosa*, remains elusive. Deciphering this link is critical, since this infection and the ensuing inflammation accounts for the majority of the morbidity and mortality in the disease.

Lungs of CF patients are often colonized or infected in infancy and early childhood with organisms, such as *Staphylococcus aureus* and *Haemophilus influenzae*, that may damage the epithelial surfaces, leading to increased attachment of, and eventual replacement by, *P. aeruginosa*. However, adequate clinical studies to determine the role of these organisms in the pathogenesis of lung disease in CF patients have never been published. The recovery of these organisms from a bronchoalveolar lavage (BAL) fluid sample from the lung would be considered a frank infection in need of therapy. However, the role that *S. aureus*, nontypeable *H. influenzae*, and similar organisms isolated from oropharyngeal cultures play in the pro-

---

* Corresponding author. Mailing address: Channing Laboratory, 181 Longwood Ave., Boston, MA 02115. Phone: (617) 525-2269. Fax: (617) 525-2510. Email: gpier@channing.harvard.edu.

gression of CF patients to respiratory failure has not been determined. Rather, the pathogenic role of *S. aureus* and nontypeable *H. influenzae* in the development of lung disease in CF patients is inferred principally from clinical anecdote but is otherwise lacking any solid support from studies in the peer-reviewed literature.

Chronic infection with *P. aeruginosa* is the main proven perpetrator of lung function decline and ultimate mortality in CF patients. Chronic *P. aeruginosa* infection leads to epithelial surface damage and airway plugging, progressively impairing airway conductance, which results in a decline in pulmonary function. Intense inflammation characterized by neutrophil sequestration in the airways contributes to impaired clearance and plugging associated with the death of senescent cells. Airway damage also arises through neutrophil release of a variety of oxidants and enzymes.

CF has not always been a disease characterized by chronic pseudomonal sinopulmonary infection. Prior to 1946, the reported prevalence of CF pseudomonal infections was low (78). However, a variety of sources indicate that during the 1960s *P. aeruginosa* became the most prevalent organism in the airways of CF patients (229). The emergence of this pathogen coincided temporally with the introduction of regional centers that specialized in CF care. The adherence to standardized principles of multidisciplinary therapy by CF centers has been lauded as an important factor responsible for increasing the median survival from 14 years in 1969 to greater than 30 years currently in the United States (247). However, studies in Denmark pointed to CF centers as potential sites of increased risk for spread of *P. aeruginosa* (223, 225). Studies in the United States have corroborated these suspicions. In a study by Farrell et al. (90), the median pseudomonas-free period of the patients attending one center was more than five times that of those attending another CF center. The center with the earlier pseudomonal acquisition time was distinguished by an urban setting, admixing of young patients with older, *P. aeruginosa*-infected patients, and more opportunity for social interactions among the patients. Further studies in Denmark (95) demonstrated a decrease in pseudomonal colonization after institution in 1981 of cohort isolation (isolation of younger, uninfected patients from older patients more likely to carry infectious agents). Thus, the acquisition of *P. aeruginosa* by CF patients can be affected by different treatment settings.

Historical Framework for the Study of Cystic Fibrosis

Prior to 1938, CF was recognized as a collection of diverse clinical syndromes of the alimentary and respiratory tracts. While defects in these systems are apparent by 6 months of age, defects of the alimentary system are the most pronounced at very early ages, when difficulty in feeding or failure to gain weight are conspicuous symptoms. Early descriptions of CF (also called fibrocystic disease of the pancreas or mucoviscidosis) were impaired by several practical obstacles. Chief among these obstacles was the small sample size of affected individuals included in these studies. This factor complicated estimates of the population frequency of CF, thus delaying the characterization of its genetic basis. Second, even after these multiple clinical manifestations of CF were recognized to represent the same disease entity, definitive diagnosis was usually possible

only at autopsy, since data on familial occurrence were frequently inaccurate or unavailable.

Our understanding of the genetic basis of CF was advanced greatly by the work of Dorothy Andersen, who in 1938 published a detailed study of 49 CF patients (6). In this study, cases were categorized into three groups based on the patients' age at death. Group I consisted of patients who died before the age of 1 week, group II consisted of patients who died between 1 week and 6 months, and group III consisted of patients who died between 6 months and 14.5 years of age. The cause of death of patients in group I was intestinal obstruction, while patients in groups II and III usually died of "respiratory complications." However, a broad range of observations demonstrated that despite these classifications, similar pathological conditions (for example, pancreatic lesions and malnutrition) could be seen in all patients. These observations led to an understanding of CF as a single disease with diverse effects rather than a loose collection of related disease states. Further evidence of the pleiotropic manifestations of CF was presented by di Sant'Agnese et al., who demonstrated that the sweat of CF patients contains abnormally high concentrations of sodium, chloride, and potassium (77), and by Shwachman et al., who made the interesting observation that seven CF patients (two males and five females), who had reached adulthood and married had universally failed to produce offspring (297).

Citing histological data from autopsy samples, Norris and Tyson (209) and Baggenstoss et al. (12) postulated that the physiological nature of the CF defect was a malformation of the pancreatic ducts, leading to defective secretion by various epithelial glands. While it was appreciated that pancreatic function could be normal in some patients with CF (pancreatic sufficient), most patients who were recognized to have the disease presented with large greasy stools (steatorrhea) due to pancreatic function that was inadequate for proper absorption of nutrients (pancreatic insufficient). Consequently, CF was viewed primarily as a disease of the digestive tract. Thus, early studies were biased toward severe cases and focused on detailed histological descriptions of the anatomical defects of the pancreas during progression of CF. While minor discrepancies exist between studies, several consistent observations warrant comment. Acini (glands) were found to contain concretions (dehydrated secretions) of various sizes; also, the acinar cells exhibited various degrees of flattening, resulting in a vaguely squamous appearance (6). The degree of flattening of the acinar cells appeared to be directly related to the size of the concretions. Furthermore, acini were often surrounded by fibrous or adipose tissue and were also occasionally infiltrated by fibroblasts, lymphocytes, plasma cells, or phagocytes (6, 209). Walters proposed that these sequelae stemmed from hyperplasia (overproliferation) of ductile epithelial cells (332). Such hyperplasia was presumed to compress the local acinar tissue, resulting ultimately in atrophy of the acini and their replacement by fibrous or adipose tissue (332). The islets of Langerhans are usually reported to be normal in terms of their architecture but have been reported at times to be less frequent in the CF pancreas than in a normal pancreas. These changes are now appreciated to be due to autodigestion of pancreatic tissue from enzymes trapped in concretions.

More recently, it has been proposed by Freedman et al. (96) that dysfunction of the acinar tissue in CF may be due to an

imbalance in the utilization of free fatty acids in the phospholipids of CF patients. These workers reported that the phospholipids of *CFTR* knockout mice contain a higher than normal proportion of arachidonic acid, at the expense of docosahexaenoic acid (DHA). Orally administering DHA to the knockout mice corrected the membrane defect and restored normal histology to the affected tissues, suggesting that physiologic defects in CF are not due to an inability of the CF intestine to absorb certain fatty acids but, rather, are due to a defect in fatty acid synthesis or utilization.

Bacteriological studies on the lungs of CF patients date to the turn of the century. In 1905, Landsteiner (169) reported that of 15 CF lung samples, 9 were culture positive. *S. aureus* was the predominant agent isolated, although *Staphylococcus albus* (i.e., coagulase-negative staphylococcus, probably *Staphylococcus epidermidis*) and *Streptococcus haemolyticus* (i.e., *Streptococcus pyogenes*) were also identified in the cultures (169). As treatment standards for CF patients improved over the years, the average mean survival of CF patients increased dramatically. Most notable among such advances was the refinement of nutritional regimens (4, 19, 290) and the advent of antibiotic chemotherapy (122, 193, 197, 228). While essentially all patients prior to the 1950s died by the age of 10 years, reports published in the 1950s (192) and 1960s (297) described a considerable proportion of CF patients surviving well beyond this age. By the 1990s, approximately one-third of CF patients were surviving to adulthood (93). This increased mean survival has had a dramatic impact on the nature of CF as an infectious disease, since the longer survival of CF patients has created opportunities for the establishment of infection by bacteria other than *Staphylococcus*. Two reports published in 1968 by Burns and by Burns and May demonstrated that the sera of CF patients contained antibodies to both *P. aeruginosa* and *Klebsiella* spp. (43, 44). Furthermore, the presence of serum antibodies to *P. aeruginosa* correlated perfectly with bacterial carriage, as assessed by sputum culture of the microorganism. Today, *P. aeruginosa* is the most prevalent pulmonary pathogen in CF patients.

CYSTIC FIBROSIS

Clinical and Biochemical Aspects

Diagnosis. The diagnosis of CF is usually made clinically, although the use of universal neonatal screening for immunoreactive trypsin in some places has allowed for very early diagnosis shortly following birth. CF often presents with a typical constellation of symptoms including chronic respiratory infections and gastrointestinal abnormalities leading to malabsorption and nutritional deficits. The definitive diagnosis is made with a sweat test. A sweat chloride concentration of more than 60 mmol/liter determined on two or more occasions by quantitative pilocarpine ionophoresis remains the "gold standard" for diagnosis (308). Interpretation may be clouded in cases of neonates whose sweat chloride levels may be transiently high and in older adults whose sweat chloride levels normally increase; both of these cases lead to false-positive tests. Similarly, false-negative tests may be obtained in malnourished patients with hypoproteinemic edema (leakage of fluid from serum due to decreased serum protein content, as encountered during

severe protein malnutrition) and in patients with hypochloremia (loss of chloride electrolytes) due to dehydration. False-negative sweat tests can also be a consequence of the particular combination of *CFTR* mutations carried by an individual CF patient. For example, patients homozygous for ΔF508 *CFTR* (which alone would cause abnormal sweat electrolyte levels) but who have a third mutation in one of their *CFTR* alleles, R553Q, may have normal sweat electrolyte levels (83), indicative of possible compensatory, second mutations in an allele that otherwise leads to elevated sweat chloride levels. For these reasons, diagnosis is confirmed by genetic analysis. While genetic screens are able to identify more than 90% of occurrences of the more than 1,000 known *CFTR* mutations, a negative screen does not ensure a normal *CFTR* genotype since the commercial screens that are currently available detect only the 70 most prevalent *CFTR* mutations. Diagnoses that remain unclear after sweat testing and genotyping may be confirmed by a test that directly measures CFTR function, such as nasal potential difference testing (a method for real-time measurement of transepithelial electrical potential resulting from ion transport through channels including CFTR [154]).

Uncovering the function of CFTR. The deciphering of the biological function of CFTR began in 1953 with the observation (77) that the sweat of CF patients contains abnormally high electrolytes levels. This observation has ultimately led to demonstrations by several researchers that CF patients have abnormalities in chloride conductance in and out of cells (243, 279). Normally, as the isotonic secretions travel from the acinus of the sweat gland to the surface of the skin, the epithelial cells lining the ducts act to reabsorb NaCl, resulting in hypotonic sweat. However, the sweat ducts of CF patients are impermeable to Cl^-. Thus, the NaCl remains in the secretions, and the sweat is salty (Fig. 1). Later studies by Sato and Sato (267) showed that unlike normal glands, CF sweat glands fail to secrete fluid in response to β-adrenergic agonists that stimulate cyclic AMP (cAMP) production, yet CF glands produced normal amounts of cAMP. Thus, the Cl^- conductance defect was located downstream from adenylate cyclase, at the level of the chloride channel or regulator. Studies utilizing the patch-clamp technique, which enables observations of single ion-channel activity, suggested that the defect lay in the regulation of a chloride channel, called the secretory channel, which was studied by multiple investigators using a variety of epithelial tissues. This channel has the following properties: outward rectification (implying a preference to transport Cl^- ions into rather than out of the cell), moderate conductance, and activation by cAMP and protein kinase A (PKA) and, under some conditions, protein kinase C (PKC). Outwardly rectifying chloride channels (ORCC) can be found in epithelial cells from CF patients, but these fail to respond to PKA and PKC. This observation prompted historical speculation that if the CF gene product did not encode the ORCC, it was perhaps a regulator of the ORCC (132, 175, 272).

The "secretory channel" designation was based on the realization in the 1970s that Cl^- channels play a pivotal role in fluid secretion by epithelial tissues (99). The ability of secretory epithelia to release fluid rests on the energy provided by the ubiquitous, basolaterally located Na^+/K^+-ATPase, which maintains a low intracellular concentration of Na^+ (Fig. 2). This low Na^+ concentration, coupled with the negative trans-
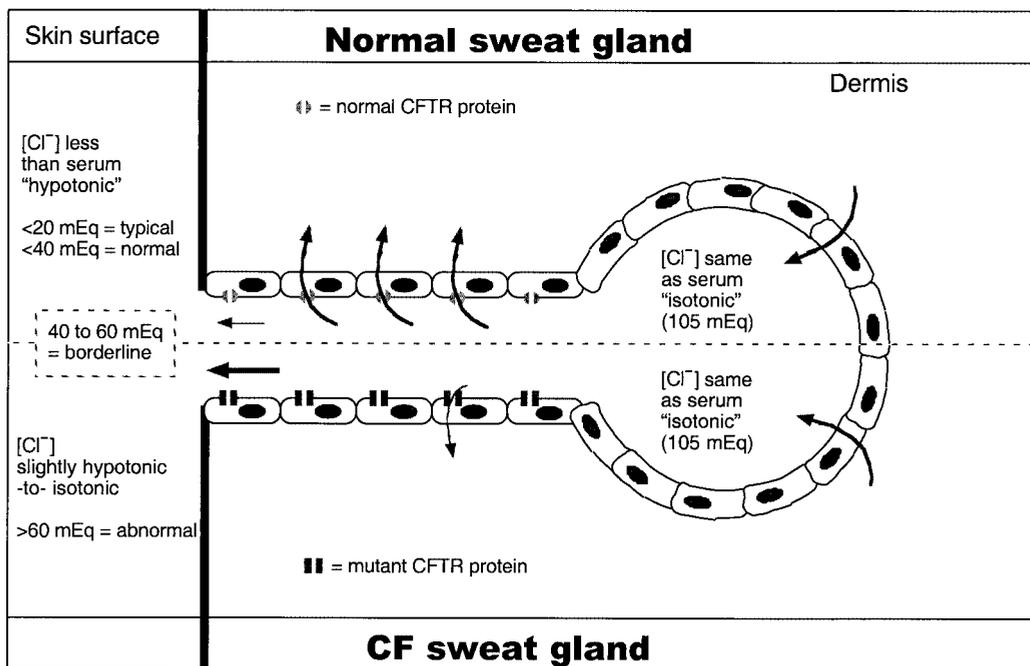
FIG. 1. Diagram of a sweat gland, showing paths taken by chloride ions (arrows) during secretion. In both normal and CF sweat glands in the dermis, chloride is present in secretions at a concentration of 105 mEq, equaling that in serum ("isotonic"). (Top) In the normal sweat gland, chloride is absorbed out of the sweat in a CFTR-dependent manner as the sweat travels from the gland to the skin surface. As a result, the chloride concentration in normal sweat is below that in serum ("hypotonic"), with <40 mEq considered normal and <20 mEq being typical. (Bottom) In the CF sweat gland, chloride absorption is hindered by defective CFTR function. As a result, sweat which reaches the skin surface has higher than normal chloride concentrations (>60 mEq).

membrane potential (the interior of the cell is negatively charged compared with the exterior), drives the passive diffusion of Na^+ into the cell, as well as the energetically unfavorable intracellular accumulation of Cl^-, through a basolaterally located $Na^+/K^+/2Cl^-$ cotransporter. When the cell is stimulated to secrete, Cl^- channels open, allowing Cl^- to exit down its electrochemical gradient. Na^+ ions follow the Cl^- ions through a paracellular pathway, and water follows the salt due to the resulting osmotic gradient. The rate-limiting step for fluid secretion is the flow of Cl^- through the regulated apical chloride channels, presumed to be either the observed secretory channels or the ORCC. However, when the gene for CF was cloned (146, 258, 260) and the gene product, CFTR, was studied, it became clear that the CFTR protein was not the expected ORCC channel. Instead, the CFTR protein acts as a cAMP-sensitive Cl^- channel of low conductance with no preference for the direction of Cl^- transport (16, 17, 144). Controversy subsequently surrounded the original work identifying the defective ORCC regulation in CF cells. However, wild-type CFTR introduced into a CF cell corrected the defective protein kinase regulation of ORCCs (84). Moreover, although ORCCs were found in cells from $CFTR^{-/-}$ mice (103), these channels were insensitive to activation by PKA or PKC. Thus, the validity of the original ORCC studies was established, as was the astute designation of the CF gene product as a conductance regulator, due to its ability to regulate other channels including the classic ORCC.

Biological function of CFTR after discovery of the gene. The list of proteins with which CFTR interacts in its role as con-

ductance regulator continues to grow and includes channels, transporters, and proteins linked to the apical cytoskeleton scaffolding of epithelial cells (Table 1). These proteins are poised to participate in the secretory functions that were originally ascribed to the ORCC but are now known to be orchestrated by CFTR. Investigations into these interactions have shed light on puzzling observations including the increased Na^+ absorption noted in CF airways (28), an abnormality that prompted a clinical trial of aerosolized amiloride, which inhibits sodium absorption, for treatment of CF (152). Cloning of the amiloride-sensitive epithelial Na^+ channel (ENaC) (47) enabled direct demonstration of the negative modulation of the ENaC current by normal CFTR (116, 135, 311) through binding of the C-terminal tail of ENaC to intracellular cytoplasmic domains of CFTR (NBD1 and the R domain) (166). Similarly, CFTR confers sulfonylurea sensitivity on the inwardly rectifying K^+ channel of the kidney, ROMK2, through interaction with the NBD1 and R domains, as well as the first transmembrane domain, of CFTR (46).

The CFTR and the ORCC appear to interact directly, as well as indirectly through CFTR-facilitated ATP release (Fig. 3). Direct interaction of CFTR and the ORCC is suggested by studies demonstrating a failure to activate ORCC when PKA and ATP are applied to either side of the channel in the absence of CFTR (140). ATP and other nucleoside triphosphates are known to stimulate Cl^- secretion when applied to the extracellular surface of cultured human airway epithelia (169). In addition, ATP and UTP were found to be equipotent in vivo Cl^- secretagogues (153, 312). Indeed, nanomolar con-
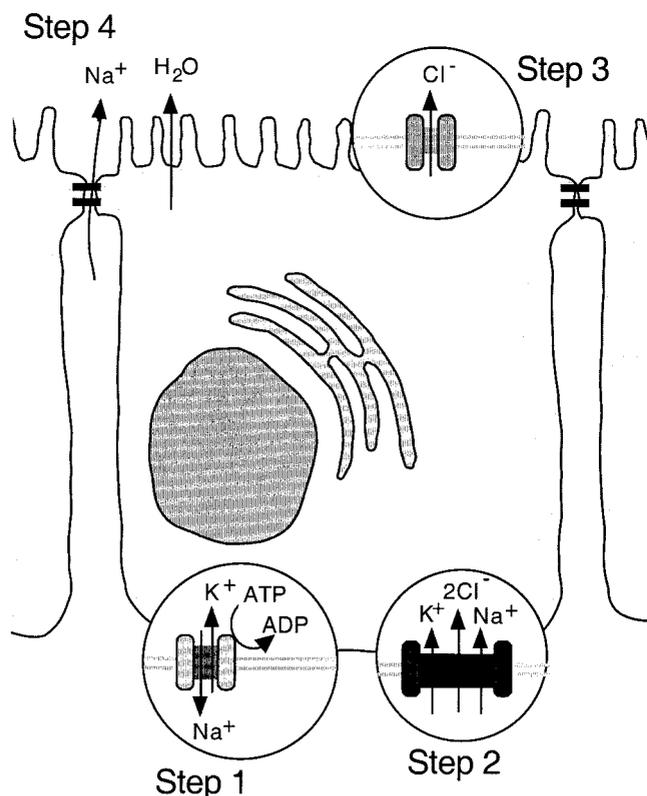
FIG. 2. Chloride secretion in a pulmonary secretory epithelial cell. The ability of secretory epithelia to secrete fluid rests on the energy provided by the ubiquitous, basolaterally located Na^+/K^+-ATPase (Step 1), which maintains a low intracellular concentration of Na^+ by actively pumping it out of the cell. The low intracellular Na^+ concentration, coupled with the high extracellular Na^+ concentration and the negative transmembrane potential, drives the passive diffusion of Na^+ into the cell down the concentration gradient. The channel through which this passive diffusion occurs (the $Na^+/K^+/2Cl^-$ cotransporter) requires concomitant transport of Na^+, K^+, and Cl^- for any transport to occur at all. Thus, the passive diffusion of Na^+ into the cell is coupled with an intracellular accumulation of Cl^- against its electrochemical gradient (Step 2). When the cell is stimulated to secrete, Cl^- channels open, allowing Cl^- to exit down its electrochemical gradient (Step 3). Sodium ions follow the Cl^- ions through a paracellular pathway, and water follows the salt due to the resulting osmotic gradient (Step 4).

centrations of extracellular ATP or UTP stimulated ORCC in both normal and CF airway epithelial cells (284), consistent with the hypothesis that ATP regulated ORCC through a purinergic receptor. However, CFTR-modulated ORCC activation by cAMP and PKA has been shown to require a physiological intracellular concentration of ATP (5 nM) (285) as well as the extracellular release of ATP (141, 284). The centrality of CFTR in the autocrine/paracrine ATP signaling between CFTR and ORCC and other epithelial anion channels (313) led to the disputed hypothesis that CFTR itself conducts ATP. Several investigators have provided evidence to support this hypothesis (254; E. H. Abraham, P. Okunieff, S. Scala, P. Vos, M. J. Oosterveld, A. Y. Chen, and B. Shrivastav, Letter, Science **275:**1324–1326, 1997), while others have failed to demonstrate CFTR-dependent ATP conductance (117, 252). More recent work has suggested that CFTR regulates a closely associated but separate ATP channel (176, 314).

Analogous debates center on the ability of CFTR to modulate or directly mediate the secretion of bicarbonate ions (HCO_3^-). Transepithelial secretion of HCO_3^-, like the transepithelial secretion of Cl^-, probably requires the coordinated activity of a variety of transporters including (i) an apical anion channel, (ii) a Cl^-/HCO_3^- exchanger, (iii) HCO_3^- uptake across the basolateral membrane, (iv) HCO_3^- production by intracellular carbonic anhydrase, and (v) paracellular transport (133). In the small intestine, HCO_3^- secretion activated by cAMP, cGMP, or Ca^{2+} requires the presence of CFTR (125, 126). Indeed, duodenal mucosal cells from CF patients display significantly lower basal secretion of HCO_3^- than do cells from patients expressing wild-type CFTR (240). However, similar to ATP stimulation of Cl^- secretion in airway cells, gallbladder epithelium lacking functional CFTR can be stimulated to secrete HCO_3^- via UTP activation of a purinergic receptor that in turn activates a Ca^{2+}-dependent channel (53). Defective CFTR results in defective HCO_3^- secretion in airway epithelial cells, in addition to gastrointestinal mucosal cells (134, 302). However, evidence for direct permeation of HCO_3^- through CFTR is equivocal. Patch-clamp studies by some groups have demonstrated that HCO_3^- permeates through CFTR, although four to seven times less well than does Cl^- (177, 239). Nonetheless, HCO_3^- uptake in CF and normal

TABLE 1. Molecules that interact with CFTR[a]

Molecule	Function	Interaction with CFTR	Reference(s)
Protein channels			
ORCC	Outwardly rectifying chloride channel	CFTR required for activation by PKA and PKC	140, 141, 284, 286
ENaC	Epithelial sodium channel	NBD-1, R domain binding inhibits ENaC function	166, 273
ROMK2	Renally derived K^+-ATP channel	TMD1, NBF1, and R domain of CFTR interact	46
Aquaporin 3 (AQP3)	Water channels in airway epithelial cells	CFTR-dependent activation via NBD1	274, 275
Transporters			
NKCC1	$Na^+/K^+/2Cl^-$ cotransporter	Wild-type CFTR upregulates expression	296
NBC-1	Electrogenic Na^+/HCO_3^- cotransporter	CFTR-Cl^- secretion potentiates NBC function	295
Other proteins			
Syntaxin 1A	Membrane traffic machinery	Binds 1:1 CFTR N terminus, inhibits CFTR function	202, 203
NHERF/EBP50	Na^+/H^+ exchanger regulatory factor/ERM-binding phosphoprotein 50	Binds to PDZ motif at CFTR C terminus	31, 119, 294

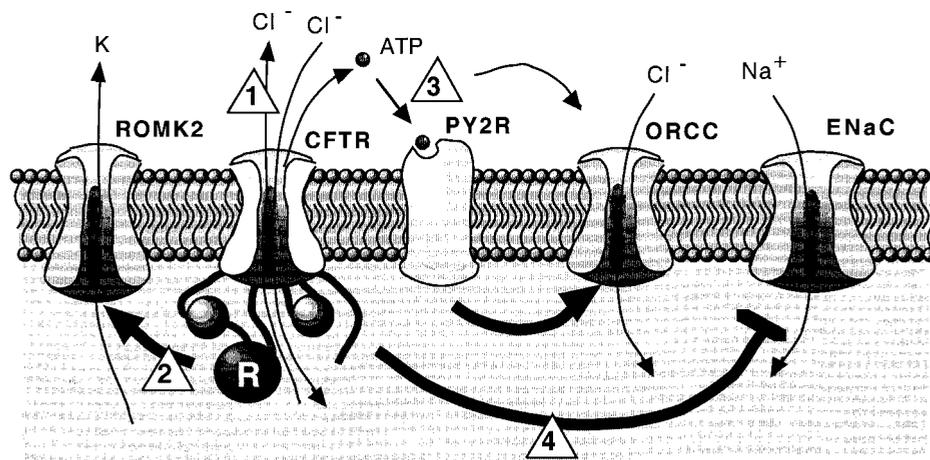[a] Reprinted from reference 229a with permission from Elsevier Science.

FIG. 3. CFTR regulates apical ion transport via several mechanisms. The first of these mechanisms is the innate function of CFTR as a chloride channel (triangle 1). Second, the R domain of CFTR associates with and regulates the activity of the potassium channel ROMK2 (triangle 2). Third, CFTR mediates transport of ATP across the plasma membrane. This extracellular ATP can then bind to the purinergic receptor (PY2R), which regulates the activity of the ORCC (triangle 3). Lastly, there is evidence that CFTR can directly activate the chloride import activity of the ORCC and repress the sodium channel ENaC (triangle 4). The plasma membrane shown in the figure represents the surface of a generic epithelial cell, with characteristics of epithelia from several tissues.

sweat duct cells is comparable (23), as is cytosolic pH regulation of CF and CFTR-corrected nasal epithelial cells (133), perhaps due to the predominance of alternate HCO_3^--conductive pathways. An important role for HCO_3^- transport in the pathogenesis of CF was provided by the recent study of Choi et al. (51), who found that *CFTR* mutations that do not support HCO_3^- transport are associated with the more severe pancreatic-insufficient phenotype, whereas *CFTR* mutations that lead only to reduced HCO_3^- transport are associated with the pancreatic-sufficient phenotype.

Clinical Manifestations of Mutations in *CFTR*

Defects in epithelial Na^+, Cl^-, and HCO_3^- transport, and accompanying abnormalities in fluid secretion, underlie many of the clinical manifestations of CF (56, 68). The destruction of the exocrine pancreas is attributed to autodigestion of the acinar tissue following plugging of the pancreatic ducts by secretions that are thickened due to decreased fluid flow by the pancreatic acinar cells. Approximately 5 to 10% of patients with CF present with a gastrointestinal blockage known as meconium ileus, which is linked to accumulation of fecal material secondary to inadequate fetal pancreatic enzyme production and diminished intestinal fluid secretion. The decrease in pancreatic enzyme production leads to malabsorption, particularly of fats, and manifests as failure to thrive, the most common feature of CF in infants and children. Older patients have an increasing incidence of diabetes mellitus, associated with destruction of the pancreas. Obstruction of the hepatic ducts has been hypothesized to be the etiology of the liver disease in CF. Abnormal fluid secretion may contribute to the cardinal respiratory features of CF, including the ubiquitous pansinusitis, through a decrease in the airway surface fluid volume, which impairs the activity of the mucocilliary escalator. Patients may present, particularly in the summer months, with hyponatremic dehydration (dehydration due to loss of sodium, as experienced by many marathon runners) and metabolic alkalosis (increased serum pH accompanying electrolyte derangements, as experienced following severe vomiting) due to electrolyte losses in the sweat.

Other features of CF are not clearly related to the role of CFTR as a coordinator of fluid secretion. For example, the link between CFTR defects and congenital bilateral absence of the vas deferens (CBAVD), which renders most males with defective CFTR infertile (55), is unclear. The role that CFTR plays in some tissues in which it is highly expressed, such as heart and kidneys, which have conspicuously normal phenotypes in CF patients (aside from the somewhat greater propensity of CF patients to develop kidney stones) is also not clear.

Genetic and Functional Aspects of Mutations in *CFTR*

The hereditary nature of CF was first demonstrated by Andersen and Hodges (7), who in 1946 published a pedigree-type analysis of CF in 20 affected families. CF was found to occur with a frequency approximating 25% in the affected families—the value expected for an autosomal recessive disorder inherited in a classical Mendelian fashion. The observation (77) that the sweat of CF patients has an abnormal electrolyte content provided an additional clue to the etiology of CF. The abnormal electrolyte content was found to be due to an absence of the normal chloride conductance in the sweat duct (243). The cause of this defect in chloride conductance and of its mode of inheritance was determined in 1989, when the *CFTR* gene was identified and cloned (146, 258, 260). The *CFTR* gene was cloned without any knowledge of its function,
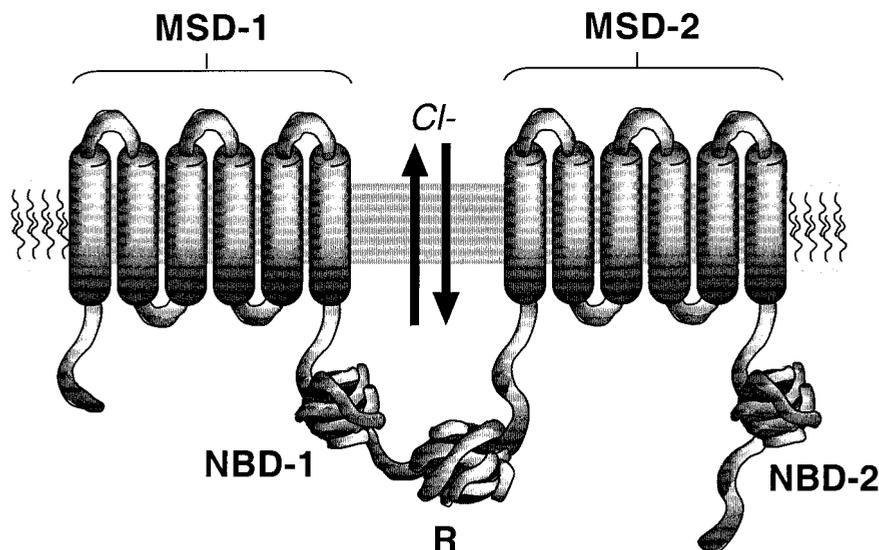
FIG. 4. Schematic diagram of the proposed structure of CFTR. A member of the ABC family, CFTR consists of a tandem repeat of the ABC motif. This motif comprises a membrane-spanning domain (composed of six transmembrane stretches of amino acids) followed by an NBD. In CFTR, the two occurrences of this motif are separated by a regulatory (R) domain. Each NBD is able to bind and hydrolyze ATP to operate chloride channel function: hydrolysis of ATP by NBD-1 opens the chloride channel, while ATP hydrolysis by NBD-2 closes the channel. Channel function is further regulated by phosphorylation of serine residues in the R domain.

by screening total RNA from various tissues with cDNA probes and by "reverse genetics" techniques based solely on the chromosomal location of the gene. The gene was identified from an mRNA which was abundant in tissues adversely affected in CF patients (258). Chromosomal walking experiments (260) confirmed the localization of the *CFTR* gene to the long arm of chromosome 7, the region to which the CF gene had previously been mapped (155, 327, 330). The identity of the *CFTR* gene as the gene responsible for the CF phenotype was further supported by the fact that CF patients were found to have homozygous loss of function at this genetic locus (258). The *CFTR* gene product has homology to a large family of transporters including the multidrug resistance protein, p-glycoprotein. Since the structure did not clearly reveal the function of the CF gene product, the protein was named cystic fibrosis transmembrane conductance regulator (CFTR) to reflect its role as either a channel, a regulator of channels, or, as is now known to be the case, both (258). The chloride channel function was later confirmed in transfection studies demonstrating that introduction of the *CFTR* gene into cell lines resulted in increased chloride flux (9).

The CFTR protein (Fig. 4) is a member of the ATP binding cassette (ABC) family of transporters. Members of this protein family are found in mammals, insects, yeast, and bacteria and include the multidrug resistance efflux pump (MDR1) (257), the transporters associated with antigen processing (TAP1 and TAP2) (307), and the bacterial histidine permease (178, 206). The highly conserved motif that defines the ABC family of proteins includes a membrane-spanning domain, containing six membrane-spanning peptides, followed by a nucleotide binding domain (NBD), which is responsible for the ATP binding and hydrolysis that supplies energy to drive the opening and closing of the ion channel. Like many members of the ABC family, CFTR consists of a tandem repeat of this motif.

The membrane-spanning domain of ABC family proteins typically consist of six membrane-spanning regions (although in some cases, five transmembrane regions have been predicted). Sequence comparisons conducted by Manavalan et al. (184) demonstrated that the amino acid sequences of the transmembrane hydrophobic stretches are only mildly conserved. However, the length of the hydrophilic loops that connect the membrane-spanning regions is highly conserved, suggesting that the spacial topology of the membrane-spanning regions is crucial for channel function. In addition, despite the low conservation of amino acids that comprise the membrane-spanning domains, specific amino acids in these regions can be important for proper functioning of CFTR. For example, *CFTR* mutations that cause proline residues to occur within transmembrane regions have been found in some cases to result in reduced chloride conductance and in other cases to alter the selectivity of transported ions (291). These data suggest that alterations in residues within the membrane-spanning regions (or alterations in the spatial arrangement of the transmembrane regions) affect the diameter of the pore for the chloride ion. This can result in a loss of channel function, when the pore size is reduced, or in the transport of ions larger than chloride, when the pore diameter is increased. Interestingly, one proline mutation caused reduced synthesis of CFTR compared to that in the wild type (291). Given the wide variety of phenotypes observed in such mutational studies, generalizations regarding the effect of mutations within the membrane-spanning domains of CFTR are difficult to make.

The NBDs of CFTR are responsible for the binding and hydrolysis of ATP and provide the energy necessary for channel activity (8). A recent study (104) reports that the two NBDs of CFTR function in a coordinated fashion to sequentially mediate the opening and closing of the ion channel pore. Thus, the N-terminal NBD (NBD-1) hydrolyzes one molecule of

TABLE 2. Classification of CFTR mutations[a]

Class	Description	Translation	Processing	Function	Regulation	Common mutations
I	No CFTR protein	−	−	−	−	G542X, W1282X, R553X
II	Defective protein	+	−	+	+	ΔF508
III	cAMP unresponsive	+	+	+	−	G551D, N1303K
IV	cAMP responsive, reduced conductance	+	+	±	+	R117H, R347P
V	Reduced synthesis/processing	±	±	+	+	5T, 621 + 1 G→T[b]

[a] As proposed by Wilschanski et al. (343).
[b] G to T at position +1 of intron 4, which starts at nucleotide 621.

ATP to open the channel and then the C-terminal NBD (NBD-2) hydrolyzes a second molecule of ATP to close the channel. The NBDs of CFTR contain motifs that are common to the nucleotide binding folds of many ABC- and non-ABC family proteins, but they also contain motifs that are restricted to the ABC family or to a subset of ABC proteins. The most widely conserved ABC family motifs are the Walker A motif (at the amino-terminal end of each NBD) and the Walker B motif (at the carboxy-terminal end of each NBD), which function in the binding and coordinate interaction of ATP and Mg^{2+}, respectively. The Walker A motif has the sequence GxxGxGK(S/T), where x is any amino acid. Mutations within the Walker A motif have a strong negative effect on channel activity (245). The Walker A motif is also referred to as the phosphate loop or P-loop, since its constituent amino acids make direct contacts with the α-, β-, and γ-phosphates of ATP. The Walker B motif has a less stringent sequence, which can be represented $R(x)^{6-8}\phi\phi\phi\phi D$, where x is any amino acid and φ is any hydrophobic amino acid. In addition to the two widely conserved Walker motifs, NBDs of the ABC family proteins contain a motif which is unique to this family—the signature motif or C motif. The C motif lies between the Walker A and B motifs, just upstream of the Walker B motif, and has the consensus sequence LSGGQ. The NBD of CFTR also contain a fourth conserved motif that appears in only a subset of ABC family proteins. This motif is the center region, which lies at the midpoint between the Walker A and B motifs. While mutations within the Walker A, Walker B, and signature motifs usually impair protein function, the importance of the center region is variable depending on the particular transporter protein. The yeast α-factor transporter STE6, for example, is tolerant of mutation within its center region (18), while other ABC proteins (CFTR, for example) are quite sensitive to mutations in this motif. For example, the most common mutation, ΔF508 *CFTR*, is a center-region mutation.

In addition to the two protein domains already described (membrane-spanning domain and NBD), CFTR contains a regulatory (or R) domain, which modulates the channel activity of CFTR (49, 318). Only a small subset of ABC family proteins contain an R domain. Other examples of R domain-containing ABC proteins are the yeast YCF1 protein, which confers cadmium resistance to yeast (317), and the MRP1 multidrug resistance gene (145). A model of R domain function has been proposed by Ma et al. (180), who suggests that when dephosphorylated, the R domain interacts with the N-terminal NBD (NBD-1), thus blocking the ATP binding site on the NBD. With ATP unable to bind, channel opening cannot occur. According to the same model, when the R domain becomes phosphorylated, it either dissociates from the NBD or

interacts with the NBD in a different way, such that the ATP binding site of NBD-1 is available. Further evidence for the important role of the R domain in channel activity was obtained through the analysis of R-domain point mutants, which demonstrated either reduced channel activity or complete loss of activity (219). Deletions in the R domain result in regulatory defects (180). The physical interaction of the R domain with NBD-1, as well as the phosphorylation dependence of this interaction, has recently been demonstrated (205), supporting the model of Ma et al. However, the actual mechanism by which the ion channel activity of CFTR is regulated is probably more complex. For example, data from other groups has shown that phosphorylation in the R domain affects channel activity differently depending on which serine residue(s) of the R domain is phosphorylated (341, 345).

CFTR Mutations

Over 1,000 naturally occurring mutations have been identified in the *CFTR* gene (see the *CFTR* mutation database maintained by the Cystic Fibrosis Genetic Analysis Consortium at http://www.genet.sickkids.on.ca/cftr/). The mutations identified to date occur throughout the *CFTR* gene and include many types of mutations including missense, nonsense, and frameshift, mutations, splice variants, and in-frame amino acid deletions. Wilschanski et al. categorized *CFTR* mutations according to the mechanism by which the protein alteration affects chloride secretion (Table 2) (343). The phenotypes generated by these mutations range in severity; some *CFTR* mutations have a completely normal phenotype while others cause severe CF impacting many organ systems. Even if one restricts one's interest only to mutations that cause clinical CF disease, one is still left with a large number of mutant alleles that results a wide variety of clinical conditions.

Early attempts to characterize *CFTR* mutations were hindered by the lack of a noticeable phenotype in *CFTR* heterozygous individuals, by the wide range of clinical presentation (resulting in the identification of mutant alleles being dependent on the prevailing clinical definition of CF), and by the extreme preponderance of one mutant allele, ΔF508. An important advance in the identification of *CFTR* mutant alleles was the refinement of molecular genetics approaches. Early attempts to track the distribution of CF alleles relied on restriction fragment length polymorphism linkage analysis, which was fraught with error due to the loose linkage of common marker haplotypes with the *CFTR* gene. The advent of techniques such as single-strand conformational polymorphism allowed the screening of large numbers of individuals for mutant alleles. This, together with a more rigorous study of the epi-

demiology of the disease, allowed the identification of many novel *CFTR* alleles, some with subtle clinical manifestations.

In addition to mutations resulting in changes in amino acids comprising the CFTR protein, other mutations affecting transcript production, such as the so-called 5T allele, can impact CFTR levels. In the 5T allele there is a variation in the length of the polypyrimidine tract in the splice acceptor site at the 3′ end of intron 8. While the normal splice acceptor site at this location has nine tandem thymidines, shortening of the polypyrimidine tract first to seven and then to five thymidines, results in inefficient splicing of the *CFTR* transcript. Although this mutation was originally characterized as the causative factor of CBAVD (58), the 5T allele has more recently been shown to manifest itself as the cause of mild but clinically detectable pulmonary dysfunction (55). Moreover, this mutation can cause pancreatic insufficiency, although the latter sequela appears to be uncommon (146). The mild and highly variable phenotype associated with the 5T allele has complicated its characterization. In a study conducted in 1997, Kerem et al. reported that of a cohort of approximately 150 subjects, those with typical or atypical CF had an approximately three-fold-higher incidence of the 5T allele than did individuals without CF (147). The subjects in this study who carried the 5T allele presented with a wide range of symptoms including asthma, bronchitis, bronchiectasis, meconium ileus, and pancreatic insufficiency. Even the sweat chloride levels of 5T allele carriers exhibited a wide range from normal to elevated, confounding the use of sweat chloride levels as the pathognomonic feature of CF. The 5T allele probably results in a more severe phenotype only when it is present in a compound heterozygous state with another mutant allele (T. Bienvenu, J. Lepercq, J. P. Allard, D. Hubert, C. Francoval, C. Beldjord, and J. C. Kaplan, Letter, Ann. Genet. **41:**63–64, 1998).

The ΔF508 *CFTR* mutation is by far the most common mutant allele, accounting for some 70% of all mutant *CFTR* alleles. This mutation is a deletion of CTT, containing the third nucleotide of the ATC codon for isoleucine at position 507 and the first two TT nucleotides of the TTT codon for phenylalanine at position 508 within the first NBD. The wild-type ATC codon becomes ATT, which also codes for isoleucine, and the normal coding sequence of a GGT codon for glycine at position 509 remains intact. Cellular localization experiments and CFTR glycosylation experiments have demonstrated that in many cell lines the ΔF508 mutant CFTR protein does not traffic to the Golgi network (335), a requirement for membrane expression. More recently, it has been shown that the ΔF508 CFTR protein is mostly retained in the endoplasmic reticulum but slowly leaks to the endoplasmic reticulum-Golgi intermediate compartment (107). This incompletely processed ΔF508 CFTR protein is eventually degraded intracellularly. Ward et al. showed that ubiquitin-ΔF508 CFTR conjugates accumulate in cells when proteosomes are inhibited (333), suggesting that degradation of ΔF508 mutant CFTR protein proceeds at least partially through a ubiquinated intermediate. Consistent with these results is the observation that ΔF508 CFTR is found in extremely small quantities at the apical plasma membrane of cultured cells (335). The low level of membrane expression of CFTR in ΔF508-homozygous cells results in low or unmeasurable chloride ion conductance (338). This reduced ion conductance is probably responsible, at least

in part, for the abnormal composition of epithelial secretions in CF patients. Processing defects similar to those described for ΔF508 CFTR have been noted in mutant *CFTR* alleles containing missense mutations in the third cytoplasmic loop (in the second membrane-spanning domain). These missense mutants migrate faster than wild-type CFTR in polyacrylamide gels, demonstrate sensitivity to the enzyme endoglycosidase H (suggesting incomplete processing of N-linked carbohydrates), and have chloride channel activity suggesting alterations in their "open probability" (287).

Countering these observations is that of Kalin et al. (142), who used a panel of monoclonal and polyclonal antibodies to CFTR to localize the expression of the ΔF508 CFTR protein in tissues from CF patients. They found a tissue-specific variation in membrane expression of ΔF508 CFTR ranging from none to levels commensurate with those of wild-type CFTR. Notably, they reported that the expression levels of the ΔF508 CFTR protein equaled that of wild-type CFTR expression in intestinal and respiratory tract tissue sections, the two tissues primarily affected in CF patients. Thus, membrane expression of ΔF508 CFTR may be normal, implicating aberrant protein function as the cardinal feature leading to disease. Smith et al. (303) showed that there was no chloride conductance activity in airway tissues from ΔF508 CF patients, consistent with a loss of CFTR function in these patients. On the other hand, Engelhardt et al. (87) reported that the submucosal glands are the site in the respiratory tract where CFTR is prominently expressed, and they could not detect any CFTR protein in patients with the ΔF508 *CFTR* allele. At this point the debate over whether the major problem with the ΔF508 CFTR protein is in its expression or function is not resolved.

Interestingly, recombinant expression of ΔF508 CFTR in either insect cells or frog oocytes resulted in levels of mutant CFTR protein in the plasma membrane similar to wild-type levels (71). This observation led Denning et al. to conclude that the ΔF508 mutation encodes a temperature-sensitive protein (xenopus oocytes and insect Sf9 cells are routinely cultured at room temperature). They also identified a second defect in ΔF508 CFTR: ΔF508-homozygous cells grown extensively at reduced temperatures did not recover a level of ion conductance commensurate with the increase observed in normally processed CFTR protein (71). Thus, in these studies, the ΔF508 CFTR exhibited a defect in chloride ion conductance. While wild-type CFTR protein has a probability of 0.34 of being in the open state, ΔF508 CFTR protein has an open probability of only 0.13. Therefore, the ΔF508 mutation affects CFTR by at least two distinct mechanisms, reducing the levels of protein reaching the plasma membrane and diminishing the ion channel activity of the CFTR protein that does reach the cell surface.

Epidemiology of CF and *CFTR* Mutations and Possible Advantages for Heterozygotes

The ΔF508 allele of *CFTR* is extremely common in certain populations; it is estimated to be carried at a frequency of 2 to 5% in Caucasians of European descent. Although the ΔF508 allele accounts for 70% of all mutant *CFTR* alleles, there is considerable regional variation, from as low as 27% of CF alleles in Turkey (131) to nearly 87% of CF alleles in Denmark

(283). Data obtained in a multicenter epidemiological study of CF show that as of 1998, approximately 50% of genotyped CF patients were homozygous for the ΔF508 mutation while an additional 25% were compound heterozygotes in whom one *CFTR* allele contained the F508 deletion (50). Cystic fibrosis occurs in males and females with approximately equal frequencies, although clinically male patients have slightly better health than do female patients (70). Although the prevalence of the CF mutation was originally theorized to be due to genetic drift (347) and was somewhat later theorized to result from a single genetic event (88), we now know that in addition to the genetic event that produced the predominant mutant *CFTR* allele, ΔF508, the more than 1,000 other mutant alleles have arisen as independent events (351). The high frequency of the ΔF508 allele of *CFTR* in specific populations suggests that selective pressure has been operative, perhaps due to heterozygote advantage. Studies aimed at discovering such an advantage have focused primarily on examination of physiological functions that are usually impaired in clinical CF: pulmonary function, intestinal absorption, and reproductive function. Results obtained from such studies have so far failed to uncover a consistent interpretation favoring the heterozygote advantage theory. Examination of pulmonary function among *CFTR* wild-type carriers and ΔF508 *CFTR* heterozygotes has, in one study, suggested that the latter are protected against asthma (276). However, these results have been refuted by others (195).

Early suggestions of increased fertility secondary to decreased fetal loss, along with a greater proportion of male births among CF heterozygotes, were not confirmed when results were analyzed after ascertaining the true parentage of affected offspring used to identify obligate carriers (139). Others have reported no overall increased fecundity in ΔF508 *CFTR* heterozygotes but have found that smoking was a potential modifier, with nonsmoking heterozygous parents showing increased family size and smoking heterozygous parents having decreased family size (63).

Another major hypothesis posits that mutations that reduce CFTR production or activity confer protection against a potentially fatal infection, including suggestions that individuals heterozygous for *CFTR* mutations are resistant to influenza (293), tuberculosis (143), cholera (102), and typhoid fever (233). The proposals for influenza and tuberculosis resistance were speculative hypotheses lacking experimental data. Although Gabriel et al. (102) found evidence for decreased fluid secretion in the intestinal lumen of heterozygous transgenic CF mice challenged with cholera toxin, Cuthbert et al. (60) could not confirm this finding. Cholera is unlikely to have been the selective factor, since *Vibrio cholerae* did not enter Europe, the site of concentration of mutant *CFTR* alleles, until 1832 during the second pandemic. It has been proposed that in order for the ΔF508 allele to reach its current level of occurrence, over two-thirds of Europeans of reproductive age would have had to die from cholera between 1832 and 1900, when public health measures bought the disease under control (20). Clearly, this disaster did not happen. However, other diarrheal toxins such as *Escherichia coli* labile toxin, could have been a factor in a heterozygous advantage for CF predicated on resistance to diarrheal disease.

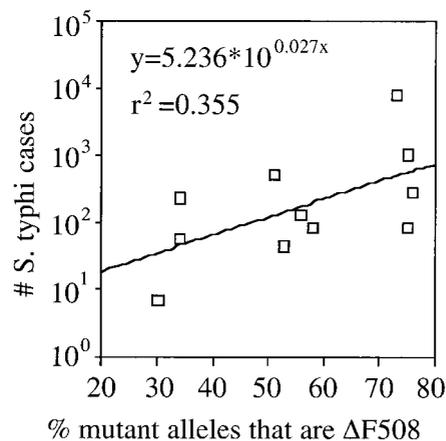Research evaluating the potential role of typhoid fever as

FIG. 5. Correlation in 11 European countries of prevalence of the ΔF508 *CFTR* allele with the incidence of *S. enterica* serovar Typhi infection 23 years earlier.

selection for a heterozygous advantage in CF was based on the finding that the CFTR protein serves as a receptor for gastrointestinal epithelial cell internalization and submucosal translocation of *Salmonella enterica* serovar Typhi. Since typhoid fever is life-threatening and typically affects individuals aged 3 to 19 years (136), resistance to this disease would probably confer a reproductive advantage. Pier et al. (233) demonstrated that the efficiency with which serovar Typhi invades intestinal epithelium is directly related to the amount of available CFTR protein on the epithelial surface. Thus, the level of bacterial invasion into ΔF508-heterozygous epithelium is 80% lower than the level of invasion observed with wild-type epithelium, probably owing to the lower level of CFTR expressed on ΔF508-heterozygous epithelia (233). In an effort to apply this model on a population scale, we examined the correlation between the incidence of serovar Typhi infection in several European countries (46) and the prevalence of the ΔF508 *CFTR* allele in the same geographic location one or two generations later (see reference 326 and references therein). The hypothesis driving this comparison was that outbreaks of serovar Typhi infection can serve as a selective pressure favoring maintenance of the ΔF508 *CFTR* allele. The results of this comparison are shown in Fig. 5 and suggest that a positive correlation may, in fact, exist. Note that the ΔF508 genotype data used in this correlation indicate the percentage of mutant *CFTR* alleles that are ΔF508 and that the genotype data represent the allele frequencies approximately 23 years after the reported incidence of serovar Typhi infection. It must be stressed that this comparison reflects the selective pressure applied by only one factor (serovar Typhi infection) over a time frame (3 years) which is very brief in evolutionary terms. However, given the potential relationship between serovar Typhi infection and the occurrence of the ΔF508 *CFTR* allele (233), the results of this comparison are provocative.

Besides the ΔF508 deletion, several other mutations have been characterized which also affect CFTR in the vicinity of phenylalanine 508, suggesting that this region of the *CFTR* gene is a mutational hot spot. Examples of these mutations are ΔI506, ΔI507, I506V, and F508C. While deletions in this region of the protein (i.e., deletions at amino acid 506, 507, or

508) result in an extremely severe disease phenotype (204, 335), missense mutations, such as I506V and F508C, are benign (157). This is consistent with the view that the severe phenotype resulting from a deletion of phenylalanine 508 is the result of a perturbation in the amino acid spacing of the protein, probably leading to misfolding of the protein and improper trafficking and maturation. Identification of these other mutations near phenylalanine 508 was hindered by two factors. The first was the mild phenotype associated with missense mutations such as I506V. Second, analytic methods such as restriction fragment length polymorphism were not able to distinguish between in-frame deletions such as ΔI506 and the far more common ΔF508 allele. Therefore, the presence of these other in-frame deletions was not realized until more sensitive genetic techniques were developed.

MICROBIOLOGIC ASPECTS OF CYSTIC FIBROSIS LUNG INFECTION

Recovery and Distribution of Microbial Pathogens among CF Patients

In the healthy respiratory system, the upper respiratory tract is colonized by a wide variety of microorganisms comprising the normal flora while the lower respiratory tract is maintained in a sterile state by the various innate defenses of the host. These defenses consist of physical barriers and endocytic/phagocytic barriers. Failure of any of these innate defenses results in susceptibility to pulmonary infection. Young CF patients, particularly before the onset of *P. aeruginosa* infection, are usually unable to expectorate sputum derived from secretions in their lower respiratory tract, and therefore oropharyngeal cultures (i.e., upper respiratory tract secretions) are usually performed to detect pathogens. However, such samples are often referred to as sputum cultures and any pathogens detected are said to be isolated from the sputum. In reality, these cultures detect organisms, including potentially pathogenic ones, present in the throat and not necessarily in the lungs.

Over the past decade, several studies have shown that there can be important differences related to the detection of CF pathogens in the lower airway when comparing results from deep throat cultures with those obtained using BAL fluid, particularly in young CF patients. Ramsey et al. (250) found, in nonexpectorating CF patients in optimal respiratory status, a high positive predictive value of oropharyngeal cultures for the presence of *P. aeruginosa* and *S. aureus* in the lower airway, which was determined by BAL cultures, but a poor negative predictive value; 46% of younger, nonexpectorating patients had *P. aeruginosa* in their BAL fluid but a negative throat culture. Similarly, 21% had *Klebsiella* spp. in their BAL fluid but not the oropharyngeal culture. Ironically, the same group later reported opposite findings (41): a high negative predictive value of the oropharyngeal cultures for presence of organisms in the lower airway, particularly if two sequential cultures were considered (85% for one culture, 97% for two), and a lower positive predictive value (69% for one, 83% for two). Similarly, for CF patients younger than 5 years, Rosenfeld et al. (261) reported a better (95%) negative predictive value of oropharyngeal cultures lacking detectable *P. aeruginosa* for ruling out the presence of this organism in the lower airway but a low

(44%) positive predictive value for detecting its presence in BAL fluid. Armstrong et al. (11) obtained a similar result in CF infants diagnosed via a neonatal screening program, with oropharyngeal cultures having a high (97%) negative predictive value for CF pathogens (*S. aureus*, *P. aeruginosa*, and *H. influenzae*) but poor positive predictive value (41%). Thus, some data indicate that CF pathogens can be present in the lower airway, but not reliably detected by throat culture, while other data, mostly from patients younger than 5 years, suggest that positive throat cultures are not necessarily indicative of pathogens in the lungs. One important caveat is that BAL fluid samples are obtained from only a small portion of the lung, leaving the possibility that pathogens might be present in parts of the lung not sampled by lavage. Therefore, it seems that the positive findings of Ramsey et al. (247), which showed detection of pathogens in the BAL at a high rate, may be more convincing than the negative findings of the other groups. Newer techniques such as fluorescent in situ hybridization (127) analysis of clinical specimens from CF patients may improve on the specificity and sensitivity of the detection of CF pathogens.

S. aureus, H. influenzae, and CF

Many of the organisms that are isolated from CF sputum are pathogens that often benignly colonize the upper respiratory tract (e.g., nontypeable *H. influenzae*) or the nose (e.g., *S. aureus*) or are common environmental organisms that behave as pathogens only under certain opportunistic situations (e.g., *P. aeruginosa*). Data collected in a 1998 multicenter study of CF patients showed that *P. aeruginosa, S. aureus*, and *H. influenzae* could be cultured from the sputum or respiratory tract secretions of 61, 47, and 16% of tested CF patients, respectively (61). A major problem inherent in understanding microbial aspects of CF lung infection is whether the presence of a potentially pathogenic organism in sputa or upper (not lower) respiratory tract secretions is indicative of a pathologic situation. The problems noted above regarding the predictive values of oropharyngeal cultures for the presence of pathogens in the lower airway bear directly on this issue. Data related to the pathogenic potential of nontypable *H. influenzae* are virtually nonexistent, yet many clinicians regard the possibility of this organism colonizing the lung as significant enough to warrant therapy.

More problematic is the definition of the contribution to CF lung disease of *S. aureus*. This organism is usually cultured only from the nose of healthy individuals, not the throat or respiratory secretions, yet it is often considered to be among the first pathogenic organisms when isolated from the CF respiratory tract (6), usually by throat culture. Clearly the presence of *S. aureus* in the lower respiratory tract is representative of a pathologic situation, but the degree of pathology associated with its presence in the lungs has never been adequately assessed in CF patients. Ulrich et al. (328) located *S. aureus* in the lungs of three infected CF patients, and this microorganism, like *P. aeruginosa*, was found predominantly in the mucus of obstructed airways. This finding is clearly indicative of a pathologic situation. What is not entirely clear is the proportion of CF patients with *S. aureus* in their lower airway causing frank disease. One likely reason why this has not been ade-

quately determined had been the routine use of anti-staphylo-coccal antibiotics in this patient population, potentially preventing the progression of *S. aureus* infection to a highly pathologic state that could be readily identified clinically.

Nonetheless, attempts have been made to evaluate the efficacy of routine or intermittent use of antistaphylococcal antibiotics in CF patients. McCaffery et al. (191) identified 13 clinical trials of antistaphylococcal antibiotic trials in CF patients. These trials used 19 different antibiotics and a variety of clinical and laboratory outcomes and involved both intermittent and continuous administration of antibiotics. While sputum clearance of *S. aureus* was achieved in most studies, none documented a positive effect on pulmonary function or other clinical outcome. On the contrary, a recent study of 3,219 CF patients in the European Registry of Cystic Fibrosis demonstrated that continuous antistaphylococcal prophylaxis increases the rate at which patients' sputum cultures converted from *P. aeruginosa* negative to *P. aeruginosa* positive (251). The patients who were monitored in this ~3-year study were categorized both according to their age and according to whether they received continuous (200 or more days per year), intermittent (only during acute exacerbations), or no antistaphylococcal therapy. The results of this study showed that *P. aeruginosa* acquisition in the group receiving continuous antistaphylococcal therapy was significantly higher than in the those receiving no or only intermittent therapy. This difference was most significant in the 0- to 6-year age group and was not significant in the > 12-year age group. Importantly, monitoring of the lung function (forced expiratory volume in 1 s [FEV1], a measure of small airway colonization) and body mass index of the same patients showed no significant differences between the three antibiotic treatment groups during this same period, suggesting that differences observed in *P. aeruginosa* acquisition were not simply a function of the patients' general health (although Ratjen et al. do not completely disregard this possibility [251]). This report bolsters previous work that had been published only in abstract form (H. R. Stutman, Abstract, Pediatr. Pulmonol. Suppl. **13**:542, 1994). Thus, while there is a consensus among clinicians about a beneficial effect from treatment of staphylococci associated with clearance of the organism from the sputum, there are no data indicating that this treatment leads to improved lung function or other clinical benefit. Indeed, several studies have shown that the presence of *S. aureus* and the absence of *P. aeruginosa* predicts long-term survival in CF patients after the age of 18 years (129, 130). In addition, the potential for increasing *P. aeruginosa* colonization as a consequence of suppression of *S. aureus* infection should not be overlooked.

Thus, most of the data implicating a pathogenic role for *S. aureus* in development of CF lung disease comes from historical findings, reasonable speculation, and the fact that the presence of *S. aureus* in the lower airway is judged to be clinically important and in need of antibiotic therapy. It has been suggested (112) that early infection "primes" the CF airway for later infections by *P. aeruginosa*. Whether there is indeed a progression from *S. aureus* to *P. aeruginosa* infection is questioned by the study of Burns et al. (41), who found evidence by culture and serologic testing for a 97.5% *P. aeruginosa* infection rate in CF children by the age of 3 years. Certainly, before the advent of antibiotic therapy, *S. aureus* was

TABLE 3. Antimicrobial susceptibility rates of *P. aeruginosa* isolated from intensive care units in 1990 to 1993[a]

Antimicrobial agent(s)	% of isolates that were susceptible
Amikacin	89
Tobramycin	93
Gentamicin	65
Piperacillin	90
Mezlocillin	78
Ticarcillin	86
Imipenem	87
Ciprofloxacin	89
Ceftazidime	86
Ceftriaxone	23
Cefotaxime	16
Aztreonam	77
Ticarcillin-clavulanate	87
Ampicillin-sulbactam	2

[a] Reprinted from reference 242 with permission of the publisher.

regarded as the chief infectious agent in CF patients, although it was not clear if this situation was due to a primary defect in the innate immune system of the lungs or was secondary to another aspect of CF, such as malnutrition (339). However, since antibiotic therapy has been extensively used to treat *S. aureus* in CF patients, recent data indicating a primary role for *S. aureus* in pathogenesis of CF lung disease are lacking. The common use of antistaphylococcal therapy for CF patients in many parts of the world has raised questions about whether such prophylactic treatment enhances susceptibility to infection by other agents such as *H. influenzae* and *P. aeruginosa* (15, 105), as suggested in the clinical trial discussed above. Interestingly, chronic colonization of CF airways by *P. aeruginosa* is reduced in regions where antistaphylococcal therapy is administered strictly on an as needed basis, rather than prophylactically (111). Thus, at the moment it is clear that conclusions supporting a pathogenic role for *S. aureus* in CF lung disease come from clinical observations, principally the judgment that oropharyngeal cultures positive for *S. aureus* might also indicate its presence in the lower airway. Definitive studies showing a positive effect from treating *S. aureus* when isolated in oropharyngeal cultures obtained from CF patients' clinical specimens are lacking.

Role of Inflammation and *P. aeruginosa* Infection

Chronic *P. aeruginosa* airway infection and the accompanying inflammatory response are clearly the major clinical problems for CF patients today. While antibiotic chemotherapy and chemoprophylaxis have reduced the morbidity and early mortality of CF patients from this infection, the intrinsic ability of *P. aeruginosa* to develop resistance to many commonly used antibiotics (36, 65, 156, 256) probably underlies the inability to eradicate *P. aeruginosa* from the CF patient's lung and ultimately allows this microbe to be highly problematic for these patients (Table 3). During the past decade, the prominent contribution of inflammation to tissue destruction and loss of function has been borne out in numerous studies (reviewed in reference 159) and anti-inflammatory therapies have been shown to produce clinical improvement in infected patients (85, 160), albeit with worrisome side effects regarding long-

term treatment. Several studies have suggested that inflammation and bacterial infection can begin at an early age, before pronounced symptoms appear. Therefore, there is considerable interest in determining the contribution of early infection and inflammation to the progression of CF lung disease.

Early aspects of inflammation. Several groups have demonstrated airway inflammation in infants with CF, but the recent study by Burns et al. (41) suggests that most of this inflammation is a result of prior or concurrent *P. aeruginosa* infection. Kirchner et al. (150) showed increased amounts of DNA and neutrophils in BAL fluid obtained from infants and young children with CF compared with non-CF controls. Birrer et al. studied 27 children with CF, including 4 who were younger than 1 year, all of whom had BAL cultures positive for typical CF pathogens. Despite the presence of normal amounts of the antiproteases α_1-antitrypsin and secretory leukoprotease inhibitor, 20 of the 27 children, including 2 of the 4 infants, had active neutrophil elastase in the BAL fluid (24). Thus, a protease-antiprotease imbalance appears early in life for CF patients, potentially contributing to lung damage. A study by Konstan et al. (161) of BAL fluid from 18 CF patients with clinically mild disease again demonstrated abundant active neutrophil elastase, even in the presence of threefold elevated levels of α_1-antitrypsin. More striking are the findings of Khan et al. (148), who studied BAL fluid samples from 16 infants with CF with a mean age younger than 6 months. Despite the young age of the patients and the absence in seven patients of pathogenic bacteria detectable in BAL fluid cultures (which may miss sampling the part of the lung where pathogens are present), the infants had increased numbers of neutrophils, as well as elevated levels of neutrophil elastase, α_1-antiprotease, and the proinflammatory cytokine interleukin-8 (IL-8). Interestingly, Freedman et al. recently reported that the infiltration of neutrophils into the airways of transgenic CF knockout mice was substantially reduced when the mice were orally given the fatty acid DHA (96), which appears to be deficient in the phospholipids of *CFTR*-knockout mice (97).

The report of elevated levels of inflammatory mediators in CF lungs in which no pathogen could be detected challenged the traditional view that the CF lung is initially normal but becomes progressively damaged by acquired bacterial infection and resultant inflammation. Researchers have begun investigating the possibility that the increase in inflammatory mediators seen in the lungs of CF patients derives from an intrinsic property of the epithelium itself, perhaps an exaggerated inflammatory response to early pathogens such as *S. aureus* that are cleared or are undetectable by culture. Bonfield et al. (26) isolated bronchial epithelial cells from healthy control subjects and from patients with CF and measured the amounts of the secreted anti-inflammatory cytokine IL-10, as well as those of the proinflammatory cytokines IL-8 and IL-6. Cells from normal patients secreted IL-10 but no detectable IL-6 or IL-8, whereas cells from CF patients did not secrete IL-10 but produced both IL-6 and IL-8. DiMango et al. (76) had demonstrated earlier that multiple gene products of *P. aeruginosa* stimulated respiratory epithelial cells to secrete IL-8 and that for a given stimulus, CF cells produced four times the amount of IL-8 than that produced by a genetically complemented control cell line. This response is linked to greater amounts of nuclear (activated) NF-κB in CF respiratory epithelial cells

compared to isogenic control cells that had been genetically complemented with episomal copies of normal *CFTR* (75). If it is true that lung disease in CF begins before any pathogen actually infects the lower airway, then the importance of early detection of CF through neonatal screening is underscored, since early detection allows early intervention. Current screening tests are based on the elevated levels of plasma trypsinogen found in most newborns with CF.

Initiation and establishment of *P. aeruginosa* infection. The ubiquity of *P. aeruginosa* (109, 115) in the environment probably underlies the high frequency of recovery of this pathogen from CF patients. The role of *P. aeruginosa* in human disease is usually opportunistic. Approximately 6 to 20% of CF patients carry *P. aeruginosa* in their gastrointestinal tracts asymptomatically (306) and without mounting a significant immune response to the organism. CF patients can acquire *P. aeruginosa* in their respiratory tracts at any time, with most studies indicating that 70 to 80% CF patients are infected by their teen years. As noted above (41), *P. aeruginosa* infection probably initially occurs within the first 3 years of life. After the onset of chronic infection, patients experience episodic exacerbations requiring antibiotic chemotherapy. Infection may result from social contacts or may be hospital acquired, but the diversity of *P. aeruginosa* clones isolated from CF patients suggests that most clinical isolates originate in the environment (41, 305).

The abnormal composition of the airway secretions of the CF lung is frequently cited as the host factor that predisposes CF patients to chronic colonization by *P. aeruginosa*. CF airway secretions may contain abnormal chloride concentrations as a result of defective CFTR Cl^- channel function (48, 344). While the actual chloride concentration in the CF lung remains the subject of intense debate, it has been proposed that alterations in ionic strength could derail the functions of several host defense mechanisms. One such mechanism is the phagocytic barrier created by macrophages and neutrophils. The results of Tager et al. (319) suggest that the neutrophils of CF patients are defective in phagocytic killing in the presence of elevated chloride concentrations. Moreover, the rate of neutrophil apoptosis was reported to be higher in the CF airway than in normal airways. In addition to their phagocytic functions, neutrophils are a primary producer of antimicrobial peptides, which play an important role in innate host defense. If abnormal, the composition of CF airway secretions may adversely affect the action of antimicrobial peptides, since antimicrobial peptide function is often sensitive to ionic strength (13, 110, 301). Although a more recent study by Brogden et al. (34) showed that the quantity of antimicrobial peptides in CF airways is reduced relative to the levels found in the normal airway, these workers examined anionic peptides, whereas salt sensitivity is found primary with cationic peptides. Other workers (190) have disputed the importance of antimicrobial peptides in innate defense of the lung, finding that there is no difference in salt concentration when normal and CF airway surface liquids are compared. Travis et al. (324), who originally proposed that antimicrobial peptides may be inhibited in the lungs of CF patients, have more recently reported that lysozyme and lactoferrin are the most abundant airway antimicrobial factors. Although they found that some of the factors were inhibited by high ionic strength, nonionic osmolytes were without effect and inhibitory conditions of ionic strength could

A

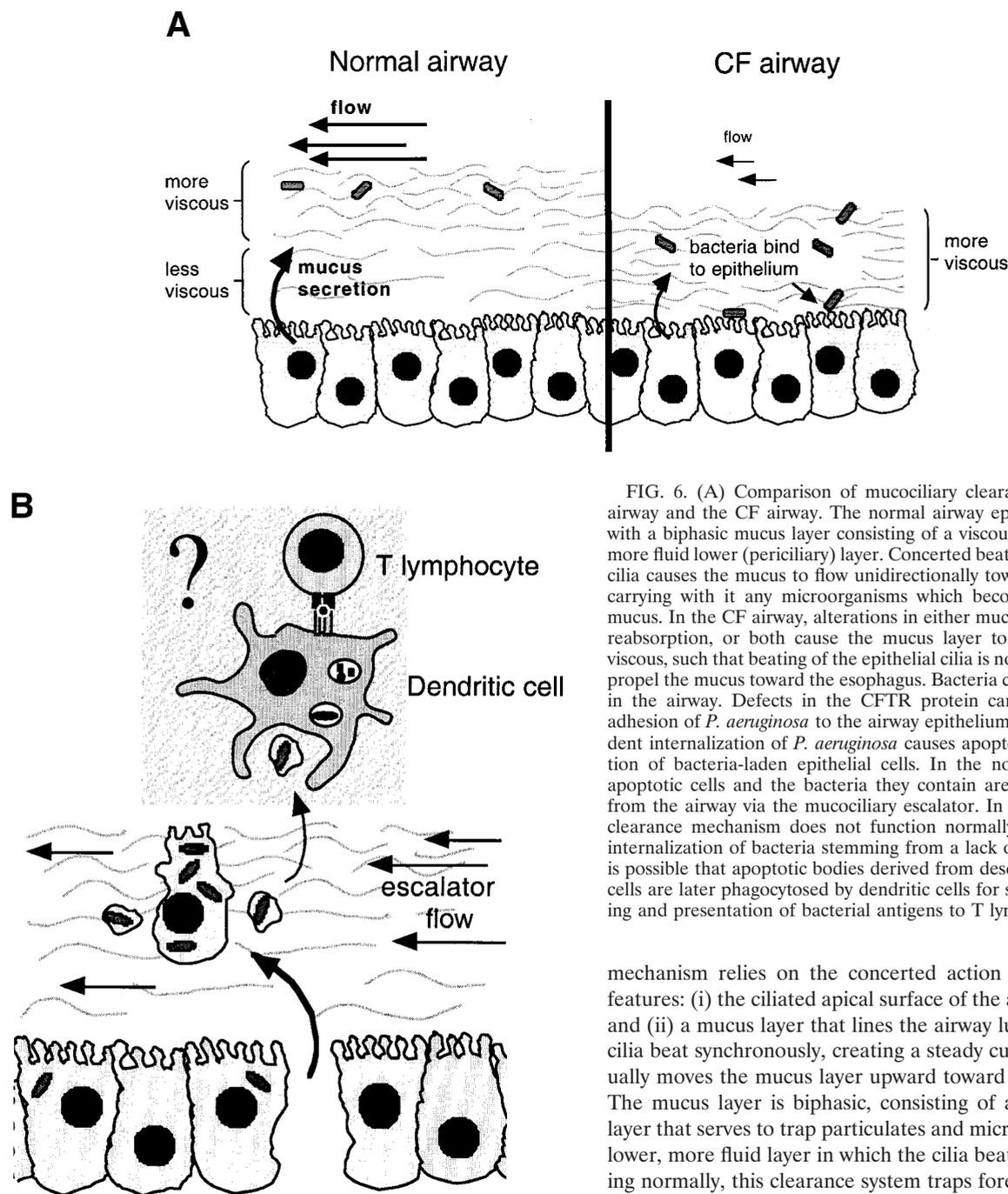Normal airway   CF airway

B

FIG. 6. (A) Comparison of mucociliary clearance in the normal airway and the CF airway. The normal airway epithelium is covered with a biphasic mucus layer consisting of a viscous upper layer and a more fluid lower (periciliary) layer. Concerted beating of epithelial cell cilia causes the mucus to flow unidirectionally toward the esophagus, carrying with it any microorganisms which become trapped in the mucus. In the CF airway, alterations in either mucus secretion, mucus reabsorption, or both cause the mucus layer to become uniformly viscous, such that beating of the epithelial cilia is no longer sufficient to propel the mucus toward the esophagus. Bacteria can therefore persist in the airway. Defects in the CFTR protein can also increase the adhesion of *P. aeruginosa* to the airway epithelium. (B) CFTR-dependent internalization of *P. aeruginosa* causes apoptosis and desquamation of bacteria-laden epithelial cells. In the normal airway, these apoptotic cells and the bacteria they contain are probably removed from the airway via the mucociliary escalator. In the CF airway, this clearance mechanism does not function normally due to inefficient internalization of bacteria stemming from a lack of CFTR protein. It is possible that apoptotic bodies derived from desquamated epithelial cells are later phagocytosed by dendritic cells for subsequent processing and presentation of bacterial antigens to T lymphocytes.

be compensated for by increased levels of the antimicrobial factors. A major problem with evaluating the contribution of antimicrobial peptides to the hypersusceptibility of CF patients to lung infection is the complexity of the antimicrobial peptides in the lung (325) and their multiple functions, including functioning as chemoattractants. Overall, the validity of the observations that suggest compromise in antimicrobial activity of airway surfaces in CF patients has not been robustly supported.

The most prominent of the innate defenses of the airway is the mucociliary action of the airway epithelium. This clearance mechanism relies on the concerted action of two anatomic features: (i) the ciliated apical surface of the airway epithelium and (ii) a mucus layer that lines the airway lumen. The airway cilia beat synchronously, creating a steady current that continually moves the mucus layer upward toward the nasopharynx. The mucus layer is biphasic, consisting of an upper, viscous layer that serves to trap particulates and microorganisms and a lower, more fluid layer in which the cilia beat. When functioning normally, this clearance system traps foreign bodies in the mucus and subsequently carries them to the nasopharynx, where they are expectorated and swallowed. While the effect of *CFTR* mutation on this clearance mechanism is not entirely understood, several possible models can be constructed based on existing observations (Fig. 6A). One possibility is that the abnormal secretory characteristics of the CF airway cells alter the viscosity of the airway fluid, such that the normally serous "periciliary" layer becomes thicker, inhibiting escalator action. Indeed, stimulus-evoked mucus secretion by CF airways is impaired relative to that by normal airway epithelium (86, 259). Alternatively, the abnormal mucus composition of the CF airway could be a result of defects in mucus clearance (342) rather than mucus production.

Another mechanism by which *CFTR* mutations could inhibit mucociliary clearance is by increasing bacterial adherence to the airway epithelial cells (Fig. 6A). The results of Prince and coworkers have shown that intracellular retention of CFTR R domains in an engineered cell line overexpressing these domains leads to alterations in the degree of sialylation of epithelial cell glycolipids (37). Similar findings have been reported when cultured nasal epithelial cells from CF and non-CF patients were compared (265). These alterations result in higher membrane levels of the ganglioside asialo-GM$_1$, an epithelial cell receptor that has been reported to bind *P. aeruginosa* via bacterial pili (57, 69, 118, 265). However, a recent report indicates that no increased expression of asialo-GM$_1$ was detected in cells overexpressing the R domain portion of CFTR if an asialo-GM$_1$-specific lectin was used for detection (165). Moreover, these studies have been carried out principally using laboratory strains of *P. aeruginosa* and not clinical isolates from patients. Schroeder et al. (278) recently reported that asialo-GM$_1$ is not a major target for binding of clinical isolates of *P. aeruginosa* to eukaryotic cells. Interestingly, the crystal structure of the *P. aeruginosa* strain PAK pilus has recently been reported (121), and these investigators have found that a sterically plausible model based on the distribution of buried and exposed surfaces leaves the C-terminal end of the pilus, reported to bind to aisalo-GM$_1$ (172), internally buried in the molecule and thus poorly positioned for multivalent binding to asialo-GM$_1$. In addition, high-affinity binding of lectins, such as pili, to carbohydrate receptors would require that multiple binding sites be available at the pilus tip, which could not be seen with the derived pilus structure. Thus, the crystal structure of the *P. aeruginosa* PAK pilus is not consistent with a role in binding to asialo-GM$_1$ or similar residues with the appropriate disaccharide residue.

While many of the above theories are plausible explanations for deficiencies in the innate defense of the CF lung and are all based on reproducible findings, these models fail to explain why infection in CF lungs is typically established by such a restricted subset of microorganisms and why *P. aeruginosa* predominates. It stands to reason that defects in such generic clearance mechanisms as mucociliary clearance or antibacterial peptides should lead to frequent infection by a very broad array of microorganisms. Nevertheless, only relatively few organisms typically cause disease in CF patients. A novel concept of host susceptibility recently emerged from the finding that the CFTR protein, besides fulfilling its function as a chloride ion channel and regulator of other ion channels, also serves as a receptor for epithelial cell internalization of *P. aeruginosa* on the airway surface (234, 235). Interaction of *P. aeruginosa* with the CFTR protein was found to be a critical step in a process leading to bacterial internalization by the airway epithelium. Prevention of this interaction by coinoculation of live *P. aeruginosa* bacteria and the purified bacterial ligand, complete outer core oligosaccharide derived from the lipopolysaccharide (LPS), led to reduced bacterial uptake by airway epithelium and to an increased bacterial burden in the airways of mice (235). A peptide composed of amino acids 108 to 117 of CFTR, identified as the portion of this molecule that binds to *P. aeruginosa*, achieved the same result in mice when coinoculated with a *P. aeruginosa* challenge; that is, prevention of epithelial cell uptake led to decreased bacterial clearance and

increased bacterial burdens in the lungs (234). Transgenic CF mice in a variety of genetic backgrounds have recently been found to have reduced lung cell internalization of *P. aeruginosa* and increased bacterial burdens 4.5 h after an intranasal or intratracheal inoculation (277). Therefore, in the normal, healthy lung, epithelial cell uptake of *P. aeruginosa* by airway epithelial cells may be a critical factor in innate host immunity to this pathogen. Current unpublished data suggest that these binding and internalization events initiate cellular pathways that lead to apoptosis, which may serve as a mechanism of removing the bacteria-laden epithelial cells from the airway surface such that they can be expectorated and swallowed. Alternatively, apoptotic bodies derived from these bacteria-laden epithelial cells may be phagocytosed by dendritic cells (2, 262–264), leading to presentation of bacterial antigens to T cells (Fig. 6B).

Another contributing factor was recently identified by Park et al. (216), who found that *P. aeruginosa* could exploit the shedding of the airway epithelial cell surface heparan sulfate proteoglycan (HSPG) known as syndecan-1 to resist innate host defenses. Four mammalian syndecans are currently known, with syndecan-1 being the most abundant in the airway. The syndecans are one of the major sources of cell surface heparan sulfate (HS). The extracellular domain of the HSPGs can be shed from the cell surface and released in a soluble form known as an ectodomain, which contains HS attached to a core protein. Previously, these workers showed that the *P. aeruginosa* elastase encoded by the *lasA* gene induced shedding of the syndecan-1 ectodomain (217) from cell surfaces. Surprisingly, syndecan-1 knockout mice were more resistant than wild-type controls to *P. aeruginosa* lung infection but became as susceptible as wild-type mice to infection when shed syndecan-1 ectodomains or HS, but not the ectodomain core protein, were added to infectious inocula. These workers postulated that the HS portion of the shed syndecan-1 ectodomains could interfere with a variety of molecular mediators of innate defenses by binding to them and inactivating them, including cationic antimicrobial peptides, neutrophil elastase, cathepsin G, chemokines, and surfactant proteins A to D (217). The surfactant proteins play a role in the pathogenesis of experimental *P. aeruginosa* lung infection (173), and HSPGs bind to chemokines and modulate receptor binding and cellular responses (167). Since shedding of syndecan-1 ectodomains is probably an early event in the interaction of *P. aeruginosa* and the respiratory epithelium, augmentation and exploitation and of this process by *P. aeruginosa* could compromise innate immune mechanisms and contribute to establishment of infection.

Emergence of the mucoid phenotype. One of the most striking and clinically important features of infection by *P. aeruginosa* is the tendency of this bacterium to change to a mucoid phenotype, probably initiating the chronic-infection stage of the disease. The mucoid phenotype results from bacterial production of a polysaccharide known as both alginate and mucoid exopolysaccharide (MEP) and plays an important role in bacterial evasion of the host immune response. Alginate overproduction by *P. aeruginosa* isolated from the respiratory tracts of CF patients was first noted by Doggett et al. (79). Later, Lam et al. (168) described a fibrous, polyanionic matrix surrounding bacterial cells in alveolar *P. aeruginosa* microcolonies

during postmortem examination of lungs from CF patients. The structure of alginate was later reported to be an acetylated random copolymer of mannuronic acid and guluronic acid (292), with the degree of acetylation varying widely among alginate samples purified from different *P. aeruginosa* isolates (241).

The mucoid phenotype is often unstable, with a large percentage of isolates reverting to a nonmucoid phenotype during in vitro culture (241). This instability has been genetically mapped to changes in the gene known as *mucA* (27). Production of MucA protein inhibits the activity of the alternative sigma factor (sigma E), which is required for the mucoid phenotype. Mutations in the *mucA* gene leading to a premature termination of the coding sequence are found in mucoid isolates. The most common mutation is referred to as the *mucA22* allele (280); nonmucoid isolates have a functional *mucA* gene.

The overall genetic regulation of alginate production has been a major focus of investigation in recent years. That the mucoid phenotype is controlled by a chromosomal genetic locus was first demonstrated in the early 1980s (101, 212). Initial attempts at cloning the genetic locus (loci) controlling MEP/alginate production were frustrated by the inherent instability of the mucoid phenotype. The creation of the mucoid-lock mutant strain 8830 allowed plasmid complementation studies in which libraries obtained from strain 8830 were transformed into mucoid-deficient mutants (64). The result of these experiments was the cloning of a minimally-essential genetic element of 6.2 kb that contained all of the genes necessary for MEP/alginate production.

Expression of the genes in the alginate biosynthetic operon is controlled by transcriptional regulation. The first gene is *algD*, which encodes a protein with GDP-mannose dehydrogenase activity. Transcription of the alginate biosynthetic locus is under control of a complex, multitier regulatory mechanism (Fig. 7) involving both constitutive (163) and inducible (72, 346) gene products. As noted above, the inducible arm of *algD* regulation relies in large part on the Sigma E product encoded by the *algU* gene (74, 281). The *algU* gene, also called *algT*, positively regulates both its own transcription (74, 186), as well as that of the transcription factors AlgB and AlgR (186, 346). The negative control of AlgU function by the antisigma factors MucA and MucB was recently proposed to be due to the antagonism of sigma E by MucB, localized to the periplasm of the cell, and MucA, localized to the inner membrane via a single transmembrane domain with amino- and carboxy-terminal domains in the cytoplasm and periplasm, respectively (189). Binding of the Muc proteins to the sigma E transcription factor sequestered it and probably prevented it from activating the alginate biosynthetic locus (282). The induction of alginate overproduction may arise following exposure of nonmucoid *P. aeruginosa* cells to hydrogen peroxide, generating the *mucA22* allele of the *mucA* gene (188). Both AlgB and AlgR are phosphorylated proteins (73, 182), but whether these phosphorylation events are important for transcriptional regulation of *algD* by AlgB and AlgR is not clear. Ma et al. (181) demonstrated that transcriptional activation of *algD* by AlgB and AlgR is not affected by the phosphorylation states of the later. Therefore, phosphorylation of AlgB and AlgR may play a yet undefined role in transcriptional regulation. For example, it is possible that these phosphorylation events are important for responses

of *P. aeruginosa* to environmental cues. Exactly which aspects of the milieu of the CF lung promote the change to a mucoid phenotype remain unclear. Interestingly, MEP/alginate production blocks the interaction of the LPS outer core pseudomonal ligand of CFTR, further inhibiting clearance that is already defective in CF patients.

Progression of chronic infection. Although there is a highly varied clinical course for CF patients once the mucoid phenotype of *P. aeruginosa* emerges, this event correlates with the onset of significant deterioration in lung function (70, 222). The small minority of CF patients who do not acquire mucoid *P. aeruginosa*, including those carrying only nonmucoid *P. aeruginosa* strains, have significantly better lung function over time compared with CF patients infected with mucoid *P. aeruginosa* (215). As lung function declines, so does isolation of organisms such as *S. aureus* and nontypeable *H. influenzae*, indicating they probably do not play an important role at this stage of the pathogenesis of CF lung disease.

While MEP/alginate overproduction is a key factor in the pathogenic picture, several other molecular changes to *P. aeruginosa* isolates from CF patients have been documented. Curiously, mucoid *P. aeruginosa* strains almost all produce a LPS defective in elaboration of O side chains (120), rendering the organisms susceptible to the bactericidal effects of complement (230, 269). Clearly, the effective complement levels in the lungs are below those capable of killing mucoid *P. aeruginosa* strains. The selective basis for the loss of O antigen production is not known, but suggestions include avoidance of host antibody responses to LPS O side chains and the instability of strains attempting to express both MEP/alginate and a smooth LPS. Other phenotypic changes have been described for mucoid *P. aeruginosa* strains, including reduced production of extracellular virulence factors and cell-bound adhesins (183).

(i) Biofilms and quorum sensing. One aspect of the pathogenesis of chronic lung infection in CF is the ability of *P. aeruginosa* to grow as a biofilm, which reportedly increases bacterial resistance to phagocytic killing (194) and antibiotic killing (35, 348). Passador et al. (218) first reported that expression of some *P. aeruginosa* virulence genes required cell to cell communication. Gray et al. (114) first noted that both *P. aeruginosa* and the fish commensal *Vibrio fischeri* had interchangeable genetic elements involved in controlling this process, which is also referred to as quorum sensing. A sensor-regulator system involving the genes *lasR* and *lasI* was found to comprise the *P. aeruginosa* genetic elements involved in sensing cell density and production of a soluble factor regulating other genes. This factor, referred to as *Pseudomonas* autoinducer, was identified as 3-oxo-*N*-(tetrahydro-2-oxo-3-furanyl)dodecanamide (220), an acyl homoserine lactone. A second autoinducer was then discovered (221) and was identified as *N*-butyrylhomoserine lactone. This factor was produced by the *rhlI* gene under the control of the *rhlR* sensor, both which are involved in the production of a rhamnolipid molecule of *P. aeruginosa* (211), as well as in control of the synthesis of other exoproducts (33). Additional studies of this system have shown that other gene products, such as that encoded by *P. aeruginosa vfr*, a homolog of the *E. coli* cyclic AMP receptor protein (3), and a quinolone-like molecule (226), also affect quorum sensing responses. Biofilm development in vitro was also found to be dependent on the quorum-sensing system (66) suggesting
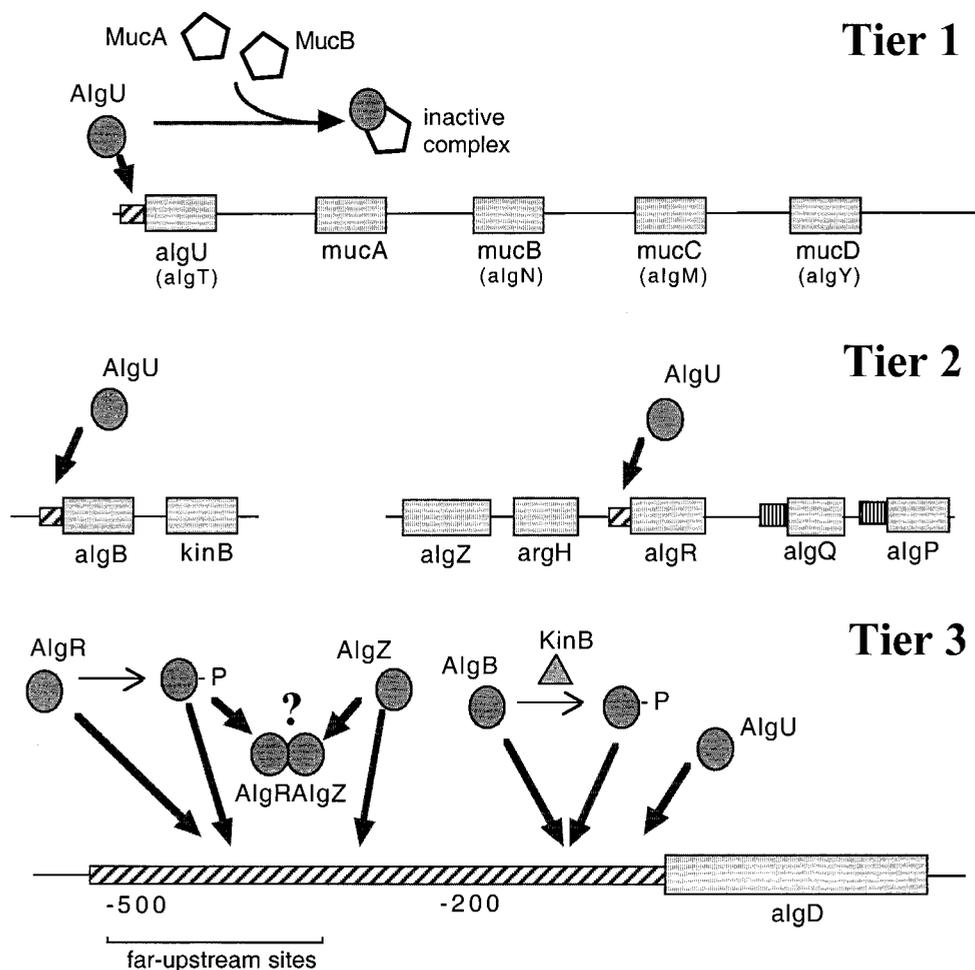
FIG. 7. The AlgU alternative sigma factor (also called AlgT) directly regulates expression of itself, of the alginate biosynthetic operon, and of several other regulatory genes. The figure depicts events leading to transcriptional regulation of the *algD* gene (encoding GDP-mannose dehydrogenase), which lies at the 5′ end of the alginate biosynthetic operon. The activity of the AlgU transcription factor is negatively regulated by the two anti-sigma factors MucA and MucB (Tier 1). When AlgU remains in an active state, it is then able to initiate transcription of the *algB* and *algR* genes, which encode transcriptional regulatory proteins (Tier 2). These transcriptional activators then positively regulate *algD* transcription. Regulation of *algD* by AlgB and AlgR may be further modified by other events such as phosphorylation of AlgB and AlgR and dimerization of AlgR with another transactivator, AlgZ (Tier 3). Symbols: ▥, constitutive (σ^{70}-like) promoter; ▨, heat shock (σ^{54}-like) promoter; ◎, transcriptional activator; ◯, transcriptional inhibitor; ▬, structural gene; ➡, positive regulation; →, posttranslational modification; ▲, kinase; -P, phosphate.

that the genes involved were critical for pathogenesis in the CF lung. Substantiation of this idea was provided by the findings of Storey et al. (309) that *lasR* transcripts could be found in the sputa of infected CF patients and by the findings of Greenberg and colleagues (298) identifying the homoserine lactone molecules in CF patient sputa. Nonetheless, the evidence that *P. aeruginosa* is growing as a biofilm in the lungs of CF patients is based principally on electron microscopic evidence (168, 298) that has yet to be adequately coupled to the major molecular mechanism of biofilm formation, which may include the elaboration of MEP/alginate (207).

In a study of the requirement for the quorum-sensing genes in the formation of a biofilm in vitro (66), it was found that mutations in the *lasI* gene did not affect the production of MEP/alginate. Moreover, there was a low correlation of transcripts for *lasR* and *algD*, the first gene in the alginate biosyn-thetic operon, in CF patient sputum samples (309), indicative of independent regulation of these two factors associated with *P. aeruginosa* biofilms. Mathee et al. (188) obtained mucoid variants from strain PAO1 growing as a biofilm after exposure of the bacteria to hydrogen peroxide, and these mucoid variants had a reduced production of quorum-sensing controlled virulence factors. More recently, Bollinger et al. (25) showed that quorum-sensing regulation of gene expression in *P. aeruginosa* was complex, dependent on the growth state of the cells and nutritional factors available, and quite dynamic. They further suggested a role for quorum-sensing products in negative regulation of gene expression in a biofilm. These data indicate there are two, probably independent, pathways to biofilm formation in *P. aeruginosa*: quorum-sensing-dependent MEP/alginate-independent and quorum-sensing-independent MEP/alginate-dependent forms.

(ii) Ineffectiveness of the innate immune response to mucoid *P. aeruginosa*. Numerous host factors representing components of the innate immune system are brought to bear on *P. aeruginosa* in the CF lung, but eventually they all fail. Failure attributable to host defects resulting from mutant *CFTR* alleles has been discussed above. However, bacterial factors also appear to contribute to the inadequacy of the innate immune system in eliminating *P. aeruginosa* from the CF respiratory tract. The ability of alginate to scavenge hypochlorite produced by phagocytic cells ingesting *P. aeruginosa* has been described previously (171). *P. aeruginosa* alginate has also been reported to reduce the chemotaxis of polymorphonuclear leukocytes (PMN) into CF lungs and by itself to inhibit activation of the complement system (224). Mucoid *P. aeruginosa* cells are inherently resistant to phagocytosis by both PMN and macrophages in comparison to nonmucoid strains (45, 164, 196). Such a situation can be exacerbated by binding of the bacteria to host factors such as respiratory mucins (329) or growth in a biofilm (194). Recently it has been shown that acetylation of the alginate of *P. aeruginosa* is essential for resistance to phagocytosis mediated by complement in the absence of antibody (231). Given that mucoid *P. aeruginosa* is clearly the pathogen associated most closely with the decline in lung function in CF patients, it is not surprising the MEP/alginate itself is able to promote bacterial survival in the face of host immune effectors.

Other bacterial factors contributing to bacterial resistance to host innate immune effectors include products of the type III secretion system (349), such as ExoS, which can inhibit phagocytic cell motility (98) and induce the death of PMNs and macrophages (62), and ExoU, which is cytotoxic to eukaryotic cells (91). ExoU knockout strains of *P. aeruginosa* show decreased virulence in animal models of acute infection (91), and strains normally lacking *exoU* that are transduced to express the ExoU protein have increased virulence in these models (5). The pathological effects of products of the type III secretion system, as well as other *P. aeruginosa* products that could compromise host defenses, may be partially mitigated by the potent immune responses to *P. aeruginosa* antigens made by CF patients (32, 81). Whether intact neutralizing antibodies are present in sufficient titer in the sputa of CF patients to counteract the bacterial factors is not clear, so that the actual contribution of these factors to the persistence of chronic infection is deduced primarily from laboratory studies.

Although defects in CFTR underlie the hypersusceptibility of CF patients to initiation of *P. aeruginosa* infection, the redundancies of many protective mechanisms in the innate immune system indicate that *P. aeruginosa* must circumvent other host responses in order to survive. Factors recently identified as being important in host resistance to *P. aeruginosa* infection include the C5a chemoattractant receptor (128), the ability of respiratory epithelial cells to undergo apoptosis in response to infection (113), and surfactant proteins in the lungs (173, 185, 255). Whether there is a compromise in the effects of any of these systems in CF patients is not known, but these examples of the variety of host factors that can contribute to innate resistance to *P. aeruginosa* infection are indicative of the multiple defense barriers that the organism must overcome or neutralize in order to establish chronic infection.

(iii) Ineffectiveness of the acquired immune response during chronic *P. aeruginosa* infection. A priori, it is apparent that the acquired host immune response to mucoid *P. aeruginosa* is ineffective at eliminating this pathogen. Indeed, this ineffective response probably contributes significantly to the inflammatory process that damages the lung tissue of CF patients. The question is, why is the immune response to mucoid *P. aeruginosa* ineffective? The principal hypothesis that has been studied is that immune responses to the MEP/alginate antigen of mucoid *P. aeruginosa* consist mostly of antibodies that fail to mediate opsonophagocytic killing of the organism: so-called nonopsonic antibodies. These nonopsonic antibodies are not unique to CF patients but have been found in all humans examined to date, albeit at higher titers in CF patients (236). Among small numbers of older CF patients lacking mucoid *P. aeruginosa* (i.e., long-term nonprogressors), the presence of opsonic antibodies to alginate has been reported and correlated with the lack of infection and better clinical status (215, 236). Although Tosi et al. (323) disputed this finding, their study did not evaluate antibodies specific to MEP/alginate. CF patients clearly do produce antibodies that can mediate the opsonic killing of mucoid *P. aeruginosa*, but these antibodies are not specific to the alginate antigen (236). These non-alginate (MEP)-specific antibodies fail to mediate the killing of mucoid *P. aeruginosa* growing in an in vitro-formed biofilm (194), potentially explaining their ineffectiveness at protecting CF patients from chronic infection. Monoclonal and polyclonal opsonic antibodies from mice and rabbits, respectively, to MEP/alginate protected rodents from endobronchial infection (237), and opsonic responses to this antigen could be engendered in a few healthy humans by vaccination (232). However, when hundreds of plasma donors were immunized with a MEP/alginate vaccine, only a minority (35%) responded with opsonic antibody, and the titer and activity were quite low (G. B. Pier, unpublished observation). Thus, nothing appears unique about CF patients regarding their inability to produce alginate/MEP-specific opsonic antibodies; this deficit is found among most humans. Those few patients with naturally occurring opsonic antibodies to MEP/alginate acquired them by some unknown mechanism; they had exceptional responses to this antigen.

The association of an ineffective immune response to MEP/alginate with progression of chronic infection and the similar association of an effective, opsonic response with resistance to infection, implicates this aspect of immunity in resistance to mucoid *P. aeruginosa* infection. These observations formed the basis for a clinical trial of a hyperimmune intravenous immunoglobulin G (IgG) preparation in infected CF patients. Unfortunately, the trial did not show any effect of monthly administration of the preparation. While the basis for failure for a trial can be attributed to a wide variety of factors, the most likely ones are related to the poor response rate of the vaccine recipients and the low titers engendered in those that did respond. Newer efforts centered on production of conjugate vaccines and human monoclonal antibodies are being pursued.

THERAPIES FOR CYSTIC FIBROSIS LUNG DISEASE

Airway Clearance

Clearance of the purulent pulmonary secretions in CF lung disease has long been enhanced through chest physiotherapy

with postural drainage. Standard chest physiotherapy is, however, time-consuming and must be carried out by a trained individual. In attempts to improve both efficacy and compliance, multiple alternative therapies have been developed in the last few decades. These therapies are all based on the supposition that clearance techniques not only relieve airway obstruction, acutely improving ventilation, but also allow increased expectoration of elastase-laden sputum that, if left undisturbed, would impair natural mucociliary clearance and mediate progressive lung damage (350). Ironically, the universal acceptance of chest physiotherapy as a beneficial therapy in CF has led to a paucity of data supporting the tenet, probably due to the ethically tenuous nature of a long-term study comparing treatment with no treatment. Consequently, most trials have compared either pairs of the various airway clearance treatment options or short-term therapy versus no treatment. For example, studies comparing high-frequency chest compression with an inflatable vest to standard physiotherapy have demonstrated similar short-term improvements in pulmonary function and sputum production in hospitalized patients (10, 30). Other common techniques include active forced expiration (334), autogenic drainage (227, 271), positive expiratory pressure (89, 199, 227), and oscillators such as the Flutter device (162). A meta-analysis of randomized trials of chest physiotherapy techniques in CF showed no difference among the techniques. All were equally capable of increasing the quantity of sputum expectorated (321). Given that comparisons have demonstrated similar efficacy in terms of sputum production, patient satisfaction and compliance may be the most important factors to consider when recommending a technique for home use (247, 331). Whether increased sputum production slows the progression of CF lung disease remains an open question.

Lending credence to the idea that improved airway clearance leads to a slowing of the progression of CF lung disease has been the study of agents that decrease the abnormally high viscosity of CF airway secretions. CF sputum contains many neutrophils that release DNA upon autolysis. The DNA aggregates into large fibrils, markedly increasing sputum viscosity (238). Purified recombinant human DNase I (rhDNase I) reduces the viscosity of CF sputum samples and is thus hypothesized to aid in sputum clearance (288). In clinical trials, rhDNase use is associated with an improvement in FEV1, as well as with a decreased frequency of pulmonary exacerbations requiring parenteral antibiotic therapy (100, 248). At the current recommended prophylactic dose of 2.5 mg of rhDNase delivered via nebulizer once daily, the therapy is well tolerated, with transient hoarseness being the only consistent side effect. The only major concern regarding the long-term use of rhDNase has been related to its high cost (P. B. Davis, Editorial, N. Engl. J. Med. **331:**672–673 1994).

Chemotherapy

Antibiotic therapy leads to improvement in lung function, the best predictor of survival in CF patients (253). While investigators generally agree that antibiotic therapy reduces the morbidity of CF lung disease, most decisions regarding when to treat, and with which antibiotics, are largely empirical and are based on the patient's age, the colonizing organisms, and

the severity of the patient's pulmonary exacerbation. Young patients with mild lung disease and colonization with *S. aureus* or *H. influenzae* are often treated with oral antibiotics, including amoxicillin-clavulanate, trimethoprim-sulfamethoxasole, and cephalexin, during mild exacerbations (Table 4). Mild exacerbations manifest as cough and congestion associated with viral upper respiratory infection and are characterized by only a modest decline in lung function (<10% decrease in FEV1) with no change on chest radiograph. Moderate or severe exacerbations with symptoms of increased cough and sputum production, changes on chest radiograph, decline in lung function by more than 10%, exercise intolerance, fever, weight loss, and possibly hemoptysis are treated with intravenous antibiotics (56). If colonized with *S. aureus* alone, patients often receive oxacillin, nafcillin, or cephalothin monotherapy. Patients colonized with both *S. aureus* and *H. influenzae* may receive combination therapy with both a narrow-spectrum penicillin and an aminoglycoside such as gentamicin.

The approach to therapy for patients colonized with *P. aeruginosa* mirrors that described for patients with *S. aureus*, namely, oral antibiotics for mild infections and intravenous ones for moderate to severe exacerbations. The effectiveness of chemotherapy against *P. aeruginosa* infection is reduced by the intrinsic resistance of this microorganism to many antibiotics. The precise mechanism of such resistance is not well understood but has been speculated to be due to the structure of the cell wall, the extensive linkage of outer membrane proteins to the LPS, or the extremely hydrophilic nature of the outer membrane, which may exclude many hydrophobic antibiotics. Oral antibiotics with activity against *P. aeruginosa* are currently limited to the quinolones, particularly ciprofloxacin. Fortunately, oral ciprofloxacin is as efficacious as intravenous therapy in improving pulmonary function (52, 124). However, the use of ciprofloxacin is limited by concern regarding both cartilage toxicity in young children (although studies have not justified the concern [38]) and rapid emergence of resistance (289). Frequently, ciprofloxacin is used in combination with an inhaled antibiotic such as tobramycin or colistin. In vitro testing shows that double-antibiotic combination therapy appears more effective than monotherapy (170). However, single-drug susceptibility testing of sputum *P. aeruginosa* isolates remains a useful guide for therapy against sensitive strains. For multiresistant strains, in vitro testing of antibiotic combinations allows the identification of synergistic antibiotics with activity against the resistant organisms. Practically, treatment of either sensitive or resistant strains involves combination therapy, typically with an aminoglycoside and a β-lactam active against *P. aeruginosa*. While monotherapy may be as effective against susceptible strains, combination therapy is clearly more effective against resistant strains and may delay the development of resistance (80).

The timing of antibiotic therapy is a matter of considerable debate. In the United States, therapy typically follows a schedule dictated by the pattern of pulmonary exacerbations. Patients receive intravenous antibiotic therapy only for moderate or severe episodes of illness. Studies from Denmark, where patients chronically colonized with *P. aeruginosa* receive regularly scheduled "maintenance" intravenous therapy, suggest that frequent antibiotic use leads to improved survival (94, 316). An extension of this concept is the use of antipseudomo-

TABLE 4. Antimicrobial therapy for CF pulmonary exacerbations

Severity	Colonizing oraganism/s	Drug(s)	Route	Dose	Comments
Mild	S. aureus	Dicloxacillin	p.o.	50–100 mg/kg/day q.i.d.	Max 500 mg q.i.d.
		Amoxicillin	p.o.	40 mg/kg/day t.i.d.	Max 500 mg t.i.d.
		Amoxicillin-clavulanate	p.o.	40 mg/kg/day t.i.d.	Max 500 mg t.i.d.
		Trimethoprim-sulfamethoxasole	p.o.	6–12 mg/kg/day b.i.d.	pt > 2 mo
		Cephalexin	p.o.	50–100 mg/kg/day t.i.d.	Max 500 mg t.i.d.
	S. aureus and H. influenzae	Drugs listed above except dicloxicillin			
	S. aureus and P. aeruginosa	Ciprofloxacin	p.o.	500–750 mg b.i.d.	Patients > 18 yr[a]
		Add drugs listed under S. aureus, as Staphylococcus becomes rapidly resistant to ciprofloxacin			
		Consider adding tobramycin inhaled 150–300 mg b.i.d.			
		Consider adding colistin[b] inhaled 80–160 mg q.d-b.i.d.			
	P. aeruginosa	Ciprofloxacin plus inhaled tobramycin or colistin			
Moderate and severe	S. aureus and P. aeruginosa	Piperacillin-tazobactam[c]	i.v.	200–300 piperacillin mg/kg/day q.6h	Max 24 g of piperacillin/day
		Ticarcillin-clavulanate	i.v.	200–300 ticarcilling mg/kg/day q.6h	Max 12 g of ticarcillin/day
		Ceftazadime	i.v.	150–200 mg/kg/day q.8h	Max 6 g/day
		Aztreonam	i.v.	150–200 mg/kg/day q.6–8h	Max 8 g/day
		Imipenem	i.v.	60–100 mg/kg/day q.6h	Max 4 g/day
		Plus tobramycin	i.v.	6–10 mg/kg/day q.8h	pk 8–12 µg/ml
		Consider adding oxacillin	i.v.	150–200 mg/kg/day q.4h	Max 12 g/day
	P. aeruginosa	Piperacillin	i.v.	200–300 piperacillin mg/kg/day q.6h	Max 24 g of piperacillin/day
		Ticarcillin	i.v.	200–300 ticarcillin mg/kg/day q.6h	Max 12 g of ticarcillin/day
		Or ceftazidime, aztreonam, imipenem as above			
		Plus tobramycin			

[a] Quinolones can be used in prepubertal children older than 5 years, although dosing standards have not been established.

[b] Colistin is used particularly for multidrug resistant organisms, or in patients who have demonstrated aminoglycoside toxicity.

[c] Piperacillin has a higher incidence of neutropenia and hypersensitivity reactions, but produces less platelet dysfunction than ticarcillin.

nal prophylaxis. Many patients, both in Denmark and elsewhere, are given long-term antibiotic therapy in an attempt to reduce the severity and frequency of pulmonary symptoms (158). However, continuous prophylaxis aimed at reducing S. aureus colonization has, at best, been shown to be equivalent to episodic therapy when clinical symptoms or lung mechanics of CF infants are compared. At worst, as noted above, decreasing the early colonization with S. aureus may increase colonization with P. aeruginosa (247). In contrast to the potentially detrimental effect of S. aureus prophylaxis, nebulization of either tobramycin or colistin is a beneficial therapy against P. aeruginosa and has no significant adverse effects (138, 340). Early treatment with inhaled tobramycin may even prevent colonization with P. aeruginosa (340), which has prompted ongoing long-term prospective trials of inhaled tobramycin in young children. A double-blind, placebo-controlled trial based on data from 520 adult CF patients showed that inhaled tobramycin taken as 300 mg on a twice-daily basis for 4 weeks followed by 4 weeks without antibiotic over a total period of 24 weeks improved pulmonary function, decreased the density of P. aeruginosa in sputum, and decreased the risk of hospitalization (249). Current prophylactic regimens in the United States utilize 300 mg of tobramycin administered on a twice-daily basis for alternating 28-day on-off cycles. The intermittent schedule

is aimed at balancing the prevention of symptomatic P. aeruginosa colonization against the development of highly resistant strains. Achieving this balance remains a major challenge of current chemotherapeutic strategies for combating CF lung disease.

Mechanisms of Antibiotic Resistance

A major problem for the treatment of infection in CF patients is the emergence of full-fledged antibiotic resistance, and most patients will eventually succumb to chronic lung infection with multiresistant P. aeruginosa. In 1984, it was reported that the application of one antibiotic could select for resistance to antibiotics that were chemically unrelated to the antibiotic that had originally been administered (266), indicating that resistance to more than one class of antibiotic could be acquired in a single event. Two well-characterized mechanisms mediate the simultaneous transfer of resistance to multiple drugs. The first is the presence of multiple resistance genes on one transferable genetic element (called an R factor or R plasmid). R factors are episomal genetic elements that encode drug resistance as well as their own conjugative transfer; they were first described in 1959 (210), although reports earlier than 1959 had described drug resistance that was probably due to the same

phenomenon (151). Several reports have suggested that many clinical isolates of *P. aeruginosa* carry R factors (137, 304, 315). R factors are transferred very efficiently among pseudomonads, with a transfer rate that approaches 0.1 event per CFU (214), and the transduction of drug resistance genes between *P. aeruginosa* strains has been demonstrated to occur under environmental conditions (198, 268). Additionally, pseudomonads can acquire plasmid-borne antibiotic resistance from nonpseudomonads, as evidenced in the case of *Serratia marcescens* resistance plasmids (213). The concatamerization of multiple drug resistance genes on a single episomal element is thought to occur through a stepwise fusion of multiple resistance plasmids. Similar fusion events have been found to occur for of R factors and TOL plasmids, the latter of which carry genes essential for the catabolism of small aromatic compounds present in soil (336).

The second mechanism by which a single genetic event can result in resistance to multiple drugs is the acquisition of genes encoding drug efflux pumps (179). The existence of such pump proteins was indicated by the finding that the acquisition of multidrug resistance could be correlated with the expression of several outer membrane proteins (187). Since that time, drug efflux pumps have been characterized in detail. They have been found to consist of a multiprotein holocomplex including (i) an energy-dependent "extrusion pump," which resides in the bacterial inner membrane; (ii) a protein that connects the inner and outer membranes; and (iii) an exit pore protein complex (201). The protein components of these holocomplexes frequently have domain structures that are analogous to those of the domains of ABC family transporters (like the CFTR protein), the difference being that in drug efflux pumps the pore proteins and nucleotide binding domains are usually present on separate proteins that are brought together by formation of the holocomplex.

EMERGING PATHOGENS AFFECTING CYSTIC FIBROSIS PATIENTS

Although *P. aeruginosa* has remained the predominant pulmonary pathogen in CF patients, recent years have seen the emergence of several new pathogens of clinical relevance to CF. An epidemic of infection with *Burkholderia cepacia* (then *Pseudomonas cepacia*) was reported in CF patients in the 1980s, and a high mortality was noted. A subsequent analysis in 1990 of 124 *B. cepacia*-infected CF patients and the same number of sex- and age-matched controls showed significantly higher mortality in the first year following detectable *B. cepacia* colonization (28 versus 6%) but no significant difference in subsequent years (174). The *B. cepacia*-infected patients seemed to have a worse clinical status up to 2 years prior to detection of this organism, suggesting that it has a predilection for more severally ill patients. In 1992, Gladman et al. reported that a notable number of CF patients were colonized with *B. cepacia* and *Burkholderia* (now *Stenotrophomonas*) *maltophilia* (108). These workers also reported a propensity for coinfection with *P. aeruginosa* in the patient population studied. The clinical significance of *B. cepacia* coinfection has also been demonstrated by Whiteford et al., in that coinfection with *P. aeruginosa* and *B. cepacia* was found to carry a poorer prognosis than was *P. aeruginosa* infection alone (337). *B. cepacia* is trans-

missible both inside and outside of the hospital setting (82, 92, 300, 320). It is capable of invading airway epithelium (42), an activity that has been cited as an explanation for its broad antibiotic resistance and its ability to cause disseminated bacteremia. Furthermore, it is intrinsically resistant to many antibiotics. This intrinsic antibiotic resistance seems to require the presence of a 30-kDa bacterial porin, and specific antibody against this porin correlates well with good prognosis (39). However, according to recent data (61), the overall age-specific prevalence of *B. cepacia* infection peaks at around 6% of patients 18 to 34 years old, while *S. maltophilia* has a slightly higher incidence but is detected in 5 to 10% of patients in all age groups. The clinical significance of *S. maltophilia* infection in CF patients is unknown.

In 1980, Boxerbaum reported the isolation of *Mycobacterium* spp. from the sputa of CF patients (29). Species implicated in such infections include *M. tuberculosis*, *M. avium-intracellulare*, *M. kansasii*, *M. gordonae*, *M. chelonei*, and *M. fortuitum* (123, 149). The prevalence of mycobacterial infection among CF patients ranges widely among studies, from approximately 4% (322) to almost 20% (123). Nevertheless, other data demonstrate no significant increase in skin test positivity in CF patients compared to non-CF control patients (200). Furthermore, a recent report of a retrospective case-control study shows no effect on prognosis attributable to mycobacterial infection (322). However, infection with *M. abscessus* may be exceptionally deleterious (59). In summary, while several studies have identified *Mycobacterium* spp. as potential pathogens of CF patients, the relative clinical importance of these infections is unclear.

Aspergillus fumigatus is a fungal pathogen that causes a wide range of pulmonary disease states, which are not restricted to CF patients but are correlated instead with immunosuppression (reviewed in reference 106). Colonization by this fungus can lead to both localized and disseminated infection and can induce an acute inflammatory response, as well as a chronic granulomatosis response (21), both of which can lead to the destruction of nearby lung tissue. In addition, allergic bronchopulmonary aspergillosis, a syndrome seen in patients with asthma and CF and marked by elevated serum IgE levels, can lead to damage to large airways (central bronchiectasis) (54). A relationship between *A. fumigatus* and CF was first suggested in the 1970s by Bardana et al., who showed that CF patients had higher serum titers of antibodies against this fungus than did healthy individuals (14). Anti-*Aspergillus* IgG levels are directly related to carriage of the microbe, while anti-*Aspergillus* IgA levels are inversely related to carriage (270). A positive correlation between fungal carriage and antifungal IgG antibodies is supported by a more recent study (299). These data seem to support a prominent role of IgA in protection in normal individuals, and they indicate that in CF patients, some host defense factor is compromised that dysregulates the humoral response, causing the production of unprotective antibodies. No relationship seems to exist between *A. fumigatus* carriage and *P. aeruginosa* infection.

Alcaligenes xylosoxidans is another pathogen found not infrequently in CF patient oropharyngeal cultures (40), but again, its finding has unclear clinical significance. Methicillin-resistant *S. aureus* is also found with increasing frequency, and this has led to concern that these strains may pose a greater

clinical problem then methicillin-sensitive *S. aureus*, or, alternatively, may be a source for dissemination of methicillin-resistant *S. aureus* in both the hospital and the community. Many other pathogens are on occasion isolated from sputum cultures from CF patients, but their clinical importance is an open question. One likely common thread to all of these unusual pathogens is that they can colonize and infect CF lungs that have been extensively damaged by years of chronic mucoid *P. aeruginosa* infection.

CONCLUSIONS

The study of CF has evolved greatly since the initial empirical characterization of this disease. While originally characterized as a collection of loosely related syndromes, CF is now recognized as a single disease entity whose diverse symptoms stem from the wide tissue distribution of the gene product that is defective in CF, the ion channel and regulator CFTR. Defective CFTR channel function impacts the function of the pancreas and alters the consistency of mucosal secretions. The latter of these effects probably plays an important role in the defective resistance of CF patients to many bacterial and fungal pathogens. As the modalities of CF research have changed over the decades from empirical histological studies to include biophysical measurements of CFTR ion channel function, the clinical management of this disease has similarly evolved to effectively address the ever-changing spectrum of CF-related infectious diseases. This changing face of CF as a disease is due to several factors but is largely attributable to the advent of antibiotic therapy and the improvement of nutritional regimens. Combined, these factors have led to the successful management of most CF-related infections, with the notable exception of chronic lung infection with the gram-negative bacterium *P. aeruginosa*. The virulence of *P. aeruginosa* stems from multiple bacterial attributes, including innate antibiotic resistance and multidrug resistance, the ability to utilize quorum-sensing signals to form biofilms, the destructive potential of a multitude of microbial toxins and secretion factors such as those of the type III secretion system, and the ability to acquire a mucoid phenotype, all of which render this microbe extremely resistant to both the innate and acquired immunologic defenses of the host. Advances in therapeutic interventions in this disease have been slow, but overall there has been a steady improvement in the quality of life and long-term survival of CF patients. With the advent of new technologies ranging from sequences of microbial and human genomes to high-throughput screening for testing of potential therapeutics, there is great hope that the treatment of CF in the next decade will improve dramatically.

ACKNOWLEDGMENTS

REFERENCES

1. Reference deleted.
2. **Albert, M. L., S. F. Pearce, L. M. Francisco, B. Sauter, P. Roy, R. L. Silverstein, and N. Bhardwaj.** 1998. Immature dendritic cells phagocytose apoptotic cells via alphavbeta5 and CD36, and cross-present antigens to cytotoxic T lymphocytes. J. Exp. Med. **188:**1359–1368.
3. **Albus, A. M., E. C. Pesci, L. J. Runyen-Janecky, S. E. West, and B. H. Iglewski.** 1997. Vfr controls quorum sensing in *Pseudomonas aeruginosa*. J. Bacteriol. **179:**3928–3935.
4. **Allan, J. D., A. Mason, and A. D. Moss.** 1973. Nutritional supplementation in treatment of cystic fibrosis of the pancreas. Am. J. Dis. Child. **126:**22–26.
5. **Allewelt, M., F. T. Coleman, M. Grout, G. P. Priebe, and G. B. Pier.** 2000. Acquisition of expression of the *Pseudomonas aeruginosa* ExoU cytotoxin leads to increased bacterial virulence in a murine model of acute pneumonia and systemic spread. Infect. Immun. **68:**3998–4004.
6. **Andersen, D. H.** 1938. Cystic fibrosis of the pancreas and its relation to celiac disease: a clinical and pathologic study. Am. J. Dis. Child. **56:**344–399.
7. **Andersen, D. H., and R. G. Hodges.** 1946. Celiac syndrome: genetics of cystic fibrosis of pancreas with consideration of etiology. Am. J. Dis. Child. **72:**62–80.
8. **Anderson, M. P., H. A. Berger, D. P. Rich, R. J. Gregory, A. E. Smith, and M. J. Welsh.** 1991. Nucleoside triphosphates are required to open the CFTR chloride channel. Cell **67:**775–784.
9. **Anderson, M. P., D. P. Rich, R. J. Gregory, A. E. Smith, and M. J. Welsh.** 1991. Generation of cAMP-activated chloride currents by expression of CFTR. Science **251:**679–682.
10. **Arens, R., D. Gozal, K. J. Omlin, J. Vega, K. P. Boyd, T. G. Keens, and M. S. Woo.** 1994. Comparison of high frequency chest compression and conventional chest physiotherapy in hospitalized patients with cystic fibrosis. Am. J. Respir. Crit. Care Med. **150:**1154–1157.
11. **Armstrong, D. S., K. Grimwood, J. B. Carlin, R. Carzino, A. Olinsky, and P. D. Phelan.** 1996. Bronchoalveolar lavage or oropharyngeal cultures to identify lower respiratory pathogens in infants with cystic fibrosis. Pediatr. Pulmonol. **21:**267–275.
12. **Baggenstoss, A. H., M. H. Power, and J. H. Grindlay.** 1951. Further studies on the pathogenesis of fibrocystic disease of the pancreas. Arch. Pathol. **51:**510–517.
13. **Bals, R., X. Wang, Z. Wu, T. Freeman, V. Bafna, M. Zasloff, and J. M. Wilson.** 1998. Human beta-defensin 2 is a salt-sensitive peptide antibiotic expressed in human lung. J. Clin. Investig. **102:**874–880.
14. **Bardana, E. J., Jr., K. L. Sobti, F. D. Cianciulli, and M. J. Noonan.** 1975. *Aspergillus* antibody in patients with cystic fibrosis. Am. J. Dis. Child. **129:**1164–1167.
15. **Bauernfeind, A., G. Emminger, G. Horl, B. Lorbeer, B. Przyklenk, and C. Weisslein-Pfister.** 1987. Selective pressure of antistaphylococcal chemotherapeutics in favour of *Pseudomonas aeruginosa* in cystic fibrosis. Infection **15:**469–470.
16. **Bear, C. E., C. H. Li, N. Kartner, R. J. Bridges, T. J. Jensen, M. Ramjeesingh, and J. R. Riordan.** 1992. Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). Cell **68:**809–818.
17. **Berger, H. A., M. P. Anderson, R. J. Gregory, S. Thompson, P. W. Howard, R. A. Maurer, R. Mulligan, A. E. Smith, and M. J. Welsh.** 1991. Identification and regulation of the cystic fibrosis transmembrane conductance regulator-generated chloride channel. J. Clin. Investig. **88:**1422–1431.
18. **Berkower, C., and S. Michaelis.** 1991. Mutational analysis of the yeast a-factor transporter STE6, a member of the ATP binding cassette (ABC) protein superfamily. Embo. J. **10:**3777–3785.
19. **Berry, H. K., F. W. Kellogg, M. M. Hunt, R. L. Ingberg, L. Richter, and C. Gutjahr.** 1975. Dietary supplement and nutrition in children with cystic fibrosis. Am. J. Dis. Child. **129:**165–171.
20. **Bertranpetit, J., and F. Calafell.** 1996. Genetic and geographical variability in cystic fibrosis: evolutionary considerations. Ciba Found. Symp. **197:**97–114; discussion 114–118.
21. **Bhargava, V., J. F. Tomashefski, Jr., R. C. Stern, and C. R. Abramowsky.** 1989. The pathology of fungal infection and colonization in patients with cystic fibrosis. Hum. Pathol. **20:**977–986.
22. Reference deleted.
23. **Bijman, J., and P. M. Quinton.** 1987. Lactate and bicarbonate uptake in the sweat duct of cystic fibrosis and normal subjects. Pediatr. Res. **21:**79–82.
24. **Birrer, P., N. G. McElvaney, A. Rudeberg, C. W. Sommer, S. Liechti-Gallati, R. Kraemer, R. Hubbard, and R. G. Crystal.** 1994. Protease-antiprotease imbalance in the lungs of children with cystic fibrosis. Am. J. Respir. Crit. Care Med. **150:**207–213.
25. **Bollinger, N., D. J. Hassett, B. H. Iglewski, J. W. Costerton, and T. R. McDermott.** 2001. Gene expression in *Pseudomonas aeruginosa*: evidence of iron override effects on quorum sensing and biofilm-specific gene regulation. J. Bacteriol. **183:**1990–1996.
26. **Bonfield, T. L., M. W. Konstan, and M. Berger.** 1999. Altered respiratory epithelial cell cytokine production in cystic fibrosis. J. Allergy Clin. Immunol. **104:**72–78.
27. **Boucher, J. C., H. Yu, M. H. Mudd, and V. Deretic.** 1997. Mucoid *Pseudomonas aeruginosa* in cystic fibrosis: characterization of *muc* mutations in clinical isolates and analysis of clearance in a mouse model of respiratory infection. Infect. Immun. **65:**3838–3846.
28. **Boucher, R. C., M. J. Stutts, M. R. Knowles, L. Cantley, and J. T. Gatzy.** 1986. Na^+ transport in cystic fibrosis respiratory epithelia. Abnormal basal

rate and response to adenylate cyclase activation. J. Clin. Investig. **78:**1245–1252.

29. **Boxerbaum, B.** 1980. Isolation of rapidly growing mycobacteria in patients with cystic fibrosis. J. Pediatr. **96:**689–691.

30. **Braggion, C., L. M. Cappelletti, M. Cornacchia, L. Zanolla, and G. Mastella.** 1995. Short-term effects of three chest physiotherapy regimens in patients hospitalized for pulmonary exacerbations of cystic fibrosis: a crossover randomized study. Pediatr. Pulmonol. **19:**16–22.

31. **Bretscher, A.** 1999. Regulation of cortical structure by the ezrin-radixin-moesin protein family. Curr. Opin. Cell Biol. **11:**109–116.

32. **Brett, M. M., A. T. Ghoneim, and J. M. Littlewood.** 1986. Serum antibodies to *Pseudomonas aeruginosa* in cystic fibrosis. Arch. Dis. Child. **61:**1114–1120.

33. **Brint, J. M., and D. E. Ohman.** 1995. Synthesis of multiple exoproducts in *Pseudomonas aeruginosa* is under the control of RhlR-RhlI, another set of regulators in strain PAO1 with homology to the autoinducer-responsive LuxR-LuxI family. J. Bacteriol. **177:**7155–7163.

34. **Brogden, K. A., M. R. Ackermann, P. B. McCray, Jr., and K. M. Huttner.** 1999. Differences in the concentrations of small, anionic, antimicrobial peptides in bronchoalveolar lavage fluid and in respiratory epithelia of patients with and without cystic fibrosis. Infect. Immun. **67:**4256–4259.

35. **Brooun, A., S. Liu, and K. Lewis.** 2000. A dose-response study of antibiotic resistance in *Pseudomonas aeruginosa* biofilms. Antimicrob. Agents Chemother. **44:**640–646.

36. **Bryan, L. E., H. M. Van Den Elzen, and J. T. Tseng.** 1972. Transferable drug resistance in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **1:**22–29.

37. **Bryan, R., D. Kube, A. Perez, P. Davis, and A. Prince.** 1998. Overproduction of the CFTR R domain leads to increased levels of asialoGM1 and increased *Pseudomonas aeruginosa* binding by epithelial cells. Am. J. Respir. Cell Mol. Biol. **19:**269–277.

38. **Burkhardt, J. E., J. N. Walterspiel, and U. B. Schaad.** 1997. Quinolone arthropathy in animals versus children. Clin. Infect. Dis. **25:**1196–1204.

39. **Burnie, J. P., E. J. al-Wardi, P. Williamson, R. C. Matthews, K. Webb, and T. David.** 1995. Defining potential targets for immunotherapy in *Burkholderia cepacia* infection. FEMS Immunol. Med. Microbiol. **10:**157–164.

40. **Burns, J. L., J. Emerson, J. R. Stapp, D. L. Yim, J. Krzewinski, L. Louden, B. W. Ramsey, and C. R. Clausen.** 1998. Microbiology of sputum from patients at cystic fibrosis centers in the United States. Clin. Infect. Dis. **27:**158–163.

41. **Burns, J. L., R. L. Gibson, S. McNamara, D. Yim, J. Emerson, M. Rosenfeld, P. Hiatt, K. McCoy, R. Castile, A. L. Smith, and B. W. Ramsey.** 2001. Longitudinal assessment of *Pseudomonas aeruginosa* in young children with cystic fibrosis. J. Infect. Dis. **183:**444–452.

42. **Burns, J. L., M. Jonas, E. Y. Chi, D. K. Clark, A. Berger, and A. Griffith.** 1996. Invasion of respiratory epithelial cells by *Burkholderia (Pseudomonas) cepacia*. Infect. Immun. **64:**4054–4059.

43. **Burns, M. W.** 1968. Precipitins to *Klebsiella* and other enterobacteria in the serum of patients with chronic respiratory disorders. Lancet **i:**383–385.

44. **Burns, M. W., and J. R. May.** 1968. Bacterial precipitins in serum of patients with cystic fibrosis. Lancet **i:**270–272.

45. **Cabral, D. A., B. A. Loh, and D. P. Speert.** 1987. Mucoid *Pseudomonas aeruginosa* resists nonopsonic phagocytosis by human neutrophils and macrophages. Pediatr. Res. **22:**429–431.

46. **Cahill, P., M. W. Nason, Jr., C. Ambrose, T. Y. Yao, P. Thomas, and M. E. Egan.** 2000. Identification of the cystic fibrosis transmembrane conductance regulator domains that are important for interactions with ROMK2. J. Biol. Chem. **275:**16697–16701.

46a.**Cambridge University Press.** 1933. The geographical distribution of *Salmonella typhi* and *Salmonella paratyphi* A and B phage types during the period 1 January 1966 to 31 December 1969. J. Hyg. **71:**59–84.

47. **Canessa, C. M., J. D. Horisberger, and B. C. Rossier.** 1993. Epithelial sodium channel related to proteins involved in neurodegeneration. Nature **361:**467–470.

48. **Chen, J. H., H. Schulman, and P. Gardner.** 1989. A cAMP-regulated chloride channel in lymphocytes that is affected in cystic fibrosis. Science **243:**657–660.

49. **Cheng, S. H., D. P. Rich, J. Marshall, R. J. Gregory, M. J. Welsh, and A. E. Smith.** 1991. Phosphorylation of the R domain by cAMP-dependent protein kinase regulates the CFTR chloride channel. Cell **66:**1027–1036.

50. **Children's Hospital.** 1999. Epidemiologic study of cystic fibrosis study center report: reporting period January 1, 1998 to December 31, 1998. Children's Hospital, Boston, Mass.

51. **Choi, J. Y., D. Muallem, K. Kiselyov, M. G. Lee, P. J. Thomas, and S. Muallem.** 2001. Aberrant CFTR-dependent HCO-3 transport in mutations associated with cystic fibrosis. Nature **410:**94–97.

52. **Church, D. A., J. F. Kanga, R. J. Kuhn, T. T. Rubio, W. A. Spohn, J. C. Stevens, B. G. Painter, B. E. Thurberg, D. C. Haverstock, R. Y. Perroncel, and R. M. Echols for the Cystic Fibrosis Study Group.** 1997. Sequential ciprofloxacin therapy in pediatric cystic fibrosis: comparative study vs. ceftazidime/tobramycin in the treatment of acute pulmonary exacerbations. Pediatr. Infect. Dis. J. **16:**97–105; discussion 123–126.

53. **Clarke, L. L., M. C. Harline, L. R. Gawenis, N. M. Walker, J. T. Turner, and G. A. Weisman.** 2000. Extracellular UTP stimulates electrogenic bicarbonate secretion across CFTR knockout gallbladder epithelium. Am. J. Physiol. **279:**G132–G138.

54. **Cockrill, B. A., and C. A. Hales.** 1999. Allergic bronchopulmonary aspergillosis. Annu. Rev. Med. **50:**303–316.

55. **Colin, A. A., S. M. Sawyer, J. E. Mickle, R. D. Oates, A. Milunsky, and J. A. Amos.** 1996. Pulmonary function and clinical observations in men with congenital bilateral absence of the vas deferens. Chest **110:**440–445.

56. **Colin, A. A., and M. E. Wohl.** 1994. Cystic fibrosis. Pediatr. Rev. **15:**192–200.

57. **Comolli, J. C., L. L. Waite, K. E. Mostov, and J. N. Engel.** 1999. Pili binding to asialo-GM1 on epithelial cells can mediate cytotoxicity or bacterial internalization by *Pseudomonas aeruginosa*. Infect. Immun. **67:**3207–3214.

58. **Costes, B., E. Girodon, N. Ghanem, E. Flori, A. Jardin, J. C. Soufir, and M. Goossens.** 1995. Frequent occurrence of the *CFTR* intron 8 (TG)n 5T allele in men with congenital bilateral absence of the vas deferens. Eur. J. Hum. Genet. **3:**285–293.

59. **Cullen, A. R., C. L. Cannon, E. M. Mark, and A. A. Colin.** 2000. *Mycobacterium abscessus* infection in cystc fibrosis. Colonization of infection? Am. J. Respir. Crit. Care Med. **161:**641–645.

60. **Cuthbert, A. W., J. Halstead, R. Ratcliff, W. H. Colledge, and M. J. Evans.** 1995. The genetic advantage hypothesis in cystic fibrosis heterozygotes: a murine study. J. Physiol. **482:**449–454.

61. **Cystic Fibrosis Foundation.** 1999. Patient registry 1998 annual data report, September 1999. Cystic Fibrosis Foundation, Bethesda, Md.

62. **Dacheux, D., B. Toussaint, M. Richard, G. Brochier, J. Croize, and I. Attree.** 2000. *Pseudomonas aeruginosa* cystic fibrosis isolates induce rapid, type III secretion-dependent, but ExoU-independent, oncosis of macrophages and polymorphonuclear neutrophils. Infect. Immun. **68:**2916–2924.

63. **Dahl, M., A. Tybjaerg-Hansen, H. H. Wittrup, P. Lange, and B. G. Nordestgaard.** 1998. Cystic fibrosis Delta F508 heterozygotes, smoking, and reproduction: studies of 9141 individuals from a general population sample. Genomics **50:**89–96.

64. **Darzins, A., and A. M. Chakrabarty.** 1984. Cloning of genes controlling alginate biosynthesis from a mucoid cystic fibrosis isolate of *Pseudomonas aeruginosa*. J. Bacteriol. **159:**9–18.

65. **Datta, N.** 1969. Drug resistance and R factors in the bowel bacteria of London patients before and after admission to hospital. Br. Med. J. **2:**407–411.

66. **Davies, D. G., M. R. Parsek, J. P. Pearson, B. H. Iglewski, J. W. Costerton, and E. P. Greenberg.** 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science **280:**295–298.

67. **Reference deleted.**

68. **Davis, P. B., M. Drumm, and M. W. Konstan.** 1996. Cystic fibrosis. Am. J. Respir. Crit. Care Med. **154:**1229–1256.

69. **de Bentzmann, S., P. Roger, F. Dupuit, O. Bajolet-Laudinat, C. Fuchey, M. C. Plotkowski, and E. Puchelle.** 1996. Asialo GM₁ is a receptor for *Pseudomonas aeruginosa* adherence to regenerating respiratory epithelial cells. Infect. Immun. **64:**1582–1588.

70. **Demko, C. A., P. J. Byard, and P. B. Davis.** 1995. Gender differences in cystic fibrosis: *Pseudomonas aeruginosa* infection. J. Clin. Epidemiol. **48:**1041–1049.

71. **Denning, G. M., L. S. Ostedgaard, S. H. Cheng, A. E. Smith, and M. J. Welsh.** 1992. Localization of cystic fibrosis transmembrane conductance regulator in chloride secretory epithelia. J. Clin. Investig. **89:**339–349.

72. **Deretic, V., and W. M. Konyecsni.** 1989. Control of mucoidy in *Pseudomonas aeruginosa*: transcriptional regulation of *algR* and identification of the second regulatory gene, *algQ*. J. Bacteriol. **171:**3680–3688.

73. **Deretic, V., J. H. Leveau, C. D. Mohr, and N. S. Hibler.** 1992. In vitro phosphorylation of AlgR, a regulator of mucoidy in *Pseudomonas aeruginosa*, by a histidine protein kinase and effects of small phospho-donor molecules. Mol. Microbiol. **6:**2761–2767.

74. **DeVries, C. A., and D. E. Ohman.** 1994. Mucoid-to-nonmucoid conversion in alginate-producing *Pseudomonas aeruginosa* often results from spontaneous mutations in *algT*, encoding a putative alternate sigma factor, and shows evidence for autoregulation. J. Bacteriol. **176:**6677–6687.

75. **DiMango, E., A. J. Ratner, R. Bryan, S. Tabibi, and A. Prince.** 1998. Activation of NF-kappaB by adherent *Pseudomonas aeruginosa* in normal and cystic fibrosis respiratory epithelial cells. J. Clin. Investig. **101:**2598–2605.

76. **DiMango, E., H. J. Zar, R. Bryan, and A. Prince.** 1995. Diverse *Pseudomonas aeruginosa* gene products stimulate respiratory epithelial cells to produce interleukin-8. J. Clin. Investig. **96:**2204–2210.

77. **diSant'Agnese, P. A., R. C. Darling, G. A. Perera, and E. Shea.** 1953. Abnormal electrolyte composition of sweat in cystic fibrosis of the pancreas: clinical significance and relationship to the disease. Pediatrics **12:**549–563.

78. **diSant'Agnese, P., and D. Anderson.** 1946. Celiac syndrome. IV. Chemotherapy in infection of the respiratory tract associated with cystic fibrosis of the pancreas. Am. J. Dis. Child. **72:**17–61.

79. **Doggett, R. G., G. M. Harrison, R. N. Stillwell, and E. S. Wallis.** 1966. An atypical *Pseudomonas aeruginosa* associated with cystic fibrosis of the pancreas. J. Pediatr. **68:**215–221.

80. **Doring, G., S. P. Conway, H. G. Heijerman, M. E. Hodson, N. Hoiby, A. Smyth, and D. J. Touw.** 2000. Antibiotic therapy against *Pseudomonas aeruginosa* in cystic fibrosis: a European consensus. Eur. Respir. J. **16:**749–767.

81. **Doring, G., and N. Hoiby.** 1983. Longitudinal study of immune response to *Pseudomonas aeruginosa* antigens in cystic fibrosis. Infect. Immun. **42:**197–201.

82. **Doring, G., S. Jansen, H. Noll, H. Grupp, F. Frank, K. Botzenhart, K. Magdorf, and U. Wahn.** 1996. Distribution and transmission of *Pseudomonas aeruginosa* and *Burkholderia cepacia* in a hospital ward. Pediatr. Pulmonol. **21:**90–100.

83. **Dork, T., U. Wulbrand, T. Richter, T. Neumann, H. Wolfes, B. Wulf, G. Maass, and B. Tummler.** 1991. Cystic fibrosis with three mutations in the cystic fibrosis transmembrane conductance regulator gene. Hum. Genet. **87:**441–446.

84. **Egan, M., T. Flotte, S. Afione, R. Solow, P. L. Zeitlin, B. J. Carter, and W. B. Guggino.** 1992. Defective regulation of outwardly rectifying Cl^- channels by protein kinase A corrected by insertion of CFTR. Nature **358:**581–584.

85. **Eigen, H., B. J. Rosenstein, S. FitzSimmons, and D. V. Schidlow.** 1995. A multicenter study of alternate-day prednisone therapy in patients with cystic fibrosis. Cystic Fibrosis Foundation Prednisone Trial Group. J. Pediatr. **126:**515–523.

86. **Engelhardt, J. F., S. S. Smith, E. Allen, J. R. Yankaskas, D. C. Dawson, and J. M. Wilson.** 1994. Coupled secretion of chloride and mucus in skin of *Xenopus laevis*: possible role for CFTR. Am. J. Physiol. **267:**C491–C500.

87. **Engelhardt, J. F., J. R. Yankaskas, S. A. Ernst, Y. Yang, C. R. Marino, R. C. Boucher, J. A. Cohn, and J. M. Wilson.** 1992. Submucosal glands are the predominant site of CFTR expression in the human bronchus. Nat. Genet. **2:**240–248.

88. **Estivill, X., M. Farrall, P. J. Scambler, G. M. Bell, K. M. Hawley, N. J. Lench, G. P. Bates, H. C. Kruyer, P. A. Frederick, P. Stanier, et al.** 1987. A candidate for the cystic fibrosis locus isolated by selection for methylation-free islands. Nature **326:**840–845.

89. **Falk, M., M. Kelstrup, J. B. Andersen, T. Kinoshita, P. Falk, S. Stovring, and I. Gothgen.** 1984. Improving the ketchup bottle method with positive expiratory pressure, PEP, in cystic fibrosis. Eur. J. Respir. Dis. **65:**423–432.

90. **Farrell, P. M., G. Shen, M. Splaingard, C. E. Colby, A. Laxova, M. R. Kosorok, M. J. Rock, and E. H. Mischler.** 1997. Acquisition of *Pseudomonas aeruginosa* in children with cystic fibrosis. Pediatrics **100:**E2.

91. **Finck-Barbancon, V., J. Goranson, L. Zhu, T. Sawa, J. P. Wiener-Kronish, S. M. Fleiszig, C. Wu, L. Mende-Mueller, and D. W. Frank.** 1997. *Exo*U expression by *Pseudomonas aeruginosa* correlates with acute cytotoxicity and epithelial injury. Mol. Microbiol. **25:**547–557.

92. **Fisher, M. C., J. J. LiPuma, S. E. Dasen, G. C. Caputo, J. E. Mortensen, K. L. McGowan, and T. L. Stull.** 1993. Source of *Pseudomonas cepacia*: ribotyping of isolates from patients and from the environment. J. Pediatr. **123:**745–747.

93. **FitzSimmons, S. C.** 1993. The changing epidemiology of cystic fibrosis. J. Pediatr. **122:**1–9.

94. **Frederiksen, B., C. Koch, and N. Hoiby.** 1997. Antibiotic treatment of initial colonization with *Pseudomonas aeruginosa* postpones chronic infection and prevents deterioration of pulmonary function in cystic fibrosis. Pediatr. Pulmonol. **23:**330–335.

95. **Frederiksen, B., C. Koch, and N. Hoiby.** 1999. Changing epidemiology of *Pseudomonas aeruginosa* infection in Danish cystic fibrosis patients (1974–1995). Pediatr. Pulmonol. **28:**159–166.

96. **Freedman, S. D., M. H. Katz, E. M. Parker, M. Laposata, M. Y. Urman, and J. G. Alvarez.** 1999. A membrane lipid imbalance plays a role in the phenotypic expression of cystic fibrosis in cftr(−/−) mice. Proc. Natl. Acad. Sci. USA **96:**13995–4000.

97. **Freedman, S. D., J. C. Shea, P. G. Blanco, and J. G. Alvarez.** 2000. Fatty acids in cystic fibrosis. Curr. Opin. Pulm. Med. **6:**530–532.

98. **Frithz-Lindsten, E., A. Holmstrom, L. Jacobsson, M. Soltani, J. Olsson, R. Rosqvist, and A. Forsberg.** 1998. Functional conservation of the effector protein translocators PopB/YopB and PopD/YopD of *Pseudomonas aeruginosa* and *Yersinia pseudotuberculosis*. Mol. Microbiol. **29:**1155–1165.

99. **Frizzell, R. A., M. Field, and S. G. Schultz.** 1979. Sodium-coupled chloride transport by epithelial tissues. Am. J. Physiol. **236:**F1–F8.

100. **Fuchs, H. J., D. S. Borowitz, D. H. Christiansen, E. M. Morris, M. L. Nash, B. W. Ramsey, B. J. Rosenstein, A. L. Smith, and M. E. Wohl for the Pulmozyme Study Group.** 1994. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. N. Engl. J. Med. **331:**637–642.

101. **Fyfe, J. A., and J. R. Govan.** 1980. Alginate synthesis in mucoid *Pseudomonas aeruginosa*: a chromosomal locus involved in control. J. Gen. Microbiol. **119:**443–450.

102. **Gabriel, S. E., K. N. Brigman, B. H. Koller, R. C. Boucher, and M. J. Stutts.** 1994. Cystic fibrosis heterozygote resistance to cholera toxin in the cystic fibrosis mouse model. Science **266:**107–109.

103. **Gabriel, S. E., L. L. Clarke, R. C. Boucher, and M. J. Stutts.** 1993. CFTR and outward rectifying chloride channels are distinct proteins with a regulatory relationship. Nature **363:**263–268.

104. **Gadsby, D. C., and A. C. Nairn.** 1999. Control of CFTR channel gating by phosphorylation and nucleotide hydrolysis. Physiol. Rev. **79:**S77–S107.

105. **Geddes, D. M.** 1988. Antimicrobial therapy against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Pseudomonas cepacia*. Chest **94:**140S–145S.

106. **Gefter, W. B.** 1992. The spectrum of pulmonary aspergillosis. J. Thorac. Imaging **7:**56–74.

107. **Gilbert, A., M. Jadot, E. Leontieva, S. Wattiaux-De Coninck, and R. Wattiaux.** 1998. Delta F508 CFTR localizes in the endoplasmic reticulum-Golgi intermediate compartment in cystic fibrosis cells. Exp. Cell Res. **242:**144–152. (Erratum, **246:**538, 1999.)

108. **Gladman, G., P. J. Connor, R. F. Williams, and T. J. David.** 1992. Controlled study of *Pseudomonas cepacia* and *Pseudomonas maltophilia* in cystic fibrosis. Arch. Dis. Child. **67:**192–195.

109. **Glazebrook, J. S., R. S. Campbell, G. W. Hutchinson, and N. D. Stallman.** 1978. Rodent zoonoses in North Queensland: the occurrence and distribution of zoonotic infections in North Queensland rodents. Aust. J. Exp. Biol. Med. Sci. **56:**147–156.

110. **Goldman, M. J., G. M. Anderson, E. D. Stolzenberg, U. P. Kari, M. Zasloff, and J. M. Wilson.** 1997. Human beta-defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis. Cell **88:**553–560.

111. **Govan, J. R., C. Doherty, and S. Glass.** 1987. Rational parameters for antibiotic therapy in patients with cystic fibrosis. Infection **15:**300–307.

112. **Govan, J. R., and J. W. Nelson.** 1992. Microbiology of lung infection in cystic fibrosis. Br. Med. Bull. **48:**912–930.

113. **Grassme, H., S. Kirschnek, J. Riethmueller, A. Riehle, G. von Kurthy, F. Lang, M. Weller, and E. Gulbins.** 2000. CD95/CD95 ligand interactions on epithelial cells in host defense to *Pseudomonas aeruginosa*. Science **290:**527–530.

114. **Gray, K. M., L. Passador, B. H. Iglewski, and E. P. Greenberg.** 1994. Interchangeability and specificity of components from the quorum-sensing regulatory systems of *Vibrio fischeri* and *Pseudomonas aeruginosa*. J. Bacteriol. **176:**3076–3080.

115. **Green, S. K., M. N. Schroth, J. J. Cho, S. K. Kominos, and V. B. Vitanza-jack.** 1974. Agricultural plants and soil as a reservoir for *Pseudomonas aeruginosa*. Appl. Microbiol. **28:**987–991.

116. **Greger, R.** 1996. The amiloride-inhibitable Na^+ conductance of rat colonic crypt cells is suppressed by forskolin. Pflugers Arch. **431:**984–986.

117. **Grygorczyk, R., and J. W. Hanrahan.** 1997. CFTR-independent ATP release from epithelial cells triggered by mechanical stimuli. Am. J. Physiol. **272:**C1058–C1066.

118. **Gupta, S. K., R. S. Berk, S. Masinick, and L. D. Hazlett.** 1994. Pili and lipopolysaccharide of *Pseudomonas aeruginosa* bind to the glycolipid asialo GM1. Infect. Immun. **62:**4572–4579.

119. **Hall, R. A., L. S. Ostedgaard, R. T. Premont, J. T. Blitzer, N. Rahman, M. J. Welsh, and R. J. Lefkowitz.** 1998. A C-terminal motif found in the beta2-adrenergic receptor, P2Y1 receptor and cystic fibrosis transmembrane conductance regulator determines binding to the Na^+/H^+ exchanger regulatory factor family of PDZ proteins. Proc. Natl. Acad. Sci. USA **95:**8496–8501.

120. **Hancock, R. E., L. M. Mutharia, L. Chan, R. P. Darveau, D. P. Speert, and G. B. Pier.** 1983. *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis: a class of serum-sensitive, nontypeable strains deficient in lipopolysaccharide O side chains. Infect. Immun. **42:**170–177.

121. **Hazes, B., P. A. Sastry, K. Hayakawa, R. J. Read, and R. T. Irvin.** 2000. Crystal structure of *Pseudomonas aeruginosa* PAK pilin suggests a main-chain-dominated mode of receptor binding. J. Mol. Biol. **299:**1005–1017.

122. **Heilesen, A. M., H. Permin, C. Koch, and N. Hoiby.** 1983. Treatment of chronic *Pseudomonas aeruginosa* infection in cystic fibrosis patients with ceftazidime and tobramycin. Scand. J. Infect. Dis. **15:**271–276.

123. **Hjelte, L., B. Petrini, G. Kallenius, and B. Strandvik.** 1990. Prospective study of mycobacterial infections in patients with cystic fibrosis. Thorax **45:**397–400.

124. **Hodson, M. E., C. M. Roberts, R. J. Butland, M. J. Smith, and J. C. Batten.** 1987. Oral ciprofloxacin compared with conventional intravenous treatment for *Pseudomonas aeruginosa* infection in adults with cystic fibrosis. Lancet **i:**235–237.

125. **Hogan, D. L., D. L. Crombie, J. I. Isenberg, P. Svendsen, O. B. Schaffalitzky de Muckadell, and M. A. Ainsworth.** 1997. Acid-stimulated duodenal bicarbonate secretion involves a CFTR-mediated transport pathway in mice. Gastroenterology **113:**533–541.

126. **Hogan, D. L., D. L. Crombie, J. I. Isenberg, P. Svendsen, O. B. Schaffalitzky de Muckadell, and M. A. Ainsworth.** 1997. CFTR mediates cAMP- and Ca^{2+}-activated duodenal epithelial HCO_3^- secretion. Am. J. Physiol. **272:**G872–G878.

127. **Hogardt, M., K. Trebesius, A. M. Geiger, M. Hornef, J. Rosenecker, and J. Heesemann.** 2000. Specific and rapid detection by fluorescent in situ hybridization of bacteria in clinical samples obtained from cystic fibrosis patients. J. Clin. Microbiol. **38:**818–825.

128. **Hopken, U. E., B. Lu, N. P. Gerard, and C. Gerard.** 1996. The C5a chemoattractant receptor mediates mucosal defence to infection. Nature **383:**86–89.

129. **Huang, N. N., D. V. Schidlow, T. H. Szatrowski, J. Palmer, L. R. Laraya-**

Cuasay, W. Yeung, K. Hardy, L. Quitell, and S. Fiel. 1987. Clinical features, survival rate and prognostic factors in young adults with cystic fibrosis. Am. J. Med. **82:**871–879.

130. **Hudson, V. L., C. L. Wielinski, and W. E. Regelmann.** 1993. Prognostic implications of initial oropharyngeal bacterial flora in patients with cystic fibrosis diagnosed before the age of two years. J. Pediatr. **122:**854–860.

131. **Hundrieser, J., S. Bremer, F. Peinemann, M. Stuhrmann, N. Hoffknecht, B. Wulf, J. Schmidtke, J. Reiss, G. Maass, and B. Tummler.** 1990. Frequency of the F508 deletion in the CFTR gene in Turkish cystic fibrosis patients. Hum. Genet. **85:**409–410.

132. **Hwang, T. C., L. Lu, P. L. Zeitlin, D. C. Gruenert, R. Huganir, and W. B. Guggino.** 1989. Cl$^-$ channels in CF: lack of activation by protein kinase C and cAMP-dependent protein kinase. Science **244:**1351–1353.

133. **Illek, B., H. Fischer, and T. E. Machen.** 1998. Genetic disorders of membrane transport. II. Regulation of CFTR by small molecules including HCO$_3$. Am. J. Physiol. **275:**G1221–G1226.

134. **Illek, B., J. R. Yankaskas, and T. E. Machen.** 1997. cAMP and genistein stimulate HCO3-conductance through CFTR in human airway epithelia. Am. J. Physiol. **272:**L752–L761.

135. **Ismailov, II, M. S. Awayda, B. Jovov, B. K. Berdiev, C. M. Fuller, J. R. Dedman, M. Kaetzel, and D. J. Benos.** 1996. Regulation of epithelial sodium channels by the cystic fibrosis transmembrane conductance regulator. J. Biol. Chem. **271:**4725–4732.

136. **Ivanoff, B., M. M. Levine, and P. H. Lambert.** 1994. Vaccination against typhoid fever: present status. Bull. World Health Organ. **72:**957–971.

137. **Iyobe, S., K. Hasuda, H. Sagai, and S. Mitsuhashi.** 1975. Drug resistance and R factors in *P. aeruginosa*, pp. 321–327. *In* S. Mitsuhashi and J. Hashimoto (ed.), Microbial drug resistance. University Park Press, Baltimore, Md.

138. **Jensen, T., S. S. Pedersen, S. Garne, C. Heilmann, N. Hoiby, and C. Koch.** 1987. Colistin inhalation therapy in cystic fibrosis patients with chronic *Pseudomonas aeruginosa* lung infection. J. Antimicrob. Chemother. **19:**831–838.

139. **Jorde, L. B., and G. M. Lathrop.** 1988. A test of the heterozygote-advantage hypothesis in cystic fibrosis carriers. Am. J. Hum. Genet. **42:**808–815.

140. **Jovov, B., I. I. Ismailov, and D. J. Benos.** 1995. Cystic fibrosis transmembrane conductance regulator is required for protein kinase A activation of an outwardly rectified anion channel purified from bovine tracheal epithelia. J. Biol. Chem. **270:**1521–1528.

141. **Jovov, B., I. I. Ismailov, B. K. Berdiev, C. M. Fuller, E. J. Sorscher, J. R. Dedman, M. A. Kaetzel, and D. J. Benos.** 1995. Interaction between cystic fibrosis transmembrane conductance regulator and outwardly rectified chloride channels. J. Biol. Chem. **270:**29194–29200.

142. **Kalin, N., A. Claass, M. Sommer, E. Puchelle, and B. Tummler.** 1999. DeltaF508 CFTR protein expression in tissues from patients with cystic fibrosis. J. Clin. Investig. **103:**1379–1389.

143. **Kapur, V., T. S. Whittam, and J. M. Musser.** 1994. Is *Mycobacterium tuberculosis* 15,000 years old? J. Infect. Dis. **170:**1348–1349.

144. **Kartner, N., J. W. Hanrahan, T. J. Jensen, A. L. Naismith, S. Z. Sun, C. A. Ackerley, E. F. Reyes, L. C. Tsui, J. M. Rommens, C. E. Bear, et al.** 1991. Expression of the cystic fibrosis gene in non-epithelial invertebrate cells produces a regulated anion conductance. Cell. **64:**681–691.

145. **Keppler, D., I. Leier, and G. Jedlitschky.** 1997. Transport of glutathione conjugates and glucuronides by the multidrug resistance proteins MRP1 and MRP2. Biol. Chem. **378:**787–791.

146. **Kerem, B., J. M. Rommens, J. A. Buchanan, D. Markiewicz, T. K. Cox, A. Chakravarti, M. Buchwald, and L. C. Tsui.** 1989. Identification of the cystic fibrosis gene: genetic analysis. Science **245:**1073–1080.

147. **Kerem, E., N. Rave-Harel, A. Augarten, I. Madgar, M. Nissim-Rafinia, Y. Yahav, R. Goshen, L. Bentur, J. Rivlin, M. Aviram, A. Genem, O. Chiba-Falek, M. R. Kraemer, A. Simon, D. Branski, and B. Kerem.** 1997. A cystic fibrosis transmembrane conductance regulator splice variant with partial penetrance associated with variable cystic fibrosis presentations. Am. J. Respir. Crit. Care Med. **155:**1914–1920.

148. **Khan, T. Z., J. S. Wagener, T. Bost, J. Martinez, F. J. Accurso, and D. W. Riches.** 1995. Early pulmonary inflammation in infants with cystic fibrosis. Am. J. Respir. Crit. Care Med. **151:**1075–1082.

149. **Kilby, J. M., P. H. Gilligan, J. R. Yankaskas, W. E. Highsmith, Jr., L. J. Edwards, and M. R. Knowles.** 1992. Nontuberculous mycobacteria in adult patients with cystic fibrosis. Chest. **102:**70–75.

150. **Kirchner, K. K., J. S. Wagener, T. Z. Khan, S. C. Copenhaver, and F. J. Accurso.** 1996. Increased DNA levels in bronchoalveolar lavage fluid obtained from infants with cystic fibrosis. Am. J. Respir. Crit. Care Med. **154:**1426–1429.

151. **Kitamoto, O., N. Kasai, K. Fukaya, and A. Kawashima.** 1956. Drug sensitivity of the *Shigella* strains isolated in 1955. J. Jpn. Assoc. Infect. Dis. **30:**403.

152. **Knowles, M. R., N. L. Church, W. E. Waltner, J. R. Yankaskas, P. Gilligan, M. King, L. J. Edwards, R. W. Helms, and R. C. Boucher.** 1990. A pilot study of aerosolized amiloride for the treatment of lung disease in cystic fibrosis. N. Engl. J. Med. **322:**1189–1194.

153. **Knowles, M. R., L. L. Clarke, and R. C. Boucher.** 1991. Activation by extracellular nucleotides of chloride secretion in the airway epithelia of patients with cystic fibrosis. N. Engl. J. Med. **325:**533–538.

154. **Knowles, M. R., A. M. Paradiso, and R. C. Boucher.** 1995. In vivo nasal potential difference: techniques and protocols for assessing efficacy of gene transfer in cystic fibrosis. Hum. Gene Ther. **6:**445–455.

155. **Knowlton, R. G., O. Cohen-Haguenauer, N. Van Cong, J. Frezal, V. A. Brown, D. Barker, J. C. Braman, J. W. Schumm, L. C. Tsui, M. Buchwald, et al.** 1985. A polymorphic DNA marker linked to cystic fibrosis is located on chromosome 7. Nature **318:**380–382.

156. **Kobayashi, F., M. Yamaguchi, and S. Mitsuhashi.** 1972. Drug resistance to aminoglycosidic antibiotics in *Pseudomonas aeruginosa* and its lability. Jpn. J. Microbiol. **16:**425–431.

157. **Kobayashi, K., M. R. Knowles, R. C. Boucher, W. E. O'Brien, and A. L. Beaudet.** 1990. Benign missense variations in the cystic fibrosis gene. Am. J. Hum. Genet. **47:**611–615.

158. **Koch, C., and N. Hoiby.** 2000. Diagnosis and treatment of cystic fibrosis. Respiration **67:**239–247.

159. **Konstan, M. W.** 1998. Therapies aimed at airway inflammation in cystic fibrosis. Clin. Chest Med. **19:**505–513, vi.

160. **Konstan, M. W., P. J. Byard, C. L. Hoppel, and P. B. Davis.** 1995. Effect of high-dose ibuprofen in patients with cystic fibrosis. N. Engl. J. Med. **332:**848–854.

161. **Konstan, M. W., K. A. Hilliard, T. M. Norvell, and M. Berger.** 1994. Bronchoalveolar lavage findings in cystic fibrosis patients with stable, clinically mild lung disease suggest ongoing infection and inflammation. Am. J. Respir. Crit. Care Med. **150:**448–454. (Erratum, 151:260, 1995)

162. **Konstan, M. W., R. C. Stern, and C. F. Doershuk.** 1994. Efficacy of the Flutter device for airway mucus clearance in patients with cystic fibrosis. J. Pediatr. **124:**689–693.

163. **Konyecsni, W. M., and V. Deretic.** 1990. DNA sequence and expression analysis of *algP* and *algQ*, components of the multigene system transcriptionally regulating mucoidy in *Pseudomonas aeruginosa*: *algP* contains multiple direct repeats. J. Bacteriol. **172:**2511–2520.

164. **Krieg, D. P., R. J. Helmke, V. F. German, and J. A. Mangos.** 1988. Resistance of mucoid *Pseudomonas aeruginosa* to nonopsonic phagocytosis by alveolar macrophages. Infect. Immun. **56:**3173–3179.

165. **Kube, D., L. Adams, A. Perez, and P. B. Davis.** 2001. Terminal sialylation is altered in airway cells with impaired CFTR-mediated chloride transport. Am. J. Physiol. **280:**L482–L492.

166. **Kunzelmann, K., G. L. Kiser, R. Schreiber, and J. R. Riordan.** 1997. Inhibition of epithelial Na$^+$ currents by intracellular domains of the cystic fibrosis transmembrane conductance regulator. FEBS Lett. **400:**341–344.

167. **Kuschert, G. S., F. Coulin, C. A. Power, A. E. Proudfoot, R. E. Hubbard, A. J. Hoogewerf, and T. N. Wells.** 1999. Glycosaminoglycans interact selectively with chemokines and modulate receptor binding and cellular responses. Biochemistry **38:**12959–12968.

168. **Lam, J., R. Chan, K. Lam, and J. W. Costerton.** 1980. Production of mucoid microcolonies by *Pseudomonas aeruginosa* within infected lungs in cystic fibrosis. Infect. Immun. **28:**546–556.

169. **Landsteiner, K.** 1905. Darmverschluss durch eingedicktes Meconium; pankreatitis. Zentralbl. Allg. Pathol. Ser. U, **16:**903.

170. **Lang, B. J., S. D. Aaron, W. Ferris, P. C. Hebert, and N. E. Macdonald.** 2000. Multiple combination bactericidal antibiotic testing for patients with cystic fibrosis infected with multiresistant strains of *Pseudomonas aeruginosa*. Am. J. Respir. Crit. Care Med. **162:**2241–2245.

171. **Learn, D. B., E. P. Brestel, and S. Seetharama.** 1987. Hypochlorite scavenging by *Pseudomonas aeruginosa* alginate. Infect. Immun. **55:**1813–1818.

172. **Lee, K. K., P. Doig, R. T. Irvin, W. Paranchych, and R. S. Hodges.** 1989. Mapping the surface regions of *Pseudomonas aeruginosa* PAK pilin: the importance of the C-terminal region for adherence to human buccal epithelial cells. Mol. Microbiol. **3:**1493–1499.

173. **LeVine, A. M., K. E. Kurak, M. D. Bruno, J. M. Stark, J. A. Whitsett, and T. R. Korfhagen.** 1998. Surfactant protein-A-deficient mice are susceptible to *Pseudomonas aeruginosa* infection. Am. J. Respir. Cell Mol. Biol. **19:**700–708.

174. **Lewin, L. O., P. J. Byard, and P. B. Davis.** 1990. Effect of *Pseudomonas cepacia* colonization on survival and pulmonary function of cystic fibrosis patients. J. Clin. Epidemiol. **43:**125–131.

175. **Li, M., J. D. McCann, C. M. Liedtke, A. C. Nairn, P. Greengard, and M. J. Welsh.** 1988. Cyclic AMP-dependent protein kinase opens chloride channels in normal but not cystic fibrosis airway epithelium. Nature **331:**358–360.

176. **Linsdell, P., and J. W. Hanrahan.** 1998. Adenosine triphosphate-dependent asymmetry of anion permeation in the cystic fibrosis transmembrane conductance regulator chloride channel. J. Gen. Physiol. **111:**601–614.

177. **Linsdell, P., J. A. Tabcharani, J. M. Rommens, Y. X. Hou, X. B. Chang, L. C. Tsui, J. R. Riordan, and J. W. Hanrahan.** 1997. Permeability of wild-type and mutant cystic fibrosis transmembrane conductance regulator chloride channels to polyatomic anions. J. Gen. Physiol. **110:**355–364.

178. **Liu, C. E., P. Q. Liu, and G. F. L. Ames.** 1997. Characterization of the adenosine triphosphatase activity of the periplasmic histidine permease, a traffic ATPase (ABC transporter). J. Biol. Chem. **272:**21883–21891.

179. **Ma, D., D. N. Cook, J. E. Hearst, and H. Nikaido.** 1994. Efflux pumps and drug resistance in Gram-negative bacteria. Trends Microbiol. **2:**489–493.

180. **Ma, J., J. Zhao, M. L. Drumm, J. Xie, and P. B. Davis.** 1997. Function of the R domain in the cystic fibrosis transmembrane conductance regulator chloride channel. J. Biol. Chem. **272:**28133–28141.

181. **Ma, S., U. Selvaraj, D. E. Ohman, R. Quarless, D. J. Hassett, and D. J. Wozniak.** 1998. Phosphorylation-independent activity of the response regulators AlgB and AlgR in promoting alginate biosynthesis in mucoid *Pseudomonas aeruginosa*. J. Bacteriol. **180:**956–968.

182. **Ma, S., D. J. Wozniak, and D. E. Ohman.** 1997. Identification of the histidine protein kinase KinB in *Pseudomonas aeruginosa* and its phosphorylation of the alginate regulator AlgB. J. Biol. Chem. **272:**17952–17960.

183. **Mahenthiralingam, E., M. E. Campbell, and D. P. Speert.** 1994. Nonmotility and phagocytic resistance of *Pseudomonas aeruginosa* isolates from chronically colonized patients with cystic fibrosis. Infect. Immun. **62:**596–605.

184. **Manavalan, P., A. E. Smith, and J. M. McPherson.** 1993. Sequence and structural homology among membrane-associated domains of CFTR and certain transporter proteins. J. Protein Chem. **12:**279–290.

185. **Mariencheck, W. I., J. Savov, Q. Dong, M. J. Tino, and J. R. Wright.** 1999. Surfactant protein A enhances alveolar macrophage phagocytosis of a live, mucoid strain of *P. aeruginosa*. Am. J. Physiol. **277:**L777–L786.

186. **Martin, D. W., M. J. Schurr, H. Yu, and V. Deretic.** 1994. Analysis of promoters controlled by the putative sigma factor AlgU regulating conversion to mucoidy in *Pseudomonas aeruginosa*: relationship to sigma E and stress response. J. Bacteriol. **176:**6688–6696.

187. **Masuda, N., E. Sakagawa, and S. Ohya.** 1995. Outer membrane proteins responsible for multiple drug resistance in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **39:**645–649.

188. **Mathee, K., O. Ciofu, C. Sternberg, P. W. Lindum, J. I. Campbell, P. Jensen, A. H. Johnsen, M. Givskov, D. E. Ohman, S. Molin, N. Hoiby, and A. Kharazmi.** 1999. Mucoid conversion of *Pseudomonas aeruginosa* by hydrogen peroxide: a mechanism for virulence activation in the cystic fibrosis lung. Microbiology **145:**1349–1357.

189. **Mathee, K., C. J. McPherson, and D. E. Ohman.** 1997. Posttranslational control of the *algT* (*algU*)-encoded sigma22 for expression of the alginate regulon in *Pseudomonas aeruginosa* and localization of its antagonist proteins MucA and MucB (AlgN). J. Bacteriol. **179:**3711–3720.

190. **Matsui, H., B. R. Grubb, R. Tarran, S. H. Randell, J. T. Gatzy, C. W. Davis, and R. C. Boucher.** 1998. Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. Cell **95:**1005–1015.

191. **McCaffery, K., R. E. Olver, M. Franklin, and S. Mukhopadhyay.** 1999. Systematic review of antistaphylococcal antibiotic therapy in cystic fibrosis. Thorax **54:**380–383.

192. **McIntosh, R.** 1954. Cystic fibrosis of the pancreas in patients over 10 years of age. Acta Paediatr. **43:**469.

193. **McLaughlin, F. J., W. J. Matthews, Jr., D. J. Strieder, B. Sullivan, A. Taneja, P. Murphy, and D. A. Goldmann.** 1983. Clinical and bacteriological responses to three antibiotic regimens for acute exacerbations of cystic fibrosis: ticarcillin-tobramycin, azlocillin-tobramycin, and azlocillin-placebo. J. Infect. Dis. **147:**559–567.

194. **Meluleni, G. J., M. Grout, D. J. Evans, and G. B. Pier.** 1995. Mucoid *Pseudomonas aeruginosa* growing in a biofilm in vitro are killed by opsonic antibodies to the mucoid exopolysaccharide capsule but not by antibodies produced during chronic lung infection in cystic fibrosis patients. J. Immunol. **155:**2029–2038.

195. **Mennie, M., A. Gilfillan, D. J. Brock, and W. A. Liston.** 1995. Heterozygotes for the delta F508 cystic fibrosis allele are not protected against bronchial asthma. Nat. Med. **1:**978–979.

196. **Meshulam, T., N. Obedeanu, D. Merzbach, and J. D. Sobel.** 1984. Phagocytosis of mucoid and nonmucoid strains of *Pseudomonas aeruginosa*. Clin. Immunol. Immunopathol. **32:**151–165.

197. **Michalsen, H., and T. Bergan.** 1981. Azlocillin with and without an aminoglycoside against respiratory tract infections in children with cystic fibrosis. Scand. J. Infect. Dis. Suppl. **29:**92–97.

198. **Morrison, W. D., R. V. Miller, and G. S. Sayler.** 1978. Frequency of F116-mediated transduction of *Pseudomonas aeruginosa* in a freshwater environment. Appl. Environ. Microbiol. **36:**724–730.

199. **Mortensen, J., M. Falk, S. Groth, and C. Jensen.** 1991. The effects of postural drainage and positive expiratory pressure physiotherapy on tracheobronchial clearance in cystic fibrosis. Chest **100:**1350–1357.

200. **Mulherin, D., M. J. Coffey, D. O. Halloran, M. T. Keogan, and M. X. FitzGerald.** 1990. Skin reactivity to atypical mycobacteria in cystic fibrosis. Respir. Med. **84:**273–276.

201. **Nakae, T.** 1997. Multiantibiotic resistance caused by active drug extrusion in *Pseudomonas aeruginosa* and other gram-negative bacteria. Microbiologia **13:**273–284.

202. **Naren, A. P., A. Di, E. Cormet-Boyaka, P. N. Boyaka, J. R. McGhee, W. Zhou, K. Akagawa, T. Fujiwara, U. Thome, J. F. Engelhardt, D. J. Nelson, and K. L. Kirk.** 2000. Syntaxin 1A is expressed in airway epithelial cells, where it modulates CFTR Cl(−) currents. J. Clin. Investig. **105:**377–386.

203. **Naren, A. P., D. J. Nelson, W. Xie, B. Jovov, J. Pevsner, M. K. Bennett, D. J. Benos, M. W. Quick, and K. L. Kirk.** 1997. Regulation of CFTR chloride channels by syntaxin and Munc18 isoforms. Nature **390:**302–305.

204. **Nelson, P. V., W. F. Carey, and C. P. Morris.** 1991. Identification of a cystic fibrosis mutation: deletion of isoleucine506. Hum. Genet. **86:**391–393.

205. **Neville, D. C., C. R. Rozanas, B. M. Tulk, R. R. Townsend, and A. S. Verkman.** 1998. Expression and characterization of the NBD1-R domain region of CFTR: evidence for subunit-subunit interactions. Biochemistry **37:**2401–2409.

206. **Nikaido, K., P. Q. Liu, and G. F. Ames.** 1997. Purification and characterization of HisP, the ATP-binding subunit of a traffic ATPase (ABC transporter), the histidine permease of *Salmonella typhimurium*. Solubility, dimerization, and ATPase activity. J. Biol. Chem. **272:**27745–27752.

207. **Nivens, D. E., D. E. Ohman, J. Williams, and M. J. Franklin.** 2001. Role of alginate and its O acetylation in formation of Pseudomonas aeruginosa microcolonies and biofilms. J Bacteriol. **183:**1047–57.

208. **Reference deleted.**

209. **Norris, R. F., and R. M. Tyson.** 1946. The pathogenesis of polycystic pancreas: reconstruction of cystic elements in one case. Am. J. Pathol. **23:**485–499.

210. **Ochiai, K., T. Yamanaka, K. Kimura, and O. Sawada.** 1959. Studies on inheritance of drug resistance between *Shigella* strains and *Escherichia coli* strains. Nippon Iji Shimpo **1861:**34.

211. **Ochsner, U. A., and J. Reiser.** 1995. Autoinducer-mediated regulation of rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA **92:**6424–6428.

212. **Ohman, D. E., and A. M. Chakrabarty.** 1981. Genetic mapping of chromosomal determinants for the production of the exopolysaccharide alginate in a *Pseudomonas aeruginosa* cystic fibrosis isolate. Infect. Immun. **33:**142–148.

213. **Olexy, V. M., D. K. Mucha, T. J. Bird, H. G. Grieble, and S. K. Farrand.** 1982. An R plasmid of *Serratia marcescens* transferable to *Pseudomonas aeruginosa*. Chemotherapy **28:**6–17.

214. **Olsen, R. H., and J. Hansen.** 1976. Evolution and utility of a *Pseudomonas aeruginosa* drug resistance factor. J. Bacteriol. **125:**837–844.

215. **Parad, R. B., C. J. Gerard, D. Zurakowski, D. P. Nichols, and G. B. Pier.** 1999. Pulmonary outcome in cystic fibrosis is influenced primarily by mucoid *Pseudomonas aeruginosa* infection and immune status and only modestly by genotype. Infect. Immun. **67:**4744–4750.

216. **Park, P. W., G. B. Pier, M. T. Hinkes, and M. Bernfield.** 2001. Exploitation of syndecan-1 shedding by *Pseudomonas aeruginosa* enhances virulence. Nature **411:**98–102.

217. **Park, P. W., G. B. Pier, M. J. Preston, O. Goldberger, M. L. Fitzgerald, and M. Bernfield.** 2000. Syndecan-1 shedding is enhanced by LasA, a secreted virulence factor of *Pseudomonas aeruginosa*. J. Biol. Chem. **275:**3057–3064.

218. **Passador, L., J. M. Cook, M. J. Gambello, L. Rust, and B. H. Iglewski.** 1993. Expression of *Pseudomonas aeruginosa* virulence genes requires cell-to-cell communication. Science **260:**1127–1130.

219. **Pasyk, E. A., X. K. Morin, P. Zeman, E. Garami, K. Galley, L. J. Huan, Y. Wang, and C. E. Bear.** 1998. A conserved region of the R domain of cystic fibrosis transmembrane conductance regulator is important in processing and function. J. Biol. Chem. **273:**31759–31764.

220. **Pearson, J. P., K. M. Gray, L. Passador, K. D. Tucker, A. Eberhard, B. H. Iglewski, and E. P. Greenberg.** 1994. Structure of the autoinducer required for expression of *Pseudomonas aeruginosa* virulence genes. Proc. Natl. Acad. Sci. USA **91:**197–201.

221. **Pearson, J. P., L. Passador, B. H. Iglewski, and E. P. Greenberg.** 1995. A second N-acylhomoserine lactone signal produced by *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. **92:**1490–1494.

222. **Pedersen, S. S., N. Hoiby, F. Espersen, and C. Koch.** 1992. Role of alginate in infection with mucoid *Pseudomonas aeruginosa* in cystic fibrosis. Thorax **47:**6–13.

223. **Pedersen, S. S., T. Jensen, T. Pressler, N. Hoiby, and K. Rosendal.** 1986. Does centralized treatment of cystic fibrosis increase the risk of *Pseudomonas aeruginosa* infection? Acta Paediatr. Scand. **75:**840–845.

224. **Pedersen, S. S., A. Kharazmi, F. Espersen, and N. Hoiby.** 1990. *Pseudomonas aeruginosa* alginate in cystic fibrosis sputum and the inflammatory response. Infect. Immun. **58:**3363–3368.

225. **Pedersen, S. S., C. Koch, N. Hoiby, and K. Rosendal.** 1986. An epidemic spread of multiresistant *Pseudomonas aeruginosa* in a cystic fibrosis centre. J. Antimicrob. Chemother. **17:**505–516.

226. **Pesci, E. C., J. B. Milbank, J. P. Pearson, S. McKnight, A. S. Kende, E. P. Greenberg, and B. H. Iglewski.** 1999. Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA **96:**11229–11234.

227. **Pfleger, A., B. Theissl, B. Oberwaldner, and M. S. Zach.** 1992. Self-administered chest physiotherapy in cystic fibrosis: a comparative study of high-pressure PEP and autogenic drainage. Lung **170:**323–330.

228. **Phair, J. P., J. S. Tan, C. Watanakunakorn, L. Schwab, and L. W. Sanders.** 1970. Carbenicillin treatment of *Pseudomonas* pulmonary infection. Use in children with cystic fibrosis. Am. J. Dis. Child. **120:**22–25.

229. **Pier, G. B.** 1985. Pulmonary disease associated with *Pseudomonas aerugi-*

nosa in cystic fibrosis: current status of the host-bacterium interaction. J. Infect. Dis. **151:**575–580.

229a.**Pier, G. B.** 2002. CFTR Mutations and host susceptibility to *Pseudomonas aeruginosa* infection. Curr. Opin. Microbiol. **5:**81–86.

230. **Pier, G. B., and P. Ames.** 1984. Mediation of the killing of rough, mucoid isolates of *Pseudomonas aeruginosa* from patients with cystic fibrosis by the alternative pathway of complement. J. Infect. Dis. **150:**223–228.

231. **Pier, G. B., F. Coleman, M. Grout, M. Franklin, and D. E. Ohman.** 2001. Role of alginate O acetylation in resistance of mucoid *Pseudomonas aeruginosa* to opsonic phagocytosis. Infect. Immun. **69:**1895–1901.

232. **Pier, G. B., D. DesJardins, M. Grout, C. Garner, S. E. Bennett, G. Pekoe, S. A. Fuller, M. O. Thornton, W. S. Harkonen, and H. C. Miller.** 1994. Human immune response to *Pseudomonas aeruginosa* mucoid exopolysaccharide (alginate) vaccine. Infect. Immun. **62:**3972–3979

233. **Pier, G. B., M. Grout, T. Zaidi, G. Meluleni, S. S. Mueschenborn, G. Banting, R. Ratcliff, M. J. Evans, and W. H. Colledge.** 1998. *Salmonella typhi* uses CFTR to enter intestinal epithelial cells. Nature **393:**79–82.

234. **Pier, G. B., M. Grout, and T. S. Zaidi.** 1997. Cystic fibrosis transmembrane conductance regulator is an epithelial cell receptor for clearance of *Pseudomonas aeruginosa* from the lung. Proc. Natl. Acad. Sci. USA **94:**12088–12093.

235. **Pier, G. B., M. Grout, T. S. Zaidi, J. C. Olsen, L. G. Johnson, J. R. Yankaskas, and J. B. Goldberg.** 1996. Role of mutant CFTR in hypersusceptibility of cystic fibrosis patients to lung infections. Science **271:**64–67.

236. **Pier, G. B., J. M. Saunders, P. Ames, M. S. Edwards, H. Auerbach, J. Goldfarb, D. P. Speert, and S. Hurwitch.** 1987. Opsonophagocytic killing antibody to *Pseudomonas aeruginosa* mucoid exopolysaccharide in older, non-colonized cystic fibrosis patients. N. Engl. J. Med. **317:**793–798.

237. **Pier, G. B., G. J. Small, and H. B. Warren.** 1990. Protection against mucoid *Pseudomonas aeruginosa* in rodent models of endobronchial infections. Science **249:**537–540.

238. **Potter, J. L., S. Spector, L. W. Matthews, and J. Lemm.** 1969. Studies on pulmonary secretions. 3. The nucleic acids in whole pulmonary secretions from patients with cystic fibrosis, bronchiectasis, and laryngectomy. Am. Rev. Respir. Dis. **99:**909–916.

239. **Poulsen, J. H., H. Fischer, B. Illek, and T. E. Machen.** 1994. Bicarbonate conductance and pH regulatory capability of cystic fibrosis transmembrane conductance regulator. Proc. Natl. Acad. Sci. USA **91:**5340–5344.

240. **Pratha, V. S., D. L. Hogan, B. A. Martensson, J. Bernard, R. Zhou, and J. I. Isenberg.** 2000. Identification of transport abnormalities in duodenal mucosa and duodenal enterocytes from patients with cystic fibrosis. Gastroenterology **118:**1051–1060.

241. **Pugashetti, B. K., H. M. Metzger, Jr., L. Vadas, and D. S. Feingold.** 1982. Phenotypic differences among clinically isolated mucoid *Pseudomonas aeruginosa* strains. J. Clin. Microbiol. **16:**686–691.

242. **Quinn, J. P.** 1998. Clinical problems posed by multiresistant nonfermenting gram-negative pathogens. Clin Infect Dis. **27**(Suppl. 1)**:**S117–S124.

243. **Quinton, P. M., and J. Bijman.** 1983. Higher bioelectric potentials due to decreased chloride absorption in the sweat glands of patients with cystic fibrosis. N. Engl. J. Med. **308:**1185–1189.

244. **Reference deleted.**

245. **Ramjeesingh, M., C. Li, E. Garami, L. J. Huan, K. Galley, Y. Wang, and C. E. Bear.** 1999. Walker mutations reveal loose relationship between catalytic and channel-gating activities of purified CFTR (cystic fibrosis transmembrane conductance regulator). Biochemistry **38:**1463–1468.

246. **Reference deleted.**

247. **Ramsey, B. W.** 1996. Management of pulmonary disease in patients with cystic fibrosis. N. Engl. J. Med. **335:**179–188. (Erratum, **335:**1167.)

248. **Ramsey, B. W., S. J. Astley, M. L. Aitken, W. Burke, A. A. Colin, H. L. Dorkin, J. D. Eisenberg, R. L. Gibson, I. R. Harwood, D. V. Schidlow, et al.** 1993. Efficacy and safety of short-term administration of aerosolized recombinant human deoxyribonuclease in patients with cystic fibrosis. Am. Rev. Respir. Dis. **148:**145–151.

249. **Ramsey, B. W., M. S. Pepe, J. M. Quan, K. L. Otto, A. B. Montgomery, J. Williams-Warren, K. M. Vasiljev, D. Borowitz, C. M. Bowman, B. C. Marshall, S. Marshall, and A. L. Smith.** For the Cystic Fibrosis Inhaled Tobramycin Study Group. 1999. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. N. Engl. J. Med. **340:**23–30.

250. **Ramsey, B. W., K. R. Wentz, A. L. Smith, M. Richardson, J. Williams-Warren, D. L. Hedges, R. Gibson, G. J. Redding, K. Lent, and K. Harris.** 1991. Predictive value of oropharyngeal cultures for identifying lower airway bacteria in cystic fibrosis patients. Am. Rev. Respir. Dis. **144:**331–337.

251. **Ratjen, F., G. Comes, K. Paul, H. G. Posselt, T. O. Wagner, and K. Harms.** 2001. Effect of continuous antistaphylococcal therapy on the rate of *P. aeruginosa* acquisition in patients with cystic fibrosis. Pediatr. Pulmonol. **31:**13–16.

252. **Reddy, M. M., P. M. Quinton, C. Haws, J. J. Wine, R. Grygorczyk, J. A. Tabcharani, J. W. Hanrahan, K. L. Gunderson, and R. R. Kopito.** 1996. Failure of the cystic fibrosis transmembrane conductance regulator to conduct ATP. Science **271:**1876–1879.

253. **Regelmann, W. E., G. R. Elliott, W. J. Warwick, and C. C. Clawson.** 1990. Reduction of sputum *Pseudomonas aeruginosa* density by antibiotics im-

proves lung function in cystic fibrosis more than do bronchodilators and chest physiotherapy alone. Am. Rev. Respir. Dis. **141:**914–921.

254. **Reisin, I. L., A. G. Prat, E. H. Abraham, J. F. Amara, R. J. Gregory, D. A. Ausiello, and H. F. Cantiello.** 1994. The cystic fibrosis transmembrane conductance regulator is a dual ATP and chloride channel. J. Biol. Chem. **269:**20584–20591.

255. **Restrepo, C. I., Q. Dong, J. Savov, W. I. Mariencheck, and J. R. Wright.** 1999. Surfactant protein D stimulates phagocytosis of *Pseudomonas aeruginosa* by alveolar macrophages. Am. J. Respir. Cell Mol. Biol. **21:**576–585.

256. **Richmond, M. H., G. W. Jack, and R. B. Sykes.** 1971. Mechanisms of drug resistance. The beta-lactamases of Gram-negative bacteria including pseudomonads. Ann. N. Y. Acad. Sci. **182:**243–257.

257. **Riordan, J. R., K. Deuchars, N. Kartner, N. Alon, J. Trent, and V. Ling.** 1985. Amplification of P-glycoprotein genes in multidrug-resistant mammalian cell lines. Nature **316:**817–819.

258. **Riordan, J. R., J. M. Rommens, B. Kerem, N. Alon, R. Rozmahel, Z. Grzelczak, J. Zielenski, S. Lok, N. Plavsic, J. L. Chou, et al.** 1989. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science **245:**1066–1073. (Erratum, **245:**1437.)

259. **Rogers, D. F., E. W. Alton, A. Dewar, M. I. Lethem, and P. J. Barnes.** 1993. Impaired stimulus-evoked mucus secretion in cystic fibrosis bronchi. Exp. Lung Res. **19:**37–53.

260. **Rommens, J. M., M. C. Iannuzzi, B. Kerem, M. L. Drumm, G. Melmer, M. Dean, R. Rozmahel, J. L. Cole, D. Kennedy, N. Hidaka, et al.** 1989. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science **245:**1059–1065.

261. **Rosenfeld, M., J. Emerson, F. Accurso, D. Armstrong, R. Castile, K. Grimwood, P. Hiatt, K. McCoy, S. McNamara, B. Ramsey, and J. Wagener.** 1999. Diagnostic accuracy of oropharyngeal cultures in infants and young children with cystic fibrosis. Pediatr. Pulmonol. **28:**321–328.

262. **Rovere, P., A. A. Manfredi, C. Vallinoto, V. S. Zimmermann, U. Fascio, G. Balestrieri, P. Ricciardi-Castagnoli, C. Rugarli, A. Tincani, and M. G. Sabbadini.** 1998. Dendritic cells preferentially internalize apoptotic cells opsonized by anti-beta2-glycoprotein I antibodies. J. Autoimmun. **11:**403–411.

263. **Rovere, P., M. G. Sabbadini, C. Vallinoto, U. Fascio, M. Recigno, M. Crosti, P. Ricciardi-Castagnoli, G. Balestrieri, A. Tincani, and A. A. Manfredi.** 1999. Dendritic cell presentation of antigens from apoptotic cells in a proinflammatory context: role of opsonizing anti-beta2-glycoprotein I antibodies. Arthritis Rheum. **42:**1412–1420.

264. **Rubartelli, A., A. Poggi, and M. R. Zocchi.** 1997. The selective engulfment of apoptotic bodies by dendritic cells is mediated by the alpha(v)beta3 integrin and requires intracellular and extracellular calcium. Eur. J. Immunol. **27:**1893–1900.

265. **Saiman, L., and A. Prince.** 1993. *Pseudomonas aeruginosa* pili bind to asialoGM1 which is increased on the surface of cystic fibrosis epithelial cells. J. Clin. Investig. **92:**1875–1880.

266. **Sanders, C. C., W. E. Sanders, Jr., R. V. Goering, and V. Werner.** 1984. Selection of multiple antibiotic resistance by quinolones, beta- lactams, and aminoglycosides with special reference to cross-resistance between unrelated drug classes. Antimicrob. Agents Chemother. **26:**797–801.

267. **Sato, K., and F. Sato.** 1984. Defective beta adrenergic response of cystic fibrosis sweat glands in vivo and in vitro. J. Clin. Investig. **73:**1763–1771.

268. **Saye, D. J., O. Ogunseitan, G. S. Sayler, and R. V. Miller.** 1987. Potential for transduction of plasmids in a natural freshwater environment: effect of plasmid donor concentration and a natural microbial community on transduction in *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. **53:**987–995.

269. **Schiller, N. L., M. J. Alazard, and R. S. Borowski.** 1984. Serum sensitivity of a *Pseudomonas aeruginosa* mucoid strain. Infect. Immun. **45:**748–755.

270. **Schonheyder, H., T. Jensen, N. Hoiby, P. Andersen, and C. Koch.** 1985. Frequency of *Aspergillus fumigatus* isolates and antibodies to *aspergillus* antigens in cystic fibrosis. Acta Pathol. Microbiol. Immunol. Scand. Ser. B **93:**105–112.

271. **Schoni, M. H.** 1989. Autogenic drainage: a modern approach to physiotherapy in cystic fibrosis. J. R. Soc. Med. **82:**32–37.

272. **Schoumacher, R. A., R. L. Shoemaker, D. R. Halm, E. A. Tallant, R. W. Wallace, and R. A. Frizzell.** 1987. Phosphorylation fails to activate chloride channels from cystic fibrosis airway cells. Nature **330:**752–754.

273. **Schreiber, R., A. Hopf, M. Mall, R. Greger, and K. Kunzelmann.** 1999. The first-nucleotide binding domain of the cystic-fibrosis transmembrane conductance regulator is important for inhibition of the epithelial Na$^+$ channel. Proc. Natl. Acad. Sci. USA **96:**5310–5315.

274. **Schreiber, R., R. Nitschke, R. Greger, and K. Kunzelmann.** 1999. The cystic fibrosis transmembrane conductance regulator activates aquaporin 3 in airway epithelial cells. J. Biol. Chem. **274:**11811–11816.

275. **Schreiber, R., H. Pavenstadt, R. Greger, and K. Kunzelmann.** 2000. Aquaporin 3 cloned from xenopus laevis is regulated by the cystic fibrosis transmembrane conductance regulator. FEBS Lett. **475:**291–295.

276. **Schroeder, S. A., D. M. Gaughan, and M. Swift.** 1995. Protection against bronchial asthma by CFTR delta F508 mutation: a heterozygote advantage in cystic fibrosis. Nat. Med. **1:**703–705.

277. **Schroeder, T. H., N. Reiniger, G. Meluleni, M. Grout, F. T. Coleman, and**

G. B. Pier. 2001. Transgenic cystic fibrosis mice exhibit reduced early clearance of *Pseudomonas aeruginosa* from the respiratory tract. J. Immunol. **166:**7410–7418.

278. **Schroeder, T. H., T. Zaidi, and G. B. Pier.** 2001. Lack of adherence of clinical isolates of *Pseudomonas aeruginosa* to asialo-GM$_1$ on epithelial cells. Infect. Immun. **69:**719–729.

279. **Schulz, I. J.** 1969. Micropuncture studies of the sweat formation in cystic fibrosis patients. J. Clin. Investig. **48:**1470–1477.

280. **Schurr, M. J., D. W. Martin, M. H. Mudd, and V. Deretic.** 1994. Gene cluster controlling conversion to alginate-overproducing phenotype in *Pseudomonas aeruginosa*: functional analysis in a heterologous host and role in the instability of mucoidy. J. Bacteriol. **176:**3375–3382.

281. **Schurr, M. J., D. W. Martin, M. H. Mudd, N. S. Hibler, J. C. Boucher, and V. Deretic.** 1993. The *algD* promoter: regulation of alginate production by *Pseudomonas aeruginosa* in cystic fibrosis. Cell Mol. Biol. Res. **39:**371–376.

282. **Schurr, M. J., H. Yu, J. M. Martinez-Salazar, J. C. Boucher, and V. Deretic.** 1996. Control of AlgU, a member of the sigma E-like family of stress sigma factors, by the negative regulators MucA and MucB and *Pseudomonas aeruginosa* conversion to mucoidy in cystic fibrosis. J. Bacteriol. **178:**4997–5004.

283. **Schwartz, M., H. K. Johansen, C. Koch, and N. J. Brandt.** 1990. Frequency of the delta F508 mutation on cystic fibrosis chromosomes in Denmark. Hum. Genet. **85:**427–428.

284. **Schwiebert, E. M., M. E. Egan, T. H. Hwang, S. B. Fulmer, S. S. Allen, G. R. Cutting, and W. B. Guggino.** 1995. CFTR regulates outwardly rectifying chloride channels through an autocrine mechanism involving ATP. Cell **81:**1063–1073.

285. **Schwiebert, E. M., T. Flotte, G. R. Cutting, and W. B. Guggino.** 1994. Both CFTR and outwardly rectifying chloride channels contribute to cAMP-stimulated whole cell chloride currents. Am. J. Physiol. **266:**C1464–C1477.

286. **Schwiebert, E. M., D. C. Gruenert, W. B. Guggino, and B. A. Stanton.** 1995. G protein G alpha i-2 inhibits outwardly rectifying chloride channels in human airway epithelial cells. Am. J. Physiol. **269:**C451–C456.

287. **Seibert, F. S., P. Linsdell, T. W. Loo, J. W. Hanrahan, J. R. Riordan, and D. M. Clarke.** 1996. Cytoplasmic loop three of cystic fibrosis transmembrane conductance regulator contributes to regulation of chloride channel activity. J. Biol. Chem. **271:**27493–27499.

288. **Shak, S., D. J. Capon, R. Hellmiss, S. A. Marsters, and C. L. Baker.** 1990. Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum. Proc. Natl. Acad. Sci. USA **87:**9188–9192.

289. **Shalit, I., H. R. Stutman, M. I. Marks, S. A. Chartrand, and B. C. Hilman.** 1987. Randomized study of two dosage regimens of ciprofloxacin for treating chronic bronchopulmonary infection in patients with cystic fibrosis. Am. J. Med. **82:**189–195.

290. **Shepherd, R., W. G. Cooksley, and W. D. Cooke.** 1980. Improved growth and clinical, nutritional, and respiratory changes in response to nutritional therapy in cystic fibrosis. J. Pediatr. **97:**351–357.

291. **Sheppard, D. N., S. M. Travis, H. Ishihara, and M. J. Welsh.** 1996. Contribution of proline residues in the membrane-spanning domains of cystic fibrosis transmembrane conductance regulator to chloride channel function. J. Biol. Chem. **271:**14995–15001.

292. **Sherbrock-Cox, V., N. J. Russell, and P. Gacesa.** 1984. The purification and chemical characterisation of the alginate present in extracellular material produced by mucoid strains of *Pseudomonas aeruginosa*. Carbohydr. Res. **135:**147–154.

293. **Shier, W. T.** 1979. Increased resistance to influenza as a possible source of heterozygote advantage in cystic fibrosis. Med. Hypotheses **5:**661–667.

294. **Short, D. B., K. W. Trotter, D. Reczek, S. M. Kreda, A. Bretscher, R. C. Boucher, M. J. Stutts, and S. L. Milgram.** 1998. An apical PDZ protein anchors the cystic fibrosis transmembrane conductance regulator to the cytoskeleton. J. Biol. Chem. **273:**19797–19801.

295. **Shumaker, H., H. Amlal, R. Frizzell, C. D. Ulrich II, and M. Soleimani.** 1999. CFTR drives Na$^+$-nHCO$_3$$^-$ cotransport in pancreatic duct cells: a basis for defective HCO$_3$$^-$ secretion in CF. Am. J. Physiol. **276:**C16–C25.

296. **Shumaker, H., and M. Soleimani.** 1999. CFTR upregulates the expression of the basolateral Na(+)-K(+)-2Cl(−) cotransporter in cultured pancreatic duct cells. Am. J. Physiol. **277:**C1100–C1110.

297. **Shwachman, H., L. L. Kulczycki, and K.-T. Khaw.** 1965. Studies in cystic fibrosis: a report on sixty-five patients over 17 years of age. Pediatrics **36:**689–699.

298. **Singh, P. K., A. L. Schaefer, M. R. Parsek, T. O. Moninger, M. J. Welsh, and E. P. Greenberg.** 2000. Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. Nature **407:**762–764.

299. **Skov, M., T. Pressler, H. E. Jensen, N. Hoiby, and C. Koch.** 1999. Specific IgG subclass antibody pattern to *Aspergillus fumigatus* in patients with cystic fibrosis with allergic bronchopulmonary aspergillosis (ABPA). Thorax **54:**44–50.

300. **Smith, D. L., L. B. Gumery, E. G. Smith, D. E. Stableforth, M. E. Kaufmann, and T. L. Pitt.** 1993. Epidemic of *Pseudomonas cepacia* in an adult cystic fibrosis unit: evidence of person-to-person transmission. J. Clin. Microbiol. **31:**3017–3022.

301. **Smith, J. J., S. M. Travis, E. P. Greenberg, and M. J. Welsh.** 1996. Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. Cell **85:**229–236. (Erratum, **87:**355, 1996.)

302. **Smith, J. J., and M. J. Welsh.** 1992. cAMP stimulates bicarbonate secretion across normal, but not cystic fibrosis airway epithelia. J. Clin. Investig. **89:**1148–1153.

303. **Smith, S. N., P. G. Middleton, S. Chadwick, A. Jaffe, K. A. Bush, S. Rolleston, R. Farley, S. J. Delaney, B. Wainwright, D. M. Geddes, and E. W. Alton.** 1999. The in vivo effects of milrinone on the airways of cystic fibrosis mice and human subjects. Am. J. Respir. Cell Mol. Biol. **20:**129–134.

304. **Sogbanmu, M. O., and H. Bialy.** 1980. Transferable drug resistance in *Pseudomonas* patients with premature rupture of membranes in Ile-Ife, Nigeria. Afr. J. Med. Med. Sci. **9:**49–51.

305. **Speert, D. P., and M. E. Campbell.** 1987. Hospital epidemiology of *Pseudomonas aeruginosa* from patients with cystic fibrosis. J. Hosp. Infect. **9:**11–21.

306. **Speert, D. P., M. E. Campbell, A. G. Davidson, and L. T. Wong.** 1993. *Pseudomonas aeruginosa* colonization of the gastrointestinal tract in patients with cystic fibrosis. J. Infect. Dis. **167:**226–229.

307. **Spies, T., M. Bresnahan, S. Bahram, D. Arnold, G. Blanck, E. Mellins, D. Pious, and R. DeMars.** 1990. A gene in the human major histocompatibility complex class II region controlling the class I antigen presentation pathway. Nature **348:**744–747.

308. **Stern, R. C.** 1997. The diagnosis of cystic fibrosis. N. Engl. J. Med. **336:**487–491

309. **Storey, D. G., E. E. Ujack, H. R. Rabin, and I. Mitchell.** 1998. *Pseudomonas aeruginosa lasR* transcription correlates with the transcription of *lasA, lasB,* and *toxA* in chronic lung infections associated with cystic fibrosis. Infect. Immun. **66:**2521–2528.

310. **Reference deleted.**

311. **Stutts, M. J., C. M. Canessa, J. C. Olsen, M. Hamrick, J. A. Cohn, B. C. Rossier, and R. C. Boucher.** 1995. CFTR as a cAMP-dependent regulator of sodium channels. Science **269:**847–850.

312. **Stutts, M. J., T. C. Chinet, S. J. Mason, J. M. Fullton, L. L. Clarke, and R. C. Boucher.** 1992. Regulation of Cl- channels in normal and cystic fibrosis airway epithelial cells by extracellular ATP. Proc. Natl. Acad. Sci. USA **89:**1621–1625.

313. **Stutts, M. J., J. G. Fitz, A. M. Paradiso, and R. C. Boucher.** 1994. Multiple modes of regulation of airway epithelial chloride secretion by extracellular ATP. Am. J. Physiol. **267:**C1442–C1451.

314. **Sugita, M., Y. Yue, and J. K. Foskett.** 1998. CFTR Cl- channel and CFTR-associated ATP channel: distinct pores regulated by common gates. EMBO J. **17:**898–908.

315. **Suzuki, S., Y. Miyoshi, and R. Nakaya.** 1978. R plasmids among Gram-negative bacteria with multiple drug resistance isolated in a general hospital. Microbiol. Immunol. **22:**235–247.

316. **Szaff, M., N. Hoiby, and E. W. Flensborg.** 1983. Frequent antibiotic therapy improves survival of cystic fibrosis patients with chronic *Pseudomonas aeruginosa* infection. Acta Paediatr. Scand. **72:**651–657.

317. **Szczypka, M. S., J. A. Wemmie, W. S. Moye-Rowley, and D. J. Thiele.** 1994. A yeast metal resistance protein similar to human cystic fibrosis transmembrane conductance regulator (CFTR) and multidrug resistance-associated protein. J. Biol. Chem. **269:**22853–22857.

318. **Tabcharani, J. A., X. B. Chang, J. R. Riordan, and J. W. Hanrahan.** 1991. Phosphorylation-regulated Cl$^-$ channel in CHO cells stably expressing the cystic fibrosis gene. Nature **352:**628–631.

319. **Tager, A. M., J. Wu, and M. W. Vermeulen.** 1998. The effect of chloride concentration on human neutrophil functions: potential relevance to cystic fibrosis. Am. J. Respir. Cell Mol. Biol. **19:**643–652.

320. **Taylor, R. F., L. Dalla Costa, M. E. Kaufmann, T. L. Pitt, and M. E. Hodson.** 1992. *Pseudomonas cepacia* pulmonary infection in adults with cystic fibrosis: is nosocomial acquisition occurring? J. Hosp. Infect. **21:**199–204.

321. **Thomas, J., D. J. Cook, and D. Brooks.** 1995. Chest physical therapy management of patients with cystic fibrosis. A meta-analysis. Am. J. Respir. Crit. Care Med. **151:**846–850

322. **Torrens, J. K., P. Dawkins, S. P. Conway, and E. Moya.** 1998. Non-tuberculous mycobacteria in cystic fibrosis. Thorax **53:**182–185.

323. **Tosi, M. F., H. Zakem-Cloud, C. A. Demko, J. R. Schreiber, R. C. Stern, M. W. Konstan, and M. Berger.** 1995. Cross-sectional and longitudinal studies of naturally occurring antibodies to *Pseudomonas aeruginosa* in cystic fibrosis indicate absence of antibody-mediated protection and decline in opsonic quality after infection. J. Infect. Dis. **172:**453–461.

324. **Travis, S. M., B. A. Conway, J. Zabner, J. J. Smith, N. N. Anderson, P. K. Singh, E. P. Greenberg, and M. J. Welsh.** 1999. Activity of abundant antimicrobials of the human airway. Am. J. Respir. Cell Mol. Biol. **20:**872–879.

325. **Travis, S. M., P. K. Singh, and M. J. Welsh.** 2001. Antimicrobial peptides and proteins in the innate defense of the airway surface. Curr. Opin. Immunol. **13:**89–95.

326. **Tsui, L. C.** 1990. Population analysis of the major mutation in cystic fibrosis. Hum. Genet. **85:**391–392.

327. **Tsui, L. C., M. Buchwald, D. Barker, J. C. Braman, R. Knowlton, J. W. Schumm, H. Eiberg, J. Mohr, D. Kennedy, N. Plavsic, et al.** 1985. Cystic

fibrosis locus defined by a genetically linked polymorphic DNA marker. Science **230:**1054–1057.

328. **Ulrich, M., S. Herbert, J. Berger, G. Bellon, D. Louis, G. Munker, and G. Doring.** 1998. Localization of *Staphylococcus aureus* in infected airways of patients with cystic fibrosis and in a cell culture model of *S. aureus* adherence. Am. J. Respir. Cell Mol. Biol. **19:**83–91.

329. **Vishwanath, S., R. Ramphal, C. M. Guay, D. DesJardins, and G. B. Pier.** 1988. Respiratory mucin inhibits the opsonophagocytic killing of *Pseudomonas aeruginosa*. Infect. Immun. **56:**2218–2222.

330. **Wainwright, B. J., P. J. Scambler, J. Schmidtke, E. A. Watson, H. Y. Law, M. Farrall, H. J. Cooke, H. Eiberg, and R. Williamson.** 1985. Localization of cystic fibrosis locus to human chromosome 7cen-q22. Nature **318:**384–385.

331. **Wallis, C., and A. Prasad.** 1999. Who needs chest physiotherapy? Moving from anecdote to evidence. Arch. Dis. Child. **80:**393–397.

332. **Walters, M. N.-I.** 1965. The ductular cell in pancreatic cystic fibrosis. J. Pathol. Bacteriol. **90:**45–52.

333. **Ward, C. L., S. Omura, and R. R. Kopito.** 1995. Degradation of CFTR by the ubiquitin-proteasome pathway. Cell **83:**121–127.

334. **Webber, B. A., and J. A. Pryor.** 1989. Respiratory physiotherapy for cystic fibrosis. J. Pediatr. **115:**167–168.

335. **Welsh, M. J., G. M. Denning, L. S. Ostedgaard, and M. P. Anderson.** 1993. Dysfunction of CFTR bearing the delta F508 mutation. J. Cell. Sci. Suppl. **17:**235–239.

336. **White, G. P., and N. W. Dunn.** 1977. Apparent fusion of the TOL plasmid with the R91 drug resistance plasmid in *Pseudomonas aeruginosa*. Aust. J. Biol. Sci. **30:**345–355.

337. **Whiteford, M. L., J. D. Wilkinson, J. H. McColl, F. M. Conlon, J. R. Michie, T. J. Evans, and J. Y. Paton.** 1995. Outcome of *Burkholderia (Pseudomonas) cepacia* colonisation in children with cystic fibrosis following a hospital outbreak. Thorax **50:**1194–1198.

338. **Widdicombe, J. H., M. J. Welsh, and W. E. Finkbeiner.** 1985. Cystic fibrosis decreases the apical membrane chloride permeability of monolayers cultured from cells of tracheal epithelium. Proc. Natl. Acad. Sci. USA **82:**6167–6171.

339. **Wiedermann, U., A. Tarkowski, T. Bremell, L. A. Hanson, H. Kahu, and**

U. I. Dahlgren. 1996. Vitamin A deficiency predisposes to *Staphylococcus aureus* infection. Infect. Immun. **64:**209–214.

340. **Wiesemann, H. G., G. Steinkamp, F. Ratjen, A. Bauernfeind, B. Przyklenk, G. Doring, and H. von der Hardt.** 1998. Placebo-controlled, double-blind, randomized study of aerosolized tobramycin for early treatment of *Pseudomonas aeruginosa* colonization in cystic fibrosis. Pediatr. Pulmonol. **25:**88–92.

341. **Wilkinson, D. J., T. V. Strong, M. K. Mansoura, D. L. Wood, S. S. Smith, F. S. Collins, and D. C. Dawson.** 1997. CFTR activation: additive effects of stimulatory and inhibitory phosphorylation sites in the R domain. Am. J. Physiol. **273:**L127–L133.

342. **Wills, P. J., R. L. Hall, W. Chan, and P. J. Cole.** 1997. Sodium chloride increases the ciliary transportability of cystic fibrosis and bronchiectasis sputum on the mucus-depleted bovine trachea. J. Clin. Investig. **99:**9–13.

343. **Wilschanski, M., J. Zielenski, D. Markiewicz, L. C. Tsui, M. Corey, H. Levison, and P. R. Durie.** 1995. Correlation of sweat chloride concentration with classes of the cystic fibrosis transmembrane conductance regulator gene mutations. J. Pediatr. **127:**705–710.

344. **Wine, J. J., and C. K. Sole.** 1990. Chloride channels in cystic fibrosis patients. Science **247:**222.

345. **Winter, M. C., and M. J. Welsh.** 1997. Stimulation of CFTR activity by its phosphorylated R domain. Nature **389:**294–296.

346. **Wozniak, D. J., and D. E. Ohman.** 1994. Transcriptional analysis of the *Pseudomonas aeruginosa* genes *algR, algB,* and *algD* reveals a hierarchy of alginate gene expression which is modulated by *algT*. J. Bacteriol. **176:**6007–6014.

347. **Wright, S. W., and N. E. Morton.** 1968. Genetic studies on cystic fibrosis in Hawaii. Am. J. Hum. Genet. **20:**157–169.

348. **Xu, K. D., G. A. McFeters, and P. S. Stewart.** 2000. Biofilm resistance to antimicrobial agents. Microbiology **146:**547–549.

349. **Yahr, T. L., L. M. Mende-Mueller, M. B. Friese, and D. W. Frank.** 1997. Identification of type III secreted products of the *Pseudomonas aeruginosa* exoenzyme S regulon. J. Bacteriol. **179:**7165–7168.

350. **Zach, M. S.** 1990. Lung disease in cystic fibrosis—an updated concept. Pediatr. Pulmonol. **8:**188–202.

351. **Zielenski, J.** 2000. Genotype and phenotype in cystic fibrosis. Respiration **67:**117–133.