Pathophysiology and Management of Pulmonary Infections in Cystic Fibrosis

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This comprehensive *State of the Art* review summarizes the current published knowledge base regarding the pathophysiology and microbiology of pulmonary disease in cystic fibrosis (CF). The molecular basis of CF lung disease including the impact of defective cystic fibrosis transmembrane regulator (CFTR) protein function on airway physiology, mucociliary clearance, and establishment of *Pseudomonas aeruginosa* infection is described. An extensive review of the microbiology of CF lung disease with particular reference to infection with *P. aeruginosa* is provided. Other pathogens commonly associated with CF lung disease including *Staphylococcal aureus, Burkholderia cepacia, Stenotrophomonas maltophilia, Achromobacter xylosoxidans* and atypical mycobacteria are also described. Clinical presentation and assessment of CF lung disease including diagnostic microbiology and other measures of pulmonary health are reviewed. Current recommendations for management of CF lung disease are provided. An extensive review of antipseudomonal therapies in the settings of treatment for early *P. aeruginosa* infection, maintenance for patients with chronic *P. aeruginosa* infection, and treatment of exacerbation in pulmonary symptoms, as well as antibiotic therapies for other CF respiratory pathogens, are included. In addition, the article discusses infection control policies, therapies to optimize airway clearance and reduce inflammation, and potential future therapies.

Keywords: cystic fibrosis, *Pseudomonas aeruginosa*, airway disease, cystic fibrosis transmembrane conductance regulator, antibiotics

CONTENTS

The cystic fibrosis (CF) scientific community has orchestrated a focused, multidisciplinary effort to understand the molecular basis of this disorder and at the same time improve clinical care for patients with CF. After identification of the CF gene in 1989, the 1990s was a decade associated with rapid expansion of knowledge regarding the structure and function of the CF gene product, CF transmembrane conductance regulator (CFTR) protein. The previous State of the Art assessment of CF in 1996 (1) provided a comprehensive review focusing on significant advances in scientific understanding of the CFTR gene. As we enter the 21st century, both laboratory and clinical investigators are applying this knowledge toward elucidating the critical factors that initiate chronic endobronchial bacterial infection in this genetic disorder and are using this knowledge to develop novel and effective therapies. This State of the Art focuses on the current understanding of the impact of abnormal CFTR function on airway surface liquid (ASL) that initiates a pathophysiologic cascade leading to progressive lung disease. The role of chronic endobronchial bacterial infection with pathogens such as *Pseudomonas aeruginosa* and the resultant intense neutrophilic inflammatory response, pathognomonic for this lung disease, will be reviewed. In addition, current and future therapies to control or eradicate *Pseudomonas* infection and slow disease progression are summarized. This review focuses only on the pulmonary aspects of this genetic disease and does not include other important aspects of this illness including gastrointestinal, endocrine, and metabolic manifestations of CF (2).

CF is an autosomal recessive disorder caused by mutations in a single gene on the long arm of chromosome 7 that encodes

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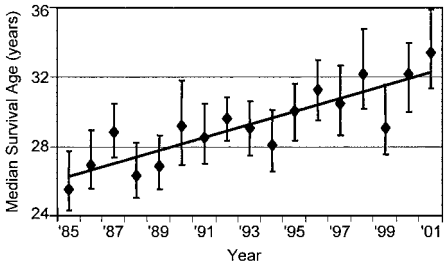

Figure 1. Median survival age in cystic fibrosis, 1985–2001. Data from the U.S. Cystic Fibrosis Foundation Patient Registry showing the age of expected death for 50% of the current Registry population, given the ages of the patients in the Registry and the mortality distribution of deaths for that specific year. The 95% confidence intervals for the survival estimate are denoted by the *vertical bars*. The median estimated survival is 33.4 years for 2001. (Reprinted by permission from Reference 6.)

the CFTR protein (2–5). Despite impressive advances in understanding the molecular basis and pathophysiology of this disorder, it remains the most common life-shortening genetic disorder in the white population with an estimated median survival age of 33.4 years in the United States in 2001 (Figure 1). This represents an increase of 6 years since the previous State of the Art was written (6). CF affects approximately 30,000 individuals in the United States and 60,000 individuals worldwide with an estimated incidence in the U.S. white population ranging from 1 in 1,900 to 1 in 3,700 (2, 7). CF is present, but less frequent, in Hispanic (8), Asian (9), and African American (7, 10) populations (1 in 9,000, 1 in 32,000, and 1 in 15,000, respectively). The CF gene is large, spans 250 kb, and is composed of 27 exons (11). As shown in Figure 2, the gene is transcribed into a 6.5-kb messenger RNA (3) that encodes a 1,480 amino acid protein. Since identification of the gene, over 800 disease-associated mutations in the CF gene have been reported to the CF Genetic Analysis Consortium database (www.genet.sickkids.on.ca/cftr/). The vast majority of mutations involves three or fewer nucleotides and result in predominantly amino acid substitutions, frameshifts, splice site, or nonsense mutations.

Although a large number of CF-causing mutations have been described, only 22 mutations have been identified with a frequency of at least 0.1% of known alleles (12). The remaining mutations are extremely rare and often limited to one or a few individuals. The most common and first identified mutation, a three base pair deletion that codes for phenylalanine at position 508 of the CFTR protein, ΔF508, accounts for 70% of CF alleles in whites (13). It is the presence of ΔF508 that increases the frequency of CF in the white population relative to other races. Although several theories have been proposed suggesting a selective advantage for ΔF508 heterozygotes such as resistance to secretory diarrhea from cholera (14) or protection against bronchial asthma (15), no confirmatory data are available. The other 21 common mutations are often found in higher frequency in particular ethnic groups, such as the W1282X mutation in Askenazi Jewish populations (16), G551D in French Canadians (17), and 3,120 + 1G → A in African/Mediterranean populations (18).

In vitro physiologic studies have demonstrated that mutations in the CF gene can disrupt CFTR function within epithelial cells in different ways, ranging from complete loss of protein to surface expression with poor chloride conductance (19). The five major mechanisms by which CFTR function is altered are summarized in Figure 3. *Class I* mutations produce premature transcription termination signals resulting in unstable, truncated, or no protein expression. *Class II* mutations, usually missense mutations including ΔF508, cause the protein to misfold leading to premature degradation and failure to reach the apical cell membrane except for special conditions such as low temperature (20). *Class III* mutations, primarily located in the two nuclear-binding domains, result in decreased chloride channel activity (21) due to abnormal adenosine triphosphate (ATP) gating. *Class IV* mutations are primarily located in the membrane spanning domains that form the chloride channel and demonstrate reduced chloride conductance (22). *Class V* mutations result in reduced amounts of functional protein (rather than no protein production seen in Class I) due to abnormal or alternative splicing (23). It is important to recognize that specific mutations may have characteristics of more than one class. Thus, these five mechanisms of CFTR dysfunction are intended to provide a framework for understanding the molecular basis of epithelial cell abnormalities in CF, help predict observed genotype–phenotype correlations, and develop treatment approaches directed to specific classes of mutations (e.g., Class I premature stop mutations [24]). A comprehensive review of genotype–phenotype relationships has recently been published (2).

CLINICAL PRESENTATION

Diagnosis

Although the genetic basis is now well understood, the diagnosis of CF remains clinical and not genetic. Until the 1990s, the diagnosis was based on clinical criteria (Table 1) and analysis of sweat chloride values (25). The availability of mutational analyses within the CF gene (12, 13) as well as an assessment of bioelectrical properties of respiratory epithelia by measurement of transepithelial potential differences (26) rapidly expanded the clinical spectrum of CF to include milder, atypical presentations. In addition, availability of newborn screening (27) in certain states and countries and prenatal diagnosis afforded the opportunity to diagnose individuals before the onset of clinical symptoms. In 1997, a consensus panel was convened to define the diagnosis of CF in the context of these newer diagnostic tools (28). This group defined the clinical parameters that support the diagnosis (Table 1) and appropriate laboratory tests to document CFTR dysfunction (28, 29). The World Health Organization has developed similar criteria (30). Of the 1,091 newly diagnosed patients from the United States in 2001 (6), only a small percentage were identified by newborn screening (9.1%) or prenatal diagnosis (3.9%). The majority of diagnoses were based on clinical features of which respiratory symptoms (43.8%), failure to thrive (29.3%), steatorrhea (24.4%), and meconium ileus (18.5%) were most common.

With these expanded criteria, the borders between normal and abnormal CFTR function have become less distinct (29). A particularly interesting group is males with obstructive azoospermia secondary to congenital bilateral absence of the vas deferens who have no other clinical features of CF. Nearly half the individuals with congenital bilateral absence of the vas deferens carry two CFTR mutations (31), and even patients with unilateral absence of the vas deferens (32) have increased incidence of CFTR mutations. Adults with chronic pancreatitis (33) and rhinosinusitis (34) have been reported to commonly carry at least one CFTR mutation. At the other end of the spectrum, CF phenotypes have been characterized in the absence of CFTR mutations (35). To address this conundrum, a continuum of diagnoses from a pre-CF to subclinical CF to classic presentation has been proposed (36). Thus, it is clear that the diagnostic criteria will continue to evolve as molecular and physiologic understanding expands.

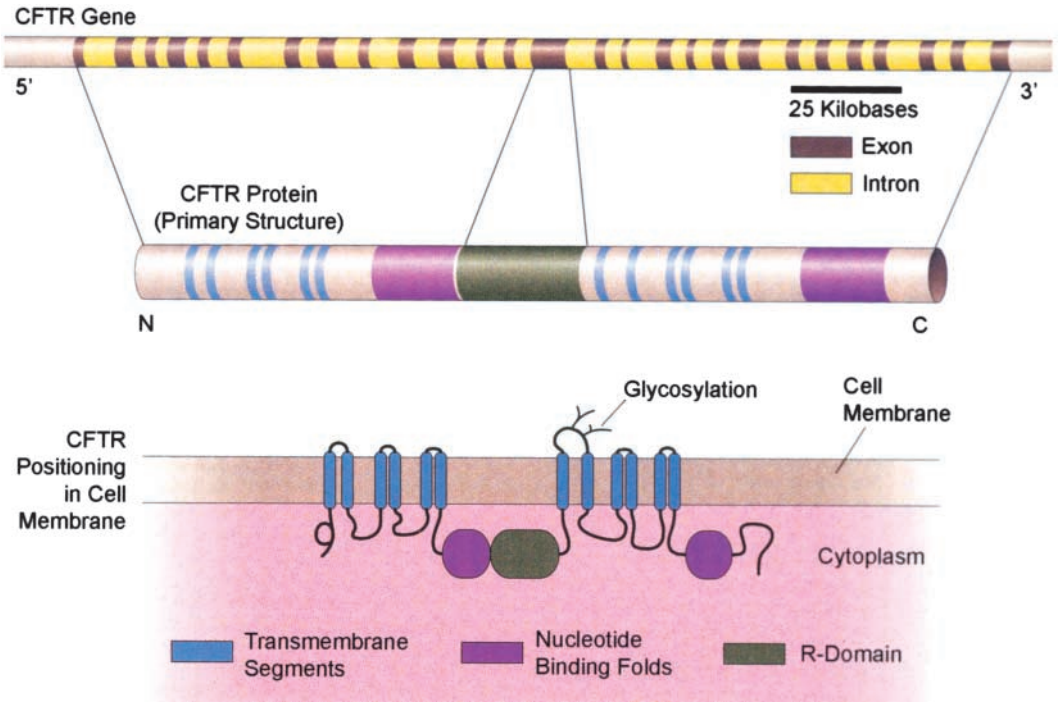

Figure 2. The cystic fibrosis (CF) transmembrane conductance regulator (CFTR) gene and its encoded polypeptide. The human CFTR gene (*top*) is located on the long arm of chromosome 7 and consists of 27 exon regions that encode the 1,480 amino acid CFTR proteins (*middle*). The mature protein after proper folding, glycosylation, and insertion into the cell membrane is shown at the *bottom*. The CFTR protein is a member of the ATP-binding cassette (ABC) family of transporters. It contains two nucleotide-binding domains that bind and hydrolyze ATP, two dual sets of membrane-spanning segments that form the channel, and a central regulatory (R) domain. The R domain, unique to CFTR, is highly charged with numerous phosphorylation sites for protein kinases A or C. (Reprinted by permission from Reference 490.)

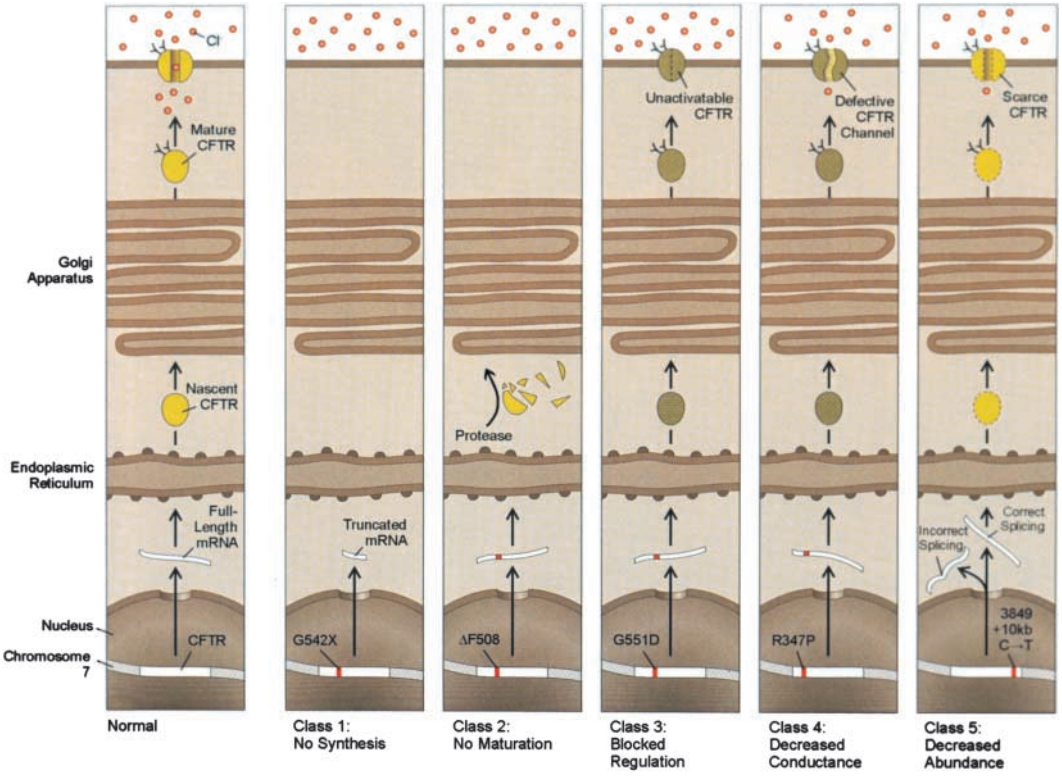

Figure 3. Functional effects of classes of CFTR mutations. Five classes of CF-related gene mutations are displayed juxtaposed to the normal maturation pathway. As shown in the *left panel*, wild-type CFTR is transcribed into messenger RNA (mRNA) followed by post-translational modifications including proper folding, glycosylation, and trafficking via the Golgi apparatus to the cell membrane where it functions as a regulated chloride channel. Class 1 mutations, exemplified by G542X, contain premature stop mutations that create truncated mRNA. Class 2 mutations, of which ΔF508 is most common, are misfolded and unable to escape the endoplasmic reticulum, where they are ubiquitinated and degraded. Class 3 mutations, such as G551D, reach the cell membrane but the channel is not properly activated. Class 4 mutations, exemplified by R347P, reach the cell surface and the channel can be activated but have decreased chloride conductance. Class 5 mutations result in decreased abundance of CFTR, as exemplified by incorrect splicing with the mutation 3849 + 10 kb C → T. With some Class 5 mutations a small percentage of correctly spliced mRNA are produced, resulting in a milder phenotype. (Reprinted by permission from Reference 490.)

TABLE 1. CLINICAL FEATURES CONSISTENT WITH THE DIAGNOSIS OF CYSTIC FIBROSIS

Chronic Sinopulmonary Disease
- Persistent colonization/infection with pathogens typical of CF lung disease, including:
  - *Staphylococcus aureus*
  - *Pseudomonas aeruginosa* (mucoid and nonmucoid)
  - Nontypable *Haemophilus influenzae*
  - *Burkholderia cepacia*
- Endobronchial disease manifested by:
  - Cough and sputum production
  - Wheeze and air trapping
  - Radiographic abnormalities
  - Evidence of obstruction on PFTs
  - Digital clubbing
- Chronic sinus disease:
  - Nasal polyps
  - Radiographic changes

Gastrointestinal/nutritional abnormalities
- Intestinal abnormalities:
  - Meconium ileus
  - Exocrine pancreatic insufficiency
  - DIOS
  - Rectal prolapse
  - Recurrent pancreatitis
  - Chronic hepatobiliary disease manifested by clinical and/or laboratory evidence of:
    - Focal biliary cirrhosis
    - Multilobular cirrhosis
- Failure to thrive (protein–calorie malnutrition)
- Hypoproteinemia–edema
- Fat-soluble vitamin deficiencies

Obstructive azoospermia in males

Salt-loss syndromes
- Acute salt depletion
- Chronic metabolic alkalosis

Definition of abbreviations: CF = cystic fibrosis; DIOS = distal intestinal obstruction syndrome; PFTs = pulmonary function tests.

Adapted by permission from Reference 167.

Pulmonary Manifestations

CF primarily affects the airways and submucosal glands with sparing of the interstitium and alveolar spaces until late in the disease (Figure 4) (37). CFTR expression has been localized to the affected regions with the most predominant expression observed in submucosal glands (38). There are limited studies describing the developmental anatomy of the CF lung in affected fetuses and newborns (39–41). The lungs, including mucus glands, appear histologically normal at birth. There may be some increase in the acinar diameter of the tracheal mucus glands, suggesting some early mucus plugging preceding any evidence of infection or inflammation (40).

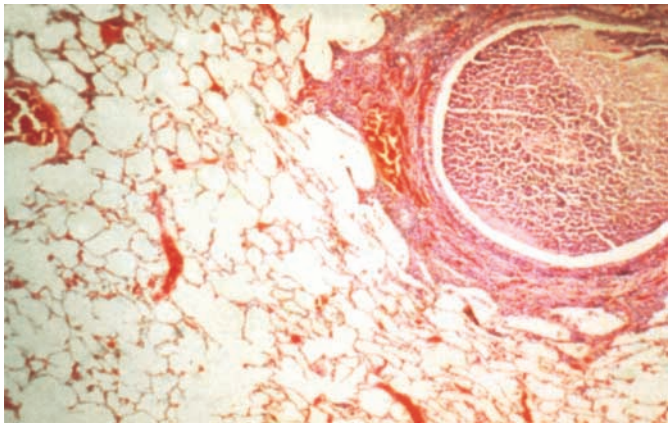

Figure 4. Mucus plugging with airway inflammation. A slightly dilated peripheral bronchus at low power, with surrounding alveolar tissue from a young adult with CF. The bronchus is filled with inflammatory cells and mucus. The peribronchial region is also filled with inflammatory cells (primarily neutrophils). By contrast, the parenchyma is spared both inflammation and scarring.

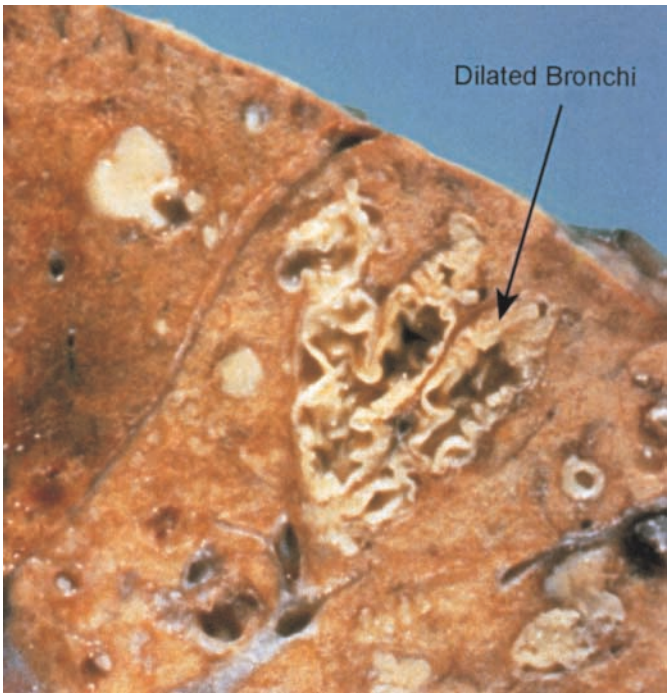

Figure 5. End-stage bronchiectasis. A postmortem, pathology slide of end-stage bronchiectasis in CF displaying dilated bronchi and mucus impaction. The bronchiectatic airways contribute to reduced mucociliary and cough clearance and the persistence of mucus inspissation and endobronchial inflammation. The parenchyma, even with this advanced bronchial disease, is not severely altered.

Soon after birth, initial infection with bacterial pathogens commences and is associated with an intense neutrophilic response localized to the peribronchial and endobronchial spaces (42–44). Early airway infection and inflammation in CF can have regional heterogeneity that complicates understanding the causal and temporal relationship between initial infection and airway inflammatory response (45–47). Several studies in toddlers and older children with CF have shown a robust inflammatory response in the airways in both bacterial culture–positive and culture-negative patients; some studies show a greater inflammatory response in those patients with at least 5×10^4 cfu/ml of bacteria in their bronchoalveolar lavage (BAL) fluid (43, 48–50). At this point, pathologic changes become more evident with mucopurulent plugging of small and medium size bronchioles (Figure 4). In older individuals with CF, persistent neutrophils dominate airway inflammation with elevated interleukin (IL)-8 and neutrophil elastase (51–53). Airways become dilated and bronchiectatic, secondary to proteolysis and chondrolysis of airway support tissue (54, 55) (Figure 5). In later stages, lung parenchyma becomes affected by atelectasis, pneumonia, and encroachment by enlarging airways. Many secondary consequences of bronchiectasis ensue, including hypertrophy of bronchial circulation and formation of bronchial cysts. A later and less common consequence is pulmonary hypertension.

In effect, the CF airway represents a prolonged primary inflammatory response usually observed in acute infections. The

CF host inflammatory response is unable to mature and promote a macrophage-driven granulomatous response seen in other chronic infections. It has been suggested that this inflammatory response remains orchestrated by local airway epithelium–pathogen interactions, rather than driven by T cells as part of the systemic immune response (52).

The critical mediators for neutrophil influx in the CF lung include IL-8, tumor necrosis factor–α and IL-1, complement-derived chemoattractants, and leukotriene B_4 (52, 56). IL-8, produced by stimulated epithelial cells, macrophages, and neutrophils, appears to be the predominant and sentinel neutrophil chemoattractant in the CF airway (52, 57). IL-1β, tumor necrosis factor–α, neutrophil elastase, LPS, and *P. aeruginosa* antigens can all stimulate further IL-8 production to sustain the neutrophilic influx. Tumor necrosis factor–α stimulates neutrophil secretory and oxidative processes, and both tumor necrosis factor–α and IL-1 can prime neutrophils for a heightened response to chemoattractants. The activated neutrophils are the primary effector cells for the pathogenesis of CF lung disease. Neutrophils release massive amounts of elastase and other proteases that overwhelm the local host defenses including α-1 antitrypsin and secretory leukocyte protease inhibitor. As the neutrophils break down, they release large amounts of high molecular weight DNA that increase the viscosity of the endobronchial secretions that contribute to reduced mucociliary clearance (58).

The clinical manifestations of CF lung disease are highly variable in onset and intensity. Affected individuals rarely demonstrate respiratory symptoms in the newborn period, but infants less than 6 months of age may demonstrate tachypnea, wheezing, increased work of breathing, hyperinflation, and cough. These symptoms may be initiated or exacerbated by respiratory viral infections (59) and, if undiagnosed, these babies may be labeled as having recurrent or persistent bronchiolitis. At some point in the course of all affected individuals' lives, cough becomes a prominent symptom. Patients with mild disease may only cough during exacerbations (*see* TREATMENT OF PULMONARY EXACERBATION), but eventually cough becomes a daily occurrence, usually associated with expectoration of sputum. With disease progression, daily sputum volume increases and becomes green to tan in color. Blood-streaked sputum and hemoptysis are not unusual in later stages of illness. Similar to other chronic obstructive lung diseases, patients experience increasing dyspnea on exertion and shortness of breath as the illness progresses. They are often oxygen dependent (at least nocturnal) with retention of carbon dioxide in the late stages of the illness and experience decreasing life quality as the frequency of exacerbations and intensity of respiratory therapy increases. Respiratory failure still accounts for over 80% of deaths for patients with CF in the United States (6).

CFTR FUNCTION AND MOLECULAR BASIS OF CF LUNG DISEASE

CFTR Structure and Function

CFTR is a member of the ATP-binding cassette transporter family of membrane proteins (2, 60). CFTR contains the characteristic two nucleotide-binding domains and two membrane-spanning domains, as well as a unique regulatory R domain with multiple phosphorylation sites (Figure 2). cAMP-dependent phosphorylation of the R domain governs channel activity (61), and ATP binding and hydrolysis by the two nuclear-binding domains controls channel gating (62, 63). CFTR structure and function, the regulatory activity of CFTR on other ion channels, and the impact of CFTR dysfunction on the composition and pH of ASL are reviewed elsewhere (2).

Impact of Defective CFTR on Airway Physiology and Mucociliary Clearance

The net impact of aberrations in transepithelial ion flow on the ionic composition and volume of airway surface fluid in CF due to dysfunctional or absent CFTR is an active topic of investigation. ASL consists of two layers above the epithelial surface—a mucus layer and a periciliary liquid layer with a height of the extended cilium (~ 7 μm) (Figure 6A) (64). The periciliary

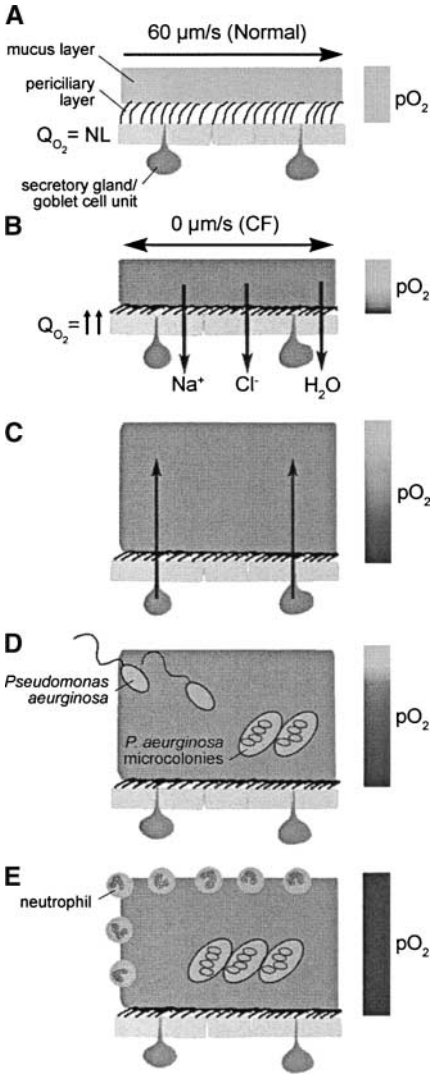

Figure 6. Pathogenic events hypothesized to lead to chronic *Pseudomonas aeruginosa*. (*A*) In normal airway epithelia, the presence of a low-viscosity periciliary layer (PCL) of normal volume promotes efficient mucociliary clearance. A normal rate of epithelial cell oxygen consumption (QO_2) results in no gradient in the partial pressure of oxygen (pO_2) within the airway surface liquid (ASL). In the CF airway, (*B*) isotonic volume depletion of the PCL (denoted by *downward arrows* and bent cilia) results in reduced mucociliary transport (*bidirectional horizontal arrow*) and (*C*) persistent mucus hypersecretion (denoted by *upward arrows* from secretory gland/goblet cell units) with time increases the height of the luminal mucus layer/plugs. Elevated CF epithelial QO_2 generates steep hypoxic gradients (*dark color* in pO_2 *bar*) in the thickened mucus layer. (*D*) *P. aeruginosa* bacteria deposited on mucus surfaces penetrate actively or passively (due to mucus turbulence) into hypoxic zones of the mucus masses. *P. aeruginosa* adapt within the hypoxic environment with increased alginate expression and the formation of microcolonies with potential evolution into biofilms. (*E*) Increased *P. aeruginosa* microcolony density and the presence of neutrophils render the mucus layer more hypoxic. *P. aeruginosa* microcolonies within the hypoxic mucus plugs resist host lung defenses, including neutrophils, and result in chronic airway infection. (Adapted by permission from Reference 97.)

liquid layer volume is tightly regulated to provide a low-viscosity solution for ciliary beat and to lubricate gel-forming mucins secreted from the cell surface (64, 65). The mucus layer consists of high molecular weight mucins whose properties are altered by water content, ion concentrations, and pH. The diversity of the carbohydrate side chains within the mucin gel is suited for binding a wide variety of particles for ultimate clearance from the airway (66).

Two competing hypotheses have been proposed: (*1*) the isotonic "low volume" hypothesis with resultant abnormalities in mucociliary clearance (64, 65), and (*2*) the "compositional" hypothesis with increased ASL salt concentrations in CF inactivating salt-sensitive antimicrobial peptides (Figure 7) (67). Both hypotheses can in part explain the early and persistent endobronchial infection in CF (68–70). In the first hypothesis, water-permeable airway epithelia regulate the volume of the ASL by isotonic transport to maintain optimal ciliary mucus layer interactions and mucociliary clearance. This hypothesis predicts the salt composition of control and CF ASL to be similar with each other and plasma. The second or "compositional" hypothesis proposes that airway epithelia regulate ASL salt concentration that is critical for optimal function of innate antimicrobial peptide defenses in the lung. This hypothesis predicts a higher ASL salt concentration in patients with CF compared with individuals who are not infected.

There is no final consensus on the tonicity of ASL in subjects with CF relative to healthy control individuals. Technical limitations of collecting and assaying ASL from the upper and lower airways are a significant obstacle. It is also uncertain if ASL composition varies along the respiratory tract (i.e., nasal epithelium to distal airways), in response to chronic inflammation and infection, and within local microenvironments such as submucosal glands or mucus plugs (68). There is increasing evidence from nasal and bronchial epithelium derived from human and animal sources that ASL is similar in healthy control individuals and subjects with CF and is isotonic (64, 71–75). However, using a novel isotopic technique, one investigator suggests that normal ASL concentrations of sodium and chloride are approximately 50 mM and that the ASL concentrations of these ions are elevated to approximately 100 mM in CF (76). Therefore, the "compositional" hypothesis has not been entirely refuted, especially when considering local microenvironments such as submucosal glands. Additional studies on the ionic composition and volume of ASL are necessary, as the answer will influence approaches to treatment of CF lung disease.

Evidence is accumulating for the important role of submucosal glands in the pathophysiology of airway disease in CF (71). CFTR is highly expressed in the serous epithelial cells of submucosal glands compared with other tissues of the lung (38, 68). Abnormalities in submucosal gland secretions are proposed to contribute to airway disease in CF. Loss of CFTR function may alter the macromolecular composition of the submucosal gland secretions and thereby change viscosity, gel hydration, and adversely effect mucociliary clearance (Figure 6D) (66, 71, 77). Submucosal gland secretions from explanted human CF airways have sodium content and pH similar to control tissue, but CF submucosal gland secretions have approximately a twofold increase in viscosity (77). Further studies are needed on submucosal gland secretions from CF airways before chronic infection, however, to determine if the increased viscosity of submucosal gland secretions in CF is due to decreased fluid secretion or altered protein/glycoprotein composition.

Mucociliary clearance is a primary innate airway defense that most studies show is reduced in CF (Figure 6) (64, 66, 78). In CF, there is abnormal regulation of the periciliary liquid volume that contributes to reduced mucociliary clearance (64, 66). Altered viscosity and regulation of submucosal gland secretion may also impair host defense (77, 79). In addition, the reduced periciliary liquid volume promotes interactions between gel mucins in the mucus layer with cell-surface mucins that hinder clearance of particles from the airways (66). Clearance of particles from normal peripheral airways by mucociliary clearance can require up to 6 hours, and this can be significantly prolonged in CF airways (66). Endogenous antimicrobial peptides can suppress bacterial growth for 3 to 6 hours (80). Thus reduced mucociliary clearance in CF may contribute to overwhelming innate antimicrobial peptides and thereby promote the initial endobronchial infection in young children with CF.

Impact of Defective CFTR on Initial and Persistent *P. aeruginosa* Infection

The abnormal composition and mechanical properties of airway secretions does not explain the propensity for the CF airway to become colonized with only a limited number of bacterial pathogens, in particular, *P. aeruginosa*. There are several hypotheses to help us understand that association.

Abnormal bacterial adherence to epithelial cells. Initial infection may be related to increased *P. aeruginosa* adherence to receptors in the CF airway (Figure 6C). CF epithelial cells demonstrate greater adherence of piliated laboratory strains of *P. aeruginosa* compared with control cells, and expression of wild-type CFTR in CF cell lines results in reduced *P. aeruginosa* binding (81–83). The degree of *P. aeruginosa* binding was greater in nasal scrapings from patients homozygous for ΔF508 compared with compound heterozygotes or carriers (84). The basis for this increased adherence of piliated *P. aeruginosa* to the apical surface of CF epithelial cells is proposed to be secondary to increased asialoganglioside-1 (81, 82, 85). Asialoganglioside-1 receptors are increased in cells expressing mutant CFTR and in areas of regenerating epithelium that are likely present in the inflamed CF airway (82, 85). The mechanism by which mutations in CFTR cause undersialylation of apical receptors is unknown but proposed to be related to hyperacidification of the trans-Golgi network in CF epithelial cells (86). Proponents acknowledge that asialoganglioside-1 is not a receptor for clinical mucoid isolates without pili or flagella (85), and therefore this host–pathogen interaction may not be relevant to chronic *P. aeruginosa* infection. Other investigators refute the role of asialoganglioside-1 as a significant *P. aeruginosa* receptor in the CF airway (87). Heparan sulfate proteoglygans on the basolateral surfaces of epithelial cells are potential receptors for nonpiliated *P. aeruginosa* in patients with chronic endobronchial infection and airway injury (88).

CFTR may serve as a receptor for P. aeruginosa *internalization.* CFTR is proposed to be a receptor for *P. aeruginosa* binding to airway epithelium for subsequent phagocytosis and clearance by desquamation (89–92). Therefore, reduced *P. aeruginosa* binding to mutant CFTR results in reduced *P. aeruginosa* clearance from the CF airway. It is postulated that this mechanism is important in the initiation of endobronchial infection. The complete LPS outer core is proposed to be the *P. aeruginosa* ligand that binds to wild-type CFTR (90, 93). This hypothesis is consistent with the observations that laboratory strains and nonmucoid clinical isolates of *P. aeruginosa*, but not mucoid isolates, bind to CFTR and are cleared more rapidly from wild-type versus transgenic CF mice (94); overexpression of CFTR in transgenic mice resulted in increased clearance of *P. aeruginosa* from the lung (92). *P. aeruginosa* adaptation within the CF airway is associated with modifications to LPS structure (95, 96); the specific LPS structures required for *P. aeruginosa* binding to CFTR have not been fully elucidated. There is no consensus on the importance of this LPS–CFTR interaction in the pathogenesis of CF lung disease.

The relative importance of epithelial cell phagocytosis in the innate defense against *P. aeruginosa* is uncertain compared with

mucociliary clearance and antimicrobial peptides. It is unlikely that epithelial phagocytosis is important in established infection, as mucoid *P. aeruginosa* and *Staphylococcus aureus* are observed primarily within endobronchial mucus and not adherent to the epithelium (97, 98).

Innate immunity and persistence of bacterial infections. Innate immune responses provide the first line of defense to airway infection in concert with mucociliary clearance. Submucosal glands, goblet cells of the large airways, Clara cells within the small airways, and epithelial cells secrete proteins and peptides into the ASL that can kill a broad spectrum of bacteria or modulate the host inflammatory response (99, 100). Classic components of the ASL with antimicrobial activity include lysozyme, lactoferrin, secretory phospholipase A2, secretory leukocyte protease inhibitor, and surfactant proteins (99, 100). Antimicrobial peptides of several classes, including α- and β-defensins and cathelicidins, are secreted from cellular components of the innate immune system. Some of these peptides are synthesized constitutively (i.e., human β-defensin 1), and others are upregulated in response to inflammatory mediators, such as human β-defensin 2 and LL-37 (2, 99–101). There is no evidence for a primary defect in the production of these antimicrobial peptides residing in the CF ASL. There have been reports, however, suggesting that decreased concentrations of a circulating serum protein, mannose-binding lectin, observed in individuals with polymorphisms in the mannose-binding lectin gene may contribute to more rapid decline in pulmonary function and poor survival in patients with CF colonized with *P. aeruginosa* and *Burkholderia cepacia* (102, 103). This serum lectin, important in innate immunity of both bacterial and viral infection, binds mannose and *N*-acetylglucosamine oligosaccharides on the surface of microorganisms activating the complement system and binding receptors on phagocytes (104). A laboratory model has also demonstrated that the mannose-binding lectin actively binds clinical strains of *B. cepacia*, activating complement, but does not bind *P. aeruginosa* isolates (105).

The central tenet of the "compositional" theory (*see* Impact of Defective CFTR on Airway Physiology and Mucociliary Clearance) is that the elevated sodium chloride content in CF ASL (Figure 7) leads to inactivation of salt-sensitive antimicrobial peptides permitting initial bacterial colonization within the CF airway (2, 67, 70). There are limited *in vivo* data to corroborate this theory (76). It has been postulated, however, that local microenvironments such as mucus plaques or submucosal glands in the CF airway, not easily reached for *in vivo* sampling, may demonstrate conditions (salt content or binding to actin/DNA) that can inactivate innate antimicrobial peptides to promote initial bacterial infection (106). New data showing steep oxygen gradients in mucus plagues within the CF airway has led proponents of the "isotonic, low ASL volume" hypothesis to propose a scheme for early and persistent endobronchial infection in CF (97). In this model, the hyperabsorption of sodium and absent chloride secretion in the CF airway result in reduced periciliary liquid volume and impaired mucociliary clearance leading to a cascade of events that provides a unique microenvironment to promote *P. aeruginosa* adaption and persistent infection as illustrated in Figure 6.

Acquired immunity. There is no evidence for a systemic immunodeficiency in CF to explain the chronic endobronchial infection. In CF, there is no increase in the frequency or severity of infections outside of the respiratory tract and patients with CF have normal immune responses to standard immunizations (52). Patients with CF mount a significant humoral response to *P. aeruginosa* antigens, and there are emerging data that serum antibodies directed against whole-cell *P. aeruginosa* lysates or specific *P. aeruginosa* antigens can be the first markers of *P. aeruginosa* infection in young children with CF (107, 108). Patients with chronic *P. aeruginosa* infection demonstrate high concentrations of antibodies directed against multiple *P. aeruginosa* antigens. Despite this early and sustained immune response to *P. aeruginosa*, the host is generally unable to clear *P. aeruginosa* from the airways.

There are multiple factors, however, contributing to the ineffective acquired immune response (109). Opsonophagocytosis of bacteria requires intact complement and F_c receptors on phagocytes. In the CF airway, with neutrophil-dominated inflammation, there is proteolytic cleavage of complement and F_c receptors resulting in reduced opsonophagocytosis (52). Local tissue destruction and reduced mucociliary clearance reduces the effectiveness of the immune response in the clearance of *P. aeruginosa* from the airway. Chronic *P. aeruginosa* antigen exposure in patients with CF appears to result in a lack of avidity maturation of anti–*P. aeruginosa* antibodies that may contribute to reduced function in *P. aeruginosa* clearance (110).

All of the proinflammatory cytokines and chemokines elevated in the CF airway have their synthesis regulated by the transcription factor nuclear factor-κB (NF-κB) (52, 111). Conflicting data exist on the degree of NF-κB activation in CF epithelia. Greater activation of NF-κB has been observed in some CF epithelial cell lines stimulated with *P. aeruginosa* or tumor necrosis factor–α compared with cells lines with wild-type CFTR (83, 112). However, not all CF airway epithelial cell culture models exhibit increased endogenous proinflammatory mediator secretion (113). The etiology of increased NF-κB activation in some CF cell culture models appears to be multifactorial. First, there is a proposed primary defect in the CF epithelium that results in increased NF-κB activation without external stimuli (57, 114). Second, there are reduced IL-10 concentrations in BAL fluid from patients with CF and reduced production of IL-10 from CF cell lines compared with control subjects (52). IL-10 causes increased production of an inhibitor of NF-κB activation IκB (52). In the setting of reduced IL-10 and IκB in the CF airway, there is unchecked NF-κB activation. Other investigators propose that a specific amino-terminus domain of CFTR is a pattern-recognition molecule for the complete LPS outer core, and binding of LPS to CFTR results in increased NF-κB activation (93).

MICROBIOLOGY OF CF LUNG DISEASE

CF has a unique set of bacterial pathogens that are frequently acquired in an age-dependent sequence. The pattern of age-specific prevalence as well as overall prevalence of these pathogens in the CF population in the United States is demonstrated in Figure 8 from the Cystic Fibrosis Foundation Patient Registry data (6). Of the organisms causing infection in CF, only *S. aureus* may be pathogenic in immunocompetent individuals. *P. aeruginosa*, *B. cepacia*, nontypeable *Haemophilus influenzae*, *Stenotrophomonas maltophilia*, and *Achromobacter xylosoxidans* are all considered opportunistic pathogens. Other organisms seen in CF that are also generally nonpathogenic in the healthy host include *Aspergillus* and nontuberculous mycobacteria.

Early Colonization and Infection

Early infections in CF airways are most frequently caused by *S. aureus* and *H. influenzae*, organisms that may be seen in other young children with chronic illnesses and in adults with non–CF bronchiectasis. *S. aureus* is often the first organism cultured from the respiratory tract of young children with CF (48). However, there continues to be debate about the significance of *S. aureus* in the pathogenesis of CF lung infection (115). Historically, significant improvements in patient longevity have been associated with the advent of antistaphylococcal therapy (116). However,

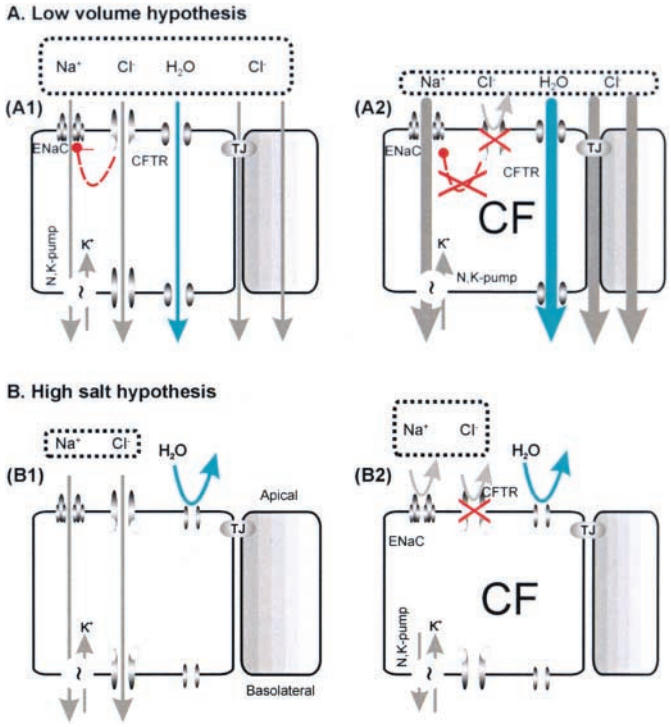

Figure 7. Two hypotheses of how ASL differs in healthy and CF lungs. (*A*) The low-volume hypothesis postulates that normal ASL (*A1*) has salt levels approximately equal to plasma. In CF (*A2*), the removal of CFTRs inhibition of epithelial sodium channels (EnaC) results in abnormally elevated isotonic fluid absorption, which depletes the ASL and leads to reduced mucociliary clearance. Key features of the low-volume model are the parallel pathway for Cl^- via shunt pathway(s) and inhibition of ENaC via CFTR. (*B*) The high-salt hypothesis postulates that normal ASL has low levels of salt as a result of salt absorption in excess of water (*B1*). Even though the epithelium is water permeable, salt is retained in thin surface films by some combination of surface tension impermeant osmolytes. In CF (*B2*), salt is poorly absorbed resulting in excessively salty ASL that inactivates endogenous, salt-sensitive antimicrobial peptides. Key features of the high-salt model are: the lack of an appreciable shunt Cl^- conductance, central importance of CFTRs channel role, no specific role for inhibition of ENaC by CFTR, and a switch from isotonic volume absorption to hypertonic salt absorption as the surface layer thins and traps residual water. (Reprinted by permission from Reference 70.)

several recently published studies of the efficacy of prophylactic antistaphylococcal antibiotics question the benefit of this therapeutic approach (*see* BRONCHODILATORS) (117, 118).

H. influenzae is also isolated from the respiratory tract early in the course of CF. In a natural history study of CF diagnosed in 40 children in the first year of life, either for clinical reasons or because of a family history, *H. influenzae* was the most common organism isolated from lower airway cultures at age 1 year (50, 107). Similar numbers have been reported in studies of children with CF identified by neonatal screening (119). The *H. influenzae* infecting patients with CF is nontypeable, thus not prevented by childhood immunization against *H. influenzae* type b. The role of *H. influenzae* in progressive airway infection and inflammation in patients with CF has not been clearly demonstrated, although it is known to be pathogenic in patients with non–CF bronchiectasis (120).

Infection with *P. aeruginosa*

P. aeruginosa is by far the most significant pathogen in CF and, based on immune responses in young children, infection appears

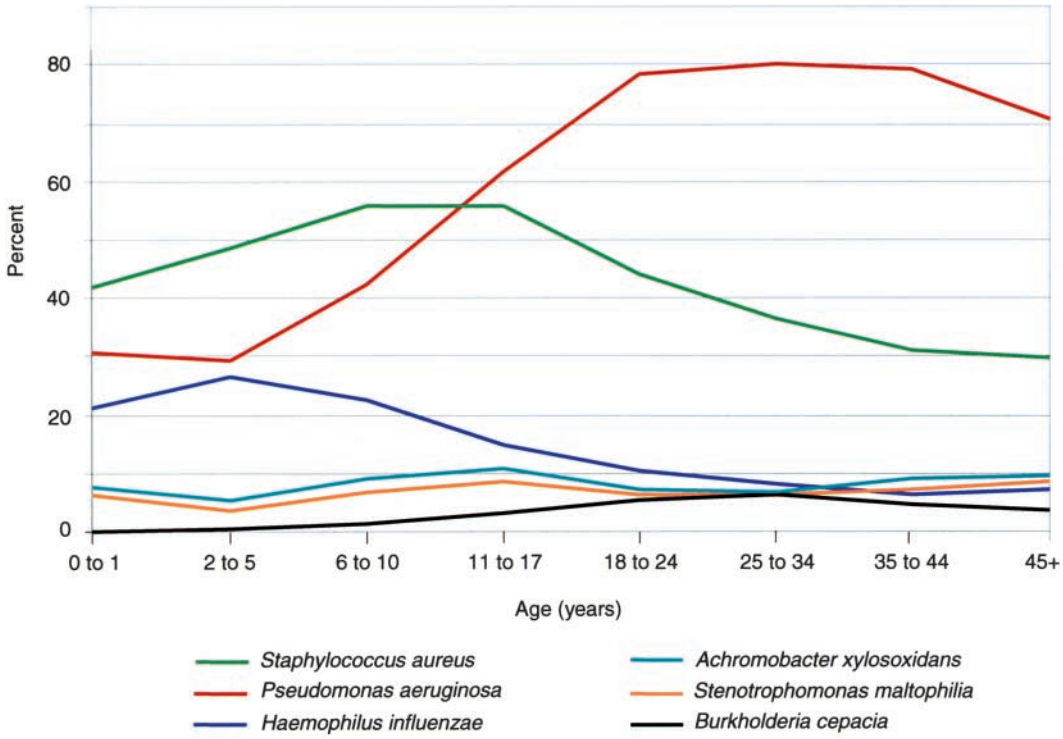

Figure 8. Age-specific prevalence of airway infections in patients with CF. Organisms reported to the U.S. Cystic Fibrosis Patient Registry, 2001. Overall percentage of patients (all ages) who had at least one respiratory tract culture (sputum, bronchoscopy, oropharyngeal, or nasal) performed in 2001 that was positive for the following organisms: *Pseudomonas aeruginosa* (*red line*), 58.7%; *Staphylococcus aureus* (*green line*), 48.0%; *Haemophilus influenzae* (*dark blue line*), 15.9%; *Stenotrophomonas maltophilia* (*yellow line*), 8.4%; *Achromobacter xylosoxidans* (*light blue line*), 4.4%; *Burkholderia cepacia* (*black line*), 3.1%. (Reprinted by permission from Reference 6.)

to occur much earlier than believed previously (107, 108, 119). In a natural history study of patients with CF in the first 3 years of life, the mean age of detection of an antibody response to *P. aeruginosa* was approximately 15 months, whereas the mean ages of first positive upper and lower airway culture were approximately 21 and 23 months, respectively (107). In a study of 68 patients with CF identified by neonatal screening, antibody responses to *P. aeruginosa* were identified, on average, nearly 12 months before positive oropharyngeal (OP) cultures (lower airway bacteriology was not available in these individuals) (108). Risk factors for initial *P. aeruginosa* airway infection in patients with CF diagnosed by newborn screening included female sex, homozygous ΔF508 genotype, and *S. aureus* isolation (121). Up to 80% of patients with CF are eventually infected with this organism (6), and acquisition of the organism is associated with clinical deterioration (50, 122–124). The source of *P. aeruginosa* isolates in patients with CF has not been clearly established. There is a wide distribution of *P. aeruginosa* genotypes that have been demonstrated in young children (107), suggesting acquisition from environmental reservoirs, and only rarely do patients with CF appear to share genotypes, generally only when they are siblings or otherwise epidemiologically linked (125, 126). Comparison of genotypes from upper and lower airway sources collected simultaneously from patients demonstrates that distinct genetic strains may colonize different anatomic sites in the CF airway (107, 127).

Characteristics of *P. aeruginosa* That Contribute to Initial and Persistent Infections

In Clinical Presentation and CFTR Function and Molecular Basis of CF Lung Disease, we have described unique characteristics of the CF airway that enhance the propensity for *P. aeruginosa* to initially colonize. Given this opportunity, the pathogens' own genetic and phenotypic plasticity enables adaptation to establish a persistent infection.

Phenotypic changes. P. aeruginosa isolates from the lungs of patients with CF are quite distinctive from those causing acute infection in other settings. These characteristics are not present in isolates causing initial colonization but appear to be selected within CF airways and occur increasingly with length of lung infection. Whereas early isolates appear much like environmental isolates in their phenotype, later isolates are more resistant to antibiotics and frequently mucoid (107). Additional phenotypic changes seen in CF isolates of *P. aeruginosa* include the loss of O-side chains on LPS making the strains nonreactive with typing sera (128), distinctive acylation of LPS (96), loss of flagella-dependent motility (129), and increased auxotrophy (130).

Although growth of *P. aeruginosa* in microcolonies has been proposed for many years (131), support for the existence of biofilms in CF has recently been reported (132, 133). Biofilms are sessile communities of bacteria that form in aggregates on surfaces using a hydrated polymeric matrix of their own synthesis (Figures 6D and 6E). Some common clinical characteristics of biofilm infections have been identified: slow growth of organisms, stimulation of production of antibodies that are ineffective in clearing bacteria, inherent resistance to antibiotics, and an inability to eradicate biofilm infections even in hosts with intact immune systems (134–137). These are characteristic of CF airway infections.

The presence of *P. aeruginosa* biofilms in infected CF airways was first suggested because of the quorum-sensing signals that the organisms produce to signal cell-density–dependent gene expression (132). In addition, both transmission and scanning electron microscopy have demonstrated organized clusters and microcolonies of *P. aeruginosa* in expectorated CF sputum consistant with biofilm formation (138). Subsequently, the presence of local hypoxia within mucus plaques in the airways has been suggested to increase *Pseudomonas* alginate production (97), which may lead to increased biofilm formation (Figure 6) (138). It has recently been reported that antibiotic-resistant phenotype variants of *P. aeruginosa* with an enhanced ability to form biofilms arise at high frequency in the lungs of patients with CF (133).

Genetic advantages. The knowledge gained from the recently available genome sequence of a laboratory strain of *P. aeruginosa*, PAO1, helps explain much of the phenotypic diversity of the organism (139). *P. aeruginosa* has a very large genome—at 6.3 Mbp it is 37% larger than the best-studied bacterial pathogen, *Escherichia coli*, which has a genome size of 4.6 Mbp. With 5,570 predicted open reading frames, the genetic complexity of *P. aeruginosa* approaches that of the simple eukaryotic organism, *Saccharomyces cereviseae*. This complete genome offers the potential for a tremendous ability to adapt to multiple different environments, including the CF airway. *P. aeruginosa* isolated from CF sputa have even larger genomes than the laboratory strain, PAO1, suggesting that they have acquired new genes during their adaptation, in addition to alterations in those already present (140).

A high frequency of hypermutability has been identified in *P. aeruginosa* isolates from patients with CF. This is likely caused by the milieu of the CF airway with large numbers of infecting organisms and compartmentalization of infection, combined with ineffective host defenses and ongoing antibiotic selective pressure (141).

Late-emerging Pathogens

Other organisms that are identified later in the course of CF airways disease include *B. cepacia*, *S. maltophilia*, *A. xylosoxidans*, fungi including *Aspergillus*, and nontuberculous mycobacteria. Of these, *B. cepacia* is the most serious because of its association with the *B. cepacia* syndrome leading to high fevers, bacteremia, rapid progression to severe necrotizing pneumonia and death. The majority of infected patients have a more chronic course with decline in lung function and increased mortality (142, 143). However, recent studies have demonstrated that *B. cepacia* is not a single species but rather a group of closely related species, termed "genomovars;" thus, the organism should be called *B. cepacia* complex. At least nine distinctive genomovars of *B. cepacia* have been identified and several have been named as distinct species (144, 145). The vast majority of CF airway infections with *B. cepacia* complex are caused by genomovars II (*Burkholderia multivorans*), III (*Burkholderia cenocepacia*) and V (*Burkholderia vietnamiensis*) (146). Although there are exceptions, most of the severe infections and strains that have been spread in epidemics are from genomovar III (147). Several clonal lineages are distributed widely across Europe and North America (146–148). In general, infection with other genomovars is associated with less severe disease.

S. maltophilia and *A. xylosoxidans* are seen more commonly than *B. cepacia* in patients with CF with advanced lung disease but are generally less virulent. Epidemiologic studies examining their association with morbidity and mortality in CF have not demonstrated a correlation between infection and outcome (149, 150). Like *P. aeruginosa*, person-to-person spread of these organisms is rarely documented in patients with CF, other than siblings (151).

Fungal colonization and infection of the CF airway late in disease progression is not surprising given the exposure of this population to frequent broad-spectrum antibiotic therapy (152). Whereas *Candida* spp. are the most frequent colonizers, isolated from almost 50 to 75% of patients with CF who were cultured (153, 154), they are usually considered to be harmless commensals. However, *Aspergillus* spp., most frequently *Aspergillus fum-*

igatus, are isolated from more than 25% of patients (154). There is not sufficient evidence to generally recommend treatment of an *Aspergillus*-postive sputum culture in the absence of allergic bronchopulmonary aspergillosis (ABPA) (155). Invasive infections caused by *Aspergillus* are rare in the immunocompetent nontransplant CF population, but ABPA can be a significant problem (156, 157).

ABPA is not an invasive fungal infection but rather a syndrome, including wheezing, pulmonary infiltrates and, potentially, bronchiectasis and fibrosis, that develops because of sensitization against allergens from *A. fumigatus* in the environment (156, 157). Exposure of the airways to high levels of *Aspergillus* allergens, due in part to reduced mucociliary clearance in CF, may be a key element in the development of ABPA. In patients with atopy, exposure to fungal spores and hyphal elements leads to the production of specific IgE and an increase in the CD4+ Th2 cell response to *A. fumigatus* (157). The overall prevalence of ABPA in CF is reported between 2 and 8% on the basis of three large clinical databases (157–159). The true prevalence of ABPA in the CF population is uncertain due to the lack of standardized diagnostic criteria and the lack of uniform surveillance and laboratory procedures (*see* Diagnosis and Treatment of ABPA).

Another filamentous fungus isolated commonly from the respiratory tract of patients with CF (8.6% of patients in one study) whose significance is unknown is *Scedosporium apiospermum* (160). For both *Aspergillus* and *Scedosporium*, no clustering of isolates has been identified by genotyping (161, 162). Other molds that have been reported from CF respiratory samples include *Wangiella dermatitidis* and *Penicillium emersonii* (163, 164).

Nontuberculous mycobacteria have been increasingly reported from the respiratory secretions of patients with CF. In a prospective prevalence study conducted at 21 CF centers across the United States, 13% of patients cultured nontuberculous mycobacteria from sputum (165). The most common species isolated were *Mycobacterium avium* complex (72%) and *Mycobacterium abscessus* (16%). Nontuberculous mycobacteria culture-positive patients were more frequently older and had a higher frequency of *S. aureus* and a lower frequency of *P. aeruginosa* compared with culture-negative control subjects. Molecular typing demonstrated a pattern of infrequent spread among patients. There appeared, however, to be a distinct geographic distribution of the prevalence of nontuberculous mycobacteria. Prevalence ranged from 7% in Boston to 24% in New Orleans, and the majority of centers with a rate greater than 15% were in coastal states.

A substudy that followed 60 nontuberculous mycobacteria–positive patients for 15 months and compared them with an uninfected control group identified no difference in the rate of decline of FEV_1 (166). Abnormalities on high-resolution computerized tomography (HRCT) scan, however, were predictive of progression. Thus, current recommendations suggest that adult patients with CF be screened on a regular basis with both acid-fast smear and appropriately processed sputum or BAL fluid culture (*see* Nontuberculous Mycobacteria). Findings suggestive of infection rather than colonization include: multiple positive cultures, a single positive culture associated with a pulmonary exacerbation that is not responsive to conventional antibacterial therapy or HRCT scan demonstrating peripheral pulmonary nodules, and/or a mucosal biopsy demonstrating granulomatous disease.

CLINICAL ASSESSMENT IN THE MANAGEMENT OF CF LUNG DISEASE

Monitoring Pulmonary Health Status

There is no approved therapy to correct the underlying genetic defect or reverse the ion transport abnormalities associated with dysfunctional CFTR. Thus, therapy is directed toward slowing the progression of secondary organ dysfunction and its sequelae such as pancreatic insufficiency with maldigestion and chronic endobronchial infection. This treatment approach has been enhanced by the establishment of comprehensive, multidisciplinary CF care centers worldwide. The Cystic Fibrosis Foundation has also established Clinical Practice Guidelines (167) followed by CF centers throughout the United States emphasizing routine quarterly monitoring of health status, patient and family education, and early intervention to slow illness progression (2).

Routine laboratory evaluations are key to assessing pulmonary status and are used to monitor disease progression and response to therapeutic interventions. These studies include radiologic examinations, pulmonary function testing, and microbiologic cultures of airway secretions. Assessment of blood oxygen and carbon dioxide values are useful in patients with more severe disease or acute pulmonary decompensation. Management of endstage lung disease including intensive care management–assisted ventilation and transplantation is beyond the scope of this review and has been reviewed (168–173).

Imaging. Chest X-rays are most helpful for defining disease progression and less sensitive in demonstrating changes during acute pulmonary exacerbations or early, mild disease. Hyperinflation with flattened diaphragms and retrosternal lucency may occur in early infancy and remain a prominent finding throughout the life of the patient. Progressive findings include nodular opacities due to mucus plugging and cystic changes due to bronchiectasis. Chest X-ray scores (174–176) have been developed for assessing disease progression but have never been widely used for routine patient management.

HRCT is more sensitive and specific than chest radiographs in identifying changes such as airway wall thickening and gas trapping in early CF lung disease and is particularly useful in identifying localized areas of bronchiectasis and parenchymal abnormalities (177–179). HRCT changes may also precede changes in pulmonary function, which assess an overall change in function rather than regional changes in structure (46, 180). For these reasons, HRCT is being used to document early bronchiectasis, localized disease, and response to antibiotic interventions during acute exacerbations (178, 181). Despite this progress, there are still no consensus guidelines for use of HRCT in CF care; the risk versus benefit ratio must continue to be addressed in terms of additional cost and radiation exposure (182).

Lung function testing. The principal measure of pulmonary status in individuals with CF older than 5 years of age is pulmonary function testing with spirometry or plethysmography. Serial measurements document stability or progression of airway obstruction and air trapping. Lung function measurements are also useful in documenting acute changes associated with pulmonary exacerbations and response to therapy. Most children are able to perform reproducible spirometric maneuvers according to American Thoracic Society guidelines (183) by age 5 to 6 years. In young children and older patients with minimal pulmonary involvement, values for FVC, FEV_1 and mean forced expiratory flow during the middle half of the FVC ($FEF_{25–75\%}$) may be normal when compared with reference values from healthy, sex, age, and height-matched individuals (6). The earliest spirometric evidence of obstructive disease (presumably due to mucus plugging, airway edema, inflammation, and increased secretions) is a decrease in expiratory flows at low volumes such as $FEF_{25–75\%}$ although these changes are highly variable (184, 185). Another early finding may be elevated residual volume (RV) and an increased ratio of RV to total lung capacity (RV/total lung capacity) consistent with gas trapping. This measurement should be determined by plethysmography as total lung capacity may be underestimated by spirometry due to air trapping.

Changes in FEV_1 will become evident as patients begin to develop obstructive lung disease. FEV_1 is the most widely used pulmonary function testing parameter of lung status (186–188) in CF because of the universal accessibility of spirometric equipment, standardized criteria for performance, availability of reference values (189–191), and reproducibility. In addition to day-to-day clinical management, FEV_1 serves two other important functions. First, it is the primary marker for disease progression identified in numerous epidemiologic studies to predict survivorship and decline in health status (122, 188, 192–195). Second, it is the primary outcome measure used for defining clinical efficacy for new therapeutic modalities in CF (187, 196, 197).

Across the entire U.S. CF population, the average decline in FEV_1 is 2% per annum (186). Factors that may negatively impact the rate of decline include nutritional status (198), comorbidity from diabetes mellitus (199, 200), colonization with *P. aeruginosa* (122, 124), and *B. cepacia* (142, 143) and frequency of pulmonary exacerbations (122). Other factors such as mild genotype and pancreatic sufficiency are associated with slower rates of decline (122, 201). Patients may be stable for many years and then show periods of more rapid progression. As FEV_1 continues to decline, patients will begin demonstrating a decline in FVC presumably due to progressive scarring, gas trapping, and increased dead space ventilation.

Infant pulmonary function testing. There is no simple, sensitive, reproducible measure of lung function in children less than 6 years of age. Techniques used for testing pulmonary function in children less than 3 years of age (202) require sedation and are only performed in specialized centers. The most promising technique developed in recent years is to raise the lung volume of infants to near total lung capacity before performing a rapid thoracic compression (203–208), yielding measurements that closely approximate the voluntary spirometric maneuvers of older children and adults. These measurements, more likely to be effort-independent and reflect underlying lung mechanics, demonstrate improved sensitivity (204, 208, 209), and decreased measurement variability (203, 210). Reference data for lung function in normal control subjects using these new measures are now published (206, 207).

Current methods of infant lung function testing cannot be extended beyond 3 to 4 years of age because of the inability to provide adequate oral sedation and loss of the Hering–Breuer reflex (relaxation of inspiratory muscles with repeated sigh breaths). Promising measures of lung function in preschool patients with CF (ages 3–6 years) that can be performed without sedation and during tidal breathing are being developed. These techniques include modified spirometry (211, 212) and respiratory resistance measured by plethysmography (202, 213), forced oscillation (214, 215), and interrupter resistance (216).

Diagnostic Microbiology

Culture of respiratory tract specimens from patients with CF can present challenges to microbiology laboratories unaccustomed to processing CF samples. Issues include nonrepresentative sampling of inhomogeneous specimens and polymicrobial infections. In addition, many of the available commercial systems for organism identification and antimicrobial susceptibility testing are inaccurate for CF pathogens (217–220).

Source of specimens. Expectorated sputum is an accurate indicator of lower airway microbiology (127, 221, 222) and the preferred source of airway secretions for management of CF lung diseases. However, sampling may be difficult in the younger patients and in patients with mild disease who do not expectorate. Two options that have been well studied are OP cultures and cultures collected by BAL. Several studies have compared these two measures of airway infection in CF (119, 127, 223, 224). One review that combined data from three prospective clinical studies (223) using simultaneous OP and BAL cultures (from 141 infants) found that the sensitivity of OP cultures in predicting lower airway *P. aeruginosa* was poor (44%; 95% confidence interval, 14–79%) but that the specificity was good (95%; 90–99%). These findings suggest that a negative OP culture is useful in ruling out lower airway infection, but that a positive culture is not reliable to make the diagnosis of *P. aeruginosa* in the lower airway. Culture of BAL fluid is considered a more sensitive measure of infection in nonexpectorating patients but the procedure is more invasive and requires sedation, thus increasing the risk and the cost. In addition, BAL is usually performed in just one lobe, which increases the risk of missing regional disease (45, 47). For these reasons, clincians generally use OP cultures as their initial source of microbiology specimens and reserve BAL for patients unresponsive to antimicrobial therapy or those with progressive disease.

Recently, hypertonic saline induction of sputum has been reported to be a good surrogate for lower airway sampling for both microbiology and inflammatory markers in both adult and older pediatric patients with CF (222, 225–227). In a comparison of culture results from expectorated and induced sputum samples and BAL fluid, similar detection rates for bacteria and fungi were identified with all three sample sources (222).

Isolation/identification techniques. Isolation and identification of bacterial pathogens from CF respiratory secretions is not straightforward for several reasons. Both expectorated and induced sputum samples are frequently very viscous requiring special processing to adequately sample the entire specimen. In addition, most CF airway infections are polymicrobial, and the organisms present may have very different growth requirements. *P. aeruginosa* is often present and, because of its mucoid phenotype, frequently overgrows both Gram-positive organisms such as *S. aureus*, and more fastidious or slower-growing Gram-negative organisms such as *H. influenzae* and *B. cepacia*. The use of selective media that inhibit the growth of *P. aeruginosa* is very helpful for the isolation of *S. aureus* and *H. influenzae* and is mandatory for the isolation of *B. cepacia* (Table 2) (153, 228–230).

Once organisms are isolated, identification, particularly of Gram-negative bacteria, may also be difficult because of the presence of a large number of unique organisms combined with the phenotypic changes that even the more common organisms may undergo. The use of standard biochemical testing rather than commercial systems has been recommended for identification of Gram-negative nonfermenting bacteria (217, 218). In addition, molecular techniques, especially polymerase chain reaction, have proved useful for bacterial identification, both directly in sputum and for isolated organisms growing in pure culture (231–233).

Antibiotic susceptibility testing. Susceptibility testing of CF isolates is also potentially difficult, for many of the same reasons that affect organism isolation and identification. Slow growth and mucoidy may impact the utility of automated systems for susceptibility testing of *P. aeruginosa* as well as for organism identification (219, 220). When compared with broth microdilution methodology, agar diffusion methodologies including disk diffusion and E-test performed well for the majority of antibiotics tested (234).

Whereas clinical laboratories have not been routinely looking for methicillin resistance in *S. aureus* isolated from patients with CF, a survey of isolates from multiple CF centers suggested that the rate of resistance in CF is comparable with that in the general population (153). Vancomycin tolerance and resistance have both been described in human isolates of *S. aureus* (235–237), and there is no reason to believe that patients with CF will be protected from acquiring them, as well.

TABLE 2. RECOMMENDED SELECTIVE MEDIA AND CULTURE CONDITIONS FOR ISOLATION OF CYSTIC FIBROSIS PATHOGENS FROM RESPIRATORY SAMPLES

Bacteria	Recommended Media and Conditions*
Staphylococcus aureus	Mannitol salt agar
	Columbia/colistin–nalidixic acid agar
Haemophilus influenzae	Horse blood or chocolate agar (supplemented or not with 300 μg/ml bacitracin); incubated anaerobically
Pseudomonas aeruginosa	MacConkey agar
Burkholderia cepacia complex†	BCSA
	OFPBL agar
	PC agar
Stenotrophomonas maltophilia	MacConkey agar
	VIA agar‡
	DNase agar used for confirmation§
Achromobacter xylosoxidans	MacConkey agar

Definition of abbreviations: BCSA = *B. cepacia* selective agar; OFPBL = oxidative-fermentative polymyxin B, bacitracin, and lactose; PC = *Pseudomonas cepacia*; VIA = vancomycin-imipenem-amphotericin.

* Media listed are commercially available.

† Detection of *B. cepacia* complex may be enhanced by prolonging incubation for as long as 4 days to allow slow-growing colonies to become apparent.

‡ Mannitol agar base media containing vancomycin–imipenem–amphotericin B as selective agents.

§ Agar containing DNA with toluidine blue as an indicator of deoxyribonuclease activity.

Other nonstandard methods for susceptibility testing in CF include synergy testing of multiply-resistant Gram-negative isolates and multiple combination bactericidal testing of *P. aeruginosa* and *B. cepacia* complex (238). Ongoing trials of the clinical utility of multiple combination bactericidal testing testing for the management of *B. cepacia* complex lung infections are being conducted in Canada. More recently, evidence of biofilm formation by organisms in the CF airway has prompted the investigation of biofilm susceptibility testing (239). Different drugs and drug combinations appear to be efficacious against *P. aeruginosa* growing in biofilms; this may help explain nonbactericidal mechanisms of activity of antimicrobial therapy. At this time, many of these nonstandard techniques cannot be recommended for routine use in CF because their clinical efficacy has yet to be tested.

Pseudomonas *serology.* The serologic diagnosis of *P. aeruginosa* infection in CF has been used clinically in Europe for many years (109, 110, 240–244) and as a research tool in the United States and Canada (107, 108, 245, 246). In early respiratory tract infection with *P. aeruginosa*, serology may be more sensitive than OP culture (108). Although in patients with established infection *P. aeruginosa* antibody levels rarely decrease in response to antimicrobial therapy (247), low titers in response to treatment with inhaled tobramycin have been documented in patients with early *P. aeruginosa* colonization (248). Despite the utility of serologic evaluation, it is considered primarily a research tool in the United States because commercial tests are not widely available. Techniques with promise include both ELISA and Western blot.

Nontuberculous mycobacteria. Mycobacteria are very slow growing organisms and require supplemented media to grow. Thus, without inhibition of the *P. aeruginosa* and other less fastidious organisms within CF specimens, they are frequently overgrown. This technique was used to examine 986 sputum specimens from patients at 21 CF centers in the United States, identifying a 13% infection rate (165). This methodology uses 0.25% *N*-acetyl-L-cysteine and 1% sodium hydroxide decontamination followed by 5% oxalic acid treatment (249). The optimum processing of CF samples for nontuberculous mycobacteria has been reported and resulted in *P. aeruginosa* contamination of only 3 to 5% of the specimens (249).

CURRENT ANTIBIOTIC THERAPIES

Appropriate antibiotic therapy directed against bacterial pathogens isolated from the respiratory tract is an essential component in the management of CF lung disease. Most clinicians prescribe antibiotic therapies in three distinct clinical settings during the lifespan of an individual with CF. First, during early lung disease patients may receive antibiotics to delay onset of chronic colonization with *P. aeruginosa*. Second, once patients are colonized with pathogens such as *S. aureus* and *P. aeruginosa*, chronic maintenance antibiotics are administered to slow decline in pulmonary function and reduce frequency and morbidity of pulmonary exacerbations. Third, at the time of periodic exacerbations in pulmonary symptoms, intensive antibiotic regimens are frequently administered during hospitalization to relieve symptomatology and restore pulmonary function to baseline values. The current recommendations for antibiotic therapy in each of these settings and the body of scientific knowledge on which the recommendations are based are outlined below. Two basic principles should be considered for all antibiotic choices in CF. First, antibiotic selection should be based on periodic isolation and identification of pathogens from respiratory secretions and review of the antimicrobial susceptibility profile for those pathogens. Second, indiscriminant use of antibiotics without thoughtful considerations of the rationale, clinical endpoint, and duration should be avoided.

Prevention of Chronic *P. aeruginosa* Infection

Characteristics of *P. aeruginosa* isolates initially infecting the CF airway appear more favorable for eradication with appropriate antibiotic therapy. They are usually nonmucoid, highly susceptible to antibiotics, and present in lower density than established infections (50, 107, 123). Thus, early aggressive antipseudomonal antibiotic therapy has been advocated to delay onset of chronic *P. aeruginosa* infection (250) on the basis of findings from several studies of eradication and delayed reinfection with this pathogen (248, 251, 252). Although encouraging, interpretation of these study results has been hampered by small sample sizes, lack of control subjects or use of historic control subjects, and different study endpoints. Despite the poor predictive value of upper airway cultures (*see* SOURCE OF SPECIMENS), only two studies (123, 246), one controlled and one uncontrolled, have examined the impact of early antipseudomonal therapy on lower airway *P. aeruginosa* infection. The controlled study (246) demonstrated

eradication of *P. aeruginosa* in all 8 patients who received 28 days administration of inhaled tobramycin (300 mg twice daily) and only 1 of 13 receiving placebo. The study was stopped by the Data Safety Monitoring Board after completion of only 25% of the proposed enrollment because of the significant microbiologic treatment effect. Although these findings are encouraging, the second study (123) reported persistent eradication of *P. aeruginosa* in the lower airway in only 25% of subjects, 12 months posttherapy.

Duration of eradication is variable and likely depends on patient selection criteria, source of respiratory secretions (upper vs. lower airway) and duration and type of therapy. This issue was recently addressed in a study that followed quarterly cultures and *P. aeruginosa* serology on 19 children after antipseudomonal treatment of their first *P. aeruginosa*–positive OP cultures (253). The mean duration of eradication was 8 months, and the majority of patients became infected with a new, genetically distinct *P. aeruginosa*. A second study reported a longer upper airway pathogen-free period after 12 months of inhaled antibiotics (248). It is likely that without ongoing antibiotic suppression, reinfection will occur. The safety of long-term antibiotics in these young patients and the impact on airway microbial flora is not known. Although accumulating data suggest that early antibiotic therapy results in *P. aeruginosa* eradication from both the upper and lower airway, there are no controlled trials providing compelling evidence of clinical benefit in this population. There is a critical need therefore for a large, adequately powered, placebo-controlled clinical trial to address the clinical efficacy and safety of antipseudomonal therapy in this early infected population.

Maintenance Therapy

Antistaphylococcal antibiotics. Continuous antistaphylococcal antibiotic usage in CF was a common practice in the latter half of the 20th century and was believed to contribute to decrease mortality in young patients (116, 254). In recent years, both randomized clinical trials (118, 255, 256) and retrospective database reviews (117, 257) have questioned the validity of this approach. In a small study, 42 infants diagnosed by newborn screening (255) were randomized to receive either continuous oral flucloxacillin or episodic antibiotics as clinically indicated for the first 24 months of life. Treated patients had fewer isolates of *S. aureus*, less cough, and shorter hospital stays, but there was no difference between groups in terms of lung mechanics. A large multicenter, randomized, placebo-controlled trial of 209 children (mean age 15 months), 119 of whom completed a 5-year course of either continuous cephalexin or placebo was recently reported (118). Subjects who received cephalexin were less likely to be culture positive for *S. aureus* but more likely to be culture positive for *P. aeruginosa*. There were no differences between the groups in any clinical outcome measures including pulmonary function, weight gain, or chest radiograph score. A large retrospective database review (117) of pediatric patients not colonized with *P. aeruginosa* demonstrated no differences in clinical outcomes between patients treated with continuous versus intermittent antistaphylococcal antibiotics. Continuously treated patients again, had lower prevalence of *S. aureus* but significantly higher rates of *P. aeruginosa* acquisition. From these accumulating data, use of continuous antistaphylococcal antibiotics is no longer recommended as there is no clear evidence of clinical benefit and may select for *P. aeruginosa* in the airway. Intermittent antistaphylococcal treatment for respiratory symptoms is appropriate. Appropriate antistaphylococcal antibiotics and dosage ranges are provided in both Tables 3 and 4.

An additional concern for indiscriminant use of antistaphylococcal antibiotics is the emergence of methicillin-resistant *S. aureus* strains in this population. Whether this concerning trend is due to antibiotic usage in CF or the dramatic worldwide increase in prevalence (258, 259) is not known. All *S. aureus* isolates should be tested for methicillin resistance. The treatment of choice for methicillin-resistant *S. aureus* is vancomycin; alternative therapies include clindamycin, trimethoprim/sulfamethoxazole, and quinolones for individuals who do not tolerate vancomycin, if susceptibility testing indicates their activity (Tables 3 and 4). Some methicillin-resistant *S. aureus* strains now demonstrate resistence to glycopeptides such as vancomycin (236, 260), and in this setting an oxazolidinone such as linezolid (261) may be effective.

The finding of slow growing isolates of both *S. aureus* and *P. aeruginosa*, called "small colony variants" in respiratory tract cultures from individuals with CF has also impacted antimicrobial therapy. These variants likely arise from antibiotic pressure combined with unique CF airway milieu (262–264). These cell wall–deficient phenotypic variants have the ability to survive intracellularly in neutrophils evading host defenses. In addition, some classes of antibiotics are less active including, the aminoglycosides, antifolate antibiotics such as trimethoprim/sulfamethoxazole, and cell wall–active agents such as the β-lactams (263, 265). Therefore, alternative nonaminoglycoside, noncell active classes of antibiotics should be considered, in consultation with experts in microbiology. Clinical data demonstrating the role of the small colony variants in disease progression is lacking, and the value of antibiotic treatment is not known.

Antipseudomonal antibiotics. Maintenance therapy for patients colonized with *P. aeruginosa* has ranged from quarterly intravenous antipseudomonal antibiotics (266) to inhaled antibiotics to oral quinolones or macrolides. Although many of these therapies are empiric, recent large, randomized, placebo-controlled trials have documented that maintenance antibiotic therapy in patients chronically colonized with *P. aeruginosa* can stabilize pulmonary function and decrease morbidity (197, 267).

Inhaled antibiotics, in particular aminoglycosides, have attracted interest for many years because of the greater therapeutic index achieved by direct delivery of high-dose antibiotics to the endobronchial space with limited systemic absorption and toxicity (268). In addition, aerosol administration of high concentrations of aminoglycosides overcomes the antagonistic effects of purulent CF airway secretions on aminoglycoside bioactivity (269, 270). Early studies of both low-dose (60–80 mg thrice daily) (271) and high-dose (600 mg thrice daily) (272) inhaled aminoglycosides were encouraging and demonstrated improved lung function and/or decreased hospitalization rate. Subsequently, a large Phase III, placebo-controlled study of 300 mg of inhaled tobramycin administered twice daily (cycling 28 days on, 28 days off therapy) for 6 months reported a 10% relative increase in FEV_1 and 36% reduction in use of intravenous antibiotics in the treatment group as compared with the placebo group (197). There was limited systemic absorption and no oto- or nephrotoxicity detected. In 2001, 60.8% of all patients with CF in the United States who were culture positive for *P. aeruginosa* received inhaled tobramycin as maintenance therapy (6).

Polypeptide antibiotics of the polymyxin class delivered by aerosol have been widely used in Europe with reports of uncontrolled clinical studies (273–275) using doses of 500,000 to 1 million IU of colistin twice daily (potency of 30,000 IU/mg) for several months. Such therapy has been associated with decreases in isolation of *P. aeruginosa* and possible slowing of decline in FVC. A randomized trial comparing inhaled tobramycin (300 mg twice daily) and colistin (80 mg twice daily) for 28 days in 109 patients older than 6 years of age demonstrated a 6.7% improvement in the tobramycin-treated group not observed in the colistin group (276). Both therapies demonstrated a comparable small reduction in bacterial density in expectorated sputum. The re-

TABLE 3. ANTIBIOTICS FOR THE OUTPATIENT MANAGEMENT OF CYSTIC FIBROSIS

Pathogen	Antibiotic	Pediatric Dose*	Adult Dose
Staphylococcus aureus	Choose one:		
	Dicloxacillin	6.25–12.5 mg/kg four times daily	250–500 mg four times daily
	Cephalexin	12.5–25 mg/kg four times daily	500 mg four times daily
	Amoxicilin/clavulanate†	12.5–22.5 mg/kg of amoxicillin component twice a day	400–875 mg of amoxicillin component twice a day
	Erythromycin (base)	15 mg/kg three times a day	500 mg twice a day
	Clarithromycin	7.5 mg/kg twice a day	500 mg twice a day
	Azithromycin	10 mg/kg initial dose followed by 5 mg/kg every day	500 mg initial dose followed by 250 mg every day
	Clindamycin‡	3.5–7 mg/kg three times a day	150–450 mg three times a day–four times daily
Haemophilus influenzae	Choose one:		
	Amoxicillin	25–50 mg/kg twice a day	500–875 mg twice a day
	Amoxicilin/clavulanate†	12.5–22.5 mg/kg of amoxicillin component twice a day	400–875 mg of amoxicillin component twice a day
	Second/third generation cephalosporins:		
	Cefuroxime axetil	15–20 mg/kg twice a day	250–500 mg twice a day
	Cefprozil	7.5–15 mg/kg twice a day	250–500 mg twice a day
	Cefixime	4 mg/kg twice a day	200–400 mg twice a day
	Cefpodoxime proxetil	5 mg/kg twice a day	100–200 mg twice a day
	Loracarbef	7.5–15 mg/kg twice a day	400 mg twice a day
Pseudomonas aeruginosa	Choose one:		
	Ciprofloxacin	10–15 mg/kg twice a day	500–750 mg twice a day
	Tobramycin via inhalation	300 mg by nebulizer, twice a day	300 mg by nebulizer, twice a day
	Colistin via inhalation	150 mg by nebulizer, twice a day	150 mg by nebulizer, twice a day
Burkholderia cepacia	Choose one:		
	Trimethoprim/sulfamethoxazole	4–5 mg/kg of trimethoprim component twice a day	160 mg of trimethoprim component twice a day
	Doxycycline	5 mg/kg initial dose followed by 2.5 mg/kg twice a day§	200 mg initial dose followed by 100 mg twice a day
	Minocycline	4 mg/kg initial dose followed by 2 mg/kg twice a day§	200 mg initial dose followed by 100 mg twice a day

Definition of abbreviation: MRSA = methicillin-resistant *S. aureus*.

This list is not intended to be exhaustive.

All doses are oral unless otherwise noted. Most doses are expressed as milligrams per kilogram of body weight.

* The dose given to children should not exceed that for adults.

† Higher doses of clavulanate are frequently associated with diarrhea.

‡ Clindamycin may be useful for community-acquired MRSA, depending on susceptibility testing.

§ Tetracycline antibiotics are not recommended for children under 8 years of age.

sults of this unblinded study may have been biased by the prior long-term treatment of most study subjects with inhaled colistin. There have been reports of bronchoconstriction and chest tightness with colistin inhalation both in adults (277) and children (278).

There is ongoing interest in developing other classes of inhaled antibiotics to complement inhaled tobramycin and colistin. To date, no randomized, placebo-controlled trials of inhaled β-lactams have been reported, and thus it is not possible to evaluate the efficacy and safety of this antibiotic class. One single center, open-label study has evaluated aerosol administration of aztreonam, a monobactam antibiotic, for treatment of patients with CF colonized with *P. aeruginosa* (279). In this study, 19 patients with CF (4–20 years of age) were treated for up to 18 months with inhaled doses of aztreonam (500 mg to 1 g) administered twice daily. Pseudomonal density declined in 15 of 16 patients who completed the trial, and several patients had improved lung function. Transient antibiotic resistance to aztreonam was observed in 10 patients, and 1 patient had a hypersensitivity reaction to the drug. Systemic administration of aztreonam for treatment of Gram-negative infections including *P. aeruginosa* has been well tolerated and efficacious in patients with CF (280, 281). Early phase trials of a new formulation of inhaled aztreonam are currently in progress (B. Montgomery, personal communication).

One of the ongoing concerns about maintenance-inhaled antibiotic regimens has been emergence of antibiotic resistance among bacterial pathogens residing in respiratory secretions of the individual receiving the therapy (282). The impact of chronic administration of inhaled aminoglycosides on respiratory microbial flora and mechanisms of aminoglycoside resistance was best studied in the Phase III–inhaled tobramycin trials (283, 284). The percentage of patients receiving inhaled tobramycin that yielded *P. aeruginosa* isolates with minimal inhibitory concentrations of at least 8 μg/ml (defined as resistant) increased from 25 to 32%. This increase was not observed in patients receiving placebo. This change in antibiotic susceptibility had no observable impact, however, on clinical improvement in 6 months of therapy (197). The primary mechanism for resistance was bacterial wall impermeability (284). There was no evidence of increased isolation of other inherently resistant Gram-negative pathogens (e.g., *B. cepacia*), but oral colonization with fungal pathogens, *Aspergillus* species and *Candida albicans*, was increased in the patients receiving inhaled tobramycin (197). Unfortunately, since the completion of this Phase III trial, no ongoing studies of antibiotic resistance in the CF population are available. The long-term impact on respiratory flora is unknown and should be monitored.

Fluoroquinolones have several characteristics that have made them appealing for oral maintenance therapy. Ciprofloxacin, the most commonly used quinolone in CF, possesses a broad-spectrum antibacterial activity with excellent bacteriocidal activity against *P. aeruginosa* strains isolated from individuals with CF (285) and may be additive with aminoglycosides (286). Phar-

TABLE 4. ANTIBIOTICS FOR THE TREATMENT OF BACTERIA ASSOCIATED WITH PULMONARY EXACERBATIONS

Prevalent Bacteria	Antibiotic	Pediatric Dose*	Adult Dose*
Staphylococcus aureus	Cefazolin	30 mg/kg intravenously every 8 h	1 g intravenously every 8 h
	OR		
	Nafcillin†	25–50 mg/kg intravenously every 6 h	2 g intravenously every 6 h
Methicillin-resistant *S. aureus*	Vancomycin‡	15 mg/kg intravenously every 6 h	500 mg intravenously every 6 h OR 1 g intravenously every 12 h
Pseudomonas aeruginosa	β-lactam (choose 1):		
	Ceftazidime	50 mg/kg intravenously every 8 h	2 g intravenously every 8 h
	Ticarcillin§	100 mg/kg intravenously every 6 h	3 g intravenously every 6 h
	Piperacillin	100 mg/kg intravenously every 6 h	3 g intravenously every 6 h
	Imipenem‖	15–25 mg/kg intravenously every 6 h	500 mg–1 g intravenously every 6 h
	Meropenem‖	40 mg/kg intravenously every 8 h	2 g intravenously every 8 h
	Aztreonam	50 mg/kg intravenously every 8 h	2 g intravenously every 8 h
	PLUS aminoglycoside (choose 1):		
	Tobramycin¶	3 mg/kg intravenously every 8 h	3 mg/kg intravenously every 8 h
	Amikacin**	5–7.5 mg/kg intravenously every 8 h	5–7.5 mg/kg intravenously every 8h
Burkholderia cepacia	Meropenem	40 mg/kg intravenously every 8 h	2 g intravenously every 8 h
	PLUS (choose 1):		
	Minocycline	2 mg/kg intravenously or orally every 12 h††	100 mg intravenously or orally every 12 h
	Amikacin**	5–7.5 mg/kg intravenously every 8 h	5–7.5 mg/kg intravenously every 8 h
	Ceftazidime	50 mg/kg intravenously every 8 h	2 g intravenously every 8 h
	Chloramphenicol‡‡	15–20 mg/kg intravenously every 6 h	15–20 mg/kg intravenously every 6 h
	Trimethoprim/sulfamethoxazole	4–5 mg/kg of trimethoprim component intravenously every 12 h	4–5 mg/kg of trimethoprim component intravenously every 12 h
	Note: Third drug may be added if synergy testing suggests efficacy		
Stenotrophomonas maltophilia	Ticarcillin/clavulanate	100 mg/kg of ticarcillin component intravenously every 6 h	3 g of ticarcillin component intravenously every 6 h
	OR		
	Trimethoprim/sulfamethoxazole	4–5 mg/kg of trimethoprim component intravenously every 12 h	4–5 mg/kg of trimethoprim component intravenously every 12 h
	OR		
	Ticarcillin/clavulanate PLUS	100 mg/kg of ticarcillin component intravenously every 6 h	3 g of ticarcillin component intravenously every 6 h
	Aztreonam	50 mg/kg intravenously every 8 h	2 g intravenously every 8 h
Achromobacter xylosoxidans	Chloramphenicol‡‡	15–20 mg/kg intravenously every 6 h	15–20 mg/kg every 6 h
	PLUS Minocycline	2 mg/kg intravenously or orally every 12 h††	100 mg intravenously or orally every 12 h
	OR		
	Ciprofloxacin	15 mg/kg intravenously or orally every 12 h	400 mg intravenously or 500–750 mg orally every 12 h
	PLUS (choose 1):		
	Imipenem	15–25 mg/kg intravenously every 6 h	500 mg–1 g intravenously every 6 h
	Meropenem	40 mg/kg intravenously every 8 h	2 gm intravenously every 8 h

Most doses are expressed as milligrams per kilogram of body weight.

* The dose given to children should not exceed that for adults.

† To minimize phlebitis, nafcillin should be diluted to a concentration of less than 20 mg/ml.

‡ Vancomycin should be infused slowly to avoid histamine release. Serum concentrations should be monitored; the peak concentration ranges from 20–40 μg/ml, and the trough from 5–10 μg/ml.

§ Ticarcillin may be associated with occasional platelet dysfunction. Its use is limited by concern about the possibility of selection for resistant organisms, such as *S. maltophilia* and *B. cepacia.*

‖ These drugs are for patients with sensitivity to cephalosporin or multidrug-resistant organisms.

¶ Serum concentrations should be monitored; the peak concentration ranges from 8–12 μg/ml, and the trough concentration is less than 2 μg/ml.

** Serum concentrations should be monitored; the peak concentration ranges from 20–30 μg/ml, and the trough concentration is less than 10 μg/ml.

†† Should not be given to patients under 8 years of age.

‡‡ Serum concentrations should be monitored; the peak concentration ranges from 15–25 μg/ml, and the trough from 5–15 μg/ml.

macokinetic studies (287) in CF demonstrate excellent oral absorption and bioavailability in airway secretions with subjects attaining sputum concentrations above the minimal inhibitory concentration of 90% of all *P. aeruginosa* isolates for up to 15 hours. Early clinical studies of ciprofloxacin monotherapy in adults with CF experiencing pulmonary exacerbation demonstrated improved pulmonary function (288) in some cases comparable with intravenous antibiotics (289, 290). Emergence of *P. aeruginosa* and *S. aureus* resistant to ciprofloxacin and other quinolones is a growing concern and appears to be associated with monotherapy for more than 3 to 4 weeks (291). Thus, prolonged treatment is discouraged. The quinolone safety profile in adults is very good (292), but the drug is not Food and Drug Administration approved for use in prepubescent children because of arthropathy demonstrated in an animal model (293). However, administration of ciprofloxacin to over 3,000 children, the majority with CF, has been associated with a very low incidence of arthropathy (294–296). Several new fluoroquinolones (moxifloxacin and gatifloxacin) have become available in recent years. These drugs may enhance activity for some ciprofloxacin-resistant pathogens, but provide little advantage for *P. aeruginosa* (297). Thus, ciprofloxacin should remain the quinolone of choice for *P. aeruginosa* infection in CF, reserving the newer quinolones for organisms unresponsive to ciprofloxacin therapy.

TABLE 5. PULMONARY EXACERBATION

I	Symptoms
A	Increased frequency, duration, and intensity of cough
B	Increased or new onset of sputum production
C	Change in sputum appearance
D	New onset or increased hemoptysis
E	Increased shortness of breath and decreased exercise tolerance
F	Decrease in overall well-being—increased fatigue, weakness, fever, poor appetite
II	Physical signs
A	Increased work of breathing—intercostal retractions and use of accessory muscles
B	Increased respiratory rate
C	New onset or increased crackles on chest examination
D	Increased air trapping
E	Fever
F	Weight loss
III	Laboratory findings
A	Decrease in FEV_1 of 10% or greater compared with best value in previous 6 months
B	Increased air trapping and/or new infiltrate on chest radiograph
C	Leukocytosis
D	Decreased Sa_{O_2}

Macrolides such as erythromycin, clarithromycin, and azithromycin have been effective in treatment of chronic airway infections with *P. aeruginosa*. This clinical effect was first demonstrated in diffuse panbronchiolitis in Japanese adults (298, 299) and more recently in studies of patients with CF (267, 300, 301). In both of these chronic progressive pulmonary disorders, *P. aeruginosa* may establish an antibiotic-resistant biofilm where organisms may evade antibiotics and host lung defenses (97, 132, 133, 137, 302). On the basis of *in vitro* studies, several antiinfective and antiinflammatory modes of action have been proposed to account for the efficacy of macrolides in this setting (303). Antimicrobial effects are augmented by excellent biofilm penetration and intracellular accumulation in *P. aeruginosa,* enabling inhibition of protein synthesis (304) and improved killing of stationary phase organisms (B. Iglewski, personal communication). Macrolides also accumulate within neutrophils impacting several key functions including oxidant production, apoptosis, inflammatory cytokine production, and macrophage-activating complex-1 expression (305). Two recent reports of Phase II, randomized, placebo-controlled trials in children (267) and adults (301) both demonstrated improved lung function (FEV_1) and fewer respiratory exacerbations, whereas only one report (301) demonstrated an antiinflammatory effect (reduced C-reactive protein serum levels). A larger, Phase III, randomized, controlled trial involving 185 subjects, mean age 20 years colonized with *P. aeruginosa* has recently been reported (306). Subjects received 6 months of thrice weekly azithromycin (500 mg thrice weekly for patients $\geq$ 40 kg and 250 mg thrice weekly for patients 25–40 kg) or placebo. The treatment effect for the primary outcome, FEV_1, was 0.098 L or 6.2% in terms of relative change. The azithromycin-treated group also demonstrated increased weight gain and decreased rate of pulmonary exacerbations.

On the basis of the results of these recent studies, the use of macrolides as a maintenance therapy in patients with CF greater than 6 years of age and colonized with *P. aeruginosa* is increasing. Future studies are necessary to understand the long-term impact of this therapy and the efficacy in younger patients with early disease. There is also a need for *in vitro* studies to better understand the mechanism of action of macrolides in this setting. Regular monitoring of airway secretions for presence of nontuberculous mycobacteria is recommended because of the concern for emergence of macrolide resistance in these pathogens.

Treatment of Pulmonary Exacerbation

Once chronically infected with bacterial pathogens such as *P. aeruginosa*, individuals with CF experience daily respiratory symptoms. However, the intensity of these symptoms vary and these patients will periodically experience pulmonary exacerbations (Table 5). The etiology of a pulmonary exacerbation is likely a variety of airway insults including respiratory viral infections, reactive airway disease, and pollutants (307). In addition to acute morbidity, repeated exacerbations may have long-term negative impact on lung function and lifespan (194). Thus, optimal treatment of these episodes of increased symptomatology is an essential component of CF care. Despite the importance of exacerbations in this illness, no standardized definition or criteria has been universally accepted (187, 308). Recent trials using frequency of exacerbation as a primary outcome measure have contributed most to a standard definition (196, 197, 308, 309).

Patients may experience some symptoms and signs of an exacerbation but are not sufficiently ill to warrant intravenous antibiotics or hospitalization. There are no controlled trials defining the optimal therapy in this setting of a mild pulmonary exacerbation. In general, patients are treated on an outpatient basis with oral or inhaled antibiotics on the basis of microbiologic culture results (Table 3) as well as augmentation of airway clearance, bronchodilators, and, at times, antiinflammatory therapy.

For patients meeting most criteria of an exacerbation, standard therapy includes intravenous administration of two antibiotics for 14 to 21 days (310). It is common for these individuals to require hospitalization and multiple therapeutic interventions including either oral or parenteral nutritional support, frequent airway clearance techniques, augmented bronchodilator therapy, and corticosteriods. Therapy is usually initiated in the hospital to monitor aminoglycoside levels and assure stability of respiratory status. Completion of intravenous therapy at home is commonplace and appears to be as effective as inpatient management with proper training and supervision (311).

Choice of appropriate antimicrobial therapy should be based on review of recent cultures of airway secretions. The most common antibiotic and appropriate dosage regimens for pathogens frequently associated with an exacerbation are listed in Table 4. In the clinical setting of an acute pulmonary infection, antibiotic efficacy is measured by eradication of the pathogen and resolution of the inflammatory response (312). With chronic infection, eradication of *P. aeruginosa* is a rare occurrence. Most patients, however, experience improvement in respiratory symptoms (313). Clinical efficacy is measured by improved pulmonary function (314, 315) reduction in sputum bacterial density (314, 316), sputum DNA content (316), and improved quality of life measures (317). A combination of an aminoglycoside and β-lactam

is recommended to provide synergy and slow emergence of resistance (318). A wide variety of β-lactams and aminoglycoside regimens have been studied, with all showing similar efficacy if administered to individuals with susceptible pathogens (313). An increasing number of patients with chronic *P. aeruginosa* infection develop multiresistant strains defined as demonstrating resistance to all drugs in at least two out of three major classes of antipseudomonal antibiotics, β-lactams, aminoglycosides, and quinolones. In this setting, clinicians are encouraged to send the pathogens to a specialized laboratory that provides synergy studies (319, 320) or multiple combination bactericidal testing (238) (*see* ANTIBIOTIC SUSCEPTIBILITY TESTING). Optimal choice in antibiotics should be based on the results from the reference laboratory.

The pharmacokinetics of intravenous antibiotics, in particular aminoglycosides, have been widely studied and reviewed (321, 322). Patients with CF have a larger volume of distribution and more rapid renal clearance of many antibiotics, requiring higher doses to achieve appropriate peak serum concentrations (323). Thus, close monitoring of serum levels is recommended to minimize risk of renal and ototoxicity. Aminoglycosides have traditionally been administered thrice daily. Because this class of antibiotics demonstrates a prolonged postantibiotic effect and bacterial killing is dependent on peak serum concentration, single administration of the total daily dose has been advocated. By contrast, toxicity is related to trough antibiotic concentration (324, 325). There have been several studies in CF populations comparing thrice-daily (3.3 mg/kg dose), once-daily (10 mg/kg dose), and in some cases twice-daily (5 mg/kg dose) regimens (324–327). All studies have shown comparable improvement in lung function, but none has been large enough to demonstrate equivalency. There has been no evidence of increased oto- or nephrotoxicity in over 100 patients receiving once-daily therapy. There is no sufficient enough long-term data to assess differences in rate of emergence of resistant *P. aeruginosa*. Once-daily aminoglycoside dosing appears to increase the peak concentration to well above the minimal inhibitory concentration of 90% of most *P. aeruginosa* but may also increase the time below the minimal inhibitory concentration (327). Which of these parameters is more important for long-term efficacy is not known.

To monitor aminoglycoside values for standard thrice-daily (every 8 hours) administration (initial dose 3.3 mg/kg), two methods may be used (328). To use the "initial dose pharmacokinetic" method, the patient is given the first intravenous dose over 30 minutes and two or three accurately timed serum levels are drawn beginning 1 hour after initiation of the infusion. Pharmacokinetic analysis can be used to predict the dose necessary to achieve the desired peak (10–12 mg/L). Use of the alternative "peak level" method is based on the assumption that patients with CF have normal renal function. After three to five dose intervals, a peak level is collected 30 minutes after a 30-minute intravenous infusion and the dose is adjusted to reach the desired peak value of 10 to 12 mg/L. By either method, a trough level drawn before the next dose should be less than 2 mg/L.

With once-daily administration aminoglycoside dosing (10 mg/kg day), the peak levels (20–60 mg/L) far exceed the customary serum targets noted previously and yet are not associated with documented increased oto- or nephrotoxicity. These data call into question the value of monitoring peak serum tobramycin levels. The Cystic Fibrosis Foundation care guidelines (167) recommend weekly tobramycin serum trough levels and creatinine for individuals receiving intravenous (not inhaled) aminoglycosides irrespective of whether the patient is hospitalized or receiving home therapy. Routine monitoring of serum tobramycin levels is not recommended for patients with normal renal function receiving only inhaled tobramycin (329). Monitoring for eighth cranial nerve toxicity should include an audiogram (500–8,000 Hz range) after every two to four courses of intravenous therapy (aerosol antibiotic) (272).

Treatment of Other Emerging Pathogens

B. cepacia *complex. B. cepacia* complex organisms are often highly antibiotic resistant. All are intrinsically resistant to the aminoglycosides (330). The rate of *in vitro* resistance to the β-lactam antibiotics, with the exception of meropenem, is also quite high (331, 332). The quinolones appear to have variable activity, but resistance can be readily induced (332). *In vitro* susceptibility testing suggests that there are combinations of antibiotics that act synergistically against *B. cepacia* complex using either synergy testing (333) or multiple combination bactericidal testing (331). Synergy testing, using two drug combinations, found that for 57% of isolates tested, no active combination could be identified. The most active combinations were chloramphenicol plus minocycline (49% of isolates) and chloramphenicol plus ceftazidime (26% of isolates) (333). Multiple combination bactericidal testing using two or three drug combinations, determined that at least one 2 or 3 drug combination could be identified for all isolates tested (331). The most active combinations are listed in Table 4. The majority of active combinations included meropenem. Unfortunately, it was not possible to predict for a given isolate whether a drug combination would be synergistic, additive, or antagonistic (331); thus, *in vitro* testing at reference laboratories is recommended (mcbt@cheo.on.ca and synergy@columbia.edu).

Other resistant Gram-negatives. Other antibiotic-resistant Gram-negative CF isolates include *S. maltophilia* and *A. xylosoxidans*. Treatment of these organisms is often complicated by resistance to the aminoglycosides and variable susceptibility to the β-lactams and quinolones. For both of these organisms, therapy should be directed by susceptibility testing. The most active single drugs *in vitro* against *S. maltophilia* are ticarcillin/clavulanate and trimethoprim/sulfamethoxazole; the most active combination in synergy studies is ticarcillin/clavulanate plus aztreonam (334). *S. maltophilia* is routinely resistant to imipenem and meropenem (335). In a study of 106 CF isolates of *A. xylosoxidans*, the most active drugs were imipenem (59% susceptible), piperacillin/tazobactam (55%), meropenem (51%), and minocycline (51%) (319). The most active additive or synergistic combinations were chloramphenicol plus minocycline, ciprofloxacin plus imipenem, and ciprofloxacin plus meropenem.

Nontuberculous mycobacteria. Treatment guidelines for the management of nontuberculous mycobacteria are evolving as more information becomes available on the epidemiology and clinical course of patients with CF culturing these organisms (165, 166). Current recommendations are that adult patients with CF be screened on a regular basis and, if there are findings suggestive of infection rather than colonization, antimycobacterial therapy of at least 1 year's duration is indicated (336). Specific drugs that are active against the most common organisms are listed in Table 6. Multiple drug therapy is recommended with sequential addition of drugs over 1 to 2 weeks to monitor side effects. Monitoring of drug levels may be useful because of altered drug metabolism in CF. *In vitro* susceptibility testing is recommended for non–*M. avium* complex organisms or if patients are not responding over a 6-month period (http://research.uthct.edu/).

Immunotherapy

Although significant advances in vaccine development directed against bacterial pathogens have occurred over the past decades (337), active immunotherapy to prevent or ameliorate *P. aeruginosa* infection in CF has not been achieved. The unique characteristics of both the host and pathogen (*see* IMPACT OF DEFECTIVE

TABLE 6. THERAPY OF NONTUBERCULOUS MYCOBACTERIA

Organism	Agents	Dosing (Route)	Monitoring
Mycobacterium avium intracellulare complex	Clarithromycin	15–30 mg/kg/d orally divided twice a day, max 1 g	Levels decreased by rifampin/rifabutin
	Rifampin	10–20 mg/kg/d orally, max 600 mg	Monitor CBC
	Rifabutin	5–10 mg/kg/d orally, max 300 mg	Monitor CBC
	Ethambutol	25 mg/kg/d orally	Monitor color vision and acuity
CONSIDER:	Streptomycin	500–750 mg two to three times/wk intravenously for first 8 weeks if severe	Monitor renal function, audiometry
Mycobacterium abscessus	Cefoxitin	200 mg/kg/d intravenously divided every 8 h, max 12 g	Monitor CBC
	Amikacin	10–15 mg/kg/d intravenously divided every 12 h	Monitor serum levels, renal function, audiogram
	Clarithromycin	15–30 mg/kg/d orally divided twice a day, max 1 g	Levels decreased by rifampin/rifabutin
CONSIDER:	Surgical debridement if infection is localized		

Definition of abbreviation: CBC = complete blood count (with differential).
Most doses are expressed as milligrams per kilogram of body weight.

CFTR on Initial and Persistent *P. aeruginosa* Infection and Characteristics of *P. aeruginosa* That Contribute to Initial and Persistent Infections) have made vaccine development challenging in this illness. Patients with CF are capable of mounting a vigorous antibody response to surface polysaccharides such as mucoexopolysaccharides (338) and exoproducts (107–109, 241) of *P. aeruginosa*. Initial antibody response to surface proteins may actually precede evidence of chronic *P. aeruginosa* infection or lung disease (107, 108) and likely prevent systemic disease. Yet, the immune response is not effective in eradicating the organism from the airway possibly because naturally acquired antibodies have low affinity with poor opsonic activity (110, 339). In fact, high antibody titers are associated with more severe lung disease (302). *P. aeruginosa* is also a challenging pathogen because most patients are initially colonized with unique environmental strains (126). Thus, developing a vaccine directed against one or a few capsular polysaccharides (e.g., *H. influenzae* type b) may not be widely effective. During chronic infection, *P. aeruginosa* also changes its surface polysaccharide structure, losing O-side chains from LPS and producing mucoexopolysaccharide, which reduces its antigenicity (115) and increases its resistance to phagocytosis.

Early studies of polyvalent *Pseudomonas* vaccines were not effective in delaying colonization with the pathogen and may have predisposed some patients to more severe pulmonary disease once infected (340). More recently, an octavalent *P. aeruginosa* O-polysaccharide toxin, a conjugate vaccine, appears to be well tolerated and capable of inducing high affinity, opsonic anti-LPS antibodies in young noncolonized patients (341). Protection against *P. aeruginosa* infection, however, was seen in only a subgroup of patients (342). No Phase III efficacy studies have been conducted to evaluate the impact of this vaccine on prevention of *P. aeruginosa* infection. A flagellar vaccine has also been shown to elicit long-lasting antibodies and a Phase III trial is currently in progress in Europe (343). Alginate-based vaccines directed against mucoid variants of *P. aeruginosa* have also been developed but have had limited immunogenicity (344). It is likely that the most efficacious approach to immunotherapy in CF in the future will be the induction of mucosal immunity in young patients before colonization with *P. aeruginosa* (337).

Patients with CF are immunocompetent and mount appropriate antibody response to currently available viral and bacterial vaccines. They should receive all routine immunizations recommended by the American Academy of Pediatrics (345). It is important that CF specialists and primary care physicians coordinate efforts to ensure that patients with CF receive age-appropriate immunizations. Although these patients do not experience increased incidence of respiratory infection associated with *Streptococcus pneumoniae* and *H. influenzae* type b, they should receive these vaccines as per American Academy of Pediatrics recommendations. It is also recommended that individuals with CF receive an annual influenza virus vaccine (345).

INFECTION CONTROL IN CF PULMONARY DISEASE

Infection control is an important topic in the management of CF airway infection. In June of 2001, the Cystic Fibrosis Foundation convened a multidisciplinary consensus conference of healthcare professionals from the United States, Canada, and Europe including infection control practitioners, CF caregivers, microbiologists, and infectious disease specialists to make CF-specific recommendations for infection control policies (346).

Transmissibility of CF Pathogens

In general, transmission of CF pathogens occurs by droplet and contact routes, thus infection control strategies must be directed at these routes. Both standard precautions and transmission-based precautions should be applied to patients with CF. One of the most important issues related to CF infection control is that infection control practices cannot be implemented on the basis of the specific microbiology results for individual patients because current bacteriologic methods are not 100% sensitive for real-time detection of the myriad of organisms that may be present.

B. cepacia. Person-to-person transmission of *B. cepacia* complex among patients with CF has been demonstrated both in healthcare and social settings (347–350). Whereas contact transmission has been clearly documented, and droplet transmission is likely, airborne transmission of *B. cepacia* complex has not been documented. Two specific virulence factors have been associated with transmission of genomovar III isolates, cable pilus, and the *B. cepacia* epidemic strain marker (148, 351). The reservoirs of *B. cepacia* include sites within the healthcare setting that may have been contaminated by patient contact (349, 352–355) and natural environments such as the soil (356). *B. cepacia* suspended in CF sputum may survive for over 24 hours on environmental surfaces such as latex or polyvinylchloride tubing of respiratory equipment (357).

The epidemiology of *B. cepacia* is perhaps the best studied of the CF pathogens and it is clear that specific infection control

TABLE 7. SUMMARY OF INFECTION CONTROL RECOMMENDATIONS FOR HEALTHCARE SETTINGS

Setting	Recommendation	Category*
General principles	Assume all patients with CF could have transmissible pathogens and apply *Standard* precautions	IA
	Implement transmission-based precautions according to CDC/HICPAC published recommendations	IA
	Healthcare workers should use approved methods of hand hygiene	IA
	Gloves should be worn when caring for patients who require *Contact* or *Droplet* precautions	IA
	Gowns should be worn as defined by *Standard* or pathogen-specific precautions	IB
	Published recommendations for sterilization and disinfection of patient care equipment should be followed	IA
	Wall humidifiers and "in-line" and hand-held nebulizers should be cleaned and dried according to manufacturer's recommendations; disposable and single-use items should be discarded after use	IB
	PFT equipment should use disposable in-line bacterial filters in between each patient; disposable mouthpieces are preferred; sterilization of the internal machinery of PFT machines is not needed between patients	II
	In ambulatory care settings, examination room surfaces should be cleaned after the room is vacated; regular cleaning of other surfaces should be on a regular basis and as soiling occurs	IB
Microbiology and surveillance	Respiratory tract cultures should be performed at least quarterly and processed for culture and susceptibility according to CF-specific guidelines	IB
	All *Burkholderia cepacia* complex isolates should be confirmed and speciated at the Cystic Fibrosis Foundation *Burkholderia cepacia* Research Laboratory and Repository (University of Michigan)	IB
	Surveillance strategies should be developed in collaboration with the institutional infection control team	IB
	Molecular typing using approved genotyping methods should be performed as epidemiologically indicated	IA
Inpatient	All patients with CF with *B. cepacia* complex, MRSA, or VRE should be housed in single-patient rooms that do not share common facilities (e.g., bathroom, shower)†	IA
	Patients with CF without the above organisms may share rooms with patients without CF who are low risk for infection†	II
	Patients not on transmission-based precautions may be evaluated for activity outside their hospital room as long as they are educated according to hand hygiene and avoidance of direct contact with other patients with CF, and with appropriate disinfection of surfaces	II
Ambulatory	Develop a reliable method for tracking patients' most recent culture and susceptibility testing results	IB
	Alert other diagnostic areas of patients' transmission precautions	IB
	Manage scheduling to minimize time in common waiting areas	II
	Encourage hand hygiene and have waterless antiseptics or other products available for use by patients	IA
	Discourage use of common items in waiting area that cannot be cleaned between patients (e.g., toys, computer)	II
	Observe *Contact* plus *Standard* precautions for epidemiologically important pathogens (e.g., *B. cepacia* complex, MRSA, multidrug-resistant *Pseudomonas aeruginosa*)	IA
	Segregate patients infected with *B. cepacia* complex, and place patients with multidrug-resistant *P. aeruginosa* in room immediately	IB

Definition of abbreviations: CF = cystic fibrosis; HICPAC = Hospital Infection Control Practices Advisory Committee; MRSA = methicillin-resistant *Staphylococcus aureus*; PFT = pulmonary function testing; VRE = vancomycin-resistant Enterococcus.

Data from Reference 346.

* Categories are based on the CDC/HICPAC system. Category IA: strongly recommended for implementation and strongly supported by well designed experimental, clinical, or epidemiologic studies. Category IB: strongly recommended for implementation and supported by some experimental, clinical, or epidemiologic studies and a strong theoretic rationale. Category II: suggested for implementation and supported by suggestive clinical or epidemiologic studies or a theoretic rationale.

† Patients with CF who sleep in the same room at home may share a hospital room (Category II).

interventions have been successful in interrupting transmission (348, 352, 353, 358–361). Interventions that have been successful (in various combinations) include segregation of patients, discouraging socializing between infected patients, emphasizing hand hygiene, and improving education of patients, families, and caregivers.

P. aeruginosa. As discussed in Phenotypic Changes, each patient is initially colonized with unique environmental strains (107, 126). *P. aeruginosa* appears to have a very different pattern of transmissibility in patients with CF compared with *B. cepacia*. Most frequently, clonal strains are shared only among siblings and patients linked socially (such as close friends or individuals in camp settings) (125, 126, 362). However, there are reports of patient-to-patient transmission in healthcare settings including clusters of multiply-resistant isolates; when documented, transmission has been successfully interrupted by implementation of infection control measures (363–365). *P. aeruginosa* in CF sputum may persist on a polyvinyl surface at least for 5 days (366).

Consensus Recommendations

The specific recommendations of the consensus conference on infection control in CF (346) are summarized in Table 7. They are "graded" according to the evidence available and are based on both general principals of infection control and CF-specific clinical situations.

These recommendations include policies for the care of patients in both the inpatient and outpatient settings as well as proper care of respiratory equipment in all settings. There are also guidelines for patients and families outside the hospital/clinic environment where multiple patients may interact. The augmentation of infection control procedures in recent years at CF care centers worldwide has and will continue to impact on the lives of patients and families. For this reason there is a need for ongoing patient, family, and staff education regarding the goals of infection control. There is also a critical need for microbiologic surveillance studies to document the impact of infection control policies on bacterial cross-infection.

CURRENT THERAPIES TO OPTIMIZE AIRWAY CLEARANCE AND REDUCE INFLAMMATION

Optimizing Airway Clearance and ASL Hydration

Assisting mechanical clearance of viscous airway secretions with airway clearance techniques is a cornerstone of managing estab-

lished CF pulmonary disease. Standard chest physiotherapy involves postural drainage with chest percussion in several anatomic positions to favor gravitational clearance of secretions from all lobes of the lungs (367). A short-term, controlled study showed standard chest physiotherapy improved sputum production and lung function in the setting of pulmonary exacerbation (368). Although, there are no randomized controlled trials in stable patients with CF comparing chest physiotherapy with spontaneous cough (369), the long-term benefits of daily maintenance chest physiotherapy are believed by many clinicians and patients to be beneficial in improving airway clearance.

Newer active and passive techniques have been developed to provide patients with more autonomy. Active techniques include the forced expiratory technique or "huff maneuver" (367, 370), autogenic drainage (370), positive expiratory pressure mask (367, 371), and oral airway oscillators such as Flutter and Acapella (372, 373). Passive techniques include high-frequency chest wall oscillator (374, 375) and an intrapulmonary percussor ventilator (376). Several small, uncontrolled studies have demonstrated some improvement in FEV_1 and/or clinical scores with several of the techniques (371, 376, 377). Therefore, with limited comparative efficacy data, patient satisfaction and compliance with a given airway clearance technique are major criteria for selecting a form of therapy.

Mucolytics. Recombinant human DNase (Pulmozyme) is the most widely used treatment to reduce sputum viscosity and is Food and Drug Administration approved for chronic maintenance therapy in all patients with CF. Purified recombinant human DNase I digests polymeric extracellular DNA and reduces the viscosity of CF sputum specimens (58, 378). The Phase III trial of Pulmozyme found 2.5 mg nebulized once daily resulted in 6% treatment effect in FEV_1 and a 22% reduction in pulmonary exacerbations requiring intravenous antibiotics (196) in patients with CF older than 5 years of age and FEV_1 greater than 40%. The primary adverse events believed to be related to the study drug were transient laryngitis and hoarseness. A subsequent study showed that daily Pulmozyme therapy in patients with CF with severe obstructive lung disease resulted in improvement in lung function over 12 weeks, but no reduction in pulmonary exacerbations (379). A 2-year randomized controlled trial in children with CF between 6 and 10 years of age and mild lung disease showed modest treatment effects in FEV_1 ($\sim$ 3%) and $FEF_{25–75\%}$ ($\sim$ 8%), but a 34% reduction in pulmonary exacerbations requiring parenteral antibiotics (380, 381). In addition, once-daily Pulmozyme therapy is safe with adequate lower airway deposition in children less than 5 years of age (382). Its effect is short lived (383) and not recommended for acute intermittent usage. There have been no published studies clarifying optimal timing and sequence for administration of DNase, bronchodilators, and chest physiotherapy. Many clincians recommend a sequence of a bronchodilator and DNase followed by chest physiotherapy on the basis of clinical experience.

Hypertonic saline. Hypertonic saline has a favorable effect on mucus rheology *in vitro* (384). Controlled trials of ultrasonic nebulization of hypertonic saline in stable patients with CF with moderate obstructive lung disease results in an acute increase in mucociliary clearance for up to 90 minutes (385, 386). The effect is dose dependent with greater increases in mucociliary clearance for increasing saline concentrations of 3, 7, and 12% compared with control subjects inhaling isotonic saline (386). Hypertonic saline challenges in stable patients with CF with mild to moderate obstructive lung disease can cause acute, transient airflow obstruction (227, 387), and the majority of clinical studies have pretreated patients with β-agonists before hypertonic saline therapy. A 2-week controlled trial of twice-daily ultrasonic nebulization of 6% hypertonic saline compared with isotonic saline showed a significant increase in FEV_1 (388). Further studies on long-term efficacy of hypertonic saline therapy, optimal hypertonic saline concentration, and mode of delivery (jet vs. ultrasonic nebulizer) are necessary (389).

Bronchodilators

Bronchial hyperresponsiveness occurs in about half the number of patients with CF (390). The etiology of bronchial hyperresponsiveness in CF is multifactorial and differs from that in asthma (391). Response to short-acting inhaled bronchodilators is variable over time and between patients with CF (391, 392); approximately 50 to 60% patients show improved FEV_1, approximately 20 to 30% patients show no change, and approximately 10 to 20% patients show reduced lung function (392). Short-term studies in stable patients with CF are not conclusive but some show acute increases in lung function especially among patients with CF with bronchial hyperresponsiveness (391, 392). A short-term study of albuterol in hospitalized patients with CF showed transient improvement in FEV_1 compared with placebo (393). Longitudinal studies of short-acting β-agonists over 1 to 2 years show that almost all patients demonstrate improvement in FEV_1 on at least one evaluation, and on average subjects had a bronchodilator response on approximately 25% of the days tested (394, 395). There was no conclusive evidence of clinically significant long-term improvement in lung function for albuterol compared with placebo (391, 394). A preliminary study of high-dose salmeterol plus albuterol compared with albuterol alone in stable patients with CF found a significant treatment benefit in lung function and respiratory symptoms after 24 weeks with salmeterol plus albuterol (396).

There are limited and conflicting data on the role of ipratroprium bromide in the treatment of CF (397–399), but most data show limited benefit for ipratropirum bromide added to β-agonist therapy. With all inhaled therapies, proper selection, use, and maintenance of meter dose inhalers and nebulizer machines is critical in ensuring optimal delivery (346, 400–403).

Antiinflammatory Therapy

Several clinical trials of antiinflammatory therapy in CF have shown clear benefit, but unacceptable adverse effects have limited their use (56). Chronic, alternate-day systemic steroids slow the decline in lung function but cause significant toxicity, including an increase in growth retardation, cataracts, and hyperglycemia (404–406). Data are lacking for short-term safety and efficacy of systemic corticosteroids for adjunctive therapy of pulmonary exacerbations in CF. High-dose ibuprofen therapy over 4 years demonstrated a significant reduction in the rate of decline in FEV_1 for patients who were 5 to 12 years old, fewer hospitalizations, and improved nutritional status (407). Despite this impressive finding, high-dose ibuprofen is used for fewer than 15% of eligible patients with CF at U.S. CF Centers (408). Concerns for the small increased risk of gastrointestinal hemorrhage and the need to monitor drug levels contribute to reduced ibuprofen use (409). The mechanism by which ibuprofen attenuates the decline in lung function is uncertain but is believed to be due to reduced neutrophil migration in the lung (56). More specifics on the mechanism by which ibuprofen preserves lung function may promote the development of better agents.

The use of inhaled steroids is common in CF (408, 409). However, unlike asthma, there is no convincing evidence for a significant antiinflammatory effect or clinical efficacy in CF (410–413). The subpopulation of patients with CF with bronchial hyperresponsiveness may derive some benefit (412). Infants with CF treated with inhaled steroids for 2 months demonstrated no adrenal suppression, no significant changes in lower airway pathogens, and a modest decrease in lower airway neutrophil

counts (414). Further studies of inhaled steroids are warranted in young children with CF.

Other antiinflammatory agents are less well studied in CF. Montelukast, a cysteinyl-leukotriene receptor antagonist, was found to reduce systemic evidence of eosinophilic inflammation in CF, but no data were presented on clinical efficacy (415). There are ongoing clinical trials of an LTB4 receptor antagonist (M. Konstan, personal communication). The burden of proteases in the CF airway far exceeds the host lung defenses (56). Initial attempts to fully inhibit the airway protease burden in CF with α-1 antitrypsin and recombinant secretory leukoprotease inhibitors have not been successful (416, 417). The oxidant burden is also high in the CF airway (56). In a pilot study, twice-daily aerosolized glutathione increased airway levels of glutathione and reduced superoxide production from cells recovered in BAL fluid (418). A pilot study of inhaled *S*-nitrosoglutathione found no significant adverse events, increased exhaled concentrations of nitric oxide, and statistically but not clinically significant increases in Sa_{O_2} (419).

Diagnosis and Treatment of ABPA

A 2001 Cystic Fibrosis Foundation Consensus Conference recently provided a defined set of clinical and laboratory diagnostic criteria (155, 157) to diagnose ABPA in patients with CF. The full diagnostic criteria are (*1*) acute or subacute clinical deterioration not attributable to another etiology, (*2*) serum total IgE concentration higher than 1,000 IU/ml in patients not receiving systemic corticosteroids, (*3*) immediate cutaneous reactivity to *Aspergillus* or *in vitro* presence of *A. fumigatus*–specific serum IgE antibodies, (*4*) precipitating antibodies to *A. fumigatus* or serum *A. fumigatus*–specific IgG antibodies by an *in vitro* test, and (*5*) new or recent abnormalities on chest radiograph (infiltrates, mucus plugging) or chest computerized tomography (bronchiectasis) that have not cleared with antibiotics and standard physiotherapy. The consensus statement also provides minimal diagnostic criteria to include: criteria (1) and (3) described previously, a total serum IgE higher than 500 IU/ml, and *either* criteria (4) *or* (5) described previously. This group recommends annual screening with a total serum IgE concentration in patients with CF 6 years and older. If the value is higher than 500 IU/ml, determine the immediate cutaneous reactivity to *A. fumigatus* or determine *A. fumigatus*–specific IgE antibodies.

The diagnosis of ABPA remains challenging because there is significant overlap in the clinical features of CF pulmonary exacerbation and ABPA, and a high proportion of patients with CF have intermittent serologic features of ABPA without the full syndrome. Some studies report elevated total IgE or serum-precipitating antibodies occur in up to 25% of patients with CF (157), and that up to 60% of patients with CF may have positive skin tests to *A. fumigatus* antigens. Availability of recombinant *A. fumigatus* antigens in the near future may improve the diagnostic accuracy for ABPA and improve our understanding of the underlying immmunopathogenesis (157).

Corticosteroids are the mainstay of ABPA therapy in asthma, and itraconozole provides additional benefit as well as a steroid-sparing effect (156, 157, 420, 421). There are limited data for either treatment of ABPA in CF (157). In addition, systemic steroids have a higher rate of untoward effect in the CF population (405). The limited data suggest that initial therapy should be prednisone, 2 mg/kg/day for 1 to 2 weeks, a taper to 1 mg/kg for 1 to 2 weeks, with further taper to alternate-day and then an attempt at discontinuation by 2 to 3 months (155, 157, 422, 423). The decision to taper prednisone is based on clinical improvement and reduction in total serum IgE (156, 157, 422, 424, 425). Itraconazole (5 mg/kg up to a maximum of 200 mg twice a day) can be added if patients show poor clinical or serologic response to steroids (157, 346, 420, 422, 426, 427). In cases where clinical response is suboptimal, it is recommended to check serum itraconazole levels (i.e, > 1 mg/L) to ensure an adequate plasma level after 1 to 2 weeks of treatment (157); the total duration of itraconazole treatment may be up to 3 to 6 months. All patients treated with itraconazole should have periodic monitoring of liver function tests and should review concomitant medications for drug interactions (346). Although other oral antifungal therapies such as voriconazole are now available, there are no data regarding their efficacy and safety for treatment of ABPA in the CF population. There are no published data showing the efficacy of inhaled corticosteroids for the treatment of ABPA in CF.

FUTURE THERAPIES FOR CF PULMONARY DISEASE

Gene Transfer Therapy

Within 1 year of discovering the CF gene, *in vitro* studies demonstrated that introduction of CFTR complementary DNA into affected cells could correct the chloride channel defect (428, 429) and provided evidence that gene transfer therapy might be effective in CF. Several aspects of this autosomal recessive disorder favored the success of this approach. First, mature wild-type CFTR is localized to the apical surface of airway epithelia and submucosal glands (430) potentially accessible by aerosol delivery and minimizing systemic exposure. Second, several lines of evidence suggested that minimal levels of CFTR would be required to correct the ion channel defect. Respiratory epithelial cells contain only 1 to 2 CFTR transcripts (431). Mixing experiments indicate that targeting less than 10% of cells could correct chloride transport (432), but normalization of raised sodium absorption may require targeting a higher percentage of cells (433). In addition, individuals with 5 to 10% wild-type CFTR transcripts have no pulmonary disease (1, 2). Third, an *in vivo* assay of ion channel activity, nasal potential difference (NPD) measurements (26), could provide real-time assessment of functional gene expression.

Early human studies using adenoviral (434–436) and nonviral cationic lipid (437–439) delivery systems were successful in demonstrating gene expression in upper and lower airway epithelia by immunocytochemistry (435, 436) and functional assay (NPD) (434, 437, 439, 440). Although these results are encouraging, barriers to effective gene transfer therapy including poor efficiency (441) and limited duration (442) of gene expression and apparent significant immune and inflammatory response to adenoviral vectors were observed (435, 441, 443).

More recent human studies have used an adeno-associated virus (AAV) serotype 2 vector to deliver CFTR complementary DNA to respiratory epithelia. Despite limited packaging capacity prohibiting the addition of a strong promoter or regulatory elements with the CFTR complementary DNA, tissue culture and animal studies have demonstrated stable expression of CFTR protein for up to 6 months without pathologic findings in animal studies (444, 445). Over 75 patients with CF have received single or repeated doses of AAV–CFTR to nasal, sinus, or lower airway epithelia (446, 447). The vector appears to be safe and well tolerated. Gene transfer at a maximum of 1 copy per 10 epithelial cells (collected by bronchial brush biopsy) was observed at 30 days after a single aerosol administration of 10^{13} DNase-resistant particles but declined to nondetectable at 90 days and no wild-type CFTR messenger RNA expression has been detected (446). A multicenter, randomized, placebo-controlled trial evaluating the safety and tolerability of three monthly repeated aerosol administrations of 10^{13} DNase-resistant particles AAV–CFTR was recently completed (448). Thirty-seven patients with mild pulmonary involvement ($FEV_1 > 60\%$)

received at least one aerosol administration (20 vector, 17 placebo). There were no safety concerns, but a fourfold or greater elevation in neutralizing serum antibodies against AAV2 was defected in all patients receiving vector (none receiving placebo). Gene transfer but not gene expression was detected. A mean improvement in FEV_1 of 0.12 L was observed at 30 days in the treatment group compared with placebo but not at subsequent time points, i.e., 60, 90, and 150 days.

Thus, initial clinical trials suggest that the AAV2 is safe, well tolerated, and may be an excellent vehicle for human gene therapy if the hurdle of poor gene expression can be overcome. Several *in vitro* approaches are currently addressing this issue. Coadministration of AAV2 with a proteosome inhibitor (449) results in improved transgene expression and augmented nuclear trafficking of the virus. Other serotypes of AAV, in particular AAV5 (450) and AAV6 (451), have demonstrated higher transduction efficiency in respiratory epithelial cells than AAV2 and likely use different cell receptors. Finally, smaller CFTR transgenes with deletions in the R domain (452) or C-terminus (453) remain functional and may better accommodate the packaging capacity of AAV and permit addition of a promoter and other regulatory elements. Gene therapy remains in an evolving, developmental stage in CF. Some initial human studies with AAV have been encouraging but significant barriers remain before efficient delivery and long-term expression of the CFTR transgene in the airway are achieved.

Pharmacologic Approaches

The current understanding of normal and mutant CFTR structure and function has directed investigators to develop therapeutic strategies to partially or fully correct the abnormal protein or bypass the loss of function through modulation of alternative ion channels. This section will summarize several of these approaches and review ongoing early human trials (Phase I/II). None of these pharmacologic approaches have reached large, Phase III trials or shown clinical efficacy. Drug development is an iterative process. It is likely that most early compounds tested will provide valuable data on safety, pharmacokinetics, and biologic effects but will not reach Food and Drug Administration approval.

Approaches to correct dysfunctional CFTR. Because the mechanism of CFTR dysfunction differs by the class of mutation (Figure 3), treatment regimens will likely be "tailored" toward each individual patient's genotype. For this reason, examples of potential strategies are presented by mutational class.

Mutations leading to defective CFTR biosynthesis (Class I) due to premature stop codons are found in approximately 10% of all patients with CF and in much higher frequency among certain populations such as individuals of Ashkenazi Jewish decent (16). Some aminoglycosides, in particular gentamicin, are effective in suppressing premature stop mutations in the CFTR gene in bronchial epithelial cell lines (454), allowing restoration of full length CFTR expression and reappearance of cAMP-activated chloride currents. Two small clinical trials of gentamicin administration to individuals with CF with at least one premature stop mutation have been reported. An Israeli study (455) of nine patients with premature stop mutation (seven with two copies, two with one copy of premature stop mutations) evaluated changes in NPD measurement after 14 days of topical gentamicin application to nasal mucosa and found significant repolarization of nasal epithelium consistent with partial correction of transmembrane chloride transport under conditions that select for CFTR function. An American study (24) reported similar trends in NPD after intravenous administration of gentamicin for 1 week. In this study, four of five patients with one copy of premature stop mutation demonstrated at least one chloride secretory response of greater than −5 mV not observed in homozygous ΔF508 patients. As further evidence of the potential benefit of gentamicin in this class of mutations, mice possessing a premature stop codon in the dystrophin gene have demonstrated restored dystrophin function in skeletal muscle (456).

Because Class II mutations (including ΔF508) are the most common, there is broad interest in identifying chemical chaperones that facilitate folding, inhibit ubiquitin-mediated degradation, and promote trafficking of these mutant CFTR proteins to the cell surface where they retain at least partial function as chloride channels (20). Many potential compounds such as glycerol (457), anthracycline derivatives (458), butyrates (459), trimethylamine oxide (460), and cyclopentyl 1,3-dipropylxanthine (461) have been tested in cell systems and have shown protein maturation and trafficking to the cell surface. Phase I randomized, double-blind human trials have been reported for oral sodium 4-phenylbutyrate (462) and cyclopentyl 1,3-dipropylxanthine (463). Oral phenylbutyrate was well tolerated for 1 week at the maximum Food and Drug Administration–approved adult dose (19 g thrice daily) and some changes in NPD suggestive of CFTR function were observed. A single-dose escalation study of cyclopentyl 1,3-dipropylxanthine did not demonstrate changes in NPD. High throughput screening efforts supported by the Cystic Fibrosis Foundation are ongoing to identify new chemical compounds that promote CFTR trafficking and will likely reach clinical trials in the near future.

For Class III/IV mutations that reach the cell surface or Class II mutations that are "assisted" to the surface by chemical chaperones, pharmacologic strategies to activate CFTR channel activity (i.e., "activator" therapies) may also be therapeutically beneficial. Compounds such as phosphodiesterase inhibitors that increase cAMP levels or flavenoid derivatives (464, 465) have been shown to stimulate chloride conductance in airway epithelium cells. Drug discovery for other CFTR channel activators by high throughput screening using cell-based fluorescence assays are currently in progress (466). There have been no human trials of channel activators completed and published.

Bypassing dysfunctional CFTR—activation of alternate channels. Dysfunctional CFTR in respiratory epithelium is associated with both increased sodium absorption and decreased chloride secretion resulting in abnormal hydration of airway secretions (64, 467). Drug therapies that do not interact with CFTR but impact one or both of these transport abnormalities by modulating alternative chloride and sodium channels in the cell membrane will likely be beneficial for airway hydration and clearance. The most well-studied sodium transport inhibitor is amiloride, which reduces the abnormally high basal NPD measurement observed in CF airways toward normal range after topical application to the apical cell surface (468). Although aerosol amiloride appeared to slow the rate of decline in lung function (468) and enhance mucociliary clearance (469) in two pilot studies, a larger Phase III study was unable to confirm this finding.

There have been extensive *in vitro* investigations of chloride secretagogues that may circumvent the CFTR channel function by activating calcium-regulated alternative chloride channels. Examples of such agents include, inositol triphosphase (470), thapsigargin (471), and triphosphate nucleotides (via the $P2Y_2$ apical receptor) (472). There has also been recent interest in ClC-2 channels as a therapeutic target (473). Although predominant in the developing lung, ClC-2 channels persist in adult lung and may be activated by treatment with the protein pump inhibitor, acid-activated omeprazole.

The alternate chloride channel activators most studied in humans are triphosphate nucleotides. Topical nasal application of ATP- and uridine triphosphate–induced chloride secretion in nasal epithelia as measured by NPD (472). An inhaled, longer-

acting uridine nucleotide, INS 365, was found to be well tolerated up to the 40-mg dose in both adults and children in an initial Phase I trial (474).

New Approaches to Treating *P. aeruginosa* Infection

Because CF lung disease is a localized mucosal infection, the role of innate immunity and endogenous cationic antimicrobial peptides in early infection has been a recent area of scientific and therapeutic interest (*see* Innate Immunity and Persistence of Bacterial Infections) (100). These peptides produced by epithelial cells and neutrophils provide broad antimicrobial activity in respiratory secretions (475, 476) and rapidly kill both Gram-positive and Gram-negative pathogens (including *P. aeruginosa*) by disrupting the microbial membrane or enzymatic digestion of microbial structures (477). These broad ranges of peptides have the common features of being cationic amphipathic molecules enabling them to bind and insert into negatively charged microbial membranes (101). As a result, the activity of cationic antimicrobial peptides is highly sensitive to the ionic milieu of the airway surface fluid where they reside. Although there is no evidence of any deficiency in antimicrobial peptides in CF, it has been hypothesized (67, 478) that either the raised ionic content of the airway surface fluid in this disorder may inactivate these salt-sensitive peptides or the slowed mucociliary clearance (97) may overwhelm the capacity of these molecules to kill pathogens. For these reasons, there has been increased interest in developing cationic antimicrobial peptides as potential antibiotic therapies for early infection (100). Treatments have been directed to topical applications because these peptides are involved in local response to mucosal infections. Two examples include the magainin-derived peptide, MSI-78 (Genaera Corporation, Plymouth Meeting, PA), that reached Phase III testing as a topical antimicrobial therapy against polymicrobic foot ulcer infections in diabetes (479), and a protegrin-derived peptide, IB-367, (IntraBiotics Pharmaceuticals, Inc., Palo Alto, CA) that reached Phase III trials as a topical therapy for oral mucositis in cancer (101). Neither product has been Food and Drug Administration approved. IB-367 is the only cationic antimicrobial peptide to reach clinical trials in patients with CF. Phase I trials of inhaled IB-367 demonstrated that this peptide was well tolerated in adult patients with CF to a maximum tolerated dose of 30 mg, but no efficacy as an antimicrobial agent has been established (480).

Developing cationic antimicrobial peptides as therapeutic candidates will likely be challenging. The cost of production may prohibit commercial development (100, 101). Optimal formulations for inhalation of antibiotics require a neutral pH (197) that will likely inactivate or precipitate these highly charged peptides. Pathogenic bacteria such as *P. aeruginosa* have acquired mechanisms to resist killing by these peptides (477) such as modifying the anionic surface charge on their LPS or employing proteases to digest these antimicrobial peptides (96, 481, 482).

Another approach to enhancing innate immunity is the use of an inhaled osmolyte with a low transepithelial permeability to lower ASL salt concentration and increase the activity of endogenous, salt-sensitive antimicrobial peptides. A promising osmolyte, xylitol, has low transepithelial permeability and has been shown to lower ASL salt concentration in CF airway epithelia *in vitro* (483). A common food additive for "sugarless" chewing gum, xylitol, has already been reported to decrease frequency of otitis media (484) and prevent dental caries (485).

One novel approach for prevention of initial bacterial colonization using the antiadhesive properties of dextrans has been proposed (486). These neutral polymers of glucose have been shown to block adherence of *P. aeruginosa* (487) and *B. cepacia* (488) to airway epithelial cells *in vitro*. Aerosolized dextran has also been demonstrated to protect mice from pneumonia due to *P. aeruginosa* (489). Dextran has several additional features that make it an attractive candidate for development as an inhaled therapy for CF; it is inexpensive and easy to manufacture, nontoxic, and readily aerosolized.

An *in vitro* study to evaluate the effect of dextran on the rheologic properties of sputum from patients with CF demonstrated a significant reduction in the sputum viscoelasticity, which may suggest improved mucociliary and cough clearance as an additional benefit (486).

Although antimicrobial peptides may play some role in the prevention of initial infection, there is a need to consider new paradigms for the treatment of *P. aeruginosa* and the development of new antipseudomonal antibiotics. First, it may be important to identify and treat *P. aeruginosa* early in the course of lung infection before the pathogen undergoes characteristic adaptive changes (*see* Prevention of Chronic *P. aeruginosa* Infection). Second, researchers must continue to define both the environmental signals and the bacterial genetic regulation that occurs during the transition from initial infection to establishment of mucoidy, biofilm formation, and chronic infection. Third, the recent availability of the *P. aeruginosa* genetic sequence (139) affords a unique opportunity for postgenomic and proteomic studies to better understand the genetic and functional diversity of this Gram-negative organism that permits it to accommodate to a wide range of environments and rapidly develop antimicrobial resistance. With this information, new strategies for drug development will evolve.

The CF community has made significant strides in the past half-century. Individuals with CF are living longer and living better. This is primarily because of both the development of new therapies and the application of new treatment strategies, at times using existing agents. For the future, it will be particularly important to develop additional therapeutic agents for CF. Key components of this effort include federal and private foundation support for basic science research, partnerships between industry and funding agencies, and development of a network of experienced investigators to perform clinical trials in CF. These pieces are falling into place and the future of individuals with CF looks bright.

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