Clinical Significance of Microbial Infection and Adaptation in Cystic Fibrosis

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“He that will not apply new remedies must expect new evils;
for time is the greatest innovator.”

—Francis Bacon, *Of Innovations*

INTRODUCTION

Our understanding of the unique subset of microbes that commonly infect the lower respiratory tracts of individuals with cystic fibrosis (CF) has evolved over time due to advances in clinical microbiology and therapeutic strategies and perhaps also due to changes in infection patterns. When CF was initially recognized as a distinct disease entity in 1938, it was linked primarily to *Staphylococcus aureus* pulmonary infections (16, 154). This pathogen was thought to play a critical role in the mortality associated with CF: “The lungs showed bronchitis, bronchiectasis, pulmonary abscesses arising in the bronchi, lobular pneumonia or any combination of these. *Staph. aureus* was the usual bacteriologic agent,” stated Dorothy Andersen in her seminal report on CF (16). Following the availability of penicillin, children with CF and staphylococcal infections were for the first time given effective antimicrobial agents, with dramatic clinical responses (17). Thus, it was recognized that antibiotic therapy could significantly modify the progression of CF lung disease. By the 1950s, however, *Pseudomonas aeruginosa* became recognized as an important CF pathogen (393). *Burkholderia cepacia* complex (BCC) organisms, previously referred to as *Pseudomonas cepacia*, emerged in the 1970s and were associated with rapid declines in pulmonary function, bacteremia, and increased mortality (349). Through the years, dramatic improvements in life expectancy in CF have been realized, but this longevity has been accompanied by the recognition of an increasingly broad and esoteric group of microbes that infect the CF airways (350). While some of these organisms are clearly harmful (114, 138, 277, 425), the roles of others in the pathogenesis of CF lung disease remain uncertain (29, 214). The decision to treat patients infected with these organisms can thus be challenging. On the one hand, prolonged courses of antibiotics are associated with inconvenience to the patient and the potential of dangerous adverse reactions. On the other hand, withholding treatment may cause short-term or long-term harm to the patient, an especially important consideration given that over 80% of individuals with CF die directly or indirectly from pulmonary disease (118, 119, 237). Here we review the microbes commonly identified in respiratory specimens from individuals with CF and, in each case, summarize the evidence for or against their impact on clinical status. For a discussion of the history, epidemiology, microbiology, pathogenesis, treatment, and infection control of respiratory infections in CF, the reader is referred to several recent reviews (129, 131, 155, 191, 198, 210, 350, 358, 486, 513, 668).

OVERVIEW OF MICROBIAL INFECTIONS IN CF

The Cystic Fibrosis Foundation (CFF) compiles results of respiratory cultures from people with CF seen at accredited CF centers and publishes these results annually in the CFF Patient Registry (118). This database paints a broad vista of the current microbial landscape in CF (Fig. 1). For example, it nicely demonstrates how the predisposition to infection by specific organisms changes with age. *S. aureus* is the most commonly isolated pathogen in infants and young children with CF, although *Haemophilus influenzae* and *P. aeruginosa* are also prevalent. The frequency of positive cultures for *P. aeruginosa* increases with age, and growth of this bacterium from respiratory specimens is observed in approximately 80% of patients by their late twenties. The decrease in *P. aeruginosa* infection observed after age 34 is probably a survivor effect, since the risk of death is higher in patients with *P. aeruginosa*. *B. cepacia* complex organisms are less common early in life but infect a substantial minority of adults. The fungus *Aspergillus fumigatus* is frequently isolated from CF patients, particularly older patients and those taking chronic inhaled antibiotics. Other organisms, such as *Achromobacter* (*Alcaligenes*) *xylosoxidans*, *Stenotrophomonas maltophilia*, and nontuberculous mycobacteria (NTM), are being reported with increasing frequency and are now more commonly isolated from CF patients than *B. cepacia* complex bacteria (400, 489). Thus, a growing and somewhat esoteric group of microbes appears to be well adapted to survival within the CF airways.

The CFF Patient Registry data also indicate that the majority of infants with CF have positive cultures for respiratory pathogens in the first year of life. To address rates of infection in early CF, Rosenfeld and colleagues performed a prospective study of bronchoalveolar lavage (BAL) fluid in infants, enrolling patients no older than 15 months of age (mean age at enrollment, 3.9 months) (506). BAL was performed at enrollment and then annually for a total of three procedures around the first, second, and third birthdays. From 63 to 70% of subjects had a positive culture at each bronchoscopy. *H. influenzae* was the most common organism isolated at 1 year of age but was surpassed by *P. aeruginosa* and *S. aureus* at years 2 and 3. The frequency of *P. aeruginosa* increased from 18% at year 1 to 33% at year 3. Importantly, 35% of positive BAL fluid cultures grew two organisms, and 10% grew three organisms. This study demonstrated that CF patients are infected at an early age and often with multiple pathogens.

Microbiological studies that rely on the culture of CF respiratory specimens suffer from several limitations. Many of the subjects in the CFF Patient Registry and other reports reviewed here are infants or children who are incapable of producing an expectorated sputum specimen. BAL, the gold standard for sampling the pulmonary airways, is an option in these situations, but it is invasive and usually is employed only when there is a compelling reason to obtain a respiratory sample and other approaches have failed. For this reason, oropharyngeal (OP) swabs are used as a surrogate for sputum or BAL fluid

FIG. 1. Prevalences of several common respiratory pathogens in CF as a function of age. (Adapted from the 2008 Annual Data Report of the Cystic Fibrosis Foundation Patient Registry, Bethesda, MD [http://www.cff.org/UploadedFiles/research/ClinicalResearch/2008-Patient-Registry-Report.pdf], with permission. © 2009 Cystic Fibrosis Foundation.)

specimens in these individuals. By definition, OP swabs sample the microbial flora of the upper respiratory tract, which is assumed to reflect that of the lower respiratory tract. The validity of this assumption was examined by Rosenfeld and colleagues (505), who reported three prospective studies of simultaneous OP swabs and BAL fluid specimens from children with CF who were less than 5 years of age. Using BAL as a gold standard, the positive predictive value for *P. aeruginosa* in OP culture was only 44%, and the negative predictive value was 95%. Similar results were seen for *H. influenzae*, while specificity was lower for *S. aureus*. A second study reported similar findings, with overall positive predictive values of 41% and negative predictive values of 97% for OP swabs (21). Other studies, however, have found higher positive predictive values (83% for *P. aeruginosa* and 91% for *S. aureus*) and lower negative predictive values (70% for *P. aeruginosa* and 80% for *S. aureus*) associated with OP swabs (482). Thus, the interpretation of OP swab culture results remains unclear, but this sampling approach has nevertheless become a routine part of CF clinical practice.

Even the use of BAL fluid or expectorated sputum samples does not ensure correct identification of infecting microbes. Organisms that are fastidious or nonculturable may not be identified, and some of the more unusual bacteria known to infect CF patients may be misidentified by automated diagnostic systems and conventional laboratory protocols (53, 264, 642). Newer molecular approaches such as ribosomal DNA sequencing (53, 615) and terminal restriction fragment length polymorphism (T-RFLP) (544) have the potential to greatly improve the sensitivity and accuracy of respiratory sampling and to allow characterization of the complete population of microbes residing in the airways of CF patients. Preliminary studies using these approaches suggest that the CF airway is home to a complex community of microbes, of which those identified by conventional methods represent only the tip of the iceberg (53, 241, 320, 495, 497, 615, 642). For example, Rogers and colleagues used T-RFLP to show that a typical CF patient harbors a mean of 13 different species of bacteria in

his/her airways (495). No control patients were included in this analysis, so it is unclear whether the polymicrobial nature of these samples is unique to CF. How interaction between these many different microbes may influence pathogenesis and disease progression in CF is a fascinating new field of study (317, 543). Likewise, the implications of the presence of previously unrecognized diverse populations of microbes in the CF lung for antimicrobial treatment strategies are unclear but have the potential to be profound (498, 545).

A final concern regarding microbial data from CF patients is that transient, minimally symptomatic or asymptomatic infection may occur and be missed even when surveillance cultures are routinely and frequently obtained. Burns and colleagues demonstrated that *P. aeruginosa* infection during the first 3 years of life is often transient and that CF patients may exhibit a serological response to *P. aeruginosa* prior to or in the absence of the culture of this bacterium (77). Thus, intermittent culturing of respiratory specimens may not accurately identify all pulmonary infections in CF.

Despite these limitations, culture-based studies of the microbiology of CF provide an important framework for assessing the impact of microbes on the clinical status of individuals with this disease. Before reviewing these reports, we will clarify a point regarding nomenclature. The use of the words “infection” and “colonization” is controversial when discussing CF. “Colonization” is often used to reflect the presence of microorganisms in the absence of overt illness, whereas “infection” is used for the corresponding situation in which signs and symptoms attributable to the organism are evident. However, since inflammation is nearly universally present in the lungs of CF patients (324, 506), and inflammation is thought to lead to pulmonary decline, it is often difficult to determine whether a particular organism is indeed causing overt manifestations of infection. Also, multiple organisms may simultaneously be isolated from the airways, making it difficult to attribute signs and symptoms to a specific microbe. For these reasons, we will follow the conventions of Casadevall and Pirofski, who define infection as “the acquisition of a microbe by a host” (79). The

term "infection" will therefore be used throughout this review regardless of whether overt signs and symptoms of infection are present.

PATHOGENESIS OF PULMONARY DECLINE IN CF

Despite constant exposure to a broad spectrum of microorganisms, individuals with CF are predisposed to infection by only a specific subset of these microbes. Although the proximal event in CF is clearly mutation of the CFTR genes, how this defect predisposes the individual to infections by this select group of organisms remains a holy grail of the field. A number of explanations have been proposed and are supported by experimental data, as follows. (i) Decreased ion transport resulting from altered or absent CFTR channels enhances fluid absorption in the airways. This in turn leads to decreased airway surface liquid and impaired ciliary transport of the mucous layer, which results in defects in microbial clearance (382). (ii) Altered salt content in the airway surface liquid inactivates antimicrobial defensins and impairs the ability of neutrophils to kill microbes (557). (iii) Abnormal CFTR channels result in increased levels of asialylated glycolipids on the surface of CF airway epithelial cells. These glycolipids serve as receptors for *P. aeruginosa*, the bacterium most commonly isolated from CF patients, and thereby increase binding of this bacterium, which facilitates the early infectious process (512). (iv) Surface-exposed CFTR molecules normally serve as a defense mechanism. CFTR binds to *P. aeruginosa* and causes this bacterium to be internalized by airway epithelial cells that are subsequently sloughed and cleared from the airways. The paucity of apically exposed CFTR molecules in CF compromises this innate immune mechanism (458). (v) Defective CFTR channels result in low levels of inducible nitric oxide synthase and nitric oxide itself. Decreased concentrations of nitric oxide, which has antibacterial properties, predisposes the individual to infection of the lung (308). (vi) Due to poorly understood mechanisms, mutations in the gene encoding CFTR cause the airways to be intrinsically hyperinflammatory. This excess of neutrophils and proinflammatory cytokines results in damage to host cells and hinders the appropriate clearance of microbes (321). In summary, despite a substantial amount of effort, the exact mechanism by which mutations in CFTR lead to specific pulmonary infections in CF remains unclear. The reader is referred to several recent reviews for more in-depth discussions of these models (129, 155, 210, 358, 359, 477, 575).

Regardless of the predisposing mechanism, it is clear that certain microorganisms are able to intermittently or chronically infect the airways of CF patients and that some of these organisms are capable of causing gradual but unrelenting decline in pulmonary function. How infection with these organisms occurs and how it leads to pulmonary deterioration have been the focus of much research. In this context, infections caused by *P. aeruginosa* have been subjected to the greatest scrutiny and will be reviewed here as an example of microbial pathogenesis in CF.

The first step in infection of the CF airways by *P. aeruginosa* is acquisition of the bacterium. *P. aeruginosa* is ubiquitous in lakes, streams, and moist soil and on vegetables (62, 491), and evidence suggests that most individuals acquire *P. aeruginosa* through casual contact with these natural reservoirs (77, 502,

503, 569). Alternatively, some CF patients may acquire *P. aeruginosa* either directly or indirectly from other individuals with CF, which has been a cause of concern for CF practitioners (24, 81, 85, 230, 337, 429, 432, 537, 569, 609, 624). In support of patient-to-patient transmission of *P. aeruginosa* in CF, reports indicated that the prevalence of commonly isolated *P. aeruginosa* strains within CF facilities decreased following cohort segregation (228, 297). For example, in one center the prevalence of *P. aeruginosa* isolates of a common genotype decreased from 21% in 1999 to 14% in 2002 following institution of strict infection control measures, including cohort segregation (228). Such observations have led to the idea that some *P. aeruginosa* strains, called "epidemic strains," are highly transmissible and thus spread more readily through populations of susceptible individuals. Supporting evidence for this notion is also found in reports showing that some epidemic strains are capable of infecting and causing disease in non-CF relatives of CF patients (388) and even in their pets (406). Attributes that may facilitate transmission, infection, or persistence of certain *P. aeruginosa* strains include antimicrobial resistance (85), type IV pili of a particular phylogenetic group (331), prophage-like gene clusters that may increase fitness (657), and the ability to persist on or within surfaces and equipment in care centers (440). Finally it should be noted that isolation of the same *P. aeruginosa* strain from multiple individuals attending the same CF clinic does not necessarily indicate patient-to-patient transmission. Exposure to unrecognized point sources within a center, such as contaminated respiratory equipment, may also account for repeated isolation of the same strain (625, 677). Likewise, some strains of *P. aeruginosa* are more prevalent and widespread within the soil, lakes, and streams of a geographical location, and the epidemiology of *P. aeruginosa* within a CF center may merely reflect random acquisition from these reservoirs by patients (501, 503). Additional characterization of the ecology of *P. aeruginosa* in its natural environment will be helpful in better understanding its epidemiology in CF.

Once *P. aeruginosa* enters the CF host, infection may ensue. Bacteria are thought to first colonize the oropharynx and then enter the lower respiratory tract by microaspiration (373, 553). Initially, infection is intermittent and results from serial acquisition of different strains rather than relapse with the same strain (77, 293). At this early stage of infection, the majority of isolates resemble environmental strains in that they are non-mucoid and highly antibiotic susceptible (77, 569). Eventually, however, one or two strains establish themselves, and chronic infection ensues (5, 215, 312, 501, 568). The molecular events that allow a particular strain to cause chronic infection are unknown but may be intrinsic to the strain itself at the time of initial infection or may reflect bacterial adaptation that occurs after acquisition. In any case, chronic infection is thought to be associated with biofilms, which are sessile communities of bacteria encased within a hydrated polymeric matrix of their own synthesis (113). Biofilms are clinically important because bacteria in this mode of growth are resistant to eradication by phagocytes (289, 394) and to killing by antibiotics (364, 665). Microscopic examinations of sputum and of the lungs from CF patients during transplantation have found *P. aeruginosa* bacteria as aggregates encased within an exopolysaccharide matrix in the CF airways (32, 54, 333, 550), consistent with a biofilm

FIG. 2. Cycle by which the presence of *P. aeruginosa* bacteria in the airways of individuals with CF leads to progressive pulmonary injury. In addition to directly damaging lung tissues, *P. aeruginosa* expresses factors that are recognized by the host immune system, resulting in release of proinflammatory cytokines. These cytokines cause the recruitment of large numbers of neutrophils that upon activation release elastase, collagenase, and oxygen radicals. The result is pulmonary injury as well as impaired bacterial clearance, which in turn leads to increased numbers of bacteria and exacerbation of the cycle.

mode of growth. It is now commonly believed that biofilms play an important role in the persistence of *P. aeruginosa* in chronically infected CF patients despite the administration of prolonged and aggressive courses of antibiotics (268).

Chronic infection with *P. aeruginosa* sets in motion an inflammatory cycle that culminates in progressive pulmonary injury (Fig. 2). At the root of this cycle is the absence of functional CFTR molecules, which confers an enhanced proinflammatory state upon cells of the respiratory tract (reviewed in references 87 and 322). It remains controversial whether the lungs of individuals with CF are autonomously prone to increased inflammation, even in the absence of recognized exogenous proinflammatory stimuli, or whether bacterial antigens simply provoke an exaggerated inflammatory response (285, 359, 423, 483). In support of the former supposition, a number of studies have demonstrated excessive pulmonary inflammation in newborns with CF prior to evidence of infection or in older individuals who lack evidence of active infection (31, 276, 313, 414, 506, 671). These findings are corroborated by *in vitro* evidence that CFTR^{-/-} cells are biased toward a proinflammatory state (166, 456, 536, 601). In contrast, others have found no difference in inflammatory markers between uninfected CF and non-CF lungs (22, 23, 123) or have argued that unrecognized infection accounts for the excessive inflammation in apparently sterile lungs (495, 496). Likewise, it has been suggested that differences in inflammatory states between CFTR^{+/+} and CFTR^{-/-} cells *in vitro* may be the result of technical issues, such as the use of adenovirus vectors to construct the cell lines (11). Regardless, once bacteria infect the CF airways, an exaggerated inflammatory response is observed relative to that

seen with bacterial infection of the normal lung (21, 123, 413, 506). Bacterial antigens, such as pili, flagella, DNA, and quorum-sensing autoinducer molecules (137, 151), are detected by the host and induce release of proinflammatory factors such as interleukin-8 (IL-8), tumor necrosis factor alpha (TNF- α), IL-1, IL-6, complement chemoattractants, and leukotriene B₄ (61, 133, 492, 651). The bacteria themselves are not merely passive bystanders in this process; rather, *P. aeruginosa* has the potential to synthesize factors that damage host cells, augmenting release of proinflammatory factors (535). For example, *P. aeruginosa* proteolytic enzymes alter host iron-containing proteins in a way that favors hydroxyl radical formation, which contributes to host tissue injury and inflammation (70). At the same time, levels of the anti-inflammatory cytokine IL-10 are decreased (60). The net result is robust recruitment of activated neutrophils to the airway lumen (324).

The prolonged presence of large numbers of mercenary neutrophils results in significant damage to the very pulmonary tissues that they were intended to protect (Fig. 2). In response to bacteria in the airways, neutrophils release large amounts of elastase, collagenase, and oxygen radicals (280, 324, 416, 463) that overwhelm the endogenous inflammatory inhibitors of the lung (48) and cause degradation of the extracellular matrix and the elastic framework of the bronchi and bronchioles (73, 161, 582, 612). Neutrophil elastase itself has been shown to induce expression of IL-8 in bronchial epithelial cells (416), resulting in a positive feedback loop that further increases inflammation and tissue damage.

Despite the associated collateral damage, the massive influx of neutrophils into the CF airways might be considered adaptive if not for one thing: they fail to eradicate *P. aeruginosa* bacteria (405). Rather than enhance bacterial clearance, neutrophils actually hamper the effectiveness of the innate immune response (Fig. 2). Through lysis and the export of "neutrophil extracellular traps," neutrophils release large amounts of high-molecular-weight DNA into the airway lumens, causing increased viscosity of endobronchial secretions (39, 69, 378). In addition, neutrophil elastase causes a decrease in ciliary beat frequency (15). Together, these effects result in a reduction in mucociliary clearance (116, 539). Neutrophil elastase cleaves and inactivates molecules important in opsonization and subsequent phagocytosis of bacteria, including complement receptor CR1, the opsonic complement component C3bi, and IgG (192, 608). Degradation of antimicrobial factors such as lysozyme and defensins by proteases from host immune cells also occurs (593). The detrimental effects of neutrophil-derived factors are compounded by substances produced by *P. aeruginosa*. *P. aeruginosa* elastase cleaves the antimicrobial factors lysozyme, surfactant proteins, and transferrin (10, 70, 71, 286) and causes a slowing of cilium beating (15). As a result, rather than being eradicated, *P. aeruginosa* bacteria actually increase in number over time (506) and may reach densities as high as 10¹⁰ to 10¹¹ CFU/ml of sputum (2, 213).

The ineffectiveness of the immune response allows the establishment of a relentless cycle whereby persistent bacteria cause increased inflammation that itself leads to increased bacterial densities and in turn more inflammation (511) (Fig. 2). Throughout this process, the patient experiences periods of relative well-being punctuated by pulmonary exacerbations (516). The net result is progressive tissue damage that eventually leads to the

FIG. 3. Chest computed tomography scan of an individual with CF showing bronchiectasis. Arrows indicate representative dilated airways that are characteristic of bronchiectasis. (Courtesy of Michelle Prickett.)

pathological consequences of CF, including mucopurulent plugging of bronchioles, chronic bronchiolitis and bronchitis, bronchial gland hyperplasia, and fibrosis (44, 517, 563). The airways become dilated and bronchiectatic due to loss of support cartilage (161, 238, 430, 441, 563) (Fig. 3). In late-stage CF, large bronchiectatic cysts and lung abscesses are common. Clinically, these pathological changes manifest initially as obstructive and later as restrictive pulmonary disease; secondary pulmonary hypertension or cor pulmonale may develop (238). As a result, in approximately 70% of individuals with CF, cardiorespiratory disease is the primary cause of death (118).

In summary, the genetic defect in CF allows persistence of *P. aeruginosa* in the face of an overly exuberant and ineffective inflammatory response that itself slowly destroys the lungs. In effect, the CF lung must bear the detrimental collateral damage of inflammation without benefiting from its sterilizing effect. The prominent role of inflammation in this process suggests that anti-inflammatory therapies may be of benefit. Indeed, studies of nonsteroidal anti-inflammatory agents (323) and corticosteroids (26, 167) have shown improved outcomes in individuals with CF. It is unclear whether other microbes follow the same steps as *P. aeruginosa* in CF. In fact, it is currently unknown whether some of these organisms adversely affect the clinical status of their hosts at all or are simply commensals inhabiting a novel niche uncovered by the CFTR mutation. Here we review the evidence regarding the impact on clinical outcomes of each of the microbes that have been linked to infection in CF. Organisms are discussed in the approximate order that they are encountered during the course of CF lung disease (Fig. 1).

INDIVIDUAL PATHOGENS AND DISEASE PROGRESSION IN CF

Haemophilus influenzae

Haemophilus influenzae is commonly cultured from the respiratory tracts of individuals with CF, with a prevalence of 16.3% in the United States (118) (Fig. 1). In CF patients, this

organism is usually unencapsulated (nontypeable) and therefore not covered by the *Haemophilus influenzae* type b (Hib) vaccine (270, 499, 638). It persists for an average of 2 1/2 months but for as long as 6 1/2 years (499). In one study of 30 CF patients, 90% of individuals were infected with two or more distinct clones over a 7-year period (499). *H. influenzae* most frequently infects CF patients early in childhood. Rosenfeld and colleagues bronchoscopically sampled the lungs of 40 infants with CF and found *H. influenzae* to be the most commonly isolated pathogen at 1 year of age, being present in 38% of patients (506). Armstrong and colleagues collected BAL fluid from 75 children diagnosed with CF by neonatal screening (21). *H. influenzae* grew from 8% of individuals sampled at a mean age of 17 months. Thus, *H. influenzae* is one of the first organisms to infect the airways of individuals with CF.

Whether *H. influenzae* is pathogenic in CF remains controversial (358) and is complicated by the fact that unencapsulated *H. influenzae* commonly colonizes the upper respiratory tracts of healthy children (409). Thus, frequent isolation of this bacterium from children with CF is to be expected. *H. influenzae*, however, is cultured more frequently from the oropharynx and sputa of children with CF than from those with other diseases such as asthma (488), and it is frequently isolated from the lower respiratory tracts of CF patients, a site not normally colonized by this bacterium (21, 506). The question is whether the presence of the organism contributes to pulmonary disease in CF. A cross-sectional analysis of 7,010 patients from the European Epidemiologic Registry of Cystic Fibrosis (EERCF) found that isolation of *H. influenzae* from the respiratory tract was not associated with lower percent predicted forced expiratory volume in 1 s (% predicted FEV₁) values (417). Several other studies, however, suggest that *H. influenzae* does play a role in CF disease progression. In sputa from stable CF patients, the organism can be isolated in large numbers comparable to those observed with known pathogens such as *P. aeruginosa* (47, 499, 506). Once in the lower respiratory tract, evidence indicates that it is capable of inducing inflammation, which has the potential to cause tissue injury. For example, Rosenfeld and colleagues reported that total leukocyte and neutrophil counts in BAL fluid from CF infants were higher when $>10^5$ CFU/ml of *H. influenzae* were present than when there were no identifiable pathogens (506). Given the uncertainty of the pathogenic role of *H. influenzae* in CF, it remains unclear whether asymptomatic patients harboring *H. influenzae* in their respiratory tracts should be treated with antibiotics (156, 465).

There is more consensus on the role that *H. influenzae* plays during acute pulmonary exacerbations in CF. Rayner and colleagues observed a rise in the rate of isolation of *H. influenzae* prior to and during acute exacerbations in CF patients (488). They also noted that clinical improvement after antimicrobial therapy coincided with a reduction in the rate of isolation of this bacterium. Most experts agree that *H. influenzae* is capable of causing exacerbations in CF patients and that these should be treated with appropriate antibiotics (222, 478).

Hypermutable phenotype. Some strains of *H. influenzae* cultured from the airways of individuals with CF acquire mutations at an unusually high rate. The ability to rapidly mutate may provide an advantage to these strains as they adapt to the harsh environment of the CF airways. In these strains, high

mutation rates occur because of disruptions in the *mutS* gene (639), which encodes a protein that repairs mismatches during DNA replication. Strains that harbor such defects are referred to as “mutator” or “hypermutable” strains and appear to be relatively common in CF. In one study, 14.5% of *H. influenzae* isolates from CF patients were hypermutable, compared to only 1.4% of isolates from non-CF patients ($P < 0.0001$) (499). Hypermutable strains were associated with long-term persistence (499), suggesting that this form of adaptation confers an advantage to *H. influenzae* in the context of CF.

Staphylococcus aureus

S. aureus is another bacterium commonly cultured from the respiratory secretions of CF patients and historically was the first bacterium noted to be associated with CF respiratory disease (16). Perhaps because it infects children with CF so early in life, *S. aureus* was the most prevalent bacterium cultured from the respiratory tracts of CF patients in series from the 1940s and 1950s (152, 275), an era when children with CF usually died before reaching the age of 10 years (131). With the development of potent antistaphylococcal therapies and increased patient longevity, *S. aureus* was surpassed in prevalence by *P. aeruginosa* in subsequent decades (393). Nevertheless, *S. aureus* remains a frequent cause of CF pulmonary infection and continues to be the organism most frequently initially isolated from the airways of most infants and children with CF (4, 20, 505) (Fig. 1). In a study of 42 children diagnosed with CF by neonatal screening, Abman and colleagues found the mean age at which oropharyngeal swabs grew *S. aureus* to be 12.4 months (4). Rosenfeld and colleagues found that *S. aureus* grew from BAL fluid specimens in nearly 50% of 141 infants with CF during the first 6 months of life (505). Currently, *S. aureus* has been cultured from the respiratory secretions of more than 70% of children in the CFF Patient Registry between the ages of 6 and 10 years (118) (Fig. 1). Furthermore, over the past decade, the prevalence of this bacterium in CF patients has increased (172, 489, 581), although this may merely reflect the use of more sensitive culturing protocols (540, 675). *S. aureus* infection in CF may be either intermittent or chronic (68, 127); one study used pulsed-field gel electrophoresis to show that strains of *S. aureus* persisted for a median duration of 37 months (303).

Historical experience supports a pathogenic role for *S. aureus* in CF. Prior to the advent of antibiotics, this organism was felt to be the major cause of death in infants and children with CF (16). For example, in a 1946 study of 13 patients with CF, *S. aureus* was the predominant organism grown from postmortem lung cultures in 12 cases and the sole organism isolated in 4 cases (152). Early use of sulfonamides and penicillin to treat these respiratory infections appeared to result in improved survival (17). Thus, it was generally assumed that this bacterium was responsible for much of the morbidity and mortality associated with CF. Later, Katz and colleagues found that the isolation of *S. aureus* at the time of CF diagnosis was associated with more severe clinical deterioration measured after 10 years of follow-up (307). However, the proportion of these patients also infected with *P. aeruginosa* was not stated. Hudson and colleagues studied the clinical significance of respiratory infections in CF patients diagnosed before 2 years of age (277). In

20 patients with *S. aureus* alone in their initial respiratory cultures, the FEV₁ was 14% lower than that in patients with pathogens other than *S. aureus* or *P. aeruginosa*. Patients with *P. aeruginosa* alone or *P. aeruginosa* with *S. aureus* in their sputa had even worse lung function measurements, although the numbers of patients in these groups were small (six and seven, respectively). Whereas *S. aureus* alone did not affect survival, patients with *S. aureus* together with *P. aeruginosa* had a significantly lower 10-year survival rate (57%) than those with either *S. aureus* alone (92%) or *P. aeruginosa* alone (100%). The authors concluded that *S. aureus* and *P. aeruginosa* contributed independently and additively to poorer outcomes in CF. A proposed mechanism consistent with these findings is that initial infection with *S. aureus* is the trigger that activates or amplifies an inflammatory cascade and leads to subsequent tissue damage. Damaged tissue may then predispose the individual to enhanced attachment of other bacterial pathogens, such as *P. aeruginosa*, in later disease and therefore contribute to worse outcomes (223). In support of this model, one study has observed that prior recovery of *S. aureus* is more frequent in infants infected with *P. aeruginosa* (4). Alternatively, *S. aureus* infection may simply serve as a marker for individuals with more severe intrinsic CF disease. Such patients may likewise be more prone to develop infections with *P. aeruginosa*. In any case, a general consensus has developed that patients experiencing pulmonary symptoms attributed to *S. aureus* should receive antimicrobial therapy; asymptomatic patients also sometimes receive treatment (478, 486).

Opposed to this view are studies that suggest that *S. aureus* in the airways of CF patients is a neutral prognostic indicator or may even be protective. In a cross-sectional analysis of the EERCF, Navarro and colleagues did not detect an association between *S. aureus* and concurrent impaired pulmonary status in children or adults (417). Huang and colleagues followed the courses of 142 patients with CF and noted that infection with *S. aureus* alone (in the absence of other pathogens) was associated with mild disease and improved long-term survival after the age of 18 years (274). Likewise, in a study of 514 individuals with CF less than 18 years of age on the lung transplant waiting list, Liou and colleagues found that infection with *S. aureus* was associated with improved survival prior to lung transplantation, albeit with reduced survival after transplantation (347). A multivariate logistic regression model based on 5,820 children and adults in the U.S. CFF Patient Registry identified *S. aureus* infection as a marker for increased survival (348). These findings have been used to argue that infection with *S. aureus* may prevent infection with more virulent pathogens such as *P. aeruginosa* and thus be protective (559). Of note, most of these studies included patients who grew *S. aureus* from their respiratory secretions only once or a few times.

Interventional studies examining the effect of antistaphylococcal treatment or prophylaxis on the clinical outcomes of CF patients do not clarify the role of *S. aureus* in disease progression (559, 581). Only limited evidence supports an improvement in lung function or other clinical benefit with antistaphylococcal therapy in the absence of exacerbations (560). Weaver and colleagues studied 38 infants diagnosed with CF at birth and randomized to receive either continuous prophylactic flucloxacillin or antibiotics as clinically indicated (640). Patients randomized to the flucloxacillin group had a lower rate of

hospital admissions and shorter admissions and required fewer antibiotic courses during the first 2 years of life than patients who received only episodic antibiotics during exacerbations. McCaffery and colleagues identified 13 clinical trials that used a variety of antistaphylococcal medications both intermittently and continuously in CF patients (387). Although most of the studies demonstrated eradication of the bacteria, none showed improvement in pulmonary function or other clinical outcomes. In a study of 42 infants randomized to receive prophylactic flucloxacillin versus antibiotics only when clinically indicated, Beardsmore and colleagues also did not observe a difference in pulmonary function between the two groups at 1 year of age (42). In a multicenter, randomized, double-blind, controlled trial, Stutman and colleagues followed CF patients receiving continuous antistaphylococcal therapy (cephalexin) for 7 years (585). No difference in the clinical and radiographic outcomes or in the anthropometric measurements between the treatment and placebo groups was observed. A concerning finding was an increased rate of *P. aeruginosa* infection in patients receiving continuous antistaphylococcal therapy. This observation was confirmed in a retrospective study by Ratjen and colleagues that examined 639 CF patients from the EERCF (485). They found that patients receiving continuous antistaphylococcal therapy had a significantly higher rate of *P. aeruginosa* infection than patients receiving only intermittent or no antibiotic therapy. This difference was especially apparent in children younger than 6 years of age. In CF patients coinfecting with *S. aureus* and *P. aeruginosa*, Bauernfeind and colleagues demonstrated that the use of antistaphylococcal antibiotics alone resulted in increased numbers of *P. aeruginosa* in sputum (41). At first glance, these findings suggest that *S. aureus* in the respiratory tract protects against infection with *P. aeruginosa*, although it is unclear whether suppression of *S. aureus* or other components of the microbial flora by antimicrobial therapy was responsible for the predisposition to *P. aeruginosa* infection. Consistent with the latter interpretation, the use of flucloxacillin (as opposed to a more broadly active cephalosporin) was not associated with a statistically significant increased risk of *P. aeruginosa* acquisition (84, 485, 560, 640).

In summary, the long-term consequences of infection with *S. aureus* appear to be less severe than those of more aggressive pathogens such as *P. aeruginosa*. Whether this pathogen even contributes to decreased survival is controversial, with some experts asserting that *S. aureus* does not have a major impact on the clinical status of individuals with CF (358, 559). Others have proposed that subsets of *S. aureus*-infected patients may experience more rapid decline in pulmonary function, such as chronically infected individuals or those with β -lactam-resistant strains.

Methicillin-resistant *S. aureus*. Although methicillin-resistant *S. aureus* (MRSA) infection of CF patients has been occurring since the 1980s (66), this bacterium has recently emerged as an increasingly common pathogen in this population (172). The prevalence of MRSA-positive cultures in CF patients has risen from 0.1% in 1995 to 22.6% in 2008 (118, 489). The prevalence figures vary by location and are substantially higher at some centers (169).

In CF, the implications of infection with MRSA, as opposed to methicillin-susceptible *S. aureus* (MSSA), are unclear. Several small studies did not find an association between MRSA

infection and increased mortality or deterioration of lung function in CF patients (208, 397, 602). Likewise, a retrospective study that evaluated a treatment protocol for eradication of MRSA carriage in 15 CF patients did not find an improvement in pulmonary function in patients who became MRSA free (564). In contrast, other studies have found an association between MRSA and worse outcomes. In a retrospective study, Miall and colleagues compared the clinical courses of 10 CF patients with MRSA in their respiratory cultures between 1992 and 1998 to those of 18 controls (some with and some without MSSA) matched for age, sex, and respiratory function (397). After 1 year of follow-up, MRSA was associated with significant worsening of height standard deviation scores and a 2-fold increase in the number of courses of intravenous (i.v.) antibiotics. Chest X-ray scores were worse in the MRSA cohort both at the time of first MRSA isolation and 1 year later. Ren and colleagues examined 2001 data from 1,834 patients enrolled in the Epidemiologic Study of Cystic Fibrosis (ESCF), a large North American observational study, with respiratory cultures positive for MRSA only versus MSSA only (490). In comparison with MSSA, MRSA was associated with lower FEV₁ values both in children less than 18 years of age (80.7 versus 89.4% predicted; $P < 0.001$) and in adults (60.9 versus 70.4% predicted; $P < 0.001$). Likewise, MRSA was associated with increased frequency of hospitalization and administration of i.v. antibiotics. In a follow-up longitudinal study, it was observed that patients who became infected with MRSA exhibited a more rapid decline in % predicted FEV₁ but that this rate of decline preceded acquisition of MRSA and was not significantly increased following acquisition (521). These authors suggested that patients who become infected with MRSA have more severe disease at baseline and that MRSA acquisition may be the consequence of more intense antibiotic exposure rather than a cause of worse disease (522). In contrast, Dasenbrook and colleagues, in a 10-year (1996 to 2005) cohort study examining 17,357 individuals from the CFF Patient Registry, found that new onset of persistent MRSA infection (defined as ≥ 3 MRSA cultures) in patients aged 8 to 21 years was associated with an average FEV₁ decline of 2.06% predicted per year, compared to 1.44% predicted per year in those without MRSA (difference of -0.62% ; 95% confidence interval [CI], -0.70 to -0.54 ; $P < 0.001$), even after adjustment for confounding factors such as % predicted FEV₁ at entry into the study (127). In a follow-up study, this same group has recently shown that MRSA infection is also associated with a higher risk of death (1.27; 95% CI, 1.11 to 1.45) (126). Thus, although studies differ as to whether chronic infection with MRSA worsens the clinical status of CF patients, more recent larger studies tend to indicate that this is the case.

Whereas acquisition of MRSA in non-CF patients was previously linked to exposure to hospital or health care-associated environments, recent MRSA infections have been community acquired (202). This epidemiological shift is the result of the rapid dissemination within communities of a new MRSA strain characterized by expression of Panton-Valentine leukocidin (PVL) toxin and susceptibility to certain antibiotics (clindamycin, trimethoprim-sulfamethoxazole, and fluoroquinolones) that are usually ineffective against hospital-acquired MRSA (672). Anecdotal reports suggest that these PVL⁺ MRSA strains may be more viru-

lent than conventional MRSA strains and capable of causing severe soft tissue infections and necrotizing pneumonia in otherwise healthy individuals (202). These community-associated MRSA strains are now infecting patients with CF. Goodrich and coworkers found that community-associated MRSA was isolated from 2.7% of 707 CF patients at the University of North Carolina (218). These isolates represented 14% of all MRSA isolates. Elizur and colleagues analyzed 40 MRSA isolates cultured from patients seen at St. Louis Children's Hospital Pediatric Cystic Fibrosis Center in Missouri (169). They found that 6 (15%) were PVL⁺ and that children infected with these strains were more likely to have evidence of severe disease, such as focal pulmonary infiltrates (including cavitary lung disease), more rapid decline in FEV₁, and higher peripheral white blood cell counts. These strains have reportedly been transmitted between family members with CF (170). If borne out by larger prospective studies, these findings regarding such a rapidly emerging and virulent pathogen are of special concern for the CF community.

Adaptation of *S. aureus*. (i) **Small-colony variants.** During persistent infections, *S. aureus* may inhabit the CF airways for years, which allows adaptation to this novel environment. One relatively common adaptation is the formation of small-colony variants (SCVs) (303). These variants have slower growth rates that result in markedly smaller colonies when grown on laboratory agar. This phenotype may result from defects in electron transport, thymidine biosynthesis, carbon dioxide generation, or regulation of the stringent response, and it appears to be adaptive for intracellular survival (204, 217, 470). SCV strains can be cultured from 17 to 49% of *S. aureus*-infected CF patients (45, 303, 529). The clinical significance of *S. aureus* SCVs is severalfold. First, SCVs are associated with a number of microbiological changes (e.g., failure to metabolize mannitol, nonhemolytic colonies, reduced coagulase production, lack of pigment, and the propensity to be overgrown by other bacteria [468]) that can make their identification problematic (626). Second, since aminoglycosides require functional electron transport to enter bacteria, some SCVs are relatively resistant to these and often other antibiotics (46, 468). In fact, exposure to aminoglycosides may lead to the emergence of SCVs (523, 629). Third, this phenotype is associated with resistance to killing by cationic proteins of the innate immune system and uptake by phagocytes (510). Fourth, SCVs have been linked to persisting and relapsing infections, including CF, osteomyelitis, and prosthetic valve endocarditis (469, 629, 630).

The impact of SCVs on disease progression in CF is unclear, although the SCV phenotype is associated with more advanced pulmonary disease and prolonged antibiotic exposure (529). *S. aureus* bacteria with the SCV phenotype persisted longer in the airways than normal *S. aureus* (302). Besier and colleagues found that isolation of *S. aureus* SCVs was associated with older age, coinfection with *P. aeruginosa*, and lower FEV₁ values compared with patients infected with normal-colony *S. aureus* (45). It remains to be determined whether these associations are causal or whether these strains are simply markers for patients with more advanced and severe disease. An association between *S. aureus* SCVs and coinfection with *P. aeruginosa*

is particularly interesting in light of a subsequent reports showing that *P. aeruginosa* releases 4-hydroxy-2-heptylquinolone-*N*-oxide (HQNO) and pyocyanin, which select for *S. aureus* SCVs during growth in mixed cultures of these two bacteria (50, 157, 259, 404).

(ii) **Hypermutable phenotype.** As with *H. influenzae*, hypermutable *S. aureus* strains have been identified in CF. Mutations in both the *mutS* and *mutL* genes have been observed and appear to account for this phenotype (46, 471). Although an association between the hypermutable phenotype and poorer clinical outcomes has not been explored, this phenotype is associated with SCVs and antibiotic resistance (46, 471).

Pseudomonas aeruginosa

Arguably the most studied microbe in the context of CF is *P. aeruginosa*. This bacterium is the most common pathogen identified in the respiratory secretions of patients with CF. According to the CFF Patient Registry, about half of individuals under the age of 18 years are infected with *P. aeruginosa*, a prevalence that rises to nearly 80% in patients ≥ 18 years of age (118) (Fig. 1). This high prevalence is due in part to the propensity of *P. aeruginosa* to cause chronic infections. Once established within the respiratory airways, *P. aeruginosa* resists eradication despite a constant assault by the host immune system and treatment with prolonged courses of antibiotics. The mechanisms by which this bacterium so successfully persists in the lungs is unclear but may involve its impressive ability to adapt to changes and stresses in its environment.

It has long been appreciated that infection with *P. aeruginosa* is associated with worse outcomes in individuals with CF. In one of the earliest studies to show a link between *P. aeruginosa* infection and increased mortality, Wilmott and colleagues followed the survival of 117 children whose *P. aeruginosa* infection status was established in 1974. Of the 31 children who were infected with *P. aeruginosa* at that time, 53% survived to age 16 whereas 84% of those not infected with *P. aeruginosa* survived to this age (652). Survival was worse in those patients who were infected with *P. aeruginosa* at a younger age. Others subsequently confirmed the association between *P. aeruginosa* infection and mortality (114, 138, 277, 425). In addition to decreased survival, *P. aeruginosa* infection is associated with poorer lung function (277, 311, 325, 326, 445, 655), worse chest radiologic imaging scores (4, 277, 326, 493, 511), slower growth of the patient (445, 511), and increased frequency of daily cough (4). For example, Schaedel and colleagues examined factors influencing pulmonary function in the entire CF population of Sweden over the age of 7 years. They found that chronic infection with *P. aeruginosa* increased the relative risk (RR) of having a severely reduced FEV₁ value (defined as less than 60% of predicted) by 1.7- to 3-fold (525). The association between *P. aeruginosa* infection and poor outcomes was not limited to studies using respiratory cultures as a marker for *P. aeruginosa* infection. In a study of 68 individuals identified as having CF through the Wisconsin CF Neonatal Screening Project, West and coworkers reported that *P. aeruginosa* seroconversion, which preceded isolation of the bacterium from respiratory cultures by 6 to 12 months, was itself associated with worsening chest radiograph scores (643). Large registry-based studies have more precisely quantified the impact of *P.*

aeruginosa infection on outcomes. Emerson and coworkers performed a CFF Patient Registry-based study of 3,323 children ages 1 to 5 years (173). Children with *P. aeruginosa*-positive respiratory cultures during the first year of the study had a 2.6-fold increase in mortality over the subsequent 8 years of follow-up relative to children who had *P. aeruginosa*-negative cultures. *P. aeruginosa* infection was also associated with lower % predicted FEV₁, lower weight percentile, and increased frequency of hospitalization for acute respiratory exacerbation. In a cross-sectional analysis of 7,010 patients from the EERCF, infection with *P. aeruginosa* at enrollment was associated with impaired lung function, defined as an FEV₁ more than 10% below predicted values (417). Konstan and colleagues used repeated-measures, mixed-model regression analysis to examine data from 4,866 children and adolescents enrolled in the ESCF for a 3- to 6-year period (325). They found that among children aged 6 to 8 years or 9 to 12 years, infection with *P. aeruginosa* was associated with an additional 0.34% and 0.22% per year absolute decline in % predicted FEV₁, respectively. Thus, numerous studies have linked *P. aeruginosa* infection with worse outcomes in CF patients.

It is important to note that studies showing worse clinical outcomes in patients infected with *P. aeruginosa* than in those free of *P. aeruginosa* suffer from a serious limitation. Rather than causing the more rapid decline in pulmonary function, it is conceivable that *P. aeruginosa* simply preferentially infects patients with more severe disease. In other words, *P. aeruginosa* infection may be the result not the cause of poor lung function. In fact, there is support for this supposition. Aebi and coworkers divided a group of 54 CF patients into two cohorts: those in whom growth of *P. aeruginosa* from respiratory samples occurred before the age of 12 years and those in whom it occurred after the age of 12 years (6). Individuals in both groups experienced rapid progression in chest radiograph changes prior to the age of 12 years, even though the second group was not yet chronically infected with *P. aeruginosa*. The authors concluded that infection with *P. aeruginosa* may be a marker rather than the cause of respiratory deterioration in CF. These results, however, must be reinterpreted in light of recent findings showing that serological evidence of *P. aeruginosa* infection in CF may occur for some time prior to the growth of the organism from respiratory samples (643).

Interventional studies have been employed to further address the question of whether the link between *P. aeruginosa* infection and worse clinical outcomes is causal. These studies have examined the effect of antipseudomonal antibiotics on the outcomes for three categories of individuals with CF: those who are (i) intermittently infected, (ii) chronically infected and stable, or (iii) chronically infected and experiencing acute respiratory exacerbations.

During the initial phase of infection with *P. aeruginosa*, this bacterium is intermittently isolated from the respiratory tracts of CF patients (77, 293). Unlike chronic infections with *P. aeruginosa*, new or intermittent infections may be amenable to eradication with antimicrobial therapy (reviewed in references 336, 343, and 487). Several small studies suggest that eradication of *P. aeruginosa* from the airways of newly or intermittently infected CF patients results in improved outcomes (660). Frederiksen and colleagues compared 48 patients treated with inhaled colistin and oral ciprofloxacin at the time of first iso-

lation of *P. aeruginosa* with 43 historical controls. After 3 1/2 years, only 7 treated patients (16%) developed chronic *P. aeruginosa* infections, compared to 19 controls (72%) (201). Furthermore, treated patients maintained or improved their pulmonary function, as measured by % predicted FEV₁ and forced vital capacity (FVC), whereas spirometry values declined in untreated patients. Taccetti and colleagues treated 58 newly infected CF patients with inhaled colistin and oral ciprofloxacin for 3 weeks (591). Eradication was achieved in 47 (81%) of these patients and was maintained for a median of 18 months. The mean annual decline in FEV₁ was significantly less in the group of patients in whom eradication was successful than in a group of age-matched and sex-matched chronically infected historical control patients (−1.63 versus −4.69; $P < 0.05$). Given the historical nature of the control groups in both these studies and the progressively improving overall status of CF patients over time (474), these findings must be viewed with caution. Kozłowska and colleagues studied 48 children with CF and 33 healthy controls between the ages of 0 and 2 years (327). They observed that infection with *P. aeruginosa* was associated with a greater reduction in lung function over the subsequent 4 years but that this reduction occurred even in children who had *P. aeruginosa* successfully eradicated from their lungs. The authors of an accompanying editorial note that one interpretation of these results is that more severe lung disease drives acquisition of *P. aeruginosa* rather than *P. aeruginosa* infection driving more severe lung disease (132). Additional studies are necessary to definitively determine whether eradication of *P. aeruginosa* during early infection results in maintenance of lung function and improved outcomes.

Once chronic infection (usually defined as growth of *P. aeruginosa* from multiple respiratory cultures over a 6-month period [293]) with *P. aeruginosa* occurs, it is very difficult to eradicate this bacterium from the CF respiratory tract (267, 319). For example, Hoiby describes a 42-year-old CF patient who had received 114 2-week courses of intravenous antibiotics over a 28-year period but remained infected with *P. aeruginosa* (266). Nevertheless, antipseudomonal therapy does appear to decrease morbidity in chronically infected individuals even in the absence of eradication. In a prospective double-blind, placebo-controlled study of 40 chronically infected CF patients, Jensen and colleagues investigated the efficacy of 3 months of therapy with inhaled colistin (290). The total decline in % predicted FVC was significantly less in the treatment group than in the placebo group (7% versus 18%; $P < 0.05$). Ramsey and colleagues performed a multicenter, double-blind, placebo-controlled trial of inhaled tobramycin for 6 months (481). A total of 520 patients with CF and *P. aeruginosa* infection were randomized to receive three cycles of either inhaled tobramycin or placebo for 4 weeks followed by 4 weeks off therapy. Although only a single *P. aeruginosa*-positive culture was necessary for enrollment, presumably the majority of these patients were chronically infected, since their mean age was 21 years. A 12% relative increase in % predicted FEV₁ ($P < 0.001$) and a 26% relative reduction in hospitalization (95% CI, 2 to 43%) in the treatment arm were observed. These clinical improvements were associated with a 0.8 log₁₀ reduction in CFU of *P. aeruginosa* per gram of expectorated sputum relative to baseline. Multiple other studies support the supposition that antipseudomonal therapy improves clinical param-

TABLE 1. Adaptations of *P. aeruginosa* observed during chronic respiratory infections of CF patients

Adaptation	Mutated gene(s)	Reference(s)
Mucoid colony morphology	<i>mucA</i>	63, 379
Antibiotic resistance	<i>mexZ</i> , <i>mexA</i> , <i>mexT</i> , <i>ampD</i>	556
Lipopolysaccharide alterations	<i>pagL</i> , O-antigen biosynthetic cluster (<i>himD/ihfB</i> to <i>wbpM</i>)	89, 176, 177, 182, 329, 570
Loss of type III secretion	<i>exsA</i> , <i>vfr</i> , <i>cyaB</i>	556
Loss of motility	<i>rpoN</i> , <i>vfr</i> , <i>fleQ</i> , <i>pilB</i> , <i>pilQ</i> , <i>cyaB</i>	82, 329, 368, 556
Auxotrophy and metabolic changes	<i>lasR</i>	37, 38, 125, 547
Small-colony variants	<i>wspF</i>	556
Defective quorum sensing	<i>lasR</i>	125, 342, 556, 647
Hypermotility	<i>mutS</i> , <i>mutL</i> , <i>uvrD</i>	96, 434
Loss of pyoverdine secretion or uptake	Deletion of pyoverdine synthetic and uptake genes	147, 176, 262
Loss of pyocin production		501
Changes in pyocyanin production		196, 197, 342, 654, 656
Peptidoglycan modification		89
Loss of type II secretion or altered activity of secreted factors (e.g., exotoxin A, phospholipase C, elastase)	<i>toxA</i> , <i>toxR</i>	75, 196, 203, 262, 342, 432, 556, 661

eters in CF patients infected with *P. aeruginosa* (258, 291, 361, 453, 479, 577, 588).

Summary. Together, these numerous observational and interventional studies support a role for *P. aeruginosa* in the morbidity and mortality of CF. Current evidence suggests that *P. aeruginosa* is not static but rather adapts to residence within CF airways, resulting in the persistence of phenotypically diverse subpopulations of bacteria. Is the mere presence of *P. aeruginosa* sufficient to cause poor outcomes, or is the emergence of specific phenotypic subpopulations necessary before the adverse effects of this bacterium are manifested? In the following sections, we will discuss adaptation of *P. aeruginosa* to the CF airways and what is known about the consequences of these adaptations for the clinical status of patients.

Does adaptation of *P. aeruginosa* impact disease progression? Given the plasticity of *P. aeruginosa* and the prolonged infections characteristic of CF, it is not surprising that this pathogen undergoes significant adaptation within the CF lung. Although initial infection may be facilitated by the large genome of *P. aeruginosa* and its superior ability to sense and respond to a broad variety of environmental conditions (584), later adaptation results at least in part from the selection of clonal lineages containing spontaneously arising mutations (556). The process by which this occurs is as follows. As with all bacteria, spontaneous mutations continuously arise in *P. aeruginosa*. The rate at which these mutations occur may be augmented by the presence of hypermutable strains (see below) (435). Likewise, downregulation of antioxidant enzymes during growth in biofilms may also enhance the mutation rate (59, 159). Mutations result in the generation of a diverse array of *P. aeruginosa* lineages, many of which exhibit altered phenotypes (556). Conditions within the CF airways then favor the growth and selection of strains with phenotypic traits that confer an adaptive advantage. Such selection is relatively common in CF but apparently differs in its nature from one portion of the respiratory tract to another, resulting in heterogeneous populations of bacteria that are closely related but possess unique sets of mutated genes (262, 421, 556). Some adaptive traits that commonly emerge during respiratory infections in CF are the mucoid phenotype, antibiotic resistance, alterations

in lipopolysaccharide (LPS), loss of type III secretion and motility, auxotrophy, SCVs, defects in quorum sensing, and hypermutability (Table 1). It is likely that many more phenotypic variants have yet to be described (281). Whether the emergence of phenotypic traits that enhance the fitness of *P. aeruginosa* causes a corresponding deterioration in the clinical status of the host is less clear but is an area of active study. Here, we review each of the common *P. aeruginosa* adaptations and discuss its impact on disease progression.

(i) Mucoid phenotype. Arguably the most studied adaptation of *P. aeruginosa* in CF is the mucoid phenotype. As early as 1963, it was noted that *P. aeruginosa* isolated from the respiratory tract of patients with CF often exhibited “a very mucoid colonial variant” (283). It is now known that this mucoid colony morphology is due to overproduction of the exopolysaccharide alginate, a polymer of D-manuronic acid and L-guluronic acid (143, 224). Most of the alginate biosynthetic genes are located in a single operon referred to as the *algD* cluster (385). This operon and the production of alginate are under the control of both positive and negative regulation. The *algD* promoter requires the alternate sigma factor AlgT (also called AlgU) (532) for expression. AlgT, though, is bound and sequestered by the anti-sigma factor MucA, which itself is encoded by the *mucA* gene. Thus, MucA normally limits the expression of the *algD* gene cluster and production of alginate. However, after extended periods in the airways of patients with CF, *P. aeruginosa* acquires mutations in the *mucA* gene (63, 379), which results in loss of production of MucA and, in turn, high levels of unsequestered AlgT. This leads to unbridled expression of the *algD* cluster of genes, overproduction of alginate, and a mucoid phenotype (Fig. 4).

The mucoid phenotype is relatively common in respiratory samples from patients with CF, although it can also be observed in cultures from the airways of individuals with bronchiectasis or ciliary dyskinesia and rarely from urine specimens, ear swabs, and sputum samples from non-CF patients, (386, 426, 472). In CF it emerges following an average of 3 years of infection (368) and at a median age of 13 years (346), but can be observed as soon as 3 months after infection (223)

FIG. 4. The mucoid phenotype of *P. aeruginosa*. The colonies on the left are a mucoid *P. aeruginosa* strain cultured from a CF patient. On the right, a nonmucoid variant of the same strain cultured from the same patient is shown.

and as early as 18 months of age (346). In the United States in 2006, 66% of *P. aeruginosa*-infected CF patients harbored mucoid strains (120).

What are the selective pressures that lead to a predominance of this phenotype in CF? Overproduction of alginate may be advantageous to *P. aeruginosa* in the context of CF in several ways (reviewed in reference 224). It may enhance biofilm formation, which prevents bacterial clearance by both host phagocytes and antimicrobial therapy (252). Alternatively, by forming a capsule around *P. aeruginosa*, alginate may impede opsonization, phagocytosis, and killing (63, 566) (Fig. 5). Finally, this exopolysaccharide may have immunomodulatory properties that lead to a dysregulated immune response (98, 206, 372, 459). Interestingly, the selective pressure driving the formation of mucoid mutants is not constant or global; up to 70% of nonmucoid isolates from chronically infected CF patients carry mutations in the *mucA* gene, suggesting that these strains at one time were mucoid but had reverted to a nonmucoid phenotype (95).

A number of studies have attempted to tease out the impact of the mucoid phenotype on outcomes in CF. Interpretation of these investigations is complicated by the fact that some experts feel that emergence of the mucoid phenotype coincides with the transition from the intermittent to the chronic phase of infection (293, 452, 464) whereas others feel that chronic infection usually precedes the emergence of mucoid *P. aeruginosa* (30). Regardless of these distinctions, it is clear that chronic infection with mucoid *P. aeruginosa* is associated with poorer outcomes in CF patients (184, 250, 269). Pedersen and colleagues performed spirometry on 73 patients with CF over a period of 13 years (453). Patients infected with mucoid *P. aeruginosa* strains had significantly lower % predicted FVC values than patients infected with nonmucoid *P. aeruginosa*. In a longitudinal study, Henry and colleagues followed 81 children with CF. Of these, 50 were infected with mucoid *P. aeruginosa*, 19 were infected with nonmucoid *P. aeruginosa*, and 12 were not infected with *P. aeruginosa*. After 8 years, 21

FIG. 5. Gram-stained sputum specimen from a CF patient infected with mucoid *P. aeruginosa*. The orange material surrounding the bacteria is alginate (magnification, $\times 1,000$). (Reprinted from reference 467 with permission of the publisher. © 2007–2010 American Society for Clinical Pathology and © 2007–2010 American Journal of Clinical Pathology.)

(42%) of those infected with mucoid *P. aeruginosa* had died, whereas 2 children (11%) infected with nonmucoid *P. aeruginosa* and 1 uninfected child (8%) had died ($P < 0.01$) (251). Demko and colleagues noted that 34 of 130 patients (26%) who were chronically infected with mucoid *P. aeruginosa* before 6 years of age died over the subsequent 10 years, whereas only 21 of 361 patients (6%) who were chronically infected with mucoid *P. aeruginosa* after 6 years of age died ($P < 0.0005$) (138). Statistically significant differences between these two groups were also noted with chest X-ray scores and % predicted FEV₁ values. In this cohort of patients, the % predicted FEV₁ was relatively constant prior to chronic infection with mucoid *P. aeruginosa* but declined at a rate of 2.5% per year after chronic mucoid *P. aeruginosa* infection. In a prospective longitudinal study of 56 patients diagnosed with CF at birth, Li and colleagues observed a significant abrupt increase in cough scores and worsening of chest radiograph scores with the first respiratory culture of mucoid *P. aeruginosa* (346). Likewise, isolation of mucoid *P. aeruginosa* was associated with abrupt declines in % predicted FEV₁ (-12.13 ; $P = 0.02$), % predicted FVC (-9.15 ; $P = 0.007$), and forced expiratory flow between 25% and 75% of FVC (FEF_{25%-75%}) (-4.65 ; $P < 0.001$). Parad and colleagues performed a mixed-model analysis of CF patients ≥ 12 years of age and found that mucoid *P. aeruginosa* infection status and gender had the greatest impact on annual rates of decline in % predicted FEV₁ (446). Together, these studies demonstrate that the emergence of mucoid *P. aeruginosa* bodes poorly for patients with CF. However, whether the mucoid phenotype actually causes poor clinical outcomes or rather is a marker for highly adapted strains that have increased virulence due to other mutations remains un-

clear. Another possibility is that the mucoid phenotype is simply selected for in the hypoxic, bronchiectatic lung environment found in advanced disease.

The association of the mucoid phenotype with poor outcomes in CF leads to a second question: does infection with nonmucoid *P. aeruginosa* cause an increase in morbidity and mortality in this patient population? Or does the mucoid phenotype account for all of the negative prognostic significance associated with *P. aeruginosa* infection in CF? Although controversial, current evidence suggests that infection with nonmucoid *P. aeruginosa* is not associated with worse outcomes. Kerem and colleagues, in a study of 502 patients with CF, found no significant change in pulmonary function parameters in the first 2 years following initial infection with *P. aeruginosa*, presumably before the emergence of the mucoid phenotype was common (311). Ballmann and colleagues directly addressed this question by following 40 patients through four stages of infection: (i) first detection of *P. aeruginosa*, (ii) chronic nonmucoid *P. aeruginosa* infection, (iii) first detection of mucoid *P. aeruginosa*, and (iv) chronic mucoid infection (30). A significant decrease in pulmonary function was not observed with first isolation of *P. aeruginosa* or with chronic infection with nonmucoid *P. aeruginosa*. In contrast, a significant decrease in mean % predicted FEV₁ was observed following first detection of mucoid *P. aeruginosa* and following chronic infection with mucoid *P. aeruginosa*. In contrast, Li and colleagues did notice a statistically insignificant trend toward worsening of cough and Wisconsin chest radiograph scores with first isolation of nonmucoid *P. aeruginosa* (346). Additional studies are necessary to determine whether infection with nonmucoid *P. aeruginosa* has a small negative impact on lung function in individuals with CF.

(ii) Antibiotic resistance. Because of the prolonged time during which *P. aeruginosa* inhabits the respiratory airways of CF patients and the repeated courses of antibiotics to which it is exposed, antimicrobial resistance is common (461). Mutations frequently occur in genes controlling production of efflux pumps and β -lactamases (556), creating antibiotic-resistant lineages of *P. aeruginosa* that expand under the selective pressure of antimicrobial therapy. Whether infection with highly resistant strains of *P. aeruginosa* is associated with poorer outcomes in CF is less clear, and surprisingly few studies have addressed this question. Al-Aloul and coworkers observed that patients chronically infected with a multidrug-resistant epidemic strain of *P. aeruginosa* had a 4.4% greater annual decrease in % predicted FEV₁ (95% CI, -8.1 to -0.9; $P < 0.02$) and 0.7 greater decrease in annual rate of change in body mass index (95% CI, -1.2 to -0.2; $P < 0.01$) than matched controls infected with nonepidemic *P. aeruginosa* strains (9). It is unclear, however, whether these differences were independently associated with antibiotic resistance or rather with other properties of the epidemic strain, such as enhanced virulence potential. Lechtzin and colleagues examined the effect of multidrug resistance on outcomes over a 33-month period in 75 CF patients infected with *P. aeruginosa* (340). They defined multidrug resistance as lack of susceptibility to all antibiotics tested in at least two of the following classes: fluoroquinolones, β -lactams, and aminoglycosides. Compared to patients infected with antibiotic-susceptible *P. aeruginosa*, those infected with multidrug-resistant *P. aeruginosa* had an increased risk of death or

lung transplantation and a more rapid decline in FEV₁. The reasons for this may be that CF patients infected with highly resistant strains derive less benefit from antimicrobial therapy, that the level of resistance simply correlates to the number of antibiotic courses a patient has received and is therefore a marker for patients with more advanced disease, or that resistance to multiple antibiotics is a marker for strains that have enhanced virulence due to other accompanying traits (1). Although the first explanation would appear to be the most likely, it should be noted that susceptibility to administered antibiotics has not been associated with improved outcomes in CF patients infected with *P. aeruginosa* (554).

Whether worse outcomes occur following lung transplantation in CF patients infected with multidrug-resistant *P. aeruginosa* than in those infected with susceptible bacteria is unclear. Two studies did not observe worse outcomes in this setting (18, 153), but a third study did. Hadjiliadis and colleagues found that 43.7% of 103 CF patients undergoing lung transplantation were infected with panresistant bacteria other than *Burkholderia* (235). All but two of these bacteria were *P. aeruginosa*. Decreased survival was observed in the patients infected with panresistant bacteria relative to those infected with sensitive bacteria (58.3% versus 85.6% at 5 years; $P = 0.016$).

(iii) Modification of LPS. Gram-negative bacteria such as *P. aeruginosa* are surrounded by an outer membrane that has an outer leaflet comprised largely of lipopolysaccharide (LPS). LPS consists of three parts (Fig. 6) (457, 476): (i) toxic and highly acylated lipid A, which replaces the phospholipids found in most plasma membranes; (ii) central core oligosaccharide, which is attached to lipid A and contains several unusual sugars; and (iii) O antigen, which is a variable and nonessential polysaccharide comprised of repeating units that extends outward from the core. Given the strategically important location of LPS at the interface of *P. aeruginosa* with the pulmonary environment, it is not surprising that this structure is modified in *P. aeruginosa* isolates from CF.

P. aeruginosa isolates frequently fail to produce normal amounts of high-molecular-weight O antigen during CF respiratory infections (239, 334) (Fig. 6). Since O antigen is responsible for resistance to the killing effects of human serum (128, 239, 527), this may explain why *P. aeruginosa* bloodstream infections so seldom occur in these patients (224). However, O antigen is also highly immunogenic and elicits a strong antibody response. Thus, the absence of O-antigen may facilitate chronic persistence within the respiratory tracts of individuals with CF. Loss of O antigen results from the accumulation of mutations such as small frameshift deletions or integration of insertion elements in the cluster of biosynthetic genes responsible for O-antigen production (182, 329, 570) or by deletion of all or large parts of this locus (176). Such mutations appear to be relatively common, in that O-antigen production was absent or decreased in 13 of 16 CF isolates examined in one study (239).

More recently, it has been shown that the lipid A portion of *P. aeruginosa* LPS is also altered in CF (Fig. 6). *P. aeruginosa* isolates from chronically infected CF patients synthesize lipid A with distinctive acylation patterns (179). Whereas lipid A from *P. aeruginosa* isolates from the environment or from non-CF infections is predominantly penta-acylated (i.e., contains five acyl groups embedded within the lipid bilayer of the

FIG. 6. Modifications of *P. aeruginosa* LPS during CF respiratory infections. (A) Structures of LPSs from strains recovered from the environment, acute infections, or bronchiectasis. The lipid A, core oligosaccharide, and O-antigen polysaccharide components are indicated. In the lower panel, a more detailed structure of lipid A is shown. The large polygons represent the diglucosamine bisphosphate backbone of lipid A, and the staggered lines represent acyl groups. (The placement of the lipid A acyl chain shown in gray is unclear [177, 179].) (B) During early infection in individuals with CF, the O-antigen polysaccharide is frequently lost. Also, palmitate (shown as a red staggered line) and aminoarabinose (shown as a red hexagon) are added to the lipid A portion of LPS. (C) In patients with advanced CF, a hydroxydecanoate chain (shown as a red staggered line) is retained, likely due to mutations in the *pagL* gene, which encodes a lipid A deacylase (89, 175).

outer membrane), lipid A from CF isolates contains substantial amounts of hexa- and hepta-acylated (containing six or seven acyl groups) lipid A (89, 177, 179). Ernst and colleagues found that 100% of 86 CF isolates from 58 CF patients contained an additional palmitate acyl group, whereas none of the 27 examined isolates from the environment, individuals with bronchiectasis, or patients with acute infections had this modification (178). Hexa-acylation was observed in initial *P. aeruginosa* isolates from infants <1 year of age, indicating that it occurs quite early during infection. In individuals with advanced CF disease, mutations in the *pagL* gene, which encodes a lipid A deacylase, are thought to cause retention of a hydroxydecanoate acyl chain (Fig. 6C), resulting in hepta-acylation (89, 175). Another alteration to lipid A observed in *P. aeruginosa* isolates from CF patients is the addition of aminoarabinose, a positively charged amino sugar residue (178) (Fig. 6). In one study, one-third of CF isolates were modified in this way, compared to no isolates from other sources (178). Lipid A modifications have important biological consequences. For instance, addition of aminoarabinose enhances resistance to antimicrobial peptides and some antibiotics (179). Acylation levels affect LPS recognition by and signaling through human Toll-like receptor 4 (TLR4) and the subsequent robustness of the induced proinflammatory response (13, 236). Indeed, LPS from a *P. aeruginosa* isolate cultured from a CF patient late in the course of disease induced less inflammation than LPS from an early isolate from the same patient (89). Thus, LPS modifications in CF may function to make *P. aeruginosa* less visible to the host immune system.

No data are currently available to assess the impact of LPS alterations on the clinical outcomes of CF patients, although

one study noted that hepta-acylated lipid A was found only in *P. aeruginosa* strains cultured from patients with “severe” CF lung disease (178).

(iv) Loss of type III secretion. The majority of *P. aeruginosa* isolates from the environment and from acute infections have the ability to secrete a set of toxic effector proteins that includes ExoS, ExoT, ExoU, and ExoY (242). Upon direct contact of *P. aeruginosa* with host cells, these proteins are injected into these cells through an elaborate apparatus called a type III secretion system. During infection of the CF lung, however, *P. aeruginosa* strains gradually lose the ability to secrete these effector proteins (122, 288, 344, 509). Although 90% of *P. aeruginosa* isolates from the environment secreted type III proteins, only 45 to 49% of isolates from newly infected children, 18 to 29% of isolates from chronically infected children, and 4 to 12% of isolates from chronically infected adults with CF secreted these proteins (287, 288) (Fig. 7). In some strains, the defect in secretion lies in the signaling networks regulating this system and not in the system itself, since complementation with an intact copy of the gene encoding ExsA, the immediate transcriptional activator of the type III system, restores secretion (121, 344). In fact, sequencing studies have confirmed that the genes encoding upstream regulators such as Vfr or ExsA itself are sometimes the sites of mutations that inactivate this system in CF (556). Even strains that retain functional type III secretion may inject proteins that have lost their toxic activities, apparently because of mutations in the genes that encode them (344). Thus, *P. aeruginosa* strains that secrete type III effector proteins appear to be at a selective disadvantage during chronic infection in CF.

The selective pressure driving the persistence of *P. aerugi-*

FIG. 7. The proportion of *P. aeruginosa* isolates with functional type III secretion systems decreases with duration of infection in CF. TTS⁺, functional type III secretion system. Error bars indicate standard deviations. (Adapted from reference 288.)

nosa clones that fail to secrete type III proteins may be the result of several factors. Individuals with CF mount an antibody response against type III proteins (33, 109, 408). The presence of antibodies against at least one of these type III proteins, PcrV, is protective and results in clearance of *P. aeruginosa* during acute non-CF pulmonary infections (185, 200, 520, 541). Therefore, it is conceivable that over time secretion-positive strains are cleared from CF patients whereas secretion-negative strains are not. Alternatively, the damage to the host induced by type III toxins may not be consistent with long-term residence within the human respiratory tract (422). This idea is supported by the paucity of CF isolates that harbor the *exoU* gene, which encodes the most cytotoxic of the type III secreted proteins (189, 288, 337).

A single study has addressed the question of whether adapted *P. aeruginosa* strains with disrupted type III secretion are associated with changes in the clinical status of CF patients. In a longitudinal study of 114 *P. aeruginosa*-infected CF patients, no overall association between the proportion of type III-secreting isolates cultured from patients and their annual change in % predicted FEV₁ was observed (287). However, in the subset of patients in whom at least one isolate had a type III secretion-positive phenotype, a statistically significant association between an increased proportion of type III-secreting isolates and a more rapid decline in FEV₁ was observed. These results are intriguing and suggest that further study of type III secretion in CF is warranted.

(v) Loss of motility. *P. aeruginosa* strains cultured from the airways of patients with CF often are defective in swimming motility; that is, they fail to produce fully functional flagella (75, 357, 368). Furthermore, this loss of motility occurs during the course of infection, since *P. aeruginosa* isolates cultured early during infection are motile (368). The basis for this gradual loss of swimming motility appears to be related to acquisition of mutations in one of several genes that regulate production of flagella, including *rpoN*, *vfr*, and *fleQ* (556), although downregulation of the *fliC* gene (which encodes flagellin, the structural subunit of the flagellum) in

response to CF airway fluid may also play a role (301, 659). The selective pressures driving this adaptation may include the resistance to phagocytosis (368, 370) or decreased immune recognition through TLR5 (673) that accompanies loss of flagella. TLR5 recognizes flagellin and signals for upregulation of the proinflammatory response (98, 282, 673).

Many strains of *P. aeruginosa* are also capable of movement over surfaces by a process called twitching motility. Twitching motility is mediated by the extension and retraction of type IV pili, proteinaceous filamentous appendages on the surface of *P. aeruginosa* (383). Chronic infection in CF is associated with loss of twitching motility by several mechanisms (342, 449). Mutations in the *pilB* gene disrupt production of the PilB protein, which is essential for pilus biogenesis (329, 556). Likewise, mutation of the *pilQ* gene, which encodes a transmembrane protein essential for extrusion of the pilus through the bacterial outer membrane, may occur (82). Finally, strains from chronically infected patients may lack RpoN, a sigma factor necessary for production of type IV pili (368, 556). It is likely that many additional mechanisms also contribute to the loss of twitching motility in isolates from CF patients.

Whether loss of motility in *P. aeruginosa* strains leads to worse clinical status in CF patients has not been examined. One study compared *P. aeruginosa* isolates cultured from CF patients with advanced disease to those from patients in “good” clinical condition (357). Isolates from patients with advanced disease were more likely to be defective in swimming motility and to lack flagella, but this may simply reflect the longer times during which these strains resided in the CF lung and underwent adaptation.

(vi) Auxotrophy and metabolic adaptations. *P. aeruginosa* respiratory isolates from CF patients frequently grow slowly on defined laboratory media, suggesting the presence of defective metabolic pathways (248). This is indeed the case. From 36 to 86% of CF patients are infected with *P. aeruginosa* auxotrophs, bacteria unable to survive in the absence of growth supplements not required by wild-type (prototroph) strains (599, 600). In an individual patient, auxotrophs comprised from 0%

FIG. 8. Examples of *P. aeruginosa* colonies of normal morphology (left) and SCVs (right). (Reprinted from reference 245 with permission of the publisher.)

to 90% of the total *P. aeruginosa* isolates recovered from the airways (196, 600). These auxotrophs most often required supplements of the amino acids methionine, leucine, and arginine (37, 600). Patients harbored auxotrophs and prototrophs of the same genotype, indicating that the auxotrophs were derived from the wild-type bacteria (37). The high concentrations of free amino acids in CF respiratory secretions apparently allow these auxotrophic strains to survive during infection and obviate the need for the biosynthetic pathways that would otherwise synthesize these molecules (38, 431, 565). Consistent with this notion is the increased expression of genes involved in arginine uptake and metabolism in some CF isolates (257). Furthermore, some *P. aeruginosa* clones within the CF airways contain mutations in the *lasR* gene, which actually enhance their ability to utilize exogenous amino acids and other nutrients and thus grow more rapidly than parental strains (125). [This will be discussed further in “(viii) Defects in quorum sensing” below.] Thus, the high amino acid content of CF respiratory secretions may supply a strong selective pressure on *P. aeruginosa*. Evidence suggests that additional metabolic adaptations also occur in CF, such as altered regulation of carbon metabolism (547).

Two studies have suggested that auxotrophic *P. aeruginosa* strains are associated with CF exacerbations or more severe disease. Taylor and colleagues noted that *P. aeruginosa* auxotrophs were more commonly cultured from CF patients experiencing exacerbations or with severe baseline lung disease than from those who were clinically stable or had mild underlying lung disease (599). Thomas and colleagues examined the sputa of 60 CF patients for the presence of auxotrophs (603). They found that the percentage of auxotrophs in a given patient was inversely correlated with FEV₁.

(vii) Small-colony variants. *P. aeruginosa* can also form SCVs in the context of CF (Fig. 8). Because they take more than 48 h to appear on culture plates, SCVs are easily missed in clinical practice, but they are thought to be present in about 10% of the respiratory specimens of CF patients (529). *P. aeruginosa* SCVs have special characteristics: they autoaggregate in liquid culture, are hyperadherent to surfaces, exhibit reduced motility, and, importantly, often have enhanced resistance to antibiotics (59, 148, 158, 244, 245). These features promote a biofilm mode of growth *in vitro* and are thought to do the same in the CF airways (148, 316). Mutations or changes in expression of chemosensory (*wspF*), exopolysaccharide (*psl* and *pel*), and two-component system (*pvrR*) response regulators may contribute to the SCV phenotype in *P. aerugi-*

nosa (124, 158, 316, 574), although current evidence suggests that individual SCVs differ significantly from one to another in their gene expression patterns (245, 316, 631). Thus, SCVs may represent a heterogeneous group of bacteria that share only a subset of their phenotypes.

The clinical relevance of *P. aeruginosa* SCVs is unclear. Schneider and coworkers compared the clinical status of 53 CF patients infected with normal-morphology *P. aeruginosa* isolates to that of 9 patients infected with SCVs. SCVs were associated with lower body mass index, % predicted FEV₁, and oxygenation (529). In a study of 88 *P. aeruginosa* culture-positive CF patients, Haussler and colleagues found that isolation of SCVs was associated with lower % predicted FEV₁ (56 versus 80; $P < 0.001$) and lower % predicted FVC (75 versus 87; $P < 0.005$) (244).

(viii) Defects in quorum sensing. Quorum sensing is a mechanism by which individual bacteria communicate with one another to alter gene expression in response to changes in population density (533). To accomplish this, bacteria secrete molecules referred to as autoinducers, the concentrations of which are detected by other bacteria within the population. *P. aeruginosa* produces several autoinducers, but two small molecules have been most extensively studied: 3-oxo-dodecanoyl homoserine lactone, which is produced by the LasI/LasR system, and butyryl homoserine lactone, which is produced by the RhII/RhlR system (448). *In vitro*, quorum-sensing systems modulate expression of 6 to 10% of the genes in the *P. aeruginosa* genome (534, 633), including several that encode important virulence determinants such as elastase, alkaline protease, phospholipase C, pyocyanin, and exotoxin A (211, 257, 524, 583).

Given the high density of *P. aeruginosa* in CF sputum, it was expected that quorum sensing would be active in the CF airways, and initial reports indicating that acyl homoserine lactones were indeed present in CF sputum were not surprising (80, 174, 398, 550, 583). It was assumed that this system played an important role in the pathogenesis of *P. aeruginosa* infection of the CF lung through its regulation of virulence factors and promotion of biofilm formation. Subsequently, however, it has been appreciated that many *P. aeruginosa* isolates from CF patients fail to produce homoserine lactones (125, 342, 556, 647) and that 3-oxo-dodecanoyl homoserine lactone is not detectable in the sputa of 22 to 46% of CF patients infected with *P. aeruginosa* (80, 174, 342, 398). It is now clear that mutations in the *lasR* and *rhlR* genes account for loss of quorum sensing in many CF isolates. These mutations occur approximately 15 years following the onset of lung infection (55). One explanation for these apparently paradoxical findings is that quorum sensing does indeed play an important role in CF pulmonary infections but that the metabolic cost of producing the large number of factors under the control of these systems encourages the emergence of “social cheaters,” clones of bacteria that do not themselves respond to autoinducers but benefit from the autoinducer-induced factors synthesized by their neighbors (150, 518). However, in some CF patients the proportion of *P. aeruginosa* isolates lacking functional quorum-sensing systems exceeds 80%, making social cheating an unlikely explanation (647). It may simply be that quorum sensing is not required (or may even be detrimental) once chronic infection is established. Since quorum sensing controls produc-

tion of several virulence determinants, this would be consistent with reports indicating that other virulence systems such as type III secretion and flagella are lost once chronic infection ensues. Alternatively loss-of-function mutations in *lasR* may confer an advantage in CF by altering gene expression patterns and leading to an increased growth rate due to better utilization of amino acids found in high concentrations in the CF airways (125), to an increased ability to utilize nitrate instead of oxygen as an electron receptor during growth in the anaerobic niches of the CF airways (261), and to increased resistance to antibiotics (125, 261). For example, *lasR* mutants produced higher levels of β -lactamases, resulting in increased tolerance to β -lactam antibiotics (125). In any case, it appears that the role of *P. aeruginosa* quorum sensing in CF is more complex than initially anticipated (253, 648, 656).

Some evidence supports a role for *lasR* mutations in progression of lung disease in CF. In a retrospective study, Hoffman and colleagues found that 31% of 166 *P. aeruginosa* isolates from 58 CF patients had colony morphologies consistent with inactivation of *lasR* (260). Analyzing a subset of 44 patients, they found that isolation of bacteria with *lasR* mutant phenotypes was associated with a more rapid decline in % predicted FEV₁ than isolation of bacteria with wild-type *lasR* phenotypes (−4.1 versus −2.3 per year of age). Additional studies are necessary to determine whether these differences are significant.

(ix) Hypermutable phenotype. As with *S. aureus* and *H. influenzae*, *P. aeruginosa* isolates from CF patients may exhibit a hypermutable phenotype. Such strains account for 37% to 54% of *P. aeruginosa* isolates in CF (compared to 1% in acute infections [232] and 6% of environmental isolates [309]) and become more common in the later stages of infection (97, 262, 435). In *P. aeruginosa*, hypermutable strains result from mutations in the *mutS*, *mutL*, and *uvrD* genes, which encode proof-reading proteins responsible for correcting errors that occur during DNA replication (395, 402, 435, 646). Hypermutable strains have mutation rates that are increased by 20- to 1,000-fold. For this reason, some investigators have argued that the hypermutable phenotype is responsible for the emergence of many of the adaptations described in the preceding sections (592), but others have suggested that the hypermutable phenotype emerges late in CF, after mutations affecting such properties as alginate production and quorum sensing have already occurred (96, 188).

Two studies have examined the relationship between hypermutable *P. aeruginosa* and clinical status in CF. In the first study of 40 adult CF patients, the hypermutable phenotype was associated with lower % predicted FEV₁ ($P = 0.008$) (634). In the second study, *P. aeruginosa* strains from 36 chronically infected CF patients were examined (190). Patients infected with hypermutable strains had significantly lower % predicted FEV₁ (43% versus 69%; $P = 0.023$) and FVC (64% versus 80%; $P = 0.025$). The results of both studies, however, may merely reflect that hypermutator strains are more likely to evolve in patients with advanced disease or that such patients may expectorate more sputum, allowing better sampling of the lower respiratory tract for the presence of hypermutator strains (634). Thus, whether the presence of hypermutable strains leads to more rapid clinical deterioration in CF is unclear, but several theoretical considerations suggest that this may be the

case. Hypermutable strains are more resistant to antibiotics (190, 360, 436, 646), more likely to be mucoid (410, 634) or defective in quorum sensing (354), more metabolically adapted to the CF airways (257), and in general more adaptable to the harsh environment of the CF airways (395, 592).

(x) Other adaptations. Table 1 lists several other adaptations that have been reported for *P. aeruginosa* strains isolated from chronically infected CF patients. Some of these are undoubtedly at least in part the consequence of the modifications described in the previous sections. For example, type II secretion is under the control of the LasI/LasR quorum-sensing system, so mutations in *lasR* would be expected to cause decreased secretion of exotoxin A, phospholipase C, and elastase (583). Others are likely to be the result of distinct mutations, such as the loss of pyoverdine production, which in at least one case was due to deletion of a large genetic locus encoding pyoverdine synthetic and uptake proteins (176).

(xi) Summary of *P. aeruginosa* adaptations in CF. As the preceding discussions demonstrate, the CF airways provide strong selective pressures against *P. aeruginosa* and lead to a number of interesting adaptations in this bacterium. Although the exact nature of these pressures remains unclear, three themes regarding adaptation have emerged. First, *P. aeruginosa* apparently does not require many of its factors to persist in the CF lung. This is perhaps most intuitive with auxotrophic mutations; the plentiful supply of certain amino acids in the CF airways obviates the need for *P. aeruginosa* to synthesize them. Second, many of the adaptations involve the gradual loss of virulence factors crucial for acute infections. Thus, it is not surprising that *P. aeruginosa* isolates from chronically infected CF patients are less virulent than other *P. aeruginosa* strains when tested in animal models of acute infection (356) or even than clonal isolates collected from the same patients at earlier stages of infection (67, 75). This attenuation of virulence may help the bacteria hide from the host immune response by eliminating factors detected by the host and by causing less tissue damage, which itself may stimulate an inflammatory response. Alternatively, strains not burdened with the production of multiple virulence determinants may be able to grow more rapidly and thus have a fitness advantage (428). A third theme is that many of the adaptive phenotypes are interrelated. For example, MucA regulates not only the mucoid phenotype but also type III secretion (295, 664) and indirectly flagellum genes (207, 596, 597). LPS modifications (175, 179), the SCV phenotype (243), and defects in quorum sensing (125, 261, 493) are all associated with increased resistance to antibiotics. A proportion of SCVs exhibit auxotrophy (574), and quorum sensing indirectly regulates type III secretion (263). Thus, a single mutation may lead to a number of adaptive phenotypes. While it is inferred from their selection that adapted strains of *P. aeruginosa* have enhanced survival in CF, it remains unclear whether these adapted strains in turn cause more severe clinical disease. Although a number of studies have demonstrated an association between adaptive phenotypes and advanced disease in CF, it is not known whether these associations are causal. Patients with advanced disease are more likely to have harbored *P. aeruginosa* strains in their lungs for many years, and these strains are therefore more likely to have acquired adaptive mutations. Similarly, strains from chronically infected patients are likely to have multiple

TABLE 2. BCC species^a

Species	Former genomovar designation	% of <i>Burkholderia</i> isolates from CF patients in the United States
<i>B. cepacia</i>	I	3
<i>B. multivorans</i>	II	40
<i>B. cenocepacia</i>	III	45
<i>B. stabilis</i>	IV	<1
<i>B. vietnamiensis</i>	V	6
<i>B. dolosa</i>	VI	4
<i>B. ambifaria</i>	VII	1
<i>B. anthina</i>	VIII	<1
<i>B. pyrrocinia</i>	IX	<1
<i>B. latens</i> , <i>B. diffusa</i> , <i>B. arboris</i> , <i>B. seminalis</i> , <i>B. metallica</i> , <i>B. contaminans</i> , <i>B. lata</i>		NR ^c
<i>B. ubonensis</i> ^b		0

^a Based on data from references 314, 350, 351, 440, 494, 622, 623, and 666.

^b Not reported to occur in CF.

^c NR, not reported.

adaptations, so attributing poor outcomes to any one adaptation is precarious. Untangling such associations will be difficult and likely will require large longitudinal studies utilizing genomic approaches.

Burkholderia cepacia Complex

The *Burkholderia cepacia* complex (BCC) is a group of Gram-negative bacteria that are widely distributed in the natural environment. They were first reported as pathogens in CF in 1972 (162). By the 1980s, the BCC had emerged as a significant problem in CF clinics around the world (110, 284, 590, 605), with some CF centers reporting prevalence rates as high as 40% (514). The institution of strict infection control practices has been accompanied by a substantial decrease in the incidence of BCC infections in CF (191, 571), although it has been argued that some of this decrease may be due to increasingly sophisticated microbiological techniques that accurately identify BCC-like organisms as other bacterial species (350). In any case, the CFF Patient Registry indicated that in 2008, respiratory cultures from 2.8% of individuals with CF grew BCC (118).

The shifting taxonomy of the BCC is the minotaur's maze of CF microbiology, but we will briefly thread our way through it. In the 1970s, this group of bacteria was thought to constitute a single species referred to as *Pseudomonas cepacia*, which was subsequently reclassified as *Burkholderia cepacia* in 1992 based on genotypic features (667). As these organisms were further characterized, it became clear that *B. cepacia* actually comprised a group of several related but distinct bacteria, many of which were previously referred to as genomovars but have now been given species designations. Currently this group of related bacteria is referred to as the BCC and consists of 17 species, all but one of which has been isolated from CF patients (Table 2) (616, 617, 622, 623). Because species within the BCC are phylogenetically related, it is difficult to separate them using biochemical tests, and limited diversity precludes the use of 16S rRNA sequencing for this purpose (105). Sequence variation of the

recA gene and multilocus sequence typing, however, do allow species discrimination (28, 365, 366). Interestingly even within a given BCC species, heterogeneity exists. For example within the species *B. cenocepacia*, *recA* gene heterogeneity has led to the identification of four phylogenetic lineages (IIIA, IIIB, IIIC, and IIID), of which only IIIC has not been cultured from patients (375). Although most BCC species have been recovered from the sputa of individuals with CF, two species, *B. cenocepacia* and *B. multivorans*, account for the majority of CF isolates (Table 2).

Another *Burkholderia* species, *B. gladioli*, is phenotypically quite similar to and often confused with BCC bacteria but is phylogenetically distinct and therefore not a member of the BCC group (644). It also causes both transient and chronic infections in CF patients (310, 653). *B. pseudomallei*, the etiologic agent of melioidosis, occasionally causes respiratory infection in individuals with CF, suggesting that this species of *Burkholderia* must also be considered, especially when a history of travel to South and Southeast Asia, China, or northern Australia is elicited (36, 112, 271, 531, 628).

BCC has acquired much notoriety for its predilection to spread rapidly among CF patients. Interpatient spread became recognized as an important clinical problem in the 1980s and 1990s, when reports describing nosocomial and social transmission of BCC appeared (221, 352, 401, 604). DNA genotyping techniques demonstrated patient-to-patient transmission both within and outside the health care setting (7, 367, 454). One strain, labeled ET-12, first infected patients in Toronto, Canada, and subsequently spread across Canada as well as the United Kingdom, probably through patient contact at CF summer camps (586). Spread even to individuals who did not have CF occurred (272). Stringent adherence to infection control practices have decreased but not eliminated the prevalence of BCC infections in CF patients (451, 489); it is now felt that a substantial proportion of the remaining BCC infections are acquired directly from the natural environment (427).

Unlike *S. aureus* and *H. influenzae* infections, BCC infections usually occur later in the course of CF pulmonary disease (Fig. 1). BCC species initially cause transient infections in the CF airways before a single strain becomes established and causes chronic infection marked by episodes of exacerbations (135, 353, 371, 576). Early reports from the 1980s suggested that *Burkholderia* species were associated with worse outcomes in CF (284, 589). In a seminal report, Isles et al. noted that the proportion of CF patients at the Hospital for Sick Children in Toronto from whom BCC (then referred to as *P. cepacia*) was isolated increased from 9.6% in 1970 to 18.1% in 1981 (284). Patients infected with BCC had lower % predicted FEV₁ and lower % predicted FVC values than patients from whom only *P. aeruginosa* was cultured or from whom neither *P. aeruginosa* nor BCC strains were cultured. In another early report, Tablan and colleagues studied 85 CF patients who grew BCC from respiratory samples for the first time between 1981 and 1983 (590). Twenty-nine (34%) of the patients died during the 3.5-year follow-up period of the study.

Subsequent studies confirmed that BCC was associated with worse pulmonary status and increased mortality, even more so than *P. aeruginosa* (171, 345, 371, 567, 598, 645). De Boeck and colleagues, for example, compared 12 Belgian CF patients infected with BCC to controls matched for sex,

pancreatic status, genotype, and infection with *P. aeruginosa* (135). Lung function was significantly decreased in BCC-infected patients relative to controls (% predicted FVC, $57\% \pm 6\%$ versus $80\% \pm 8\%$ [$P < 0.01$]; % predicted FEV₁, $41\% \pm 7\%$ versus $64\% \pm 8\%$ [$P < 0.05$]). Lewin and colleagues studied 124 CF patients infected with BCC and compared them to sex- and age-matched controls not infected with BCC (345). In the first year following BCC infection, 32 BCC-infected patients (26%) died, compared to only 8 control patients (6%) ($P < 0.001$). Thus, it appeared that BCC was associated with alarming clinical deterioration.

BCC infections, however, tended to occur in patients with more severe preexisting pulmonary disease, so it was unclear whether BCC was causing poor outcomes or whether it was simply a marker for patients with especially severe disease who were destined to do poorly. Subsequent studies that controlled for baseline pulmonary function supported the former as the true explanation. Muhdi and colleagues followed 16 BCC-infected patients and 16 control CF patients matched for baseline FVC as well as age and gender (412). They noted significantly higher rates of decline in mean FEV₁ (-29.2 versus $+7.0$ ml/month, respectively; $P < 0.05$) and in mean FVC (-41.4 versus $+16.5$ ml/month, respectively; $P < 0.02$) in BCC-infected patients than in controls. In a study by Frangolias and colleagues, 36 BCC-infected CF patients from the adult CF clinic at St. Paul's Hospital in Vancouver were matched at the time of acquisition of BCC with controls for gender, age, % predicted FEV₁, height, weight, and pancreatic sufficiency (199). BCC-infected patients had increased long-term mortality (9 BCC patients [25%] versus 4 controls [11%]; $P = 0.04$) and a higher frequency of intravenous antibiotic therapy. Trends toward more rapid decline in long-term pulmonary function were also noted but did not achieve statistical significance. Ledson and colleagues followed for 5 years 107 patients who attended the Liverpool adult CF clinic, 37 of whom were infected with an epidemic strain of BCC (341). Cox proportional hazards analysis indicated that infection with BCC (hazard ratio, 7.92; 95% CI, 2.65 to 23.69; $P < 0.001$) and lower % predicted FEV₁ (hazard ratio, 1.1; 95% CI, 1.06 to 1.14; $P < 0.001$) were both significant and independent risk factors for death.

It is important to note that many of these reports were heavily influenced by BCC outbreaks, and the clinical outcomes associated with them may be representative not of BCC species in general but rather only of the particular outbreak strain. Since different strains harbor different sets of putative virulence determinants, such as the cable pilus (515), the *Burkholderia cepacia* epidemic strain marker (BCESM) (369), and antibiotic resistance determinants (674), it is quite possible that single-site studies fail to reflect the overall properties of the BCC species. Several investigators have attempted to remedy this by examining populations from large CF patient registries. A multivariate logistic regression model of 5-year survivorship in CF was constructed by Liou and colleagues using patients in the CFF Patient Registry. Their model indicated that infection with BCC had the greatest negative impact on mortality and that this impact was independent of baseline % predicted FEV₁ (348). In fact, infection with "*B. cepacia*" had an impact on mortality equivalent to a 48% drop in % pre-

dicted FEV₁. Similarly, Corey and Farewell analyzed 3,795 CF patients in the Canadian Patient Data Registry between 1970 and 1989 and found that of the factors they examined, BCC most highly associated with mortality (hazard ratio, 3.22; 95% CI, 2.33 to 4.46; $P < 0.001$) (111); BCC increased the risk of mortality at all levels of lung function, as measured by % predicted FEV₁.

Cepacia syndrome. In 1980, Rosenstein and Hall reported a CF patient with *B. cepacia* pulmonary infection complicated by bacteremia (507). The report was notable because spread of bacteria from the lungs to the bloodstream is quite unusual in CF. Shortly thereafter, Isles and colleagues reported seven patients with previously mild to moderate pulmonary disease who had a fulminant infection associated with BCC, characterized by acute respiratory failure with extensive destruction of lung tissue and microabscess formation (284). It later became apparent that a minority of BCC-infected patients develop severe disease characterized by bacteremia, necrotizing pneumonia, fevers, hemodynamic instability, and rapid deterioration to death after a period of weeks to months but occasionally years (56). This dreaded clinical constellation of signs and symptoms, referred to as "cepacia syndrome," was subsequently noted at many, although not all (199), centers with large numbers of BCC-infected patients. Given that some BCC strains are highly transmissible, these cases caused particular angst within the CF community, as illustrated by this excerpt from an article by Govan and colleagues (221): "In October, 1991, patient E16 became colonized by a strain of *P. cepacia* that showed no phenotypic or genotypic relation to the epidemic strain. In March, 1992, however, in addition to the original *P. cepacia* strain, his sputum cultured the epidemic strain for the first time, an event that caused considerable anxiety to himself and to his girlfriend (patient E22) who was *P. cepacia* negative... After much discussion, and aware of the risks, the couple decided to continue their relationship. In May, 1992, patient E22 became colonized with the epidemic strain. She died 6 weeks later. . . Patient E16 experienced grief and a period of self-neglect after the death of E22 and became profoundly unwell. Despite aggressive antibiotic therapy, the epidemic strain continued to be isolated from his sputum and subsequently from blood cultures. He died 1 month later. Patient E16 had only been in hospital once, 3 years before his terminal illness." While some of the increased mortality associated with BCC is attributable to the cepacia syndrome, it is likely that other consequences of infection, such as chronic decline in pulmonary function, also contribute (187).

Individual species and outcomes. Although the bulk of early studies on *Burkholderia* and CF simply refer to "*B. cepacia*" or BCC, more recent reports have begun to tease out the contributions to disease of individual species within the BCC.

B. cenocepacia is the species that has been most closely associated with high rates of morbidity and mortality as well as the development of the cepacia syndrome, and many of the epidemic strains originally described as "*B. cepacia*" are now known to be *B. cenocepacia* (296, 341). A retrospective study compared 31 patients chronically infected with *B. cenocepacia* to matched *P. aeruginosa*-infected controls (296). Five-year survival was stated to be 67% in the *B. cenocepacia* group and 85% in the *P. aeruginosa* group, a difference that was statistically significant ($P < 0.01$). Two patients with *B. cenocepacia*

infection died of the cepacia syndrome. A trend toward greater decline in % predicted FEV₁ in the *B. cenocepacia*-infected group was noted. A second study also found the rate of % predicted FEV₁ decline to be greater in *B. cenocepacia*-infected patients than in *P. aeruginosa*-infected matched controls (115). In an 18-year observational study, cumulative mortality of *B. cenocepacia*-infected patients was 43%, compared to 16% of those infected with *B. multivorans* (371). The incidence of cepacia syndrome was 13% with *B. cenocepacia* versus 5% with *B. multivorans*. It is important to note that in the studies referenced above, *B. cenocepacia* infection at each center was usually due to a single epidemic strain. Thus, one cannot assume that all strains of *B. cenocepacia* are as virulent. In other words, it is not clear whether these results apply to patients infected with different strains of *B. cenocepacia*.

As suggested by the above-mentioned study, the data on clinical outcomes with the BCC species *B. multivorans* are less discouraging. Three reports compared *B. multivorans*-infected patients to non-BCC-infected controls and found no significant differences in mortality or decline in % predicted FEV₁ (115, 296, 304), whereas one small case-control study of seven BCC-infected patients (six of whom were infected with *B. multivorans*) showed significantly higher 6-month mortality (57% versus 16%; $P = 0.02$) in the BCC patients (187). Still, *B. multivorans* has been anecdotally reported to cause the cepacia syndrome (56, 371, 538, 669), pulmonary hypertension (187), respiratory failure requiring lung transplantation (146), and death (187). In summary, the impact of *B. multivorans* on outcomes in CF remains poorly defined but appears to be less severe than that of *B. cenocepacia*.

One report examined the outcomes for *B. dolosa*-infected patients who were part of an epidemic (304). A total of 31 patients infected with *B. dolosa* were compared to 58 age- and sex-matched but non-BCC-infected controls. Patients were well matched for % predicted FEV₁ at baseline and were followed for 2.5 years following acquisition of *B. dolosa*. The % predicted FEV₁ declined on average by 7.1% in the *B. dolosa* group compared to 0.5% in controls, and the hazard ratio for death was 10.8 (95% CI, 1.3 to 92.8) at 18 months for the *B. dolosa* cohort. The cepacia syndrome was also noted. This study suggests that at least some strains of *B. dolosa* are capable of dramatically impacting the disease course in CF patients.

Very little is known about the clinical impact of other BCC subspecies, though there are anecdotal reports of *B. stabilis* causing the cepacia syndrome (443) and of *B. vietnamiensis* leading to lung transplantation (146).

Are there common traits that explain why some BCC species or strains appear to be more virulent than others? Recent data suggest that the nonmucoid phenotype may be such a trait. Many species of BCC, like *P. aeruginosa*, have the potential to produce excessive amounts of exopolysaccharide (distinct from alginate), resulting in a mucoid phenotype (679). A naturally occurring nonmucoid isolate was shown to overexpress several putative virulence factors relative to an isogenic mucoid isolate, suggesting that nonmucoid strains may be more virulent than mucoid strains (680). Likewise, a survey by Zlosnik and colleagues showed that strains of *B. cenocepacia*, arguably the most virulent of the BCC species, were also most frequently nonmucoid (679). To further investigate the association between a nonmucoid phenotype and virulence, this group per-

formed a retrospective review of 100 CF patients and found that patients infected exclusively with nonmucoid BCC had a more rapid decline in % predicted FEV₁ than those infected with mucoid BCC ($-8.51\%/year$ versus $3.01\%/year$; $P < 0.05$) (678). Intriguingly, BCC strains may convert from a mucoid to a nonmucoid morphology during the course of chronic infection, and *in vitro* growth in the presence of ceftazidime or ciprofloxacin but not meropenem facilitated this conversion (678). Additional studies are necessary to confirm these findings and further explore the relationship between exopolysaccharide and virulence in BCC, especially in the context of antimicrobial therapy.

Impact of *Burkholderia* spp. on lung transplantation. BCC's impact on the survival of CF patients following lung transplantation is viewed as so detrimental that infection with these organisms is considered a relative or absolute contraindication for transplantation (234). Posttransplant survival rates are 15 to 63% lower than those of CF patients not infected with BCC (12, 142, 164, 234, 562), with death usually resulting from BCC sepsis in the early postoperative period (144). Chaparro and colleagues reported a 1-year posttransplant survival rate of 67% for individuals from whom BCC was isolated pretransplant versus 92% for BCC-negative patients (83). This increased mortality appears to be largely attributable to *B. cenocepacia* strains (64, 145, 146). Aris and colleagues reported a retrospective study of 121 CF patients who underwent lung transplantation, 21 of whom were infected with BCC prior to transplant (19). Mortality at 6 months posttransplant was 33% in the BCC group versus 12% in patients not infected with this organism ($P = 0.01$). Again, all the deaths in the BCC group were of patients infected with *B. cenocepacia*. Using a cohort of 528 CF lung transplant recipients, Murray and colleagues found that patients infected with *B. cenocepacia* or *B. gladioli* had significantly higher posttransplant mortality, whereas patients infected with *B. multivorans* did not have increased mortality compared to patients not infected with BCC (415). Furthermore, the risk appeared to differ even within *B. cenocepacia* species, as infection with (surprisingly) nonepidemic *B. cenocepacia* strains was associated with higher mortality. These results suggest that not all BCC species cause worse outcomes following lung transplantation and that even within the *B. cenocepacia* species itself, strain-specific differences in virulence exist.

Summary. The available evidence indicates that BCC causes substantial morbidity and mortality in CF, more so than other pathogens such as *S. aureus*, *H. influenzae*, and even *P. aeruginosa*. The negative impact of BCC on outcomes has become recognized to the extent that infection with this organism is grounds for exclusion from many clinical treatment trials and is considered a relative or absolute contraindication for lung transplantation. The ability of this bacterium to readily spread from patient to patient only enhances its potential to do harm.

Stenotrophomonas maltophilia

Stenotrophomonas maltophilia is a Gram-negative bacillus that frequently causes infection in immunocompromised patients (140) but was first reported to cause infection in CF patients in 1979 (57). This organism, which may prove challenging for some clinical microbiology laboratories to correctly

identify, was initially classified as *Pseudomonas maltophilia* (278, 279) before being renamed *Xanthomonas maltophilia* in 1983 (587) and finally *S. maltophilia* in 1993 (444). *S. maltophilia* is highly resistant to many antibiotics, including carbapenems (140), and it has been postulated that repeated broad-spectrum antibiotic exposure is a risk factor for acquisition of this bacterium (130). In 2008 its prevalence was 12.5% among individuals with CF in the United States (118) (Fig. 1) and up to 24 to 33% in some U.S. and European centers (120, 613); evidence suggests that its prevalence is increasing (172, 219, 376, 489, 594). The reservoir for this bacterium is the natural environment, including soil (especially in association with plant roots), streams, and rivers (140). Patient-to-patient transmission appears to be relatively rare (141, 330).

S. maltophilia infections in CF occur at all ages (118) (Fig. 1). It frequently is transiently isolated from the airways of CF patients but may become more firmly established and cause chronic airway infection, although this appears to be much less common than with *P. aeruginosa*, occurring in only 1 in 10 cases (139, 330, 613).

The clinical importance of *S. maltophilia* infections in CF has been debated (29, 214), and relatively few studies have examined the issue. Marchac and colleagues compared 63 *S. maltophilia*-infected CF patients to controls matched for age, sex, and FEV₁ (376). They found no difference in survival or change in lung function between the two groups. Talmaciu and colleagues matched 51 *S. maltophilia*-infected CF patients with 102 CF controls not infected with *S. maltophilia* (594). Patients were matched for age at the time of first infection with *S. maltophilia*. Patients infected with *S. maltophilia* had lower % predicted FEV₁ values and nutritional indices. The design of the study did not allow one to draw conclusions regarding whether *S. maltophilia* infection played a pathogenic role in worse clinical outcomes or whether this bacterium had a predilection for infecting individuals with preexisting severe disease. Demko and colleagues analyzed 773 individuals at the Cleveland CF Center from 1982 to 1994; *S. maltophilia* was cultured at least once from the respiratory secretions of 211 (139). Infection with this bacterium was not associated with worse clinical status except in patients with preexisting severe pulmonary disease. In this subset of patients, *S. maltophilia* was associated with a 5-year survival of only 40%, compared to 72% for those not infected with *S. maltophilia*. Waters and colleagues examined whether chronic infection with *S. maltophilia* (defined as ≥ 2 positive respiratory specimens in a year) was associated with pulmonary deterioration (637). They developed a serologic assay that identified CF patients chronically infected with *S. maltophilia*. This assay was then retrospectively applied to serum samples from a cohort of 692 CF patients, 7% of whom were chronically infected with *S. maltophilia*. Over a mean follow-up of 8.3 years, no difference in the rate of pulmonary decline was noted, but chronically infected patients had an increased risk of pulmonary exacerbation relative to patients who had never had *S. maltophilia* (RR, 1.63; $P = 0.0002$), even after adjustment for other factors associated with worse lung disease.

A much larger cohort study examined the role of *S. maltophilia* infection in survival using the CFF Patient Registry (220). Of the 19,255 patients included in the study, 1,673 (8.7%) had at some point produced respiratory secretions pos-

itive for growth of *S. maltophilia*. Relative to patients in the *S. maltophilia*-negative cohort, those in the *S. maltophilia*-positive cohort tended to be older, to be more likely female, and to have more severe baseline disease. For example, the *S. maltophilia*-positive cohort had a baseline % predicted FEV₁ of 63.0%, versus 72.3% for the *S. maltophilia*-negative cohort ($P < 0.001$). After a median follow-up of 3 years and after correcting for differences in baseline characteristics such as lung function, sex, age, and gender, no statistically significant difference in survival between the two cohorts was noted. In a second, similar study, this same group did not find an increased rate of decline in pulmonary function associated with *S. maltophilia* infection (219).

In summary, *S. maltophilia* has a predilection to infect CF patients with more advanced disease, perhaps because these individuals are more frequently exposed to broad-spectrum antibiotics that select for this bacterium. The role of *S. maltophilia* in disease progression, however, remains unclear, and more studies are needed to examine individuals chronically infected with this bacterium. Nevertheless, approximately half of physicians treat CF patients with antibiotics targeting *S. maltophilia* when it is cultured from respiratory secretions (578).

Achromobacter (Alcaligenes) xylosoxidans

Achromobacter xylosoxidans is an aquatic Gram-negative bacillus that is a pathogen in immunocompromised hosts. Like *Burkholderia* and *Stenotrophomonas* species, it is difficult to correctly identify and it suffers from a confusing nomenclature, having previously been referred to as *Alcaligenes xylosoxidans*, *Alcaligenes denitrificans* subsp. *xylosoxidans*, and *Alcaligenes xylosoxidans* subsp. *xylosoxidans*. Reports identifying this bacterium in the respiratory secretions of CF patients appeared in the 1980s (318, 466). Currently the prevalence of *A. xylosoxidans* in CF ranges from 2% to 11% (76, 306), and it appears to be increasing (172, 489). Infection is frequently transient, although approximately 2% of CF patients are chronically infected with this bacterium (595). Chronic infection is often due to isolates of the same genotype (306), though different genotypes have also been reported (330), suggesting reinfection. Consistent with cross-infection or a common-source outbreak, multiple patients at a single CF center may harbor the same strain (306, 504).

Relatively little is known about the clinical significance of *A. xylosoxidans* in CF. This bacterium has been associated with acute pulmonary exacerbations (407), although in some cases coinfection with *P. aeruginosa* may have accounted for these symptoms (160). Likewise, chronic infection with *A. xylosoxidans* has been associated with higher concentrations of the proinflammatory cytokine TNF- α in sputum than are seen in patients chronically infected with BCC or *P. aeruginosa* (240), suggesting that the organism does elicit an inflammatory response. However, a pathogenic role for this organism in CF has not been borne out by controlled studies. In a retrospective case-controlled study by Tan and colleagues, 13 patients chronically infected with *A. xylosoxidans* were compared to controls matched for age, gender, respiratory function, and *P. aeruginosa* infection status. No difference in change in pulmonary status, chest radiograph scores, or clinical condition between

the two groups was noted in the 2 years following *A. xylosoxidans* infection (595). In a small case-control study that compared CF patients chronically infected with *A. xylosoxidans* to age- and gender-matched *P. aeruginosa*-infected patients, *A. xylosoxidans*-infected patients had worse lung radiographic scores and lower FVC values at the time of initial infection, suggesting that individuals with more advanced disease may be predisposed to infection by this bacterium (134). However, no difference between the groups in body mass index or rate of lung function decline was noted over a mean follow-up period of 1.5 years. Another retrospective, case-control study enrolled 15 CF patients chronically infected with *A. xylosoxidans* and matched them for age, FEV₁, and body mass index to control CF patients not infected with *A. xylosoxidans* (504). The period of follow-up varied from 3 to 11 years. No significant difference between the two groups in overall rates of decline in % predicted FEV₁ or FVC or in change in body mass index was observed, although the authors did report a more rapid decline in lung function in a subgroup of patients with a marked increase in specific precipitating antibodies.

Despite the overall rather benign nature attributed to *A. xylosoxidans* by these studies, anecdotal cases of dramatic deterioration in pulmonary status following infection with *A. xylosoxidans* have been reported (500). At the Cincinnati CF Center, chronic infection of CF patients with a single clone of *A. xylosoxidans* (referred to as CNAX) was associated with worse outcomes relative to infection with unrelated *A. xylosoxidans* strains (390). The rate of decline in % predicted FEV₁ was significantly higher in nine patients infected with CNAX than in 16 patients infected with non-CNAX *A. xylosoxidans* (10.8% versus 3.8%; $P < 0.005$). Alarming, 10 of 11 patients in the original CNAX cohort from that institution died or received lung transplantation within 5 years of CNAX infection.

In summary, *A. xylosoxidans* infection occurs most commonly in CF patients with advanced lung disease, but the current evidence is insufficient to attribute a major role for most strains of *A. xylosoxidans* in disease progression. Additional studies are necessary to determine whether specific strains of *A. xylosoxidans* are capable of causing dramatic clinical deterioration.

Nontuberculous Mycobacteria

Nontuberculous mycobacteria (NTM) are increasingly recognized as inhabitants of the respiratory tracts of individuals with CF. An initial report of NTM isolated from CF sputum appeared in 1974 (265), which led to increased testing of respiratory secretions from CF patients for mycobacteria. As a result, several series were later published showing that these organisms were indeed relatively commonly isolated from CF patients (65, 256, 314, 460, 475, 558). More recently, a multicenter study of nearly 1,000 CF patients in the United States found an overall NTM prevalence of 13% but prevalences as high as 24% at some centers (439). A study of 1,582 CF patients attending centers in France found a prevalence of 6.6% (508). In both studies, *Mycobacterium avium* complex and *M. abscessus* were the species most commonly isolated, but many different mycobacterial species have been cultured from the respiratory secretions of patients with CF (Table 3). At least in

TABLE 3. Species of NTM cultured from the respiratory secretions of patients with CF^a

Species	% of NTM isolates from CF patients
<i>M. avium</i> complex.....	21–72
<i>M. abscessus</i> ^b	16–48
<i>M. goodii</i>	6–18
<i>M. chelonae</i>	3–8
<i>M. kansasii</i>	1–6
<i>M. fortuitum</i> ^c	1–4
<i>M. scrofulaceum</i>	3
<i>M. szulgai</i>	3
<i>M. xenopi</i>	2–3
<i>M. lentiflavum</i>	1–3
<i>M. simiae</i>	1
<i>M. malmoense</i>	1
<i>M. terrae</i> complex.....	1

^a Based on data from references 440, 460, and 508.

^b Includes *M. massiliense* and *M. bolletii*.

^c Includes *M. peregrinum*.

the case of *M. abscessus*, strains causing infections in CF are predominantly nonclonal, suggesting acquisition from the environment rather than patient-to-patient spread (298).

The prevalence of NTM in CF is highest in older patients, reaching 40% in those older than 40 years in one study (8, 439, 460). Interestingly, unlike many other bacteria that infect CF patients later in the course of their disease, NTM appear to preferentially infect patients with milder lung disease. For example, in a study of 55 CF patients over the age of 40 years, those diagnosed with CF later in life had milder disease and were less likely to be infected with *P. aeruginosa* but were three times more likely to have respiratory secretions that grew NTM than CF patients diagnosed early in life (494). In fact, one study found that 20% of 50 adults aged 28 to 82 years with bronchiectasis and/or pulmonary NTM infection had undiagnosed CF (676), which has led to the recommendation that individuals who present with bronchiectasis and are culture positive for NTM should be screened for CF regardless of age (494). The reason for the association between NTM and milder lung disease in CF is unclear and does not appear to be related to specific CFTR mutations (439).

The issue of whether NTM affects lung function in CF is complex and is complicated by the ubiquity of NTM in the environment (183). Thus, these bacteria frequently contaminate clinical samples or may be found transiently in the respiratory tract in the absence of disease. Anecdotal cases of dramatic declines in lung function, clinical deterioration, and even death following NTM infection in CF have been reported (65, 163, 246), as has improvement in clinical status following antimicrobial therapy directed against NTM (117, 186, 195, 437). Likewise, recurrent or fatal disseminated NTM infections in CF have occurred following lung transplantation (315, 519), while other patients have cleared this organism and done quite well posttransplantation (194). In other CF patients, NTM are isolated only once from respiratory secretions or are not associated with clinical deterioration even if repeatedly present over a period of years (8, 117, 256, 439). In this regard, a case report by Cullen and colleagues is particularly intriguing (117). They describe a CF patient who persistently grew *M. abscessus* from her sputum for 11 years while remaining clinically stable

but then developed clinically apparent disease. Her disease consisted of several exacerbations and a left upper lobe cavitary lesion that responded to anti-NTM treatment. The authors conclude that, "the repeated isolation of mycobacteria from the sputum of these patients should alert the clinician to the possibility of indolent disease."

Few studies have directly examined the impact of NTM on clinical status in CF. A retrospective study of 372 patients attending the Leeds CF clinics identified 14 individuals with NTM in their respiratory secretions (607). Each of these patients was matched with two control patients for gender, age, and respiratory function at the time of first NTM isolation. Over the 2-year follow-up period, NTM-positive patients and controls did not differ significantly in change in FEV₁ or FVC, nutritional status, chest radiographic scores, or clinical well-being. In contrast, a retrospective study of children with CF by Esther and colleagues did observe differences between NTM-positive and -negative CF patients (181). A total of 17 patients with NTM identified in their respiratory secretions were divided into two groups: (i) those with three or more positive cultures or two positive cultures and a positive smear and (ii) those with two positive cultures without a positive smear or only one positive culture. Patients in the first group had a higher annual rate of decline in % predicted FEV₁ over an average follow-up period of 5 years ($-4.9\% \pm 1.4\%$ versus $-2.5\% \pm 1.4\%$; $P < 0.035$). Olivier and colleagues addressed the impact of NTM on clinical status in CF by performing a larger prospective nested cohort study of 60 NTM-positive patients and 99 patients who were culture negative for NTM (438). NTM-positive patients were divided into two groups using the same criteria as in the study by Esther et al. (181). During a 15-month follow-up period, the annual rate of decline in FEV₁ did not differ between NTM-negative patients and either of the two NTM-positive groups. However, more patients from group i with baseline high-resolution computed tomography (CT) consistent with NTM infection had progression of their CT findings, compared to patients from group ii. As pointed out by Griffith, the short follow-up period of 15 months may have prevented detection of more rapid decline in pulmonary function caused by NTM (226). Also, analyzing all NTM species as a group may mask the more virulent nature of some NTM species, such as *M. abscessus*. In support of this interpretation, Esther and colleagues examined a cohort of 38 CF patients chronically infected with NTM (180). They noted a trend toward more rapid decline in pulmonary function associated with *M. abscessus* infection than with NTM excluding *M. abscessus* (excess annual decline in % predicted FEV₁ of 0.78 versus 0.57, respectively). These results add to the body of literature suggesting that under appropriate conditions, certain NTM species can cause invasive disease in CF and negatively affect patient outcomes. They also support autopsy findings indicating that necrotizing pulmonary granulomas associated with granulomatous organizing pneumonia are present in some CF patients with respiratory cultures repeatedly positive for NTM but absent from CF patients with a single positive culture (606).

In an attempt to aid clinicians in the difficult process of managing patients with positive NTM cultures, the American Thoracic Society has established guidelines for distinguishing NTM infection from contamination or colonization (227). Ac-

cording to these guidelines, diagnosis of actual NTM disease requires compatible clinical symptoms, radiographic findings, and microbiologic features. Although developed for non-CF patients, these guidelines are stated to be applicable to CF patients as well, but this is challenging given that CF patients not uncommonly fulfill the clinical and radiographic criteria in the absence of NTM. According to the guidelines, growth of NTM from a single sputum sample is insufficient microbiologic evidence to make the diagnosis of NTM disease. This, however, is the situation in approximately 70% of CF patients who have sputum specimens that are positive for NTM growth (439). The significance of isolation of NTM from these patients is unclear, and most authorities recommend that they not be presumptively treated but rather be followed closely and that additional sputum samples be obtained periodically (227, 438).

In summary, the current evidence suggests that NTM are capable of causing worsening of pulmonary status in CF patients when present over long periods of time and in high numbers. However, when present only in a single positive respiratory culture, NTM may merely represent contamination or transient colonization. The significance of the intermittent presence of small numbers of NTM (i.e., negative smear for acid-fast bacilli) in the respiratory tracts of CF patients is less clear and requires further study.

Aspergillus Species

Species of the fungus *Aspergillus* are natural inhabitants of soil, plants, and decomposing organic matter (34). They are also commonly cultured from the respiratory tracts of individuals with CF. Reported prevalences vary from 9% to 57% (43, 168, 300, 338, 411, 418, 530), with most isolates being *A. fumigatus*, although *A. niger*, *A. terreus*, *A. versicolor*, and *A. flavus* are also found (94, 418). The high prevalence of *Aspergillus* species in the airways of CF patients may reflect the predilection of this organism to inhabit bronchiectatic lungs (43). Initially, multiple genotypes of an *Aspergillus* species may be present, but the establishment of chronic infection is associated with the emergence of a single dominant genotype (93, 420, 621).

As is the case with non-CF patients, CF patients may experience a number of disease manifestations caused by *Aspergillus* species. The majority of patients appear to only transiently harbor this organism. Rarely, invasive pulmonary aspergillosis (72, 380) or aspergilloma (362) may occur. A recent report describes six CF patients who developed *Aspergillus* bronchitis that responded to antifungal therapy (542). A substantial proportion of CF patients who chronically harbor *Aspergillus* in their airways will develop allergic bronchopulmonary aspergillosis (ABPA), an allergic reaction to *Aspergillus* (usually *A. fumigatus*) that is characterized by transient pulmonary infiltrates and episodic wheezing that is unresponsive to bronchodilator therapy. The implications of *Aspergillus* growth from a respiratory culture of a CF patient must be examined with regard to each of these clinical contexts.

Is growth of *Aspergillus* species from respiratory cultures in and of itself of any consequence to the CF patient? Milla and colleagues compared lung function in 45 CF patients with *Aspergillus* grown from their respiratory secretions to that in 167 patients who were culture negative (399). After adjustment

for age and gender, no significant difference was noted between the two groups in % predicted FEV₁, FVC, or FEF₂₅₋₇₅ or in chest radiography scores, although 26% of the patients in the *Aspergillus* group were receiving corticosteroids that may have obscured the impact of this fungus. Bargon and associates isolated *Aspergillus* species from the sputa of 43 of 104 adult patients (41%) in their clinic (35). The *Aspergillus*-positive and -negative groups were fairly equivalent with regard to age and gender distribution, and no difference in % predicted FEV₁ was observed between them. Furthermore CF patients with respiratory cultures positive for *Aspergillus* species did not appear to be at an increased risk for complications from lung transplantation (194), although other authors have noted anastomotic dehiscence due to *Aspergillus* in this setting (249, 473). On the other hand, Shoseyov and colleagues reported six CF patients with sputum cultures that grew *A. fumigatus* and who experienced pulmonary exacerbations (542). Each patient did not meet criteria for ABPA but improved with antifungal therapy. Amin and colleagues performed a retrospective cohort study examining 230 CF patients (14). After adjusting for baseline pulmonary function, persistent infection with *A. fumigatus* (defined as the presence of two or more positive sputum or BAL fluid cultures in a given year) was associated with a trend toward increased risk of pulmonary exacerbations (RR = 1.40; *P* = 0.065). Thus, more studies are necessary to determine whether growth (in particular, repeated growth) of *Aspergillus* species from respiratory cultures has a substantial impact on the pulmonary disease of CF patients.

Is evidence of an immune response to *Aspergillus* species associated with more rapid pulmonary deterioration in CF? An immune response would suggest a more invasive *Aspergillus* infection rather than mere colonization or contamination. Studies differ markedly in how frequently such a response occurs, with *A. fumigatus* precipitins or *Aspergillus* immediate skin test sensitivity being reported in 10 to 84% of CF patients versus 0% for healthy controls (168, 338, 377, 384, 411, 418, 424, 670). Some investigators have suggested that immune responses to *Aspergillus fumigatus* were associated with increasingly severe lung damage (338), whereas others have not (74). Schonheyder and colleagues found that CF patients with high IgA antibody titers against *Aspergillus* antigens (but negative cultures for *A. fumigatus*) had a median % predicted FEV₁ value of 49%, compared to 63% in those with low antibody titers (*P* < 0.02) (530). Wojnarowski and colleagues studied 118 CF patients and found that 31 (26%) were sensitized to *A. fumigatus* (658). After adjusting for gender, age, height, and weight, sensitized patients still had lower FEV₁ and FEF₂₅₋₇₅ values. Nicolai and colleagues studied 148 CF patients aged 6 to 34 years and noted that 46% had IgE to *Aspergillus* (424). Multiple linear regression analysis showed the *Aspergillus* IgE titers were negatively correlated with lung function. In summary, the presence of *Aspergillus* in CF to a degree that leads to recognition by the immune system appears to be associated with poorer lung function, although it remains unclear whether this relationship is causal.

Allergic bronchopulmonary aspergillosis. ABPA in CF patients was first described in the 1960s (391, 392) and subsequently has been recognized as relatively common, with reported prevalences of 2% to 11% (209, 338, 381, 411, 418, 419, 549, 551). The prevalence of ABPA increases with age (209),

TABLE 4. Diagnostic criteria for ABPA in CF^a

Criterion
Acute or subacute clinical deterioration not attributable to another etiology
Elevated serum total IgE concn (in the absence of corticosteroid therapy)
Immediate cutaneous reactivity to <i>Aspergillus</i> or presence of serum IgE antibody to <i>A. fumigatus</i>
One of the following:
Precipitating antibodies to <i>A. fumigatus</i> or serum IgG antibody to <i>A. fumigatus</i>
New or recent abnormalities on chest imaging studies that have not cleared with antibiotics and standard physiotherapy

^a Based on data from reference 580.

and it is more common in individuals over 6 years of age (381). The diagnosis of ABPA requires that specific clinical and laboratory criteria be met (Table 4); unfortunately, some of these criteria overlap with the manifestations of CF, making diagnosis difficult in this context. Importantly, not all CF patients with ABPA have growth of *Aspergillus* species from their respiratory cultures (209, 411). The pathogenesis of ABPA is complex (484), but individuals with CF appear to be especially predisposed to this hypersensitivity reaction to *Aspergillus*. For this reason, ABPA may suggest undiagnosed CF (627) or heterozygosity for a CFTR mutation (403).

Numerous anecdotal reports and clinical experience indicate that ABPA in CF indeed is associated with acute episodes of pulmonary decompensation that respond to treatment with steroids (49, 363, 374, 377, 411, 548, 549, 627, 632). In fact, Nepomuceno and colleagues reported that acute episodes of ABPA were associated with 10% of all admissions for CF exacerbations at their hospital (419). Thus, ABPA is clearly capable of causing short-term deterioration in lung function, and current guidelines recommend treating ABPA during CF exacerbations (580).

Opinions differ on the question of whether ABPA itself is associated with irreversible long-term pulmonary decline in CF (549, 552), although there is a consensus that uncontrolled ABPA is associated with the development of bronchiectasis and pulmonary fibrosis in non-CF patients (450). Nepomuceno et al. reported a 3.3% average annual rate of decline in % predicted FEV₁ among 13 CF patients with ABPA, compared to a general average decline of 1.1% for children and 1.9% for adults in the CFF Registry database (419). However, no statistical analysis was provided to assess the significance of these differences. Mroueh and Spock found no difference in clinical status (as measured by Shwachman-Kulczycki scores) between CF patients with ABPA and those with positive cultures for *Aspergillus* species but who did not meet ABPA criteria (411). Mastella and colleagues examined data from the EERCF on 12,447 CF patients in Europe and found that patients with ABPA had on average 10% lower % predicted FEV₁ values than those without ABPA (381). However, in the same study a mixed-model regression analysis failed to show more rapid decline in FEV₁ in ABPA patients during a median follow-up period of 25 months. Kraemer and colleagues observed 122 children with CF born between 1978 and 1999 and followed through 2005 (328). They found that ABPA was associated

with more rapid decline in a number of measures of lung function relative to those in control patients with CF. Thus, although ABPA can clearly cause acute decompensation, the evidence on whether it causes an accelerated chronic decline in pulmonary function in CF patients is inconclusive.

Viruses

As with the general population, individuals with CF are prone to acquire viral respiratory infections. These infections appear to be no more common in children with CF than in healthy control children (254, 480, 618, 635). The viruses most commonly implicated in CF are influenza virus, parainfluenza virus, adenovirus, respiratory syncytial virus, and rhinovirus (reviewed in reference 619). A recent report suggests that metapneumovirus infection is also a frequent occurrence in these patients (205). Newer, highly sensitive metagenomic approaches are now being applied to CF respiratory samples and are likely to greatly expand the types of viruses known to be associated with the CF airways (650).

Several observations suggest that viral infections are more severe in CF patients than in control subjects and contribute to exacerbations. These infections are more likely to involve the lower respiratory tract in CF than in non-CF patients (254, 618) and therefore can cause acute reductions in pulmonary function (433) and perhaps pulmonary exacerbations (526). Along these lines, Wat and colleagues reported a higher incidence of viral infections in children with CF exacerbations (46%) than in CF children without exacerbations (17%) (636). Interpretation of this study, however, is complicated by the fact that the presence of cold-like symptoms was sufficient to define an exacerbation. Examining a cohort of 21,506 patients from the CFF Patient Registry, Ortiz and colleagues found an excess of only 2.1% in the number of exacerbations occurring during influenza season, suggesting at best a minor role for this virus in CF pulmonary exacerbations (442). Several studies have examined whether repeated viral respiratory infections can lead to permanent declines in pulmonary function. Most but not all (480) studies have found an association between the frequency of viral infections and the rate of pulmonary deterioration in CF over periods ranging from 3 weeks to 26 months (3, 108, 480, 561, 635). An intriguing explanation for these findings is that viral infections in the CF lung may prepare the way for subsequent attachment and persistence of bacterial pathogens such as *P. aeruginosa* (108, 455, 620). Thus, respiratory viruses may contribute to exacerbations in CF, but more studies are necessary to conclusively determine whether they independently contribute to a long-term decline in pulmonary function in these individuals.

Other Microbes

Several other organisms have occasionally been identified from the respiratory secretions of CF patients. Some are environmental bacteria that rarely cause human infections, while others are components of the normal human flora.

Anaerobic bacteria. Oxygen tension is low in CF airway secretions (663), suggesting a niche suitable for the growth of anaerobic bacteria, and several reports have indicated that anaerobic bacteria do indeed reside in CF airways. Frequently

identified anaerobes include *Prevotella*, *Bacteroides*, *Veillonella*, *Propionibacterium*, *Peptostreptococcus*, and *Actinomyces* species as well as *Staphylococcus saccharolyticus* (193, 292, 610, 662). Newer molecular approaches are dramatically expanding this list (231, 241, 495). Using 16S rRNA gene sequencing with 25 sputa samples, Bittar and colleagues identified 53 different bacterial species, 30% of which were anaerobes (53). Anaerobic bacteria may be quite common in the CF lung. Tunney and colleagues, for example, found substantial numbers of anaerobic bacteria ($\geq 10^4$ CFU/g of sputum) in 64% of 66 sputum samples from adults with CF (610), and Worlitzsch and colleagues detected anaerobes in 91% of the 45 CF patients they examined (662). Very little work has been done to examine whether these bacteria influence outcomes for CF patients. In the study by Worlitzsch et al., lung function was not associated with the presence or absence of anaerobic bacteria (662). Although additional studies are required to determine the clinical relevance of anaerobes in CF patients, it is noteworthy that they are present in concentrations similar to those of *P. aeruginosa* (292, 610, 611) and persist up to 11 months in the CF airways (662).

Streptococci. Streptococci may also cause respiratory infections in CF patients (229). Unlike most of the microbes that have a predilection for CF patients, the *S. milleri* group of bacteria frequently disseminate and cause extrapulmonary infections (447). In a provocative study by Sibley et al., T-RFLP identified *S. milleri* group bacteria in samples from 7 of 18 acute pulmonary exacerbations in patients at their institution (544). These bacteria, which are commensals of the mouth and nasopharynx, were not detected by routine culture approaches. Resolution of the symptoms of clinical exacerbation was associated with antistreptococcal therapy and reduction in the density of this bacterium in sputum, suggesting that these bacteria may be a cause of pulmonary exacerbations in CF (544). Genotyping did not show evidence of patient-to-patient transmission, suggesting that patients are infected by their own endogenous flora (546). Other streptococci, such as *S. agalactiae*, have also been associated with CF. This bacterium, which normally infects neonates and immunocompromised patients, was found in the respiratory tracts of 16% of 185 CF patients at one center (165).

***Pandoraea apista*.** *Pandoraea* is a recently described genus of environmental Gram-negative bacilli (99). Studies of the epidemiology and pathogenicity of *Pandoraea* in CF have been hindered by difficulties in distinguishing it from BCC organisms or *Ralstonia* species (see below), but newer PCR-based techniques may prove to be helpful in this regard (102, 103). The species most often linked to CF is *P. apista*, although other species have also been cultured from CF patients (99). There is limited information on the clinical significance of *Pandoraea* infection in CF patients, but this bacterium has been associated with chronic infection (25) and patient-to-patient spread (299). In addition, anecdotal reports suggest that it may cause rapid pulmonary deterioration (299) and even bacteremia (294).

***Inquilinus limosus*.** *Inquilinus limosus* is another recently described bacterial species that has been linked to CF (101, 462). This organism is capable of chronic persistence in the airways of CF patients and may assume a mucoid phenotype (86, 247, 641). In one study of 365 sputum samples from 145 children and adults with CF, the incidence of *I. limosus* was 4.9% in

TABLE 5. Other unusual microbes identified from the respiratory tracts of patients with CF

Microbe(s)	Description	Method(s) of identification	Reference(s)
<i>Acinetobacter</i> spp.	Gram-negative coccobacillus	Biochemical and molecular approaches	101
<i>Acrophialophora fusispora</i>	Fungus	Culture	92
<i>Bordetella</i> spp. (primarily <i>B. bronchiseptica</i> / <i>parapertussis</i>)	Gram-negative coccobacillus	16S rRNA gene sequencing, whole-cell protein analysis	101, 572
<i>Brevundimonas diminuta</i>	Gram-negative bacillus	16S rRNA gene sequencing	396
<i>Candida albicans</i>	Fungus	Culture	27, 88, 614
<i>Chryseobacterium</i> spp.	Gram-negative bacillus	Biochemical and molecular approaches	101, 335
<i>Comamonas testosteroni</i>	Gram-negative coccobacillus	Biochemical and molecular approaches	101
<i>Corynebacterium pseudodiphtheriticum</i>	Gram-positive bacillus	Molecular methods, mass spectrometry	51
<i>Coxiellaceae</i> spp.	Gram-negative bacillus	rRNA gene sequencing	241
<i>Craurococcus roseus</i>	Gram-negative coccus	Terminal restriction fragment length polymorphism	495
<i>Cupriavidus</i> spp.	Gram-negative bacillus	16S rRNA gene sequencing	305
<i>Dialister pneumosintes</i>	Anaerobic Gram-negative bacillus	16S rRNA gene sequencing	53
<i>Dolosigranulum pigrum</i>	Gram-positive coccus	16S rRNA gene sequencing	53
<i>Exophiala dermatitidis</i>	Fungus	Culture	149, 225, 233, 273, 332
<i>Geosmithia argillacea</i>	Fungus	Culture	40, 212
<i>Herbaspirillum</i> sp.	Gram-negative bacillus	Biochemical and molecular approaches	101, 573
<i>Klebsiella</i> spp.	Gram-negative bacillus	Culture	578
<i>Lysobacter</i> spp.	Gram-negative bacillus	rRNA gene sequencing	241
<i>Moraxella osloensis</i>	Gram-negative coccobacillus	Biochemical and molecular approaches	101
<i>Nocardia asteroides</i>	Gram-positive bacillus	Culture	355
<i>Ochrobactrum anthropi</i>	Gram-negative bacillus	16S rRNA gene sequencing	396
<i>Paenibacillus cineris</i>	Gram-negative bacillus	Culture	339
<i>Paracoccus halodenitrificans</i>	Gram-negative coccobacillus	Terminal restriction fragment length polymorphism	495
<i>Penicillium emersonii</i>	Fungus	Culture	90
<i>Rhizobium radiobacter</i>	Gram-negative bacillus	Biochemical and molecular approaches	101
<i>Rickettsiales</i> spp.	Gram-negative coccobacillus	rRNA gene sequencing	241
<i>Scedosporium</i> spp.	Fungus	Culture	58, 91, 136, 649
<i>Segniliparus rugosus</i>	Acid-fast bacillus	Culture	78
<i>Serratia marcescens</i>	Gram-negative bacillus	Culture	76
<i>Trichosporon mycotoxinivorans</i>	Fungus	Culture	255
<i>Xanthomonas</i> spp.	Gram-negative bacillus	Biochemical and molecular approaches	101

adults and 1.2% in children (52). The clinical significance of this bacterium is unclear, but in one report new acquisition of *I. limosus* was associated with worsening respiratory status in one patient (52). Also, culture of the organism is often associated with the presence of a specific antibody response (528), which supports a role in infection.

***Ralstonia* spp.** Another group of Gram-negative bacilli occasionally isolated from individuals with CF belong to the genus *Ralstonia* (76, 100, 101, 107, 495). Although the presence of this bacterium is often transient, in some CF patients the same strain of *Ralstonia* may be recovered for weeks or months, indicating chronic infection (106, 579). Again, identification of these bacteria can be problematic, but new molecular approaches show promise of being more satisfactory (104, 106). The clinical significance of *Ralstonia* bacteria in CF is unclear.

Rarely isolated microbes. Many other microbes have been identified in samples from the CF respiratory tract (Table 5). Molecular studies indicate that many additional bacteria are likely common inhabitants of the CF airway but have not been previously appreciated because of fastidious growth requirements (241, 495–497). For some of these microbes, it is unclear whether isolation represents true infection or merely coloni-

zation or contamination. In any case, the roles of these bacteria in the clinical courses of CF patients remain to be explored.

INTERPRETATION OF CLINICAL STUDIES

As the preceding discussions clearly indicate, a large number of studies have examined the relationship between microbial infection and clinical outcomes in CF. Several limitations must be kept in mind when interpreting the results of these studies. First, an association between the presence of a microbe and worse outcomes does not necessarily imply that the microbe caused the worse outcomes. Rather, the microbe may simply have a predilection for lungs with more advanced disease. Second, because the airways of CF patients are rarely sterile, control groups for studies of a particular microbe consist of CF patients not infected with that microbe, but such control patients are usually infected with other bacteria or fungi. Thus, these studies do not determine whether the microbe is detrimental to the patient but rather whether it is more detrimental than the potpourri of organisms infecting the control patients. Third, interventional studies in which antibiotics are administered to measure the effect of eradication of a particular microbe on clinical status suffer from the lack of specificity of

antibiotics. Recent investigations indicate that the CF lung is populated by a large number of culturable and nonculturable bacteria (53, 241, 320, 495, 497, 615, 642), many of which may be susceptible to the broad-spectrum antibiotics frequently given to individuals with CF (610). Thus, it is difficult to attribute improved outcomes to the eradication of any one microbial species. Conversely, clinical or functional improvement may occur in the apparent absence of a microbiological response (216, 389, 554, 555). These and other complexities may account for the often apparently contradictory results of CF outcome studies.

CONCLUSIONS

The impact of traditional CF pathogens on lung function and survival is being more precisely defined by large clinical studies. Likewise, as diagnostic technologies become more powerful, a larger spectrum of organisms are being identified within the airways of individuals with CF. In many cases, microbes in CF patients are not static but are constantly adapting to the selective pressures applied by the respiratory tract. The characteristics of these adaptations are now being more fully appreciated at a molecular level, but additional work is required to elucidate the natures of the selective pressures and the impact of these adaptations on clinical outcomes. A more complete understanding of these processes has the potential to dramatically improve survival in CF.

ACKNOWLEDGMENTS

We thank Michelle Prickett for supplying Fig. 3.

We acknowledge support from the Cystic Fibrosis Foundation (CFF) (M.J. and S.A.M.), CFF Therapeutics, Inc. (A.R.H., M.J., and S.A.M.), the CFF Therapeutics Development Network (M.J. and S.A.M.), and the National Institutes of Health (grants R01 AI053674, R01 AI075191, K02 AI065615, and R21 AI088286) (A.R.H.).

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