

# Pathogenesis of Early Lung Disease in Cystic Fibrosis: A Window of Opportunity To Eradicate Bacteria

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## Clinical Principles

Early lung disease in patients with cystic fibrosis may be clinically silent.

Bacterial colonization, inflammation, or both can be detected before other signs or symptoms of lung disease develop.

## Pathophysiologic Principles

The early pathophysiology of lung disease in patients with cystic fibrosis has several interconnected deleterious cycles leading to impaired innate immunity.

Patients with cystic fibrosis typically experience transition from sterile lower airways, to transient infections (with organisms including nontypeable *Haemophilus influenzae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*), to chronic nonmucoid *P. aeruginosa* infection, to mucoid biofilm *P. aeruginosa* infection.

The time before chronic colonization with *P. aeruginosa* represents a window of opportunity to eradicate bacteria and delay persistent infection. Increasing evidence suggests that early eradication can slow progression of lung disease.

**T**he patient, a previously asymptomatic 18-year-old man with cystic fibrosis, presented with a chronic, nonproductive cough. His medical history was significant for pancreatic insufficiency and homozygosity for the  $\Delta F508$  CFTR mutation. Pancreatic insufficiency was well controlled by enzyme supplementation, and the patient achieved a normal height and weight. He had *Pseudomonas aeruginosa* detected by bronchoscopy at the age of 5 years and by throat culture at the age of 16 years, both of which were successfully eradicated by prolonged courses of antibiotics. Chest radiographs were normal, and he did not have chronic respiratory symptoms until cough 2 months earlier. Bronchoscopy was done to evaluate the patient for respiratory infections and inflammation. The culture of the bronchoalveolar lavage fluid tested positive for 1 strain of nonmucoid *Pseudomonas aeruginosa* in high numbers (1.2 million colony-forming units/mL of bronchoalveolar lavage fluid) and showed increased neutrophils (86% of leukocytes [normal values <5%]). Because the patient was not known to have chronic *P. aeruginosa* infection, he was treated with inhaled and oral antibiotics for 3 months in an attempt to eradicate this pathogen.

How to appropriately treat newly diagnosed *P. aeruginosa* infection is a frequent question facing clinicians caring for patients with cystic fibrosis.

## EARLY LUNG DISEASE IN CYSTIC FIBROSIS

Second only to sickle-cell anemia, cystic fibrosis is the most common genetic disease causing early death (1). Although pulmonary disease causes most of the morbidity and mortality associated with cystic fibrosis, the lungs are thought to be anatomically normal at birth (2). In patients with cystic fibrosis, progression of lung disease is insidious, and patients may be relatively asymptomatic before irre-

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versible changes and chronic bacterial colonization occur. Cough is the predominant symptom in the early stages of cystic fibrosis, occurring in as many as 50% of patients by 10 months of age (3); however, many patients do not have pulmonary symptoms. The first detectable evidence of lung disease in patients with cystic fibrosis is infection and/or inflammation in bronchoalveolar lavage fluid, denoted by elevated counts of interleukin-8 and neutrophils and the presence of microorganisms (4–7). However, detecting bacteria is complicated by regional heterogeneity of inflammation and infection (8, 9). *Haemophilus influenzae*, *Staphylococcus aureus*, and *P. aeruginosa* are the most prevalent early pathogens, and most patients have colonization with at least 1 of these bacteria by 1 year of age (10, 11). Early infections with *P. aeruginosa* can be transient, and approximately half clear spontaneously (12–14). However, by their teenage years, most patients have colonization with *P. aeruginosa* (11).

Chronic colonization with *P. aeruginosa* is associated with a more rapid decline in lung function (15, 16), especially if the isolate becomes mucoid (see Glossary) (17–19). Although most patients are initially infected with nonmucoid *P. aeruginosa*, it later transitions to a mucoid state. In a recent study of patients with cystic fibrosis identified by neonatal screening in Wisconsin, acquisition of nonmucoid and mucoid *P. aeruginosa* occurred at median ages of 1.0 and 13.0 years, respectively (17). Early acquisition of mucoid *P. aeruginosa* was associated with a 4-fold greater decrease in cumulative survival (18). Mucoid *Pseudomonas* is much more difficult to treat and eradicate because it lives in a defensive mode of growth called a biofilm (20, 21). Biofilms are communities of bacteria, enclosed in a self-produced matrix, attached to a surface (22). Common examples of biofilms include dental plaque, endocarditis, and slime on river stones. Biofilms are increasingly recognized as contributing to disease pathogenesis in cystic fibrosis and in other bacterial diseases (23). Bacteria in a biofilm state exhibit increased resistance to antibiotics (24) and host defense factors (25). Communal bacteria in a biofilm can survive antibiotic concentrations as much as 1000-fold higher than the same bacteria in an individual, free-living, planktonic (see Glossary) state (26). Therefore, clinically attainable antibiotic concentrations may not adequately clear biofilm infections, allowing the bacterial population to recover, persist, and spread. Microscopic and physiologic evidence supports biofilm formation by *P. aeruginosa* in sputum from patients with cystic fibrosis (27). Antibiotic-resistant, biofilm-forming mucoid *P. aeruginosa* are believed to play a dominant role in the progression of lung disease in patients with cystic fibrosis (17, 19).

## LUNG DISEASE PATHOGENESIS IS MULTIFACTORIAL IN PATIENTS WITH CYSTIC FIBROSIS

The reason for the high prevalence of bacteria in the early stages of cystic fibrosis is currently unclear. There are

### Glossary

**Biofilm:** Communities of bacteria enclosed in a self-produced matrix that are adherent to a surface. Biofilms display increased resistance to antibiotics and host immune factors.

**CFTR:** Cystic fibrosis transmembrane conductance regulator.

**Eradication:** Treatment of a first or new colonization of *Pseudomonas aeruginosa* with intensive oral, inhaled, or intravenous antibiotics with the goal of eliminating the pathogen from the lower airway.

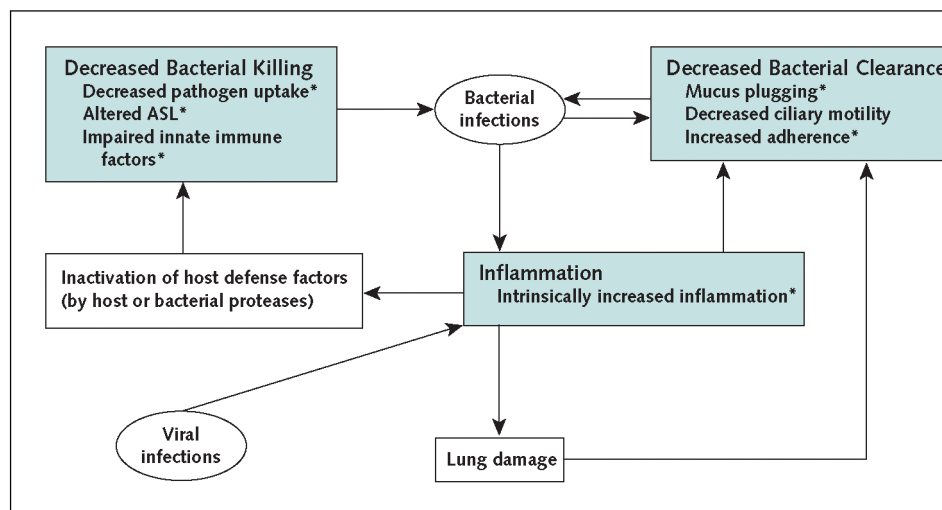
**Mucoid:** Bacteria producing an extracellular matrix of glycoproteins structurally similar to the mucins.

**Planktonic:** Free-living, individual bacteria.

many hypotheses related to how mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) (see Glossary) gene product cause increased susceptibility to bacterial infections. These can be simplified into problems in 3 areas: decreased bacterial clearance, intrinsic hyperinflammation, and decreased bacterial killing (Figure 1). Decreased bacterial clearance may result from defective CFTR by increasing bacterial adherence through altered epithelial cell glycosylation (28) or by increasing the availability of receptors, such as asialoGM1 (29, 30). Furthermore, altered ion and liquid transport is hypothesized to cause thickened and dehydrated mucus that impairs mucociliary clearance of bacterial pathogens (31). Although most methods of comparison do not find differences in submucosal gland development or anatomy in patients with cystic fibrosis during the first months of life (2, 32) infants with this disease had significantly dilated acini before 4 months of age (32). Supporting the second possibility, that lower-airway epithelia in patients with cystic fibrosis may have intrinsic hyperinflammation, some cell culture (33–35), murine model (36, 37), and xenograft (38) studies found decreased anti-inflammatory cytokines, increased pro-inflammatory cytokines, and increased inflammatory airway damage in response to infection. However, studies done on primary cell cultures found little evidence of intrinsic hyperinflammation (39, 40). Mutations of CFTR are also hypothesized to reduce airway surface liquid glutathione levels, which may enhance oxidant injury (41, 42). There is limited in vivo evidence of an intrinsic hyperinflammatory state (43, 44). Hypotheses for the third possibility, that defective bacterial killing results from mutations of CFTR (see Glossary), include reduced uptake of pathogens by airway epithelia (45); alterations in airway surface liquid composition (46); and impaired activity of innate immune factors, such as nitric oxide (47) or defensins (46, 48). Although a controversial area, these hypotheses are not mutually exclusive, and it is likely that CFTR has a multifactorial influence on host defense in patients with cystic fibrosis.

Regardless of the mechanisms initiating airway infection, once the airway is colonized by bacteria, a vicious cycle of infection, inflammation, and airway damage begins. Most children with cystic fibrosis have lower-airway colonization with bacteria by 1 year of age (10). Although

Figure 1. Pathogenesis of lung disease in patients with cystic fibrosis.



Regardless of the initial inflammatory insult or defective process, once this cycle is started, it will lead to all elements being increasingly perturbed. Several interconnected cycles perpetuate and further impair host defense, leading to persistent bacterial infections and progressive lung disease. Possible primary factors resulting from mutant cystic fibrosis transmembrane conductance regulator also are indicated by asterisks. ASL = airway surface liquid.

*P. aeruginosa* is considered the major pathogen in later stages of the disease, early infections with viruses and other gateway bacteria may cause substantial inflammation and pave the way for subsequent colonization with *P. aeruginosa*. Eighty-two percent of patients have colonization with *S. aureus* or *H. influenzae* before they have colonization with *P. aeruginosa* (49). Studies of bronchoalveolar lavage fluid of single-pathogen infections with *P. aeruginosa*, *S. aureus*, and *H. influenzae* in patients with cystic fibrosis did not reveal clinically significant differences in interleukin-8 or neutrophil levels (50), indicating that all of these early pathogens are associated with high levels of inflammation. Other studies of bronchoalveolar lavage fluid found similarly increased inflammatory cells and cytokines regardless of the pathogen (10). Viral illnesses may also play key roles in priming the airway for bacterial colonization. In a retrospective Danish study, 68% of patients had chronic colonization with *P. aeruginosa* during the winter viral season (51).

Chronic infection and inflammation impair innate immunity by several mechanisms (Figure 1). Although cilia structure and function are normal in patients with cystic fibrosis, factors involved in infection (52, 53) and inflammation (54, 55) can slow the beat frequency of cilia. Bronchiectasis decreases mucociliary clearance, especially from chronic disease starting early in life (56). Researchers do not agree whether impaired mucociliary clearance is a primary defect resulting in infection (31) or whether mucociliary clearance is initially normal and is secondarily impaired by infection and inflammation (57, 58). It is clear, however, that mucociliary clearance progressively worsens with advancing lung disease and contributes to disease pathogenesis. High numbers of neutrophils in the airway lumen are characteristic of lung disease in patients with cystic fibrosis. Neutrophils may survive longer in patients

with cystic fibrosis because of excess production of granulocyte macrophage colony-stimulating factor and decreased interleukin-10 (59, 60). These neutrophils release enormous amounts of neutrophil elastase, which overwhelms airway surface liquid antiprotease activity and damages surrounding airway parenchyma (61, 62). This may be further exacerbated by bacterial proteolytic enzymes (63). Oxygen radicals released from neutrophils also damage the airways (64). Neutrophil elastase also cleaves molecules used to opsonize and phagocytize bacteria, including IgG, CR1, and C3bi (ligand for CR-3) (65, 66), leading to increased bacterial persistence. Proteolytic cleavage of antimicrobial proteins and peptides, such as lysozyme, lactoferrin, defensins, and surfactant proteins, also occurs (67–70). Neutrophil elastase also increases airway secretions (71) and release of epithelial interleukin-8 (72), further stimulating neutrophil influx. Deoxyribonucleic acid released from dead neutrophils and from bacteria additionally increases mucus viscosity. Mucus plugging, in turn, further exacerbates bacterial persistence and airway damage and may also serve as a site for bacterial adherence (73). Thus, several interconnected processes, including bacterial infection, exuberant host response, and airway damage, perpetuate the vicious cycle of bacterial persistence and progressive obstructive lung disease in patients with cystic fibrosis.

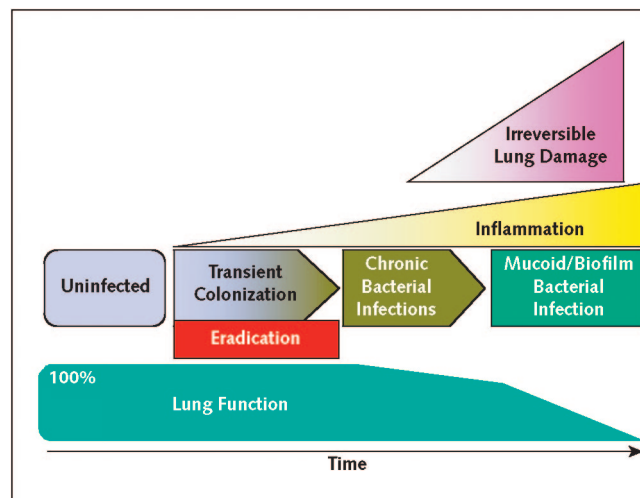
## EARLY INTERVENTION

Although *P. aeruginosa* infection is clearly associated with increased morbidity and mortality, it usually does not pose an immediate threat (Figure 2). In the early years of intermittent *P. aeruginosa* colonization, patients generally have few signs or symptoms of infection. It is increasingly recognized that there may be a window of opportunity to

eradicate nonmucoid and antibiotic-sensitive *P. aeruginosa* during these initial intermittent infections (14), when they may be present in lower numbers and associated with less inflammation (10, 74). Eradication (see Glossary) may be impaired in chronic infections, when *P. aeruginosa* has genetically adapted to the airways (10, 75, 76), or may be extremely difficult when the bacteria has become mucoid (77). The rationale for eradication therapy is elimination of bacteria at first detection to break the vicious cycle of infection, inflammation, and airway damage before it becomes irreversible.

First used as the standard of care by cystic fibrosis centers in Denmark (12), several eradication strategies using different combinations of oral, inhaled, and intravenous antibiotics have been successfully implemented (Table) (12, 13, 78–85). Although the antibiotics, methods of delivery, and dosages vary, each of these approaches decreased colonization with *P. aeruginosa*, at least in the short-term (138 of 161 patients [86%] in the treatment groups became *P. aeruginosa*-negative by culture vs. 31 of 72 patients [43%] in the control groups). Of interest, early intervention has not changed the rate of acquisition of intermittent colonization with *P. aeruginosa*, but it has dramatically affected the transition to chronic infection. In a longitudinal study of DNA genotyping of *P. aeruginosa* in young children, approximately 50% had transient colonization with multiple genotypes of *P. aeruginosa* (14). This study also found that the time from initial isolation to the second genotype averaged less than 10 months, which indicates that once a patient initially has positive cultures for *P. aeruginosa*, acquisition of a new genotypic isolate is relatively common. This complicates verification of eradication because acquisition of a new genotypic isolate may occur even with successful eradication of the pathogen that

Figure 2. Diagram showing the relationship between the window of opportunity for eradication of *Pseudomonas aeruginosa* and the progression of lung disease over time.



Inflammation may be reversible during the transient infection period, but increasing inflammation and irreversible lung damage occur with chronic and mucoid infections. There is evidence that some inflammation and lung damage may occur at times earlier than indicated. “100%” indicates the top of the y-axis for the Lung Function box.

was initially isolated. Only 2 studies have used DNA evidence for eradication, verification, and longitudinal monitoring of *P. aeruginosa* isolates (80, 83). These 2 studies found subsequent recurrence of the same genotype in 26% and 25% of patients, respectively. In addition to verifying colonization by obtaining a culture of throat swabs, sputum, or bronchoalveolar lavage fluid samples, serologic diagnosis may be a more sensitive approach (14). Potential negative effects from eradicating initial isolates of *P. aerugi-*

Table. Summary of *Pseudomonas aeruginosa* Eradication Trials\*

Study, Year (Reference)	Treatment	Treatment Duration	Patients Negative for <i>Pseudomonas aeruginosa</i> at the End of Therapy, n/n (%)	Duration of Eradication
Valerius et al., 1991 (12)	Ciprofloxacin plus inhaled colistin	3 wk	Treatment group, 12/14 (86) Control group, 5/12 (42)	Not studied
Fredericksen et al., 1997 (13)	Ciprofloxacin plus inhaled colistin	3 wk or 3 mo	Treatment group, 41/48 (85) Historic control group, 24/43 (56)	Not studied
Weisemann et al., 1998 (78)	Inhaled tobramycin, 80 mg BID	12 mo	Treatment group, 8/9 (89) Placebo control group, 1/4 (25)	Not studied
Ratjen et al., 2001 (79)	Inhaled tobramycin, 80 mg BID	12 mo	Treatment group, 14/15 (93)	14/15 at 12 mo
Munck et al., 2001 (80)	IV antibiotics, then inhaled colistin	21 d 2 mo	Treatment group, 19/19 (100)	8 mo (SD 6)
Nixon et al., 2001 (81)	IV antibiotics, then ciprofloxacin	14 d 3 mo	Not studied	6/24 > 12 mo
Griese et al., 2002 (82)	Age < 5 y, inhaled tobramycin; age ≥ 5 y, ciprofloxacin plus inhaled colistin†	28 d 3 wk	Treatment group, 7/8 (88) Treatment group, 6/9 (67)	≥ 2 y
Gibson et al., 2003 (83)	Inhaled tobramycin, 300 mg BID	28 d	Treatment group, 8/8 (100) Placebo control group, 1/13 (8)	Not studied
Lee et al., 2004 (84)	Ciprofloxacin plus inhaled colistin	3 mo	Treatment group, 23/31 (74)	Not studied

\* The number of patients who continued to have positive cultures for *P. aeruginosa* at the end of therapy in each experimental group is shown. The percentage of patients with negative cultures is shown in parentheses. BID = twice per day; IV = intravenous.

† If patients did not respond to initial attempts at eradication, they received an additional course of oral ciprofloxacin plus inhaled colistin or intravenous tobramycin/ceftazidime. Modified from Rosenfeld et al. (85).

*nosa* include tobramycin resistance and emergence of new cystic fibrosis pathogens; however, when investigated, no evidence of these effects has been found (83). Other potential problems with these early intervention trials include small numbers of patients, limited follow-up, and unclear effects of treatment on other pathogens. The ongoing Early *Pseudomonas* Infection Control (EPIC) study in the United States and EarLy Inhaled Tobramycin for Eradication (ELITE) trial in Europe should help to address some of these issues (86, 87). With the longest history of eradicating *P. aeruginosa* when first detected, Denmark has now decreased the prevalence of chronic *P. aeruginosa* infection among its patient population from 60% in 1980 (88) to 45% in 1995 (88) and 36% in 2000 (89). Of interest, with 15 years of eradication experience in Denmark, no infant or child younger than 14 years of age with cystic fibrosis has experienced chronic colonization with *P. aeruginosa* since 1990 (90). Early interventions that clear infections may also decrease airway inflammation. In a study of 46 infants who received cystic fibrosis diagnoses by neonatal screening, those who initially had at least  $10^5$  bacteria/mL or viral respiratory pathogens but had clearance of these infections 1 year later had decreased levels of bronchoalveolar lavage fluid, neutrophils, and interleukin-8 levels, although persistent infections were associated with further augmentation of these pro-inflammatory factors (4). Although no data are yet available to judge the impact of early eradication (see Glossary) on mortality or hospitalization rates, eradication of *Pseudomonas* infection correlates with improved pulmonary function (13). Early intervention will probably increase the population of patients transitioning from the pediatrician to the internist with near-normal lung function and without chronic *P. aeruginosa* colonization. Although studies of eradication to date have been done in children, the rationale and potential benefit of eradicating newly diagnosed *P. aeruginosa* colonization should extend to adult patients with cystic fibrosis.

## SUMMARY

Mutations of CFTR impair the innate immunity of the pulmonary system. The initially “normal” lungs of a patient with cystic fibrosis are predisposed to infection and inflammation. Defective CFTR may contribute to bacterial infections by 1 or more means. The end result is a web of interconnected processes that cause progressive lung damage (Figure 1). Although there are academic and therapeutic implications to identifying the primary links between CFTR mutations and innate immunity, the cascade of affected processes co-exist early in lung disease pathogenesis and collectively contribute to airway infection and inflammation. Infants and children with cystic fibrosis experience transient *P. aeruginosa* infections within the first years of life, although they usually have few symptoms. These infections eventually become chronic, with nonmucoid *P. aeruginosa* that later transition to mucoid–biofilm infec-

tions, which are associated with increased morbidity and mortality (Figure 2). The question now facing the clinician is how to best manage these mostly asymptomatic younger patients with cystic fibrosis who have evidence of lower-airway bacterial infection or inflammation. There is increasing evidence of a window of opportunity to eradicate *P. aeruginosa* infection when it is first detected and thereby prevent or delay transition to chronic infection. We should continue to focus our efforts on how to best exploit this opportunity to benefit patients with cystic fibrosis.

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