Current Understanding of the Inflammatory Process in Cystic Fibrosis: Onset and Etiology

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Summary. Although airway obstruction and chronic endobronchial infection have long been recognized as major factors in the pathogenesis of lung disease in cystic fibrosis (CF), only recently has it been recognized that the inflammatory process itself may be responsible in a major way for destroying the lungs. The most characteristic feature of inflammation in the CF lung is the persistent infiltration of massive numbers of neutrophils into the airways. Although neutrophils help to control infection, when present in great excess, they cause more harm than good. Major advances in our understanding of the inflammatory process in the CF lung have come from the use of bronchoscopy and bronchoalveolar lavage (BAL) to analyze the inflammatory process in patients who are relatively symptom free and/or do not regularly produce sputum. Recent BAL studies suggest that neutrophil-rich inflammation begins very early, even in infants without clinically apparent lung disease. A number of chemoattractants from epithelial cells, macrophages, neutrophils themselves, and bacterial products contribute to the neutrophil influx. Surprisingly, some infants have inflammation even in the apparent absence of infection, leading to the speculation that inflammation may precede infection. Links between the basic defect in CF and inflammation have been postulated, with dysregulation of cytokine production and abnormal epithelial host defenses being implicated as causal factors of sustained inflammation. Regardless of the details of how this process is initiated and/or perpetuated, it has become clear that inflammation begins at a very early stage and progresses throughout life, gradually worsening and destroying the lungs. For these reasons, anti-inflammatory therapy should be initiated in early life. Additional studies are necessary to define the optimal anti-inflammatory drugs and regimens, and to confirm their long-term safety and efficacy. Pediatr. Pulmonol. 1997; 24:137–142.

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INTRODUCTION

Progressive pulmonary disease, resulting in lung destruction, continues to account for most of the morbidity and nearly all the mortality in CF.1 Thus, much effort is being directed toward discerning the pathogenesis of CF lung disease, with the hope that new treatment strategies will be devised to ameliorate the destructive processes. The currently accepted pathogenic scheme begins with the defective CF gene resulting in absent or deficient CFTR. The primary pathophysiologic effect of this is believed to be alteration of the airway environment such that abnormal mucus causes airway obstruction (Fig. 1). Infection with organisms with a predilection for the CF airway, particularly Pseudomonas aeruginosa, soon follows. It should be noted that some investigators have presented evidence suggesting that alterations in cell surface glycoconjugates, also due to defective CFTR function, may contribute to the peculiar predilection of CF patients to develop infection with P. aeruginosa, which is not generally pathogenic in the normal host. The inflammatory response to this infection is excessive and persistent and sets the stage for a vicious cycle of airway obstruction, infection, and inflammation that ultimately leads to lung destruction.2 Recent observations suggest that inflammation may occur in the absence of infection, and that there may be a direct link between deficient or defective CFTR and both infection and inflammation, prompting a re-evaluation of the current pathogenic scheme (see below). Regardless of the intermediary steps, there is little doubt that inflammation plays a cen-
tral role in the vicious cycle that leads to lung destruction. Discerning the onset and etiology of inflammation will ultimately guide the development of new therapeutic strategies aimed at retarding the progression of CF lung disease.

CHARACTERISTICS OF INFLAMMATION IN THE CF LUNG

Histopathology of the lungs from infants who have died from pulmonary and non-pulmonary complications of CF has provided important information concerning the characteristics of inflammation in the CF lung early in life.3 Except for mild dilatation of the acini of tracheal submucosal glands, the airways are usually histologically normal during the first few months of life. Plugging of the small airways by abnormal mucus is the most prominent early manifestation, followed by endobronchial infiltration of massive numbers of neutrophils into the airways.3 Although neutrophils help to control infection, when present in great excess, they cause more harm than good. Neutrophils infiltrating into the airways and decomposing in situ are the major source of the DNA that makes CF sputum so tenacious. Indeed, studies have shown that exacerbations of lung infection are accompanied by increased amounts of DNA in the sputum, which is primarily of human rather than bacterial origin.4 Neutrophils also release an arsenal of oxidants and proteases, including elastase. The huge excess of elastase overwhelms the antiprotease screen in the airways and results in uninhibited proteolytic enzyme activity. Elastase directly damages the airway wall by digesting elastin and other structural proteins, leading to bronchiectasis. In addition, elastase alters a number of airway functions. It has potent secretagogue activity, which increases mucus secretion, worsening airway obstruction. It cripples host defenses by cleaving vital opsonins and receptors necessary for phagocytosis, thus contributing to persistence of infection. Finally, elastase promotes the generation of chemoattractants, particularly IL-8 and LTB4. These and other chemoattractants arise from the airway epithelial cells themselves, as well as from macrophages and infiltrating neutrophils. Bacteria and their products also promote the generation of chemoattractants (Table 1). These chemoattractants recruit more neutrophils into the airways, fueling the vicious cycle of inflammation that leads to lung destruction. Although all these chemoat-

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**Abbreviations**

- BAL: Bronchoalveolar lavage
- CD: Cluster designation
- CF: Cystic fibrosis
- CFTR: Cystic fibrosis transmembrane conductance regulator
- ELF: Epithelial lining fluid
- FEV1: Forced expiratory volume in 1 second
- FVC: Forced vital capacity
- ICAM: Intercellular adhesion molecule
- IL: Interleukin
- LPS: Lipopolysaccharide
- LT: Leukotriene
- TNF: Tumor necrosis factor

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**Fig. 2. Understanding how and when the CF airway progresses from being structurally normal without infection or inflammation (A), to one that is characterized by luminal and peribronchial inflammation predominated by neutrophils, with relative sparing of the alveoli (B), is critical to the successful development of anti-inflammatory therapy.**

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tractants likely contribute, recent studies have focused on IL-8, which is produced by many types of cells in the CF lung (Table 2), and has been shown by some investigators to correlate with the number of neutrophils in BAL fluid.

Early in the course of disease inflammation may be found without evidence of infection (see below). In most patients, infection and inflammation are intimately linked, with each exacerbating the other. Thus, studies of the onset and regulation of inflammation invariably focus on factors that promote infection in CF and/or prevent its eradication. Early observations showed that corticosteroid therapy in CF patients with asthma and/or allergic bronchopulmonary aspergillosis ameliorated rather than exacerbated lung disease. It was also shown that CF patients with hypogammaglobulinemia had milder rather than more severe lung disease, suggesting that inflammation in the lungs was excessive relative to infection. Subsequent clinical trials with corticosteroids and non-steroidal anti-inflammatory drugs further supported this concept and have led to more in-depth studies of the regulation of inflammation in the CF lung.

**HOW EARLY DOES INFLAMMATION OCCUR?**

Major advances in our understanding of the inflammatory process in the CF lung have come from the use of bronchoscopy and BAL to analyze the inflammatory process in patients who are relatively symptom free and/or do not regularly produce sputum. Recent studies suggest that inflammation begins very early in terms of both the chronological age of the patient and the degree of airway dysfunction (or obstruction) as indicated by the decline in FEV₁ or FVC. Newborn screening programs have afforded opportunities to use BAL to assess the lungs of...
very young infants who have been diagnosed with CF before the lung disease is clinically apparent. Studies in the United States (Colorado) and Australia (Victoria) have shown that even infants without symptomatic lung disease have significant endobronchial bacterial infection associated with inflammation and large numbers of neutrophils. The 16 infants in the Colorado study had a mean age of 6 months, while the 45 infants in the Australia study had a mean age of slightly less than 3 months. Inflammation was present in some infants as early as 4 weeks of age. Active elastase and elevated concentrations of IL-8 were also present in many of these infants. In a separate study of a subset of 17 infants from the Colorado study, elevated levels of DNA were also present. Surprisingly, some infants in both the Colorado and Victoria studies had inflammation in the apparent absence of infection. In the Colorado study, 7 of the 16 infants with CF had no evidence of bacterial, viral, or fungal infection at the time of BAL. Although the degree of inflammation was less in these infants compared with the CF infants who were positive for microorganisms, it was significantly greater than that observed in control infants. In the Victoria study, only 17 of the 45 infants were infected at the time of BAL. Although inflammation was present in some of the uninfected infants, there was considerable overlap between their neutrophil counts and those in control infants. Aspiration lung disease was felt to be responsible for inflammation in the absence of infection in two infants.

The results from these two studies may lead one to conclude that inflammation may precede infection. This, in turn, leads to speculation that defective CFTR function could, in some way, directly contribute to inflammation. Alternative explanations could be that infection was not detected due to insufficient sensitivity, or that infection indeed preceded inflammation. It may be that the inflammatory response and/or treatment effectively cleared the infection, but that the inflammation then persisted without an ongoing infectious stimulus. In support of this latter possibility are data from a 5-month-old CF infant we recently had the opportunity to study with serial bronchoalveolar lavages. Infection with  P. aeruginosa, Haemophilus influenzae, and Klebsiella pneumoniae was present at the time of the first study but was eradicated by 2 weeks of broad-spectrum antibiotic therapy. Bronchoalveolar lavage 6 weeks later revealed the continued presence of large numbers of neutrophils and high concentrations of IL-6 and IL-8, even though all cultures were negative (Table 3).

The airways of older CF patients are essentially impossible to sterilize. Results from our previous BAL studies demonstrate that even the most clinically mild adolescent and adult patients, with FEV₁ values that are not significantly different from the predicted values for healthy control individuals, have large numbers of neutrophils and active elastase in their airways. These injurious factors are present even in patients who are remote from clinical exacerbations and who have never been hospitalized. Studies in younger children with minimal lung disease support these observations. Taken together, all these bronchoalveolar lavage studies support the notion that inflammation begins very early in the course of disease, both in infants and in older patients without clinically apparent lung disease. This inflammation continues relentlessly and eventually destroys the lungs.

### POSSIBLE LINKS BETWEEN DEFECTIVE CFTR AND INFLAMMATION

No direct link has been established between the basic CF defect and systemic immunologic or neutrophil dysfunction. It is important to note that there is no evidence of increased infection outside of the sinopulmonary tract and, with the exception of arthritis in some patients in the late stages of their disease, no other distinct abnormalities of inflammation have been reported. However, recent studies focusing on epithelial cells have suggested ways in which defects in CFTR function may influence the infectious and inflammatory processes in the lung. Our studies of cytokines in the CF airways show that in addition to dramatically increased concentrations of pro-inflammatory cytokines like IL-1, IL-6, IL-8, and TNF,
CF airways are relatively deficient in the anti-inflammatory cytokine IL-10. The latter finding may be particularly important, since IL-10 inhibits the production of IL-1, IL-8, and TNF by inflammatory cells. The CF infant presented in Table 3 typifies these findings. Even after infection was eradicated, there was still no detectable IL-10 in the BAL fluid, and only a trace amount was secreted by her epithelial cells in vitro, but markedly elevated concentrations of IL-8 and IL-6 persisted in vivo and were secreted in vitro. Further studies have shown that normal bronchial epithelial cells constitutively produce IL-10 in the healthy lung. Decreased IL-10 production by CF epithelial cells may allow excessive and/or persistent inflammatory responses to transient infection. The ensuing inflammatory damage may predispose to further infection and/or may help to select for pathogens like Pseudomonas.

Normal airway epithelial cells are in an anti-inflammatory state: they do not produce IL-8 and do not express the important pro-inflammatory adhesion molecule ICAM-1. By contrast, CF epithelial cells actively produce IL-8 and express large amounts of ICAM on their membranes. ICAM is a ligand for the neutrophil’s major adhesion molecule, CD11b/CD18, and laboratory studies suggest that neutrophil adhesion mediated by ICAM may increase IL-8 production and lead to persistence of the neutrophils in and/or on the epithelium, prolonging their injurious effects in situ. Thus, the airway epithelial cells in CF, by shifting to a pro-inflammatory phenotype in which they express ICAM on their surface and secrete IL-6 and IL-8 instead of the anti-inflammatory cytokine IL-10, are likely to make a major contribution to the excessive local inflammation. The realization that the epithelial cells themselves may be a major determinant of the local immunologic and inflammatory milieu leads to the speculation that defective CFTR function, which is expressed most importantly in the epithelial cells, may be directly related to excessive inflammation. Preliminary studies with transformed cell lines in vivo suggest that alterations in IL-8 and IL-10 secretion may indeed be directly due to defective CFTR function.

Recent results from other laboratories suggest that alterations in cell surface glycoconjugates, which may also be directly attributed to defective CFTR function, contribute to increased adherence of pathogenic bacteria such as P. aeruginosa to epithelial cells and increased secretion of IL-8 by epithelial cells cultured in the presence of these bacteria. This is one of the few hypotheses that could explain the specific association of P. aeruginosa with CF, and warrants further investigation. A number of other studies have focused on determining whether the altered ionic milieu of CF airway epithelial cells could impair local host defenses, allowing persistent infection to stimulate inflammation. These would also tie CFTR dysfunction to the abnormal inflammation in the lung. On one hand, it has been suggested that phagocytosis and bactericidal activity of neutrophils is impaired in the hypotonic milieu in CF, while other investigators have proposed that the hypertonic milieu interferes with the function of bactericidal peptides that can kill Pseudomonas organisms.

Clearly, additional data on the actual milieu in vivo is necessary before the importance of these in vitro observations can be fully understood. The recent report suggests that normal airway epithelial cells can ingest and kill P. aeruginosa, and that cell lines expressing mutant forms of CFTR are defective in cellular uptake of this organism. These observations, taken together, suggest a direct link between primary defects in CFTR and impairments of host defense in CF. As mentioned earlier, any local host defense defect that contributes to persistent infection will also likely contribute to the chronic inflammation.

CONCLUSIONS

Regardless of how the inflammatory process is initiated and/or perpetuated, it has become clear that inflammation begins at a very early stage and progresses throughout life, gradually worsening and destroying the lungs. Eventually, this relentless inflammatory process claims the life of the patient. For these reasons, anti-inflammatory therapy should be initiated early in life, and infection must be controlled to the maximum extent possible. Therapeutic strategies aimed at decreasing neutrophil influx or countering injurious neutrophil products are under active investigation, and promising therapies are being added to the comprehensive treatment regimens for CF lung disease. Recent success with chronic high-dose ibuprofen underscores the benefits of anti-inflammatory therapy, particularly in young patients with mild lung disease. Additional studies are necessary to define the optimal anti-inflammatory drugs and regimens and confirm their long-term safety and efficacy.

REFERENCES

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