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Anti-inflammatory Therapies for Cystic Fibrosis-Related Lung Disease

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Abstract Cystic fibrosis (CF) is an autosomal recessive disease affecting many organ systems. In the lung, the underlying ion transport defect in CF establishes a perpetuating cycle of impaired airway clearance, chronic endobronchial infection, and exuberant inflammation. The interrelated nature of these components of CF lung disease makes it likely that the most effective therapeutic strategies will include treatments of each of these. This chapter reviews the preclinical and clinical data focused on ways to better understand and particularly to limit inflammation in the CF airway. Anti-inflammatories are an attractive therapeutic target in CF with a proven ability to decrease the rate of decline in lung function. However, the inherent complexity of the inflammatory response combined with the obvious dependency on this response to contain infection and the side effect profiles of common anti-inflammatories have made identifying the most suitable agents challenging. Research continues to discover impairments in signaling events in CF that may contribute to the excessive inflammation seen clinically. Concurrent with these findings, promising new therapies are being evaluated to determine which agents will be most effective and well tolerated. Available data from studies commenced over the last two decades, which have generated both encouraging and disappointing results, are reviewed below.

Keywords Cystic fibrosis · Lung · Inflammation · Anti-inflammatory therapy · Corticosteroids · Ibuprofen

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Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations on chromosome 7 resulting in defective and/or deficient chloride transport through the CF transmembrane conductance regulator (CFTR) protein [1]. In exocrine glands, this protein resides in the apical membrane of epithelial cells and primarily serves to regulate ion and water content at the luminal surface. In the lung, dysfunctional regulation of ion and water content causes tenacious secretions to accumulate within the airways, impairing the natural process of airway clearance. These secretions trap bacteria and other pathogens and initiate a self-perpetuating cycle of airway obstruction, endobronchial infection, and exuberant inflammation. Pulmonary manifestations are largely responsible for the morbidity and mortality of CF and are a primary treatment focus [2]. For over 40 years, attention has been given to therapies directed at improving airway clearance of secretions and treating endobronchial infection. Recently, there is a growing appreciation of the role of inflammation in CF and an interest in developing therapies capable of mitigating the excessive inflammatory response in the airways. As a key component of the pathophysiology of CF-related pulmonary disease, inflammation, or more specifically, its overabundance should be addressed in a comprehensive therapeutic strategy. The preponderance of evidence indicates that this process begins early in life, is of greater magnitude than is observed

in patients without CF, and persists beyond apparent eradication of infectious stimuli [3–14]. Both prospective and retrospective studies of anti-inflammatory therapies in CF demonstrate a slowing of lung function decline—a measure closely correlated with survival [15–20]. This paper will review the past, present, and potential future anti-inflammatory therapeutic options (Table 1). To better understand these therapies, a brief review of the current understanding of pulmonary inflammation in CF-related lung disease is necessary.

Inflammation in CF lung disease

Airway inflammation in CF appears to begin in early infancy. At birth, the lungs are structurally normal and not inflamed. Within the first several months of life, secretions plug the bronchioles and ducts of submucosal glands [3]. Bronchoalveolar lavage (BAL) studies from infants with CF show high concentrations of neutrophils and proinflammatory mediators in the airways, often in the absence of identifiable pathogens [4, 10–12]. When bacteria are found, the inflammatory response relative to the bacterial burden is exaggerated in CF infants compared to infants without CF [13, 14]. In vitro experiments using respiratory epithelial cells corroborate these findings by demonstrating increased inflammatory responses in cells lacking CFTR function [21]. Matched cell studies employing chemical CFTR inhibitors or correctors both show that impairment of CFTR function correlates with increased basal and inducible inflammation [21, 22]. The inflammatory response in CF cells is not only heightened but is also prolonged, which may explain why inflammation can be detected in BAL samples of infants with CF in the apparent absence of microbial pathogens [4, 11]. Airway inflammation observed in these infants may reflect the prolonged response to previously cleared pathogens.

Table 1 Targets of anti-inflammatory therapies in CF

Target in inflammatory cascade	Therapies
Stimuli	
Bacteria/Bacterial Products	Antibiotics
Receptors	TNF-α-RA, LTB4-RA, montelukast, zafirlukast, anti-ICAM-1
Intracellular signaling mechanisms	Corticosteroids, ibuprofen, IL-10, interferon-γ1B, pioglitazone, simvastatin, parthenolide, synthetic triterpenoids, p38MAPK inhibitors, NF-κB inhibitors
Products	
Cytokines	Corticosteroids, ibuprofen, anti-TNF-α, IL-10, anti-IL-17, anti-IL-8, IFNγ, azithromycin
Eicosanoid Modulators	Corticosteroids, zileutin, DHA, omega-3 fatty acids
Oxidants	*N*-acetylcysteine, glutathione, β-carotene, vitamin C, vitamin E
Proteases	α1-protease inhibitor, secretory leukoprotease inhibitor, MNEI, EPI-hNE4
Other	Dornase-alpha, hydroxychloroquine, methotrexate, cyclosporine-A, azathioprine

Past, current, and potential future anti-inflammatory therapies organized by their biological targets. Many of these therapies are yet unproven in adequate clinical trials to warrant their widespread use.

The inability to properly terminate the inflammatory response in CF is intensified by the fact that the process continues as an acute, neutrophil-dominated phenotype rather than converting to that of chronic inflammation populated with mononuclear cells (e.g., lymphocytes, macrophages). The perpetual neutrophilic activation is typically sequestered in and around the airways but spreads to involve the submucosa, airway wall, and supporting structures in advanced stages. Notably, the alveoli are relatively spared until late in the disease course. Airway inflammation works to prevent the systemic spread of resident bacteria, but it also causes significant collateral damage to normal tissue thereby becoming a pathologic force.

This inflammation is not independent from infectious stimulation, and the concentrations of neutrophils and inflammatory mediators increase significantly during times of pulmonary exacerbation triggered by increased infectious burden. For reasons that are not fully understood, a limited and remarkably consistent collection of distinct bacteria colonize and infect the CF lung. *Staphylococcus aureus*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa* predominate in CF, although these patients occasionally become infected with other distinctive organisms including *Burkholderia cepacia* complex organisms, *Stenotrophomonas maltophilia*, and *Alcaligenes xylosoxidans*. Many believe that the heightened inflammatory response in CF is closely related to abnormal CFTR function. This theory is supported by the observation of increased inflammatory responses of airway cells treated with chemical CFTR inhibitors and decreased response in CF epithelial cells treated with CFTR correctors [21, 22]. Other investigators have shown proinflammatory signaling in response to misfolded CFTR protein in the endoplasmic reticulum [23]. This, however, does not adequately explain the excessive inflammation seen in patients with two stop mutations and, therefore, no misfolded protein. CFTR dysfunction conveys multiple downstream effects within the cell, many of which may contribute to the inflammatory response (Fig. 1). Available data regarding the nature of

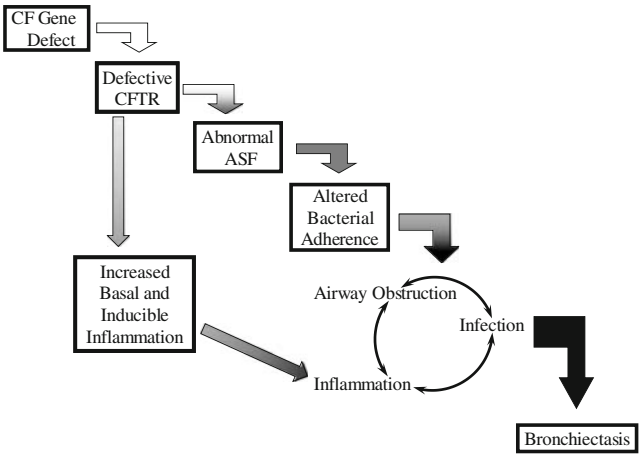

Fig. 1 Flow chart depicting the stepwise pathological progression of lung disease in cystic fibrosis beginning with the basic genetic defect and ending with irreversible airway damage. Note the role of heightened inflammation, which is part of an interdependent cycle along with airway obstruction and chronic endobronchial infection. Defective CFTR function likely influences intracellular proinflammatory signaling, contributing to the exaggerated inflammatory state

these proinflammatory alterations with a focus on therapeutic intervention is reviewed below.

Bronchoalveolar lavage (BAL) fluid samples from CF airways contain high concentrations of proinflammatory mediators, including interleukin (IL)-8, which drives the intense neutrophil recruitment to the pulmonary space [4–14]. Whereas BAL from healthy lung typically contain <3% neutrophils, BAL from CF lung often contain >20% and, at times, >80% neutrophils. The synthesis of many proinflammatory mediators is promoted by activation of the intracellular transcription factor nuclear factor kappa-B (NFκB). Cytokines and chemokines regulated by NFκB activity include IL-8, tumor necrosis factor-alpha (TNF-α), IL-1β, IL-6, and granulocyte-macrophage colony-stimulating factor—all of which are increased in CF [5]. In an inactive state, NFκB is sequestered in the cytoplasm by the inhibitory protein kappa-B (IκB). Cellular interaction with bacteria, bacterial products, and proinflammatory cytokines causes IκB to dissociate and allows NFκB to translocate to the nucleus and promote the transcription of these proinflammatory mediators. This transcription factor does not appear to be inherently defective in CF, and the increased NFκB activation may be a response to the abundant stimuli present in the CF lung. Alternatively, there is evidence of increased IκKinase (IKK) activity in CF cells. IKK is responsible for phosphorylating IκB, an important step in targeting IκB for degradation. Once released from IκB, NFκB is free to translocate into the nucleus and activate transcription of proinflammatory genes [24–26] (Fig. 2). Regardless of whether or not increased activation of NFκB is a primary or secondary process in CF cells, its central role as a regulator of many downstream inflammatory responses makes it an obvious target for potential anti-inflammatory drugs.

Studies have suggested that the local environment of airway secretions surrounding CF respiratory epithelial cells enhances NFκB activation by increasing local calcium-dependent signaling of this transcription factor [27]. The observation that the presence of CF airway mucus enhances NFκB activation supports the belief that improved airway clearance can indirectly reduce inflammation in the CF airway. Several studies demonstrate increased oxidative stress in the CF airway, which also results in increased NFκB activation. This is exacerbated by low levels of nitric oxide (NO) from a deficiency of inducible nitric oxide synthase in epithelial cells [28, 29] and low concentrations of glutathione in extracellular lining fluid, possibly due to deficient transport of this antioxidant through CFTR channels [30, 31]. In CF animal models and human cell culture studies, mitochondria have decreased glutathione reserves and increased levels of reactive oxygen species (ROS) [31, 32]. The proteome of human CF and non-CF respiratory epithelial cells likewise shows an imbalance in proteins related to redox control, favoring increased oxidative stress in CF [33]. ROS, as intracellular messengers, are complicit in NFκB activation in respiratory epithelia [34]. Thus, compounds with antioxidant properties may be effective anti-inflammatories in a state of increased oxidative stress such as CF-related lung disease.

Other immunoregulatory molecules appear deficient in CF. IL-10, an important anti-inflammatory cytokine produced by many cell types, including macrophages, epithelial cells, and T and B lymphocytes, is reduced in CF [35, 36]. This decrease in IL-10 is peculiar because lipopolysaccharide (LPS) and TNF-α, which are abundant in the CF airway, stimulate IL-10 production in subjects without CF [37]. Nonetheless, we and others have reported that CF patients' BAL contains relatively little IL-10 as compared to normal BAL [5, 38]. Both IL-10 and NO limit NFκB activation by preserving IκB. By this and other mechanisms, IL-10 induces neutrophil apoptosis, decreases antigen presentation and T-cell stimulation, and helps to terminate the inflammatory response [39, 40]. Therefore, the apparent deficiency of this immunoregulatory cytokine in CF may be an important component of both the heightened and prolonged airway inflammatory response.

Activation of peroxisome proliferator-activated receptor gamma (PPARγ), a transcription factor that limits NFκB activity, also is reduced in CF [41, 42]. Abnormalities in the fatty acid content of CF cells, with deficiencies of both docosahexaenoic acid (DHA) and linoleic acid, may contribute to the impaired PPARγ signaling and the hyper-inflammatory state [41]. Lipoxin A4 (LXA_4) is an important endogenous anti-inflammatory lipid mediator that

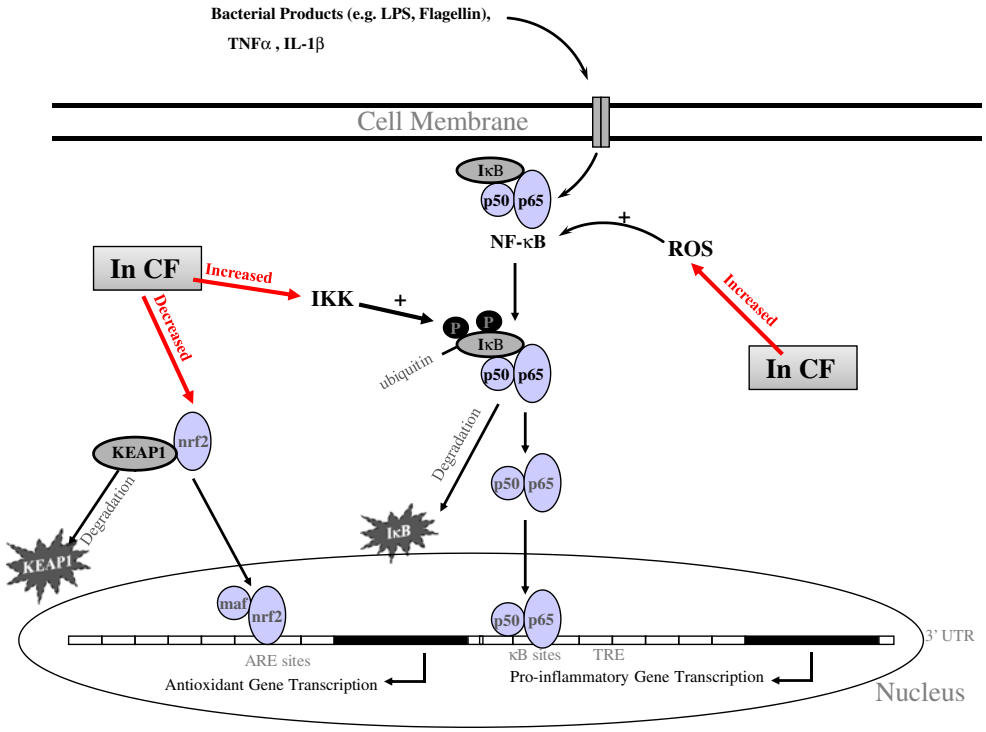

Fig. 2 Simplified depiction of intracellular signaling believed to be pivotal in the CF inflammatory response. Multiple stimuli are capable of inducing the activation of NFκB transcription factor. This is achieved when IKK (I kappa kinase complex) phosphorylates IκB, causing it to dissociate from NFκB and allowing the transcription factor to translocate to the nucleus to promote the transcription of multiple proinflammatory genes. Nrf2 is a transcription factor also typically sequestered in the cytoplasm by an inhibitory protein (KEAP1). When nrf2 is activated, it translocates to the nucleus to induce the transcription of multiple antioxidant and cytoprotective genes, often as part of the phase II inflammatory response. This response is believed to be central to mitigating and ultimately haulting acute inflammation. In CF, abnormalities exist in these signaling pathways, including increased NFκB activation through increased IKK activity. Contrary to other conditions with increased oxidative stress, CF epithelial cells have decreased nrf2 activity. These abnormalities likely help to explain the heightened and prolonged inflammatory state

promotes the resolution of acute neutrophilic inflammatory responses [43]. It has been reported that LXA_4 concentrations are reduced in BAL fluid from stable CF patients compared to non-CF patients with pulmonary inflammation [43]. Although the reasons for the decreased LXA_4 concentrations have not been elucidated, administration of a metabolically stable lipoxin analog in cell culture and mouse models of chronic airway infection and inflammation decreased IL-8 production and neutrophilic inflammation without increasing infectious burden [43]. The role of LXA_4 deficiency in CF lung disease remains controversial, and a more recent study found no difference in LXA4 concentration in BAL from CF patients compared with non-CF disease-matched controls [44]. Finally, nuclear factor E2-related factor 2 (nrf2), a transcription factor active in respiratory epithelia and pivotal to mitigating the acute inflammatory response, has recently been shown to be deficient in CF cells [33] (Fig. 2). Thus, many regulatory pathways responsible for limiting the inflammatory response in CF are dysfunctional. While no unified pathway connecting CFTR dysfunction to exuberant inflammation has been found, the combined effects of the many alterations in cellular processes help explain the exaggerated and prolonged inflammatory phenotype.

Recent work proposes a role for adaptive immunity in CF, as a newly described subset of T cells known as TH-17 cells were shown to be involved in CF pulmonary inflammation [45]. IL-23 and IL-17 are proinflammatory cytokines involved in TH-17 cell signaling and are elevated in CF airways (human and mouse) in response to common stimuli [46, 47]. Viscous secretions at the apical surface of airway epithelia trap bacteria and may magnify the likelihood of antigen presentation to dendritic cells and macrophages, which, in turn, triggers the IL-23/IL-17 signaling pathway. Work has shown that IL-17 is important in recruiting neutrophils to the airway in response to bacterial products such as LPS and the relative importance of IL-17 in the CF airway is a focus of continued research [48].

To summarize, when considering potential anti-inflammatory strategies, several aspects of CF airway inflammation are notable. (1) The process is primarily endobronchial rather than alveolar or systemic. (2) It is

characterized by persistent neutrophil influx. (3) Proinflammatory signaling pathways have significant redundancy. (4) NFκB activation is a pivotal transcriptional regulator of inflammation within cells. (5) The inflammatory response does not terminate properly once initiated and exists in a state of heightened oxidative and proteolytic stress.

The endobronchial location makes CF pulmonary inflammation potentially amenable to inhaled therapies, particularly if started before the development of significant architectural damage and airway obstruction. Because the neutrophil is the primary effector cell mediating the damaging inflammation, anti-inflammatory therapies must address, either directly or indirectly, the neutrophil itself or its products. Therapies targeting proinflammatory signaling may require multiple sites of action to overcome redundancy. Compounds that work to terminate the inflammatory process, such as those that activate IL-10 or the late phase inflammatory response, may be effective in CF. Likewise, antioxidant and protease antagonists may be important as well. Many potential anti-inflammatory targets exist, and a combination of therapies may be most effective. In developing such therapies, one must not forget that this inflammation is undoubtedly protective from resident bacterial infection and that anti-inflammatory therapies will have to be balanced against such protective effects.

Therapies directed at the inflammatory response of the cystic fibrosis airway

The regular use of airway clearance therapies and antibiotics limit the inflammatory stimulation within CF airways and are mainstays of patient care. Additionally, other medications have been used to directly suppress the overabundant inflammatory response. To date, corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) have garnered the most attention. Because of concerns by clinicians regarding safety or efficacy, these therapies are not widely prescribed. Investigators continue to evaluate other potential anti-inflammatory agents in CF including immunoregulatory cytokines, antibodies to proinflammatory cytokines, intracellular signaling inhibitors, eicosanoid modulators, antioxidants, protease inhibitors, and antibiotics with anti-inflammatory properties.

Corticosteroids

Corticosteroids have broad and potent anti-inflammatory effects. They limit edema and mucus formation, inhibit leukocyte chemotaxis, adhesion and activation, and inhibit NFκB activation. Systemic corticosteroids have selectively been used for some time in CF to treat allergic bronchopulmonary aspergillosis and asthma. In the 1980s, investigators observed that CF patients with hypogammaglobulinemia treated with systemic corticosteroids had preserved lung function [49], and trials were undertaken to evaluate the systemic administration of corticosteroids on CF lung disease. Separate 4-year, double-blind, placebo-controlled clinical trials testing the effects of alternate day oral prednisone administered at 1–2 mg/kg/dose demonstrated preserved lung function with steroid treatment [15, 16]. In one study, 21 patients age 1 to 12 years with mild to moderate lung disease had better lung function, improved weight gain, fewer hospital admissions, and stable immunoglobulin G (IgG) concentrations with no apparent adverse effects during the study period compared to patients receiving placebo [15]. This led to a larger, multicenter study in which two alternate-day dosing regimens of prednisone (1 and 2 mg/kg) were compared with placebo over a 4-year period in 285 CF patients, aged 6 through 14 years, with mild to moderate lung disease [16]. Although beneficial effects on lung function were observed, particularly in patients infected with *P. aeruginosa*, so were adverse effects, including glucose intolerance, growth impairment, and cataracts. The 2 mg/kg group was halted halfway through the study due to a high incidence of steroid-related side effects [50]. By the end of the trial, these side effects were also seen in the 1 mg/kg group [16]. Follow-up 6 years after the completion of this trial showed persistent deficits in growth, even after prednisone had been discontinued [51]. Subjects in the 1 mg/kg prednisone group also seemed to have an accelerated rate of decline in forced expiratory volume in 1 s (FEV_1) after discontinuation of the drug to the point that there no longer was a difference between placebo and prednisone-treated patients. A 5-year follow-up analysis from the earlier trial [15] found that 14 of the 17 patients assigned to the prednisone group and who completed the trial developed growth retardation, two developed cataracts, and two developed glucose abnormalities [52]. Due to the significant risk of adverse events and data indicating that corticosteroids worsen osteopenia/osteoporosis [53–55] and promote proximal skeletal muscle weaknesses [56] in CF patients (who are already at high risk for such conditions), long-term use of prednisone at 1 or 2 mg/kg every other day for slowing the progression of lung disease is not recommended. However, it is worth noting both the benefits to lung function and the lack of increased infection in treated patients again suggesting that the inflammatory response in the CF airway is excessive relative to the burden of bacteria. Thus, limited and selective use of corticosteroids may be warranted and beneficial in certain patients, and effective anti-inflammatory medications with safer adverse effects profiles would likely benefit the majority of CF patients.

Shorter courses of systemic corticosteroids have been studied. Prednisolone (the bioactive form of prednisone) was given at 2 mg/kg/dose (maximum, 40 mg) daily for 2 weeks and tapered to 1 mg/kg per dose every other day for an additional 10 weeks in a placebo-controlled, double-blind design in 24 children with CF [57]. Compared with placebo, the prednisolone group had increased forced vital capacity (FVC) and FEV_1 that were associated with decreased serum IgG and cytokine concentrations. These findings suggest that short courses of steroids may be useful, possibly as adjunct to the treatment of exacerbations, when inflammatory activity is greatest.

Following trials of systemic corticosteroids, investigators evaluated inhaled corticosteroids (ICS) in hopes of finding similar benefits to pulmonary health while avoiding the prohibitive adverse effects. These medications have been shown to inhibit NFκB activation [26], and a recent study showed that fluticasone proprionate decreased LPS-induced release of IL-6 and IL-8 in CF tracheal xenografts implanted in nude mice [58]. Unfortunately, significant clinical benefits of ICS have been difficult to demonstrate in patients with CF. Available clinical trials of ICS in CF do not convincingly or consistently demonstrate an effect on lung function or inflammatory markers in airway secretions [59–65]. Conclusions from these studies are limited due to relatively small study populations and short observation periods. Most dosing regimens used in these trials were extrapolated from use in patients with asthma, which may be inadequate to penetrate the large amount of mucus present in the CF lung, although larger doses may be associated with adverse effects [66]. Although clinical trials have failed to demonstrate benefit from ICS, preliminary results from an analysis of observational data from CF patients aged 6–12 years participating in the Epidemiologic Study of Cystic Fibrosis revealed that the institution of ICS was associated with a marked reduction in subsequent rate of FEV1 decline [67]. However, the most recent Cochrane Review of ICS in CF concluded that there is insufficient evidence to determine if they are beneficial or harmful [68]. Since that review, a withdrawal study of ICS in CF patients was performed in UK and did not detect a significant harm in discontinuing these medications during a relatively short observation period [69]. These investigators recommend against the use of ICS in most patients with CF due to a lack of proven benefit and increased time and financial burdens from daily medication use.

While the administration of ICS to infants with CF suggests that they cause no adrenal suppression when administered for up to 2 months [65], case reports documenting significant adrenal suppression in CF and non-CF patients emphasize the importance of designing studies of sufficient duration and power to evaluate the effects of ICS on the adrenal glands [70]. Moreover, other long-term complications of ICS, including growth failure, glucose intolerance, cataract formation, and decreased bone mineral density, have not yet been adequately evaluated in patients with CF. A preliminary report that ICS may be associated with earlier acquisition of *P. aeruginosa* is also concerning [71]. The lack of convincing data regarding efficacy and lingering concerns over safety in CF patients gives pause when considering the use of ICS in young children with CF. Unfortunately, this may be the time when anti-inflammatory therapy would be most efficacious, before the onset of irreversible damage to the airway wall. Additional trials of ICS in CF using larger treatment groups, longer observational periods, and possibly higher doses will be necessary to better determine safety and efficacy of this class of drugs as anti-inflammatory agents.

Ibuprofen

Though used by <10% of CF patients in the USA, high-dose ibuprofen is the only anti-inflammatory agent that has proven efficacy with an acceptable safety profile. Several animal and human studies support the use of ibuprofen as a chronic therapy for CF lung disease. In a rat model of chronic pseudomonas endobronchial infection, ibuprofen inhibited neutrophil migration into the lung without worsening infection [72]. This finding led to a 4-year, double-blind, placebo-controlled clinical trial of twice daily high-dose ibuprofen in 85 CF patients, aged 5 to 39 years with mild lung disease [17]. Individual doses were determined by pharmacokinetics to reach the desired peak plasma concentrations between 50 and 100 μg/ml, which generally required doses between 20 to 30 mg/kg. Compared to placebo, ibuprofen-treated patients had significantly less decline in pulmonary function measures, better preservation of body weight, fewer hospital admissions, and better Brasfield chest radiograph scores [17]. The effect was most pronounced in the youngest age group (5 to 13 years old), in which the annual rate of decline in FEV_1 was reduced by 88% compared to the placebo group. There were no significant differences in adverse effects between the treatment groups (although the sample size was too small to detect even common adverse effects), but two subjects could not tolerate long-term ibuprofen use because of increased epistaxis in one and conjunctivitis in another. The incidence of gastrointestinal (GI) complications was actually lower in the ibuprofen group compared to the placebo group. Interestingly, 18 years after the start of the 4-year trial, survival has been better in patients randomized to ibuprofen (81%) compared to those randomized to placebo (70%; unpublished data).

A second randomized placebo-controlled trial of high-dose ibuprofen conducted in Canada in 142 CF patients demonstrated significant preservation of FVC over a 2-year observational period [19]. There was a trend toward better

preservation of FEV_1 in patients treated with ibuprofen. A pre-study power analysis revealed that 200 patients would be required to demonstrate a significant effect on FEV_1, but study enrollment fell short. An important occurrence in this study was that nearly twice as many patients in the placebo group withdrew due to adverse event as did in the ibuprofen group (7 vs. 4), for reasons that otherwise could be attributed to ibuprofen. This nicely demonstrates the importance of controlled trials, particularly when known adverse effects of a drug are similar to those caused by the disease itself.

The precise anti-inflammatory mechanisms of action of ibuprofen in CF lung disease are unknown. High concentrations of ibuprofen induce expression of heat shock protein 70 (HSP70), an important inhibitor of proinflammatory cytokine production [73], and inhibit the activation of NFκB and AP-1, two important proinflammatory transcription factors [74–76]. These intracellular effects help to explain the selective activity of high-dose ibuprofen against neutrophils. It should be noted that doses below the targeted 50 μg/ml are ineffective and may even have proinflammatory actions by increasing neutrophil influx to the site of inflammation [77]. Therefore, individual pharmacokinetics are required when starting this therapy and periodically (on average every 2 years) while taking ibuprofen. In addition to direct anti-inflammatory effects, ibuprofen may enhance mucociliary clearance in CF by counteracting the ability of *P. aeruginosa* to downregulate P2Y receptors found on the apical surface of airway epithelia [78].

The most common reasons cited for not using ibuprofen include concerns over safety, particularly GI hemorrhage and nephrotoxicity. In a survey of US CF centers shortly after ibuprofen was advocated as a therapy for CF lung disease, providers cited these concerns as well as the need to establish the dose in each patient through a pharmacokinetic test and the need for more data regarding effectiveness as impediments to use [79]. A recent analysis of the US CF Foundation (CFF) Patient Registry revealed a 29% reduction in the rate of FEV_1 decline in 6- to 17-year olds with mild lung disease (FEV_1 > 60% predicted) compared to those not treated with this therapy [20]. The incidence of GI hemorrhage requiring hospitalization was significantly higher in patients treated with ibuprofen (0.37% vs. 0.14% per year) but, overall, was low in both groups. The incidence of GI hemorrhage due to ibuprofen might be reduced by concomitant administration of antacids (e.g., proton pump inhibitors) or misoprostol (a PGE_1 analogue). These gastroprotective strategies have proven effective in trials of long-term NSAID use but have not been directly studied in CF patients receiving high-dose ibuprofen. Renal failure associated with ibuprofen therapy has been the subject of a few case reports, but based upon US CFF Registry data, the incidence is not increased among CF patients treated with ibuprofen.

The additional clinical trial data showing effectiveness and generally good tolerability of ibuprofen taken twice daily on a chronic basis were sufficient for a recent Cochrane Review to conclude that high-dose ibuprofen can slow the progression in lung disease in children with CF with an acceptable safety profile [80]. Real-world clinical use of ibuprofen, as shown by the CFF Patient Registry, supports this conclusion [20]. The CFF now recommends considering high-dose ibuprofen for CF patients with mild lung disease [81]. This therapy received a grade of "B" reflecting fair strength of evidence demonstrating effectiveness and a moderate estimated net benefit from use (projected benefit minus harm). It is not known whether CF patients with more advanced lung disease would derive the same benefit from this therapy nor has a safety profile been established for these patients. Overall, the available data thus far suggest that the health benefits of high-dose ibuprofen outweigh the risks in young patients with mild to moderate lung disease; however, continued monitoring is necessary to further determine the efficacy and safety of long-term use of ibuprofen in CF.

Macrolides

Studies demonstrating benefit in patients with diffuse panbronchiolitis led researchers to investigate the use of these medications, particularly azithromycin, in CF patients. Trials in CF have now demonstrated that the chronic use of this drug, typically given thrice weekly at 250 or 500 mg based on body weight, decreases the rate of pulmonary exacerbations and improves lung function in CF patients [82–85]. Significant debate remains regarding the mechanisms by which azithromycin is effective in CF and whether it is truly an anti-inflammatory drug. Pseudomonas may be more susceptible to azithromycin in a stationery growth phase, which is likely the most common state of this bacterium in the CF airway [86]. Additionally, the macrolides may exert antibiotic effects by inhibiting virulence factors in *P. aeruginosa*, including biofilm formation and quorum sensing [86–93]. Immunomodulatory capabilities may also play a role as macrolides appear to inhibit neutrophil migration and elastase production and to reduce the production of proinflammatory mediators including TNF-α, IL-1β, IL-8, and NO_2^- [94–99], possibly by inhibiting the activity of transcription factors regulating cytokine production. For example, clarithromycin and azithromycin were shown to decrease IL-8 production in bronchial epithelial cells by inhibiting the NFκB and AP-1 signaling pathways [100, 101]. Thus, the macrolides may convey anti-inflammatory effects through a combination of antimicrobial and immunomodulatory capabilities.

Positive results from a multi-center placebo-controlled trial in the USA in 185 CF patients colonized with *P. aeruginosa* with mild to moderate lung disease led to the recommendation that chronic administration of azithromycin be considered in patients infected with *P. aeruginosa* [85]. Patients who were randomized to receive thrice weekly azithromycin (250 mg for weight < 40 kg, 500 mg for body weight ≥40 kg) showed improvements in lung function, weight gain, and quality of life, as well as a reduction in CF pulmonary exacerbations compared to patients who received placebo [85]. A multicenter, placebo-controlled trial conducted in Europe involving younger CF patients without *P. aeruginosa* colonization also found pulmonary benefits. Of the 82 patients enrolled, those randomized to thrice weekly azithromycin had fewer pulmonary exacerbations and less need for other oral antibiotics [102]. No significant effect on FEV1 was observed over the 12 month observational period. Azithromycin was found to be safe and well tolerated in these trials, though nausea, diarrhea, and wheezing were more common in the patients who received this drug [85]. A trial of azithromycin in patients not infected with *P. aeruginosa* is underway in the USA. From available data, the CFF has recently given macrolide antibiotics a recommendation of grade "B," reflecting fair evidence of effectiveness and substantial potential for net benefit from use [81].

Antioxidants

As discussed above, general oxidative stress and specific deficiencies of glutathione have been found in the CF lung. Oxidative stress has been shown to affect many intracellular pathways, including increased activation of NFκB [103–109]. Therefore, antioxidants may have anti-inflammatory benefits in conditions of increased oxidative stress, such as the CF airway. *N*-acetylcysteine (NAC) is an antioxidant that both inhibits H_2O_2 and increases glutathione concentrations by donating cysteine. Doses of NAC up to 3 g per day given to patients with CF for 4 weeks significantly increased glutathione levels in whole blood and peripheral neutrophils [110]. Decreased levels of sputum IL-8, sputum neutrophil content, and sputum neutrophil elastase activity were also observed. No significant change in pulmonary function was observed over the 4-week study, but these encouraging results have led to a trial of longer duration. One challenge to NAC therapy may be identifying a dosing strategy that both significantly impacts the oxidative state in the lung and is broadly acceptable to CF patients.

As decreased lung levels of glutathione, particularly reduced glutathione, have been demonstrated in CF mice [32] and CF patients [30], it may be beneficial to directly augment the concentration of this antioxidant in the CF lung. Glutathione levels in BAL fluid may be increased by twice-daily treatment with aerosolized glutathione. This treatment also reduces superoxide production in response to phorbol myristyl acetate by inflammatory cells recovered from the BAL fluid [111]. Small studies assessing BAL fluid from CF patients treated with inhaled glutathione have not detected significant changes in markers of oxidative stress, despite beneficial effects on PGE2 and lymphocyte content and modest improvements in lung function [112, 113]. These data raise the possibility that inhaled glutathione is operating through currently unidentified yet potentially beneficial mechanisms. Inhaled glutathione therapy for CF has attracted much attention in the lay press. However, its therapeutic and adverse effects have not been studied sufficiently in controlled clinical trials to warrant clinical use, and establishing an optimal formulation, route of delivery, dose, and dosing schedule of glutathione will be required before long-term efficacy and safety studies can commence. Failure to do so may result in serious consequences for patients who elect to treat themselves with unapproved formulations.

Others have also tried to impact the oxidative state in CF through oral supplementation of antioxidants [114]. Forty-six patients with CF were randomized to receive low-dose antioxidant supplementation (10 mg vitamin E and 500 μg vitamin A) or high-dose antioxidant supplementation (200 mg vitamin E, 300 mg vitamin C, 25 mg β-carotene, 90 μg selenium, and 500 μg of vitamin A). Serum concentrations of vitamin E, β-carotene, selenium, and glutathione peroxidase improved in the high-dose group but not the low-dose group [114]. Although there was no difference in lung function between the two groups, within the high-dose group, β-carotene (a vitamin A precursor) correlated with FVC and selenium correlated with FEV_1 [114]. Supplementation of oral β-carotene in CF has also been evaluated in three observational studies and one placebo-controlled trial [115–118]. In an uncontrolled 16-month observational study, oral β-carotene supplementation in 33 vitamin-E-sufficient CF patients decreased plasma levels of elastase/α_1-protease inhibitor complex (a marker of lung inflammation) and plasma lipid levels [115]. A second observational study in 18 children with CF and 15 patients with bronchiectasis found that, after 6 months of β-carotene supplementation, the plasma levels of β-carotene and vitamin E increased while the plasma levels of TNF-α and malondialdehyde decreased [116]. Recently published data report that a novel micellar formulation of fat-soluble nutrients and antioxidants (including β-carotene) was capable of increasing serum levels of antioxidants while decreasing sputum concentrations of myeloperoxidase in a small number of patients [118]. In a placebo-controlled trial of 24 subjects with CF, high-dose β-carotene supplementation (1 mg/kg/day, maximum 50 mg) increased serum levels of β-carotene and decreased

the need for antibiotics for treatment of a pulmonary exacerbation [117]. Vitamins E (alpha tocopherol) and C (ascorbic acid) also have antioxidant properties and are often prescribed to patients with CF, especially vitamin E. Their antioxidant effects are only beginning to be rigorously studied in CF [119–122]. Although the evidence is limited, these results suggest that antioxidant supplementation could attenuate lung inflammation and therefore deserves further study.

Docosahexaenoic acid

Investigators have shown that oral administration of the omega-3 fatty acid DHA to *cftr* −/− mice corrected inherent lipid imbalances in the lung and GI tract [123]. DHA administration also decreased airway neutrophils and eicosanoids in CF mice but not wild-type mice after aerosol exposure to *Pseudomonas* LPS [124]. Current essential fatty acid supplements may not contain enough bioactive DHA, and some preparations contain fatty acids that may antagonize the effects of DHA. Studies of DHA supplementation in CF patients, unfortunately, vary widely in source and dose of DHA supplementation, study size, duration of treatment, and outcome measures. Few are placebo-controlled, and many do not demonstrate a clinical effect on lung function—possibly due to short study periods. Despite these drawbacks, the cumulative data from these studies indicate that DHA oral supplementation may effectively increase serum and phospholipid concentrations of this essential fatty acid and that this may have beneficial health effects, including improved pulmonary function. To validate this claim, a larger, placebo-controlled trial with an adequate duration of study is necessary. Until such a study is performed, DHA supplementation should be given with caution, as high-dose supplementation is believed to increase oxidative stress and may have deleterious effects. A thorough review of DHA supplementation in CF has recently been published [125].

Eicosanoid modulators

Eicosanoids, including LTB_4, a potent neutrophil chemoattractant, are increased in the sputum and BAL from patients with CF [8, 126]. It is difficult to determine the relative contribution of each neutrophil chemokine present in the CF airway. However, once present, neutrophils release LTB_4 that contributes to further neutrophil accumulation [127]. Neutrophil products such as elastase also may contribute to the chemotactic load by stimulating alveolar macrophages to release even more LTB_4 [128]. Therefore, it seems logical that limiting LTB_4 effects should be beneficial in CF. 5-Lipoxygenase inhibitors such as zafirlukast decrease LTB_4 production and are in clinical use for asthma [129]. However, concerns about safety in patients with underlying liver disease and the need for frequent dosing regimens have limited its use in CF. An alternative drug amelubant [130], a specific LTB_4 receptor antagonist, was tested in a randomized, placebo-controlled, double-blind phase II clinical trial in both pediatric and adult CF patients. The trial was halted early due to increased risk of serious pulmonary-related adverse events, primarily hospitalizations for pulmonary exacerbation in adults receiving amelubant [131]. Although antagonists of the cysteinyl leukotriene receptor, such as montelukast and zafirlukast, may benefit CF patients with airway reactivity, their potential impact on the airway inflammatory response in CF may be limited because the cysteinyl leukotrienes (LTC4, LTD4, and LTE4) are not viewed as important in fueling the neutrophil dominant inflammatory response. However, in one study, 26 children with CF treated with montelukast in a randomized, placebo-controlled, double-blind crossover fashion for 8 weeks seemed to be of benefit [132]. Spirometry and biomarkers of inflammation in serum and sputum were monitored, reflecting significant improvement in FEV1, FEF25/75, and markers of inflammation (sputum IL-8, eosinophil cationic protein, myeloperoxidase; serum IL-8, and eosinophil cationic protein) [132]. Moreover, montelukast also was associated with increased concentrations of IL-10 in serum and sputum, when compared to placebo in this study. Another small study in CF, including a report of long-term follow-up, also indicates that montelukast therapy may have an anti-inflammatory benefit with improved small-airway function in CF patients [133, 134]. It is unclear whether montelukast is responsible for a direct attack on the inflammatory response in CF or if its effects occur indirectly, providing improved bronchodilation and enhancing airway clearance of secretions laden with proinflammatory mediators. Despite these promising results in small studies, multicenter trials with greater numbers of patients and longer observational periods are needed before recommendations regarding the use of montelukast as an anti-inflammatory agent in CF are made.

Cytokines and anti-cytokines

Pharmacological therapies have been developed which target both proinflammatory and regulatory cytokines that are important in CF lung inflammation. The most widely used agents in other inflammatory conditions include blockers of TNF-α receptor activation (e.g., infliximab, etanercept, and adalimumab). These agents are effective anti-inflammatories in rheumatoid arthritis, psoriasis, and inflammatory bowel disease (reviewed in [135]) but may be too potent for use in CF. Concerns have been raised that these drugs might overly suppress the inflammatory

response leading to secondary infectious complications. The use of these drugs is associated with reemergence of latent tuberculosis, and many worry that their use in CF may make it difficult to contain both bacterial and mycobacterial infections. No published data regarding their use in CF lung disease is available.

Several other biological-based anti-inflammatory therapies have been developed for autoimmune diseases and cancer. These drugs have numerous targets, including IL-6, IL-15, IL-17, B-lymphocyte stimulator, and the CD20 B-lymphocyte receptor. No published data reporting their use in CF is available. A central question yet to be answered is whether the inherent redundancy of the inflammatory cytokine networks active in CF lung disease will preclude the use of such specific, albeit potent inhibitors. Many investigators believe that the broad effects of drugs such as corticosteroids and NSAIDS are important to their effectiveness in CF lung disease. Further research in animal models and well-designed clinical trials will be necessary to answer these questions. More practically, the cost of these biological drugs in their current forms and the need for subcutaneous injections may also limit their widespread use as chronic therapy for CF.

Most cytokines are pleiomorphic, and while some are primarily proinflammatory, others, like IL-10, are primarily anti-inflammatory or regulatory. The relative deficiency of IL-10 in CF may contribute to the persistent neutrophilic inflammation seen even after a stimulus has been eradicated. *iL-10* −/− mice have much worse endobronchial inflammation for the same inoculum of *P. aeruginosa* compared to their wild-type littermates, and administration of IL-10 to mice with chronic endobronchial pseudomonas infection is beneficial [136, 137]. Thus, restoring IL-10 to the CF airway or administering it in pharmacologic quantities would seem advantageous. A planned clinical trial of the drug was stopped for reasons unrelated to CF, but this remains an interesting therapeutic option, and other drugs that increase IL-10 production may be of benefit to CF patients.

One cytokine that has come to trial in CF is interferon-γ. The rationale for its use is several-fold. In CF, the Jak-Stat signaling pathway in airway epithelial cells is inefficient, reducing messenger RNA (mRNA) production for interferon regulatory factor, nitric oxide synthase (NOS)-2, regulated on activation normal T cell expressed and secreted (RANTES), and other Stat-1 dependent products [138–140]. This signaling deficiency is likely related to increased concentrations of RhoA and the protein inhibitor of activated stat-1 found in these cells [141]. Due to the deficiency of NOS-2, epithelial cells produce less NO, which likely contributes to the inflammatory response by destabilizing IκB. These functions can be restored in cell models by application of excess gamma interferon [142]. To help determine if this effect is achievable in vivo, 66 CF patients were studied in a randomized, placebo-controlled, double-blind trial of interferon-γ1B [143]. The medication was nebulized thrice weekly for 12 weeks at either 500 mcg or 1,000 mcg doses. There was a trend toward improvement in FEV_1 in the 500 mcg dose group but an increased rate of hospitalization for pulmonary exacerbations in the 1,000 mcg group, although it should be noted that this group had greater sputum bacterial density at enrollment [143]. Ultimately, nebulized interferon-γ1B did not significantly alter lung function, sputum bacterial density, or sputum biomarkers of inflammation over the 12-week study and is no longer being considered a potential therapy in CF.

Antibodies to proinflammatory mediators, like intercellular adhesion molecule-1 (ICAM-1) and IL-8, have been evaluated in preclinical studies but never came to fruition in CF clinical trials. One recent antibody, anti-IL-17, has generated much excitement and hopefully will be evaluated in a comprehensive research program. Work has shown that IL-17 is important in recruiting neutrophils to the airway in response to bacterial products such as LPS and that anti-IL-17 antibodies can effectively reduce airway neutrophilia in mice stimulated with LPS [48]. IL-17 appears to be an important mediator of inflammation in human chronic inflammatory diseases such as rheumatoid arthritis and psoriasis [144, 145]. Clinical trials of anti-IL-17 in rheumatoid arthritis and psoriasis have recently completed. Given the similarities between CF lung inflammation and these hyper-inflammatory conditions, clinical trials of anti-IL-17 are being considered for CF.

Inhibitors of intracellular signaling

As part of the exuberant airway inflammation in CF, proinflammatory cytokines, chemokines, and other mediators of inflammation are present in excess. Finding ways to interrupt the signaling pathways responsible for their production may be an effective strategy. Knowledge of genomic and non-genomic intracellular events involved in the inflammatory response is rapidly growing but assuredly incomplete. Furthermore, how these pathways relate to the basic defect in CF is unknown. As a somewhat simplistic view of a collection of highly complex interactions, NFκB activation has a well-accepted role as a central regulator of proinflammatory signaling. Many effective anti-inflammatory therapies in CF limit NFκB activation to some degree, thereby decreasing production of several proinflammatory mediators involved in CF airway inflammation at the transcriptional level. For example, dexamethasone in vitro reduces the excess cytokine production by CF airway epithelial cells [146]. Glucocorticoids have several intracellular effects including the ability to bind to and inhibit NFκB directly and to induce transcription of IκB [147, 148].

High-dose ibuprofen and IL-10 also inhibit NFκB activation by preserving IκB, thereby downregulating the inflammatory response [147, 148]. Other intracellular therapeutic targets include inhibiting the mitogen-activated protein kinase (MAPK) pathways. The p40/p42 and p38 MAPK pathways are activated when bacteria interact with airway epithelial cells. Activation of the p38 MAPK pathway may prolong the half-life of cytokine mRNA molecules. This may have significance in CF, as there seems to be sustained production of inflammatory cytokines, even after a stimulus has been removed. Therefore, inhibiting the p38 MAPK pathway may help to decrease the inflammatory response. However, altering the activity of intracellular signaling pathways is not without risk. In addition to their role in exuberant inflammation, these transcription factors possess other important activities such as mounting an appropriate response to infection, maintaining cellular homeostasis, activating differentiation and maturation of certain cell types, and stimulating production of immunoregulatory molecules. Complete inhibition of these pleomorphic effects would likely result in significant detrimental consequences, which far outweigh their potential anti-inflammatory benefit in CF. Clearly, extensive studies in preclinical models would be necessary before proceeding to a trial in humans.

One small molecule group currently in preclinical trials in CF is the synthetic triterpenoids. These compounds are synthetic derivatives of naturally occurring compounds used in Asian medicine for centuries. The natural compounds possess relatively modest anti-inflammatory and anti-carcinogenic properties, and the synthetic derivatives potently limit inflammatory responses by inhibiting both IKK complex and Kelch-like ECH-associated protein 1 (KEAP1) [149–152]. By inhibiting IKK, these drugs prevent the degradation of IκB, thereby inhibiting NFκB activation. KEAP1 binds the nrf2 transcription factor in the cytoplasm, marking it for proteasomal degradation. Therefore, by inhibiting KEAP1, the triterpenoids preserve nrf2 activity, a central transcriptional promoter of many antioxidant and cytoprotective genes. The battery of genes regulated by nrf2 is central to the cells redox capability, influencing the initiation and propagation of inflammatory signaling. Recently, in vitro work has demonstrated specific deficiencies of both IKK and nrf2 in CF models [24–26, 33, 153]. Therefore, small molecules, like the synthetic triterpenoids that appear to target known proinflammatory signaling abnormalities in CF cells, may be excellent candidates for anti-inflammatory therapy. To this end, early work in CF mice has demonstrated the ability of 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO), a synthetic triterpenoid, to greatly reduce the inflammatory response after direct airway administration of either LPS or flagellin, two inflammatory components of *P. aeruginosa*. Cell culture studies show that nanomolar concentrations of CDDO can limit NFκB activation by ~50% in CF-like epithelial cells stimulated with TNF-α and that this mitigating effect is greater in CFTR-deficient cells than in matched non-CF cells [153]. Much research is still needed to better determine the potential utility of these compounds, but early data support the theory that compounds affecting transcriptional regulators of inflammation (both pro- and anti-inflammatory) are a promising therapeutic approach. In further support of this concept is a recent report demonstrating the ability of parthenolide, another small molecule compound derived from the medicinal plant feverfew. In CF cell culture and mouse models, parthenolide decreased the inflammatory response by inhibiting IKK and thereby decreasing NFκB activation [154].

Protease inhibitors

Early in life, neutrophils infiltrate the CF airway and release massive amounts of active proteases that overwhelm local anti-protease defenses, particularly alpha-1-protease inhibitor (α_1-PI) and secretory leukocyte protease inhibitor (SLPI). α_1-PI diffuses into the smaller airways and alveoli from the blood and is carried up the airway by the mucociliary escalator. SLPI is produced and secreted by airway mucosal cells and is the predominant protease inhibitor in the conducting airways. The concentration of α_1-PI in the BAL fluid of CF patients is several-fold higher than in healthy subjects, but even in mild patients, there is a several hundred to several thousand-fold excess of elastase and other neutrophil proteases, which vastly exceeds the capacity of the inhibitors [2, 7, 9]. The imbalance between the proteases and their inhibitors becomes worse during exacerbations and undoubtedly contributes to the patients' deterioration. The challenge of inhibiting the enormous burden of active proteases in the CF airway is substantial. As neutrophils reach the airway by migrating through the epithelium, much of the release of proteases will occur beneath the thick layer of mucus, which is difficult for inhibitors to penetrate. However, in model systems, this can be overcome by using the polymeric immunoglobulin receptor to deliver systemically administered α_1-antitrypsin to the luminal side of the airway epithelium [155].

Exogenous administration of anti-proteases to supplement the inadequate amount of endogenous anti-proteases present in CF airways has been under investigation since the early 1990s. In a rat model of chronic *P. aeruginosa* infection, exogenous α_1-protease inhibitor has been shown to inhibit the effects of elastase and to decrease bacterial burden [156]. In a preliminary study in 12 patients with CF, aerosol delivery of plasma derived α_1-antitrypsin (1.5–3 mg/kg twice daily for 1 week) was shown to effectively suppress active elastase in the airways [157]. A multicenter dose

escalation clinical trial studying the efficacy and safety of plasma derived α_1-antitrypsin administered by aerosol twice daily for 4 weeks also revealed a reduction in active elastase [158]. Another study of inhaled plasma derived α1-AT has recently been published [159]. In this multicenter trial, 52 CF patients were randomized to receive 25 mg Prolastin® (Bayer Corporation, USA) through a nebulizer generating particle size to target either peripheral or larger bronchial airways. No significant difference in outcome measures was seen between the two groups treated with different nebulizer systems; thus, the data were combined and compared with pre-treatment indices. In this study, nebulized α1-AT treatment given daily for 4 weeks significantly decreased free neutrophil elastase activity in sputum and the concentrations of several proinflammatory mediators (TNF-α, IL-1β, IL-8, and LTB4). Treatment with α1-AT also decreased the percentage of neutrophils and concentration of *P. aeruginosa* in sputum. No significant change in spirometry was observed, but one would not necessarily expect to see a change in lung function in a trial of only 4 weeks duration. The results from these trials are encouraging. However, the use of this therapy is limited by expense, supply, and the inherent risks associated with receiving a plasma derived product. To overcome some of these barriers, transgenic and recombinant α1-AT are being considered in CF. A phase II trial of transgenic α_1-antityrpsin in CF patients demonstrated modest results [160]. Subjects who received 250 or 125 mg of study drug once daily by inhalation experienced a trend toward improvement in time to first exacerbation and fewer total hospitalizations compared to subjects who received 62.5 mg of study drug or placebo, but the results did not reach statistical significance [160]. A trial of recombinant α1-AT in CF, the only placebo-controlled trial performed in CF thus far, involved relatively few patients but demonstrated trends toward decreased concentrations of sputum-free myeloperoxidase and IL-8 and increased lung function by spirometry [161]. Although the majority of these outcome measures did not reach statistical significance, possibly due to sample size and length of treatment, it appears that inhaled α1-AT has been well tolerated in these multiple studies. The frequency of drug administration could be reduced by conjugating recombinant human α_1-protease inhibitor with polyethylene glycol to extend its in vivo half-life [162], thereby helping to bring this therapy to clinical trial in CF.

Aerosolization of recombinant human SLPI (rhSLPI) has also been studied. Administration of 100 mg of rhSLPI twice daily to 16 CF patients decreased active elastase and IL-8 in the airways [163]. Lower doses (50 mg twice daily for two weeks) failed to significantly decrease elastase [164]. Unfortunately further development of rhSLPI has been stalled, likely due to the inability to produce sufficient quantities at a less than prohibitive cost. EPI-hNE4 is a highly specific and potent inhibitor of human neutrophil elastase and is also being considered as an anti-protease therapy in CF [165]. This drug effectively decreased neutrophil migration in both cell culture and animal models and inhibited active neutrophil elastase present in sputum from CF children in vitro, but clinical trials have not been performed [166]. Phase II trials in CF are currently underway in Europe.

To date, promising data demonstrate that anti-protease therapy has the potential to significantly impact inflammation in CF. To be effective, however, it may be necessary to inhibit all of the active elastase in the airways because even minute amounts interfere with opsonophagocytosis. Determining the optimal agent, dosing, and best route of administration will require further investigation.

Other agents

Other anti-inflammatory compounds currently undergoing clinical trials as part of the CFF therapeutics pipeline include low-dose methotrexate, hydroxychloroquine, pioglitazone, and simvastatin. Although chemotherapeutics have been studied in refractory asthma, there have been few studies in CF. In one recent pilot study, methotrexate was found to increase FEV_1 and decrease total serum immunoglobulins in five CF patients after 1 year of treatment [167]. However, another recent pilot study in CF patients indicated that methotrexate may be detrimental to pulmonary function and difficult to tolerate long-term [168]. Low-dose cyclosporin A was found to decrease the need for systemic corticosteroids in four out of six pediatric CF patients in one case series [169]. Chest radiograph scores and height velocity also improved. The most common side effects were transient renal impairment, mild hypertrichosis, and gingival hyperplasia. No definitive conclusions can be made from the available data, but further evaluation of chemotherapeutics in CF is warranted. However, due to their toxicities, studies in animal models and carefully designed phase I studies are required before beginning large clinical trials. As with all treatments, utility will largely depend on the balance between potential risks and benefits.

Hydroxychloroquine, which has demonstrated anecdotal efficacy in some rare interstitial lung diseases, is also being evaluated in CF as a potential anti-inflammatory. In addition, HMG-CoA reductase inhibitors (i.e., statins) and thiazolidinediones (i.e., PPARγ agonists) are in early CF clinical trials. Statins may be beneficial by reducing RhoA activation (upregulated in CF) and subsequently increasing production of NO, which has potent anti-inflammatory. PPARγ is a transcription factor with largely anti-inflammatory effects, including the ability to inhibit NFκB signaling [170]. Its activity appears to be relatively

impaired in CF animal models [42], and in vitro supplementation in CF cell culture systems is capable of decreasing NFκB activation [171]. These drugs, including pioglitazone, are used clinically for type II diabetes as oral hypoglycemic agents. Whether doses great enough to effect inflammatory signaling in the lung can be achieved with oral supplementation remains to be determined. Results from early studies of both simvastatin and pioglitazone in CF are pending.

Therapies with indirect effects on inflammation

Airway obstruction, endobronchial infection, and airway inflammation, as major components of CF-related lung pathophysiology, are often interrelated, and therapies targeting one (e.g., airway obstruction) may have beneficial effects on another (e.g., inflammation). As neutrophils necrose, they release DNA and actin. The highly anionic nature of the free DNA causes it to bind with other constituents in CF sputum. Due to the prominent neutrophilia in CF airways and impaired phagocytosis of dying leukocytes, these necrotic cells can release large amounts of DNA into the airway lumen, contributing to the hyperviscosity of CF sputum. This process adds to the dehydrating effects of impaired ion transport at the apical surface to greatly impair the airway clearance component of innate host defense. Additionally, DNA may bind aminoglycoside antibiotics and decrease their efficacy. Recombinant human DNase (dornase alpha) hydrolyzes DNA released from neutrophils, decreases the viscoelasticity of purulent secretions, and increases the ability of mucus to be cleared from the airway [172, 173]. In a pivotal 6-month phase III trial that led to approval of DNase as a therapy for CF, this aerosolized drug resulted in significant improvement in pulmonary function and a decreased rate of pulmonary exacerbation in patients with CF [174]. In a 2-year placebo-controlled clinical trial, dornase alpha was also associated with improved pulmonary function and fewer hospitalizations for treatment of pulmonary exacerbations in young CF patients with mild lung disease [175]. Preliminary analysis of data from the Epidemiologic Study of Cystic Fibrosis showed that administration of dornase alpha was associated with a slower rate of decline in FEV1 after controlling for confounding variables [176]. These effects may be due to improved removal of the damaging inflammatory products during airway clearance maneuvers. Data from another clinical trial demonstrated that treatment with dornase alpha was associated with decreased accumulation of IL-8, neutrophils, neutrophil elastase, and DNA in the BAL fluid [177, 178]. Thus, dornase alpha may be considered an anti-inflammatory therapy, although these effects are probably indirect.

Hypertonic saline inhalation therapy has gained much attention in recent years as a means of improving airway clearance in CF patients. The effects of this therapy on airway inflammation have not been well-studied. One could postulate that inhaled hypertonic saline, much like dornase alpha, may indirectly decrease airway inflammation by improving airway clearance. Trials of hypertonic saline have documented reduced frequency of pulmonary exacerbations and preservations of pulmonary function in CF patients, similar to trials of dornase alpha [179]. Studies of hypertonic saline (4.5%) for sputum induction in chronic obstructive pulmonary disease and asthmatic patients find no differences in many markers of airway inflammation, when compared to isotonic saline [180, 181]. However, these studies do not evaluate potential effects of long-term use, as recommended for improved airway clearance in CF patients. A randomized crossover trial of 48 children with CF treated with dornase alpha or 7% hypertonic saline found no differences in sputum content of several inflammatory markers after 12 weeks of twice daily hypertonic saline compared to baseline [182]. Data from CF cell and mouse models indicates that hypertonic saline exposure to respiratory epithelial cells significantly increases the release of glutathione [183]. Whether this is cautionary evidence of osmotic stress on the epithelia or a mechanism by which hypertonic saline can reduce sputum viscosity and protect the airway from oxidative damage is unknown. This therapy has been widely adopted by CF care providers, and long-term efficacy and safety analysis will help to better determine the benefits and potential safety concerns of chronic daily hypertonic saline.

Drugs targeting the basic defect in CF (i.e., ion channel function) may have indirect benefits, as they affect abnormalities that occur early on in the pathophysiologic cascade (Fig. 1). As noted, research with specific inhibitors and correctors points to a close correlation between CFTR function and inflammatory response; however, the precise intracellular events connecting the two has not yet been identified. Therapies under investigation that work by increasing ion channel function (e.g., CFTR activators, CFTR potentiators, and gene therapy) would be expected to correct the airway surface fluid abnormality and improve airway clearance. It is possible that correcting the basic defect might not fully obviate the host inflammatory response, especially in patients with existing damage to the airway wall architecture. In these patients, anti-inflammatory agents might continue to be beneficial, even once a cure is found. Therapies correcting the basic defect in CF have great potential and are the focus of much research. If realized, it will be interesting to see how this correction affects airway inflammation in patients.

Conclusion

In summary, exaggerated basal and inducible airway inflammation is a major component of the pathophysiology of CF lung disease. This process begins early in life and is interrelated with other airway conditions, including impaired airway clearance and chronic endobronchial infection. Comprehensive treatment targeting each of these areas will likely be the most effective in preserving lung function and thereby reducing morbidity and mortality. Among proven therapies, high-dose ibuprofen and azithromycin are the most widely used chronic anti-inflammatory agents at present. Several other compounds have been studied or are under investigation in hopes of identifying an agent that is effective and more acceptable to the CF community. If realized, this would be a significant advance in the care of patients with CF.

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