Review

Inflammation in cystic fibrosis lung disease:
Pathogenesis and therapy☆

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Abstract

Lung disease is the major cause of morbidity and mortality in patients with cystic fibrosis (CF). Although CF lung disease is primarily an infectious disorder, the associated inflammation is both intense and ineffective at clearing pathogens. Persistent high-intensity inflammation leads to permanent structural damage of the CF airways and impaired lung function that eventually results in respiratory failure and death. Several defective inflammatory responses have been linked to cystic fibrosis transmembrane conductance regulator (CFTR) deficiency including innate and acquired immunity dysregulation, cell membrane lipid abnormalities, various transcription factor signaling defects, as well as altered kinase and toll-like receptor responses. The inflammation of the CF lung is dominated by neutrophils that release oxidants and proteases, particularly elastase. Neutrophil elastase in the CF airway secretions precedes the appearance of bronchiectasis, and correlates with lung function deterioration and respiratory exacerbations. Anti-inflammatory therapies are therefore of particular interest for CF lung disease but must be carefully studied to avoid suppressing critical elements of the inflammatory response and thus worsening infection. This review examines the role of inflammation in the pathogenesis of CF lung disease, summarizes the results of past clinical trials and explores promising new anti-inflammatory options.

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Keywords: Lung inflammation; Cystic fibrosis; Neutrophils; Anti-inflammatory therapies; Bronchiectasis; Mucosal immunity

Contents

1. Introduction ............................................................................................................. 420
2. Linking the CF basic defect to inflammation ......................................................... 420
   2.1. Airway surface liquid and mucociliary clearance ............................................ 420
   2.2. Mucus layer hypoxia ....... 420
   2.3. Low ASLM pH, low bicarbonate content ..................................................... 421
   2.4. Lipid abnormalities .............................................................................. 421
   2.5. Other dysregulated immune pathways ..................................................... 422
3. Secondary inflammatory defects in the CF airways ............................................. 422
   3.1. Serine proteases .............................................................................. 422
   3.2. Calgranulins ...................................................................................... 423
4. Anti-inflammatory therapies ................................................................................. 423
   4.1. Proof of concept: systemic corticosteroids ................................................. 423

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1. Introduction

Cystic fibrosis lung disease is characterized by early colonization and infection of the airways. Although structural changes in the CF airways can be observed at birth in both humans and the CF pig, little inflammation is observed [1,2]. However, infection occurs very rapidly and the inflammatory response to pathogens is severe [3]. Free and bound airway neutrophil elastase is detected very early in CF infants and predicts the development of bronchiectasis later in life [4]. No other lung disease is known to induce such an early, sustained and intense inflammatory process as seen in the CF airway. Individuals with CF also suffer from an intense systemic inflammation characterized by increased serum acute phase reactants, high antibody titers to numerous exogenous and endogenous antigens, a high incidence of ileitis including Crohn’s disease, atopy and heightened Th2 responses [5,6].

CF is caused by a severe functional deficiency of the cystic fibrosis trans-membrane conductance regulator (CFTR) protein [7]. CFTR is largely expressed in the apical membranes of epithelial cells that line the cylindrical structures of tissues that secrete fluids often rich in mucus and other proteins. The airways are among the tissues with the highest expression of CFTR. The lack of functional CFTR causes deficient cAMP-dependent chloride and bicarbonate secretion into airway secretions. Consequently, mucus is tethered to the bronchial apical surfaces, and airway surface fluid pH is decreased. Recent findings indicate that the degree of acidification of the CF airway surface is sufficient to induce significant defects in host anti-bacterial defenses [8]. Furthermore, defective secretion of both chloride and bicarbonate prevents the release of mucus strands from glands, leading to the tethering of mucus to the gland ducts [9]. Persistent bronchopulmonary infections with *Staphylococcus aureus*, *Pseudomonas aeruginosa* and several other pathogens ensue, leading to chronic airway and systemic inflammation, tissue destruction, and respiratory insufficiency [10–12]. But why is the inflammation so severe? The link between CFTR deficiency and inflammation likely involves several CFTR-related abnormalities (Fig. 1) that are discussed in the paragraphs below.

2. Linking the CF basic defect to inflammation

2.1. Airway surface liquid and mucociliary clearance

The airway surface liquid and mucus layer (ASLM) is a complex and dynamic structure that is continuously changing in response to signals from the environment and the host. Two of the major functions of airway mucus are to clear pathogens through the mucociliary escalator, and when needed, to provide a protective barrier against toxic endogenous and exogenous products. The former function requires a sufficiently fluid mucus layer to allow evacuation of particles and pathogens, whereas the latter requires a more abundant and viscous mucus layer. CFTR is key to defining the changing properties of the airway surface liquid and mucus layer [13].

Recent work has provided evidence supporting a model to explain how airway surface liquid and mucus hydration are regulated [14]. Tethered mucins present on the cilia of airway epithelial cells form a mesh network ensuring that the water content between cilia is constant under most conditions. The secreted mucins (primarily MUC5AC, MUC5B and MUC2) attract water and form a reservoir from which water can be drawn into the periciliary space to ensure its constant hydration. By secreting chloride and either directly or indirectly regulating sodium absorption, CFTR plays a key role in providing the water needed to balance the hydration of these compartments. The function of CFTR is also highly susceptible to cues from the environment. CFTR function is suppressed by oxidant stress (ex: cigarette smoke) and by certain bacterial products (ex: *P. aeruginosa*) [15,16]. The loss of chloride secretion from CFTR deficiency results in changes in osmotic pressures and electro-neutrality which likely lead to excessive sodium and water absorption. Loss of the critical hydration of ASLM favors chronic retention of pathogens and a secondary inflammatory response.

2.2. Mucus layer hypoxia

Histopathological examination of end stage CF lungs reveals extensive plugging of the small airways by purulent mucus. Furthermore the lung of the newborn CF pig is characterized by abundant thick mucus streaming out of goblet cells and adhering in several other layers to the epithelial airway surface [2]. The oxygen tension in mucopurulent “masses” present in the CF *P. aeruginosa* infected airway is very low [17]. Low oxygen tensions may hinder normal host anti-bacterial defenses and favor bacterial growth. Hypoxia initiates a cascade of cell-signaling events that begin with the stabilization of the hypoxia inducible factor-1 (HIF-1), a transcription factor affecting angiogenesis, inflammation and fibrosis [18]. HIF-1 stability may be altered in CFTR-deficient cells [19], however HIF-1 has not been studied in the newborn CF airway when inflammation begins.
2.3. Low ASLM pH, low bicarbonate content

The pH of CF airway surface liquid is 8-fold more acidic than that of individuals without CF [20]. CFTR is essential for normal bicarbonate secretion from cultured bronchial epithelial cells and from native small airways [21–24]. The low pH at the airway surface results in the inactivation of ASLM antimicrobial peptides thus creating a host defense defect [8]. Furthermore, growing evidence suggests that CFTR-dependent bicarbonate is important in defining the expansion and solubilization of mucin granules as well as the density of airway mucus [25–27]. Secreted mucins are packaged as highly condensed granules sequestered by an abundance of cations, mainly calcium and hydrogen. As the granules are released from the crypts of small airways, CFTR secretes bicarbonate that displaces Ca++ and H+ from mucins and allows them to deploy and disaggregate [25–27]. The absence of bicarbonate at the time of mucin secretion yields a thick dense layer of mucins that strongly adheres to epithelial cell apical surfaces [27]. The bicarbonate-free mucinous layer traps particles and adheres so tightly that it cannot be aspirated form the apical surface of cells even with vigorous suction in ex vivo tissues. Correction of defective bicarbonate secretion restores the fluidity of the secreted mucins in vitro [27]. It remains unknown whether increasing airway bicarbonate in vivo would have an anti-inflammatory effect in CF airways.

2.4. Lipid abnormalities

Ceramide is a metabolite of sphingomyelin and is normally metabolized to sphingosine through acid ceramidase. An increase in vesicular pH of CF cells is hypothesized to result in inhibition of acid ceramidase, and the accumulation of lung epithelial ceramide [28]. High cellular concentrations of ceramide have been suggested to increase cell death, stimulate release of DNA, increase bacterial binding to extracellular DNA, and initiate IL-1ß and chemokine release [28,29]. Amitriptyline, a sphingomyelinase inhibitor is being investigated as a means to decrease CF cell ceramide, lung infection and inflammation. A phase 2 trial of amitriptyline in a small number of CF patients (n = 40) over 28 days indicates a trend towards an improved FEV1 [30]. Other investigators have reported a deficiency of ceramide in CF cells and associated this deficiency with an intense lung inflammatory response [31]. Clinical trials of fenretinide to increase CF cell ceramide levels and decrease lung disease in CF patients are currently being developed [31–33]. The objectives of these studies are clearly opposite. One study aims to increase and the other to decrease CF cell ceramide levels. Both studies are based on solid experimental data. However, several factors may contribute to the contrasting results. First, the methods used to measure lung ceramides vary between studies. Second, the denominators for expressing tissue ceramide levels are different between studies and therefore not easily compared.
Finally, ceramide itself is both beneficial and harmful for host defenses, depending upon the compartment in which it is located and its abundance in each compartment. Clearly ceramide in lipid rafts is essential to clear bacteria and control infection [29]. This is why investigators have indicated that ceramide levels must not be decreased below a critical level otherwise essential host defenses may be affected [34]. The benefit of ceramide in lipid rafts for host defense provides a rationale for attempting to increase CF cell ceramide. In contrast, accumulation of ceramide at the cell surface clearly leads to increased apoptosis, DNA release and increased bacterial adherence, suggesting a rationale for decreasing extracellular ceramide accumulation. Resolution of the ceramide controversy in CF will most likely not be possible without well-designed definitive clinical trials.

Another CFTR-related lipid abnormality reported in CF is an abnormally high arachidonic acid to docosahexaenoic acid (AA/DHA) ratio in CF cell membranes [35]. A high AA/DHA ratio is associated with an increased inflammatory response and is found in both CF patients and their parents who have ratios intermediate between CF and non-CF individuals. DHA is a precursor of several anti-inflammatory lipids including the resolvins [36]. Restoration of a normal cell AA/DHA ratio decreases lung inflammation in an animal model of airway hyperreactivity [37].

2.5. Other dysregulated immune pathways

Host-pathogen interactions that are critically important in defining the resolution or persistence of inflammation in cystic fibrosis are discussed in the accompanying review by Yonker et al. [38]. Several other immune pathways have been reported to be dysregulated in individuals with CF. Among these pathways thought to be directly affected by a deficiency of CFTR are: decreased NO synthesis in the lower lung [39], altered STAT1 signaling that leads to NOS2 inactivation [40], an increased differentiation of T lymphocytes to the Th17 phenotype in CF patients [41], a decrease in efferocytosis [42], abnormal Nrf2 signaling that prevents proper anti-inflammatory and antioxidant responses [43], increased epithelial ER stress from a misfolded protein response [44], abnormal TLR5 responses [45], and increased MAPK and NFkB-dependent pathways [46]. Systemic and lung epithelial lining fluid glutathione deficiency has also been reported in CF [47,48], and is speculated to be due to the lack of CFTR-mediated GSH transport, although CFTR is poorly selective for glutathione [49]. Regardless of its exact cause, glutathione deficiency can favor NFkB-dependent inflammatory gene transcription and may be relevant to CF lung inflammation [50].

CFTR dysfunction, or its absence has been associated with several defects of key immune pathways in inflammatory cells. For example, neutrophils lacking CFTR do not transport halide in the phagolysome were oxidative killing occurs [51]. Macrophages with dysfunctional CFTR show delayed phagolysosomal fusion and bacterial clearance after ingestion of *Burkholderia cepacia* [52]. CF macrophages also display decreased caveolin-1 expression and enhanced TLR4-dependent responses to LPS [53]. Finally, CFTR deficiency has been reported to induce an intrinsic predisposition of naïve T lymphocytes to differentiate towards a Th17 phenotype [41]. These and several other immune cell defects associated with CFTR deficiency strongly suggest that the intense inflammation observed in the CF lung is multifactorial and involves many immune pathways intrinsic to both epithelial and inflammatory cells.

3. Secondary inflammatory defects in the CF airways

CF lung disease is characterized by chronic non-resolving inflammation, driven by continuous recruitment of immune cells into CF airways [54]. In this section we summarize and discuss the key cell populations and their effector mechanisms that contribute to inflammation, tissue damage and remodeling in the CF airway microenvironment.

Neutrophils (or polymorphonuclear leukocytes, PMNs) represent the first cells migrating into the pulmonary compartment. While recruited there to combat bacterial and fungal pathogens, activation of neutrophils bears the potential to harm the surrounding lung tissue through the release of oxidants and proteases [55]. Both oxidants and proteases have been shown to interfere with CFTR expression and/or function [56,57]. Consequently, the key question arises, whether airway neutrophils in the context of CF lung disease are beneficial, as they protect against *P. aeruginosa*, A. fumigatus and other bacterial/fungal pathogens, or rather harmful by releasing proteases such as elastase that cleave extracellular matrix and immune components [58]. In fact, there is evidence supporting both of these concepts, rendering a clear vote for or against neutrophils in CF lung disease difficult. The main rationale supporting a harmful role of neutrophils builds on the protease–antiprotease hypothesis, which basically states that the local balance of protease and opposing antiproteases regulates the proteolytic activity in a certain organ compartment, such as the lung [59]. Translated into the context of CF lung disease, the protease burden overwhelms the anti-protease shields, liberating mainly serine proteases and matrix metalloproteases (MMPs) that cause a variety of harmful effects by degrading tissue components, such as elastin, or by cleaving immune receptors, such as T cell receptors, complement receptors and the IL-8 receptor CXCR1 [58,60].

3.1. Serine proteases

Recently, the serine protease elastase has been demonstrated to degrade and disable CFTR itself [57] and to predict the development of bronchiectasis in CF patients [4]. Therefore, we will discuss the source and potential role of this protease class in more detail. Neutrophils are the major source of serine proteases in the human body, which are stored in primary/azurophil granules. The neutrophil granule serine proteases elastase, cathepsin G, proteinase-3 and the recently discovered neutrophil serine protease-4 [61] are highly similar in sequence. Besides their destructive role, these proteases play a physiological role in a variety of processes, particularly tissue remodeling, chemotaxis and microbial killing. To prevent harmful free proteolytic activities, all serine proteases are tightly regulated by protease inhibitors, prototypically alpha-1-antitrypsin [62].
Neutrophil elastase has been shown to mediate killing of the CF pathogen P. aeruginosa by degradation of outer membrane protein F (OprF) [63]. In contrast, cathepsin G-deficient mice were not more susceptible to P. aeruginosa [64], suggesting a non-redundant role for elastase in killing P. aeruginosa. A recent in vivo study in a CF-like mouse model further shows that elastase plays an essential role in modulating inflammation, mucus hypersecretion, and emphysema, but was dispensable for antibiotic host defense [65]. Elastase has several potential roles in CF lung disease including degradation of structural airway matrix proteins [66], increased mucus secretion [67,68], cleavage of opsonophagocytosis proteins [69,70], degradation of the macrophage phosphatidyserine receptors with failure to resolve inflammation because of the lack of efferocytosis [71,72], and cleavage of the neutrophil TIM3 receptor leading to decreased galectin-9/TIM3 interactions [73].

Besides serine proteases, matrix metalloproteinases, primarily MMP-9 and MMP-12, also have been involved in CF lung disease and chronic neutrophilic inflammation [74–76]. Other studies further implicate MMP-8 [74], meprin [77], prolyl endopeptidase (PE) [78] and, most recently, cathepsin S [79] in CF lung disease pathologies. Based on these protease-driven disease concepts, neutralizing unopposed proteolytic activities by supplementation of anti-proteases, prototypically alpha-1-antitrypsin, has the potential to counterbalance the viscous proteolytic loop of tissue destruction and immune cell impairment [80].

### 3.2. Calgranulins

Calgranulins, including S100A, S100A9 and S100A12 represent another family of pro-inflammatory proteins derived form neutrophils, macrophages and monocytes. The levels of these calgranulins are clearly increased in CF sputum and recent evidence indicates that these proteins can activate bronchial epithelial cells through TLR4, elicit ERK phosphorylation, facilitate NF-κB translocation to the nucleus and induce MUC5AC production, all of which are key features of CF lung inflammation (Fig. 1) [81,82].

Immune cells other than neutrophils have been described to modulate CF lung disease, particularly macrophages, dendritic cells and T cells. For a more in-depth discussion of the distinct roles of these cell types, we refer to the recently published review [58]. Importantly, T cells, the major orchestrators of adaptive immune responses exhibit abnormal function in CF patients, cells and mice [58]. A series of publications now supports the concept that CF T cells are skewed towards a Th2/Th17 immune response that is associated with or even primes for infection with P. aeruginosa [5,83–85]. Overshooting T cell responses are counter-regulated by regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) and recent studies support the notion that MDSCs are enhanced [86], while Tregs are reduced [87] in CF with P. aeruginosa infections, but the underlying mechanisms and clinical implications remain to be defined.

### 4. Anti-inflammatory therapies

The inflammatory response in CF lung disease begins early in life, becomes persistent, appears excessive relative to the bacterial burden, and ultimately results in bronchiectasis [88]. Interrupting the pathophysiologic cascade early in the disease process with drugs such as CFTR correctors and modulators [89], mucolytics [90], and antibiotics [91] may have subsequent beneficial effects on inflammation. However, a direct assault on the airway inflammatory response seems warranted given that drugs that target inflammation have been shown to slow the decline in lung function and improve survival [92–94]. Ibuprofen is the only anti-inflammatory drug currently recommended for the long-term treatment of CF airway inflammation [95]. Despite this recommendation, less than 10% of eligible patients are prescribed with ibuprofen. New anti-inflammatory drugs are needed for the treatment of CF airway inflammation. This section will provide an update on anti-inflammatory drug development in CF. Length restrictions prohibit a thorough review of all anti-inflammatory drugs evaluated in CF. A more complete list of anti-inflammatory drugs can be found in Table 1. For more information regarding specific drugs, the reader is referred to the published literature.

#### 4.1. Proof of concept: systemic corticosteroids

Two general strategies for decreasing inflammation in the CF lung include the administration of broad spectrum anti-inflammatory drugs or multiple drugs targeted towards specific inflammatory mediators. Corticosteroids represent the former approach. The first study of long-term use of systemic corticosteroids was initiated in the 1980s [96]. In a single-center, 4-year clinical trial in 45 pediatric CF subjects with mild to moderate lung disease, alternate-day prednisone (2 mg/kg) was associated with better lung function, improved weight gain, and fewer hospital admissions compared to placebo [96]. In a subsequent 4-year multi-center study, 285 CF subjects aged 6 to 14 years with mild to moderate lung disease were randomized to receive alternate day placebo or prednisone (1 mg/kg or 2 mg/kg) [97]. The 2 mg/kg group was halted at two years because of increased adverse events. At study end, subjects receiving 1 mg/kg of prednisone every other day had better lung function, particularly if they were infected with P. aeruginosa, but they also had multiple corticosteroid-associated adverse events [97]. The percentage of patients that acquired respiratory P. aeruginosa during the trial was significantly increased in the prednisone-treated subjects although the rate of acute infectious complications remained the same. Six years after the completion of the original trial, subjects in the 1 mg/kg prednisone group had persistent growth defects, and they had the same percent of predicted forced expiratory volume in 1 s (FEV1%) as subjects in the placebo group, thus suggesting that not only were the benefits of prednisone lost after discontinuation of the drug, but that the lungs may have been smaller in the prednisone-treated group [98]. This implies that preservation of lung function requires continued administration of prednisone. Given their toxicities, systemic corticosteroids are not recommended for use in CF [95]. Although clinical trials of
systemic corticosteroids for the treatment of CF airway inflammation did not pan out, these studies did demonstrate that drugs used to dampen the airway inflammatory response could maintain lung function without resulting in infectious complications.

Many investigators have evaluated inhaled corticosteroids (ICS) for CF. An observational study demonstrated that consistent ICS use resulted in slowing of lung function decline but they also decreased weight and height for age and increased use of insulin and oral hypoglycemic agents [99]. Unfortunately, a benefit of ICS has not been demonstrated in randomized placebo-clinical trials [100]. Small study populations and short observation periods hampered these trials. In a large prospective, multi-center study, withdrawal of ICS was not associated with significant worsening of CF lung disease [101]. Due to the lack of supportive evidence, the long-term use of ICS for CF airway inflammation cannot be recommended at this time [95].

4.2. Ibuprofen

Ibuprofen also has broad anti-inflammatory effects. In a 4-year clinical trial, patients receiving twice daily high-dose ibuprofen had slower lung function decline, better preservation of body weight, fewer hospital admissions, but no increase in adverse events [92]. A 2-year multicenter trial in Canada also demonstrated a beneficial effect of ibuprofen on lung function [102]. Results from an analysis of observational data from the CF Foundation Patient Registry revealed that ibuprofen reduced the annual rate of FEV1 decline by 29% [103]. Despite these beneficial effects, ibuprofen has not been widely adopted, largely due to the challenges associated with obtaining a pharmacokinetic study for appropriate dosing and concerns over adverse effects [104]. While ibuprofen is associated with gastrointestinal bleeding, the occurrence is rare (annual incidence 0.37% vs. 0.14%) [103]. Concomitant use of antacids, proton pump inhibitors, or PGE1 analogues would likely limit this adverse event. The benefits of ibuprofen outweigh its risks. A Cochrane review concluded that “high-dose ibuprofen can slow the progression of lung disease in people with CF, especially in children, and this suggests that strategies to modulate lung inflammation can be beneficial for people with CF” [105]. Ibuprofen is the only anti-inflammatory drug recommended for chronic use in CF [95].

4.3. Antibacterial therapies that have anti-inflammatory properties

Because bacteria stimulate the inflammatory response, therapies directed towards the chronic infection in CF should reduce inflammation. Intravenous antibiotics may represent the best short-term strategy to reduce lung inflammation since this approach markedly decreases airway and systemic inflammatory biomarkers [106]. However since chronic continuous intravenous antibiotic therapy is not feasible, other chronic antibiotic strategies have been developed that effectively decrease lung inflammation. Inhaled tobramycin decreases inflammation as demonstrated by lower blood neutrophil counts [107], although the anti-inflammatory effect is less than that of systemic antibiotics [108]. Azithromycin is a macrolide antibiotic believed to have anti-inflammatory effects. In a 6-month study, thrice weekly azithromycin was associated with improvements in lung function, weight gain, and quality of life; decreases in pulmonary exacerbations and stabilization of sputum elastase in CF patients with mild to moderate lung disease who were infected with *P. aeruginosa* [109]. A subsequent study in subjects without *P. aeruginosa* infection found a significant reduction in pulmonary exacerbations but no change in lung function [110], whereas another study in CF subject without *P. aeruginosa* infection reported that azithromycin decreased blood neutrophil counts and serum inflammatory markers. The mechanism of action of azithromycin in CF is unclear. Perhaps the effect of azithromycin in CF is due to a combination of effects including bronchodilatation, antibacterial and anti-inflammatory. Despite its unknown mechanism of action, azithromycin appears to be of benefit to patients with CF, and the CF Foundation recommends the chronic administration of azithromycin to CF patients aged 6 years and older who are infected with *P. aeruginosa* and that it be considered in those patients who are not infected with *P. aeruginosa* [95].

A humanized monoclonal antibody directed towards the Type III secretion system of *P. aeruginosa* has demonstrated beneficial effects in a Phase I/II study. A single dose of this compound was
associated with a significant decrease in sputum neutrophils and neutrophil elastase and a dose-dependent trend towards decreased sputum myeloperoxidase, IL-1β, and IL-8 [111]. A subsequent Phase II repeat dose study is ongoing and results are expected in 2015. The results of all of these studies suggest that therapies directed towards the chronic bacterial infection have beneficial effects on the inflammatory response.

4.4. Modulators of intracellular signaling

Transcription factors responsible for upregulating the airway inflammatory response in CF provide an attractive target for therapeutic intervention. IL-10 inhibits pro-inflammatory cytokine production by down regulating NF-κB. In pre-clinical models, IL-10 deficiency was associated with increased airway inflammation [112] and administration of IL-10 was associated with beneficial effects on airway inflammation [113]. A subsequent clinical trial in CF was abandoned for reasons unrelated to CF. Thiavelodinediones or glitazones inhibit NF-κB activation by upregulating of peroxisome proliferator activating receptor (PPAR). CF tissues appear to be deficient in PPAR [114,115], resulting in an imbalance between NF-κB and its inhibitor IκB. Activation of PPAR may decrease the CF inflammatory response. A preliminary report of a 28-day clinical study of pioglitazone did not demonstrate a beneficial effect on sputum inflammatory mediators [116]. However, this may have occurred because the dose of pioglitazone was inadequate, the duration of the study was too short, or the number of subjects was too small (N = 20). Although this study did not demonstrate a beneficial effect, further evaluation of the glitazones in CF should be considered.

Nitric oxide (NO) is decreased in exhaled breath from patients with CF. Decreased NO may lead to bacterial infections and increased inflammation [117,118]. The Jak-Stat signaling pathway is inefficient in CF, which reduces mRNA production of nitric oxide synthase (NOS)-2 [39,117,119]. These functions can be restored in vitro by application of gamma interferon. A trial comparing inhaled interferon-γ (actimmune) (500 and 1000 μg) did not improve pulmonary function, alter sputum bacterial density, or affect sputum inflammatory markers [120]. Subjects who received 1000 μg of inhaled interferon-γ had a higher percentage of hospitalizations for pulmonary exacerbations. Pathways responsible for activating the host inflammatory response share many processes with pathways that terminate the inflammatory response. It is possible that targeting one step in the pro-inflammatory arm may result in untoward effects in the anti-inflammatory arm. The impact of such an intervention may depend upon which aspect of the inflammatory response is impacted the most.

Upregulation of RhoGTPase, as seen in CF, may also account for decreased NO [121]. RhoGTPase can be inhibited by the statins [121]. A preliminary clinical study of simvastatin demonstrated a trend towards increased exhaled NO, but had no effect on sputum inflammatory markers [122]. Other agents that increase NO production have also been studied. Arginase activity is increased in blood and sputum of CF patients and may further decrease NO by degrading L-arginine, a NO substrate [123]. A small study of L-arginine in CF was associated with increased eNO [124]. Further studies of L-arginine are warranted in CF.

Synthetic triterpenoids are small molecules that increase Nrf2 activity. Nrf2, which is deficient in CF, is pivotal to mitigating the acute inflammatory response [125,126]. Pre-clinical studies demonstrate the ability of triterpenoids to reduce the inflammatory response [126]. Much research is still needed to determine their utility in CF, but early data suggest that these compounds have potential as therapeutic agents.

4.5. Inhibition of neutrophil influx and neutrophil products

Because the neutrophil is responsible for most of the damage to the airway in CF, investigators have sought to either limit neutrophil influx or inhibit neutrophil products. Antibodies to IL-8, an important neutrophil chemokine, and ICAM-1, an adhesion molecule important in neutrophil migration, were studied in pre-clinical models but were never studied in a clinical trial. CXCR2 is a receptor expressed on the surface of neutrophils that recognizes neutrophil chemoattractants such as IL-8. A clinical trial of a CXCR2 antagonist in CF was found to be safe and well-tolerated [127]. Although no effect on lung function was seen, there was a trend towards decreased sputum inflammatory markers. Curiously, increases in systemic markers of inflammation were seen. Further investigations into why this occurred and whether CXCR2 antagonists may be beneficial in CF are under consideration. IL-17 is a potential target for limiting airway neutrophilia. IL-17, a cytokine elevated in CF tissues, may be linked to neutrophil influx or inhibit neutrophil products. Antibodies to IL-17 may have utility in CF. LTB4, a metabolite of arachidonic acid metabolism, is a potent neutrophil chemoattractant present in the CF airway [129]. A study in CF of BIIL 284 BS (Amelubant), a specific LTB4 receptor antagonist, was terminated early due to an increase in pulmonary-related serious adverse events including pulmonary exacerbations in adults [130]. It is unclear as to why this occurred. Perhaps the inhibitory effect of BIIL 284 BS on the LTB4 pathway was too potent, resulting in impaired anti-microbial defenses and increasing the risk of an exacerbation. In a murine model of *P. aeruginosa* lung infection, BIIL 284 BS was associated with an increase in the lung bacterial burden [131]. Regardless of the etiology, the results of this study demonstrate that caution must be used when selecting anti-inflammatory agents for clinical trials.

Another approach to limiting the impact of neutrophils in CF lung disease would be to inhibit their products. Proteases and oxidants are two neutrophil products that have been targeted. Free proteases are present in the CF lung in far excess of their anti-protease inhibitors. Anti-proteases have been under investigation in CF since the early 1990s. Multiple small studies of various anti-proteases have demonstrated some positive effects. However, further development of this therapy has been limited by expense, supply, and the inherent risks associated with administering a plasma derived product. Nonetheless, enthusiasm for the development of plasma derived α1-antitrypsin (AAT) in CF has not diminished. In a multicenter, randomized, open-label
multicenter trial, 4-weeks of AAT inhalation was associated with a significant reduction in neutrophils, pro-inflammatory cytokines, and levels of elastase activity [132]. A placebo-controlled, multicenter clinical trial to assess tolerability of inhaled α-1 proteinase inhibitor has recently completed. Results are expected in 2015.

In addition to proteases, neutrophils also release large amounts of oxygen radicals that can overwhelm antioxidant defenses and damage the tissues. Therefore potential therapeutic approaches to dampen inflammation in CF include antioxidants [133,134]. Because glutathione, an important lung antioxidant, is decreased in CF, it seems reasonable to increase its concentration via exogenous administration. Twice daily glutathione reduced superoxide production by inflammatory cells in one study [135]. In another study, inhaled glutathione demonstrated no detectable change in BAL markers of oxidative stress [136,137]. Because glutathione, an important lung antioxidant, is decreased in CF, it seems reasonable to increase its concentration via exogenous administration. Twice daily glutathione reduced superoxide production by inflammatory cells in one study [135]. In another study, inhaled glutathione demonstrated no detectable change in BAL markers of oxidative stress [136,137].

A Phase II study found that inhaled glutathione could be delivered to the airway, but this was not associated with changes in markers of oxidation, proteolysis or inflammation or clinically relevant improvements in lung function, pulmonary exacerbations or patient-reported outcomes [138]. A 12-month randomized, single-blind, placebo-controlled clinical trial of inhaled glutathione has completed recently, and results should be published soon. Initially developed as a mucolytic, N-acetyl cysteine is receiving interest as an anti-oxidant because it inhibits H2O2 and increases glutathione. In a pilot study, oral N-acetyl cysteine was associated with increased glutathione levels in whole blood and decreased sputum neutrophils, IL-8, and elastase activity [139]. The study was of short duration and not powered to detect changes in pulmonary function or other clinical outcome measures. A subsequent large randomized double-blind placebo controlled trial demonstrated that N-acetyl cysteine stabilized lung function but had no effect on neutrophil elastase activity or other inflammatory biomarkers [140]. N-acetyl cysteine is converted to cysteine, an essential precursor of glutathione synthesis. In the pilot study, the dose of N-acetyl cysteine (1 g tid) was slightly different from that of the definitive study (0.9 g tid). Although whole blood glutathione increased in the initial pilot study, no difference was observed in the larger clinical trial. The explanation for this discrepancy may be related to differences in dose or formulation. It therefore remains unknown whether a dose and formulation of oral N-acetyl cysteine sufficient to increase whole blood glutathione would result in measurable anti-inflammatory effects. In general, anti-oxidants are safe and well tolerated, and they have potential to impact CF airway inflammation.

4.6. Upregulation of inflammation resolving factors

Deficiencies in some fatty acids may contribute to CF pulmonary inflammation. In CF, there is an increase in AA and a decrease in DHA. There have been a few clinical trials of DHA supplementation in CF [141,142]. Unfortunately, the dose of DHA, study size, duration of treatment, and outcome measures vary widely. Few are placebo-controlled, and some do not demonstrate an effect on lung function. One of the difficulties with many anti-inflammatory drugs, including DHA, is being able to deliver enough of the drug to the lung to impact the inflammatory response. Despite these drawbacks, the cumulative data from these studies indicate that DHA may have beneficial health effects. While DHA may never come to fruition as an anti-inflammatory drug, the data do suggest that augmenting the lipid mediators of resolution may be beneficial in CF.

Lipoxin A4 (LXA4) is an omega-6 polyunsaturated fatty acid derived from arachidonic acid metabolism that promotes the resolution of inflammation by inhibiting NF-κB, pro-inflammatory cytokine production, and neutrophil migration. LXA4 is decreased in BAL fluid from patients with CF and in murine models of P. aeruginosa lung infection [143]. Decreased neutrophils, bacterial burden, and lung inflammation were seen when an exogenous lipoxin analog was administered to P. aeruginosa infected mice [143]. Like LXA4, the resolvins and protectins are also lipid-derived mediators involved in the resolution of inflammation. Resolvins and protectins inhibit neutrophil migration and reduce lung inflammation. In addition, some resolvins antagonize the LTB4 receptor, inhibit pro-inflammatory cytokine production, and inhibit the expansion of Th17 cells. To date, most approaches to treating inflammation in CF have focused on directly downregulating the pro-inflammatory arm of the inflammatory response. Perhaps a more prudent approach for limiting the inflammatory response in CF would be to upregulate the anti-inflammatory regulatory mechanisms, like fatty acid-derived mediators.

5. Future directions

Anti-inflammatory drug trials have been conducted in CF for over 30 years. Overall, the inability to develop any new anti-inflammatory drug besides ibuprofen has been frustrating. The inflammatory response in CF provides a host of potential targets for new drug development. Determining which targets will have the most impact without resulting in untoward effects remains the objective. Most anti-inflammatory drugs studied in CF have targeted the pro-inflammatory arm of the inflammatory response. However, new drugs that “turn on” the body’s own anti-inflammatory mechanisms may be a more practical approach. Clinical trials of these drugs will likely begin in the next 1–2 years.

6. Summary

An appropriate inflammatory response to infection is critical to clear pathogens and preserve normal tissue function. Functional CFTR is essential to ensure that pathogen-associated airway inflammation is appropriate. Unfortunately, the inflammatory response to lung infection in CF is ineffective, severe and persistent. High-intensity inflammation that does not resolve, induces permanent structural damage of the CF airways, impairs lung function and, if unchecked, eventually causes respiratory failure and death. The research of many investigators has provided an abundance of knowledge that links CFTR deficiency to numerous innate and acquired immune dysfunctions. Clinical trials of anti-inflammatory therapies have provided information that dictates caution but also holds much promise. It is now
our collective responsibility to conduct well-designed clinical trials that will ensure that novel, safe and effective anti-inflammatory therapies change the course of CF lung disease.

References


