ANTI-INFLAMMATORY THERAPY IN CYSTIC FIBROSIS

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The inflammatory response in Cystic Fibrosis

An exuberant and persistent acute inflammatory reaction is commonly observed in the lung from cystic fibrosis (CF) patients, representing a key pathogenetic event of lung damage and respiratory insufficiency. CF inflammation is characterized by massive infiltration of activated neutrophils and is sustained by over expression of a repertoire of pro-inflammatory mediators and receptors (1). The mechanisms responsible of lung inflammation in CF are still under debate. One line of evidence suggests that inflammation is triggered by bacterial infection, which develops early in life and is promoted by reduced bacterial clearance, due to mucous viscosity (2). More recent results indicate, instead, that CF airway is prone to inflammation, even in the absence of appreciable bacterial colonization (3). It appears that a dysfunctional Cystic Fibrosis Transmembrane conductance Regulator (CFTR) may trigger over-production of pro-inflammatory cytokines by respiratory epithelial cells either at basal or in response to bacterial infection (4). In vitro studies have shown that inhibition of CFTR activity increases interleukin-8 (IL-8) secretion and NF-κB nuclear translocation in normal human respiratory epithelial cells (5). On the other side, CFTR may regulate functions of circulating cells involved in the immune-surveillance and in the inflammatory response. In particular, polymorphonuclear neutrophils (PMNs) express a functional CFTR protein, which regulates chlorination of bacterial proteins (6). Also, CFTR controls phagosome acidification and bacterial killing in alveolar macrophages (7).

It is becoming clear that, under physiological circumstances, the resolution of the inflammatory response is a well timely-ordered, active process which involves the participation of a number of mediators (8). Thus, pathology may develop also because of alterations in the resolution phase of the inflammatory reaction. Indeed, it has been reported that a defect in generation of the endogenous pro-resolution lipid mediator lipoxin A_4 (LXA_4) can be observed in CF (9). In aggregate, these observations indicate that a number of factors concur to maintain an acute inflammatory state in CF lung.

This article reviews the anti-inflammatory protocols employed in CF, with the introduction of novel potential experimental approaches.

Classic anti-inflammatory drugs

Steroids

Although steroids can improve lung function and retard parenchyma deterioration, their use in CF has been undermined by the serious side-effects (cataracts, glucose intolerance, hypertension and growth retardation) associated with long-term administration (10). To minimize such effects, inhaled corticosteroids have been used for both short and long term treatments. Unfortunately, clinical results with aerosolized steroids have been modest (11). Recently, a novel strategy for steroid administration has been proposed. Erythrocytes from patients are loaded with dexamethasone and then reinfused (12). Preliminary data from a small number of patients indicate that erythrocyte-mediated delivery of steroid is relatively safe and is associated with a statistically significant improvement of FEV_1 (12). Larger studies are however required to assess the real efficacy of this treatment to control lung inflammation in CF.

NSAIDs

High doses of ibuprofen, to reach plasma concentrations between 50 and 100 μg/ml, have been administered to CF children for 4 years in a randomized double-blind trial (13). Initial results showed a slower progression of lung disease in patients treated with ibuprofen (13). A ten-year follow-up of this study showed the persistence of the improvement in lung function in patients taking ibuprofen (14). Furthermore, children with mild lung disease treated with ibuprofen had 33% less reduction in FEV_1 compared with children randomized to placebo (15). The risk of side effects (mainly GI bleeding) and the need for an accurate monitoring of plasma concentrations may limit the use of this drug, although benefits may prevail over the risks. The use of more selective cyclooxygenase-2 (COX-2) inhibitors is not recommended because of their documented cardiovascular side effects in long-term treatments (16).

Alternative anti-inflammatory strategies

Deoxyribonuclease

DNA released by apoptotic neutrophils infiltrating the CF airways, contributes to mucus viscosity, which in turn promotes bacterial infection and exacerbation of respiratory inflammation. Therefore, treatment with human recombinant deoxyribonuclease (rhDNase) was proposed. 105 CF patients with normal lung function were randomized to receive 2.5 mg/day of nebulizedi rhDNAse or no treatment for three years (17). Untreated patients displayed over time an increase in the number of neutrophils as well as in elastase activity and IL-8 levels in bronchoalveolar lavage fluids (BALF). Such increments were not observed in BALF from patients treated with rhDNAse (17). However, no significant variations in FEV_1 were observed between untreated and rhDNAse-treated patients. On the other hand, in a 96-week double-blind placebo-controlled randomized study involving a total of 474 CF patients, treatment with dornase alpha reduced the decay in lung functions and the risk of exacerbations (18). Similar

results emerged from the analysis of data from Epidemiologic Survey of Cystic Fibrosis (19). Thus, rhDNAse may exert anti-inflammatory actions in CF patients with mild lung disease, although the impact of this treatment on survival should be better evaluated in longer follow up studies.

Leukotriene receptor antogonists

Leukotrienes (LTs) are generated by 5-Lipoxygenase (LO)-catalyzed conversion of arachidonic acid (20). They are potent regulators of the immune-inflammatory response. In particular, LTB_4, which is produced by PMNs and macrophages, is a potent chemoattractant for PMNs (21). It activates specific membrane receptors belonging to the G-protein-coupled receptor (GPCR) family and denominated BLT_1 and BLT_2 (22). Increased levels of LTB_4 can be measured in epithelial lining fluid and exhaled breath condensate from CF patients (23,24). Given that BLT_1 plays a key role in the regulation of PMN recruitment in the respiratory tract, contributing to inflammation progression (22), a multi center randomized, double blind, placebo-controlled phase II clinical trial with the BLT_1 antagonist BIIL 284 BS was started. Unfortunately, this trial had to be interrupted because of serious pulmonary adverse effects in patients taking the drug (25).

5-LO generates an additional class of leukotrienes termed cysteinyl leukotrienes (Cys-LT), which regulate vascular tone and permeability and exert pro-inflammatory functions (26). CysLT activate two GPRC receptors, $CysLT_1$ and $CysLT_2$ (27). $CysLT_1$ receptor antagonists are routinely used for treatment of allergic rhinitis and asthma (28). $CysLT_1$ receptor antagonists have been administered also to CF patients. In one study, montelukast was given to 11 clinically stable CF patients for two weeks (29). Patients displayed some improvement in exercise tolerance (29). In another study, montelukast was administered to 16 patients for three weeks (30). A reduction in levels of eosinophilic cationic protein levels and number of eosinophils was denoted, although functional tests remained unchanged (30). Improvement in respiratory parameters as well as decrease in cough, wheezing, levels of IL-8 and myeloperoxidase in sputum, was instead observed in other clinical investigation with montelukast in CF patients (31). Zafirlukast was also given to thirty CF patients with some improvement in clinical score (32).

Taken together, studies with $CysLT_1$ inhibitors do not conclusively show a real efficacy of this class of drugs as anti-inflammatory agents in CF.

N-acethylcysteine

Oxidant stress may significantly contribute to progression of lung damage in CF. Expression of genes regulating the redox/anti-oxidant systems was altered in the $CFTR^{\Delta F508}$ mice (33). *N*-acethylcysteine is a sulfhydryl group donor with anti-oxidant properties, which has been largely used as a mucolityc agent. In phase 1 clinical investigation, high doses of *N*-acethylcysteine (0.6 to 1.0 g three times per day) were administered to 18 CF patients for four weeks (34). A significant reduction in sputum elastase activity as well as neutrophil burden was observed, whereas no changes in

pulmonary functions were detected (34). A phase II trial is underway to determine whether, by improving the neutrophil burden, *N*-acethylcysteine can also ameliorate lung function in the long-term.

Nitric oxide regulators

Generation of nitric oxide (NO) by the inducible form of nitric oxide synthase (iNOS) is impaired in CF respiratory epithelial cells (35). This contributes to enhance bacterial infection and to exacerbate airway inflammation. Thus, drugs that increase NO generation and/or bioavailability may be a useful tool to combat inflammation and infection. In this respect, statins, which inhibit the 3-hydroxy-3-methylglutaryl-CoA reductase pathway, restored iNOS expression in CF airway cells, with a mechanism involving the Rho GTPase pathway (36). Statins may also regulate additional anti-inflammatory mechanisms, namely inhibition of cytokine, chemokine production and of chemokine receptor expression (37), NF-κB activation (38), MIP-1α expression in monocytes (39), inhibition of PMN activation and recruitment (40). The impact of simvastatin administration to CF patients is being currently evaluated.
On the other hand, NO formation may be increased by providing iNOS with L-Arginine, which is enzymatically converted to NO. Administration of inhaled or oral L-Arginine to CF patients resulted in increased NO formation, although a beneficial effect on FEV_1 could not be observed (41).

PPAR activators

Peroxisome proliferators-activated receptors (PPARs) represent a class of transcription factors involved in the regulation of lipid metabolism and of the inflammatory response. Among the three isoforms (α, β, γ) so far identified, PPAR-γ is highly expressed in the respiratory epithelium as well as in alveolar macrophages (42). In addition, a decrease in PPAR-γ expression was denoted in the lung from $cftr^{-/-}$ mice compared to lung from normal mice (43). Activation of PPAR-γ by endogenous (15-deoxy-$\Delta^{12,14}$-prostaglandin J_2) or exogenous (thiozolidinediones) agonists results in down-regulation of pro-inflammatory cytokine expression in airway cells (44) as well as in alveolar macrophages (45). Thus, PPAR-γ activators have been proposed for treatment of chronic airway inflammation (46). Together, these observations support the hypothesis that PPAR-γ agonists may be beneficial in CF. A clinical trial with pioglitazone in CF patients in underway (47).

Cytokines

Cytokines actively concur to the development and persistence of the inflammatory reaction in CF. In particular, increased production of pro-inflammatory cytokines as well as reduced formation of anti-inflammatory cytokines can be observed in CF patients. Therefore, treatments aimed at regulating the cytokine network may be proposed for CF

therapy. In this respect, IL-10 plays a key role in the resolution of the inflammatory response. Interestingly, IL-10 knockout mice display prolonged inflammation in response to *Pseudomonas aeruginosa* challenge (48). Moreover, treatment with IL-10 improved survival of these mice (49). Therefore, administration of recombinant IL-10 could be a therapeutic option for CF patients. On the other hand, interferon gamma (IFN-γ) can modulate a number of mechanisms of inflammation and alterations in the IFN-γ pathway have been detected in CF (50). Inhaled IFN-γ was administered to 66 patients randomized to receive 500 or 1000 μg IFN-γ1b. Results from this study did not show changes in inflammatory parameters or improvements in respiratory functions (51).

Protease inhibitors

The massive infiltration of PMNs in CF airways is associated with the release of large amounts of proteases stored in PMN granules. These proteases greatly contribute to the development of inflammation and tissue damage. Thus, it has been proposed that supplementation of the airway with anti-proteases may counter-balance the increment in airway protease concentration which occur in CF. Early studies showed that inhaled α1-antitrypsin suppressed neutrophil elastase in the airway lining fluid from 12 CF patients (52). In a more recent prospective randomized investigation, 52 CF patients were given 25 mg inhaled α1-antitrypsin for four weeks (53). A reduction in pro-inflammatory cytokines and PMNs in the induced sputum was observed, although there were no changes in lung function (53). Aerosol preparation of engineered protein inhibitor human neutrophil elastase (EPI-hNE4) revealed potent inhibitory capability of PMN elastase (54). EPI-hNE4 protected rats from acute lung injury and reduced by 64% PMN influx in rat airway after tracheal instillation of pooled sputum from children with CF (55). Phase II clinical trials of engineered protein inhibitor human neutrophil elastase (EPI-hNE4), which has been shown to efficiently reduce elastase concentrations as well as PMN migration, are being carried out in Europe (47).

Antibiotics

Anti- bacterial therapy may also decrease the inflammation burden in CF airway. A number of evidence indicates that macrolides reduce formation of pro-inflammatory cytokines in vitro and in vivo and also inhibit PMN migration and activation (56). In a multicenter, double-blind, placebo-controlled trial, 87 CF patients were randomized to oral azithromycin (250 or 500 mg) 3 days a week for 168 days, whereas 98 CF patients received placebo. Patients on azithromycin showed a significant improvement in FEV_1 and experienced less exacerbation in comparison with patients on placebo (57). In another study, 27 CF patients were treated with oral clarithromycin (250 mg every other day) for 12 months (58). A reduction in TNF-α and IL-8 levels in plasma and sputum associated with a significant amelioration of lung functions was observed (58).

Stop signals and pro-resolution mediators of inflammation

The inflammatory response is a well ordered sequelae of events that physiologically culminates with resolution and restitution at integrum. It is now clear that the resolution phase of inflammation is an active process, tightly regulated by a wide number of mediators. Thus, the understanding of mechanisms involved in resolution, may provide the basis for alternative anti-inflammatory treatments.
Metabolism of unsaturated fatty acids generates a number of pro-resolution compounds. In particular, arachidonic acid (AA) is converted by cooperation between lipoxygenases into lipoxins (LX), whereas aspirin-treated cyclooxygenase 2 (COX-2) in cooperation with 5-LO yields the 15-epi-LX (59). On the other side, eicosapentaenoic acid (EPA) is converted by aspirin-acetylated COX-2 into resolvins of the E-series (RvE1), whereas docosahexanoid acid (DHA) can give rise to protectins/neuroprotectins (PD1) or 17*S*-resolvins of the D-series (RvD1) when attacked by LO or 17*R*-resolvins of the D-series (AT-RvD1) in the presence of aspirin-acetylated COX-2 (60). LX and epi-LX as well as resolvins and protectins exert potent anti-inflammatory bioactions in vitro and in vivo (59,60). Interestingly, BALF from CF patients display decreased lipoxin A_4 (LX_4) levels (61). In addition, a LXA_4 stable analog suppressed neutrophilic inflammation and reduced disease severity in a mouse model of CF (61). On the other hand, a deficiency in DHA, which is the precursor of protectins and resolvins, has been observed in CF (62), providing a rationale for DHA trials in CF (63). Administration to 5 CF patients of 70 mg of DHA/kg body weight/d for six weeks, determined a significant increase in tissue DHA levels (64). Long-term administration of DHA to *Cftr*-/- mice resulted in a significant improvement of inflammatory liver disease (65).
These observations indicate that alterations in endogenous pro-resolution signals may be also involved in CF inflammation. It may be therefore hypothesized that administration of pro-resolution drugs may represent an alternative therapeutic option to combat inflammation in CF.

Concluding remarks
It is now clear that controlling inflammation is a challenging, however crucial, task in CF therapy. A variety of approaches have been experimented with mixed results (Table 1). It is essential to decode the key and peculiar circuits of CF inflammation to establish a successful treatment, since conventional anti-inflammatory drugs have shown limited therapeutic efficacy. If, on one side, there is a need for more, prospective clinical trials, on the other side the indications provided by basic science should be translated into novel approaches. In this respect, the synthesis and the use of pro-resolutions drugs may be worth pursuing.

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TABLE 1

ANTI-INFLAMMATORY DRUGS IN CF			
Drug	**Administration route**	**Results and Remarks**	**Reference**
STEROIDS	Oral Inhaled Erythrocyte	Improvement in lung function. Serious adverse effects in long-term treatment Insufficient evidence of benefit or risk. Small populations studied. Some improvement in FEV_1. Evidence is too preliminary.	[10] [11] [12]
NSAIDs *Ibuprofen* *Selective COX-2 inhibitors*	 Oral	 Consistent slow progression of lung disease. Modest risk of GI bleeding. No data available in CF. Cardiovascular risk in long-term administration.	 [13-15] [16]
DEOXYRIBONUCLEASE	Inhaled	Reduction in inflammatory markers in BALF. Reduction in decay of lung functions and risk of exacerbations. Longer follow up trials are needed.	[17-19]
LEUKOTRIENE-R ANTAGONISTS *BLT_1* *$CysLT_1$*	 Oral Oral	 Trial stopped because of serious respiratory adverse effects. Modest improvement in exercise tolerance, respiratory symptoms and inflammatory parameters. No conclusive evidence of benefit.	 [25] [29-32]
ANTI-OXIDANTS *N-acethylcysteine*	 Oral	 Significant reduction in sputum elastase activity and neutrophil burden. No changes in pulmonary functions. More consistent evidence needed. Phase II	 [34]

		trial is underway.	
NITRIC OXIDE REGULATORS			
Statins	Oral	Under evaluation.	
L-Arginine	Oral or Inhaled	Increased NO formation. No changes in FEV_1.	[41]
PPAR ACTIVATORS			
PPAR-γ	Oral	Down-regulation of pro-inflammatory cytokine expression in airway cells and in alveolar macrophages. Under clinical evaluation.	[44-45]
CYTOKINES			
IFN-γ	Inhaled	No changes in respiratory functions or inflammation markers.	[51]
IL-10		Prolonged inflammation in response to *Pseudomonas aeruginosa* in IL-10 knockout mice. IL-10 improved survival of IL-10 knockout mice.	[48-49]
PROTEASE INHIBITORS			
α1-antitrypsin	Inhaled	Suppression of neutrophil elastase. Reduction in pro-inflammatory cytokines and PMNs in the induced sputum. NO changes in lung function.	[52-53]
EPI-hNE4	Inhaled	Protection of rats from acute lung injury. Reduction of PMN influx in rat airway after tracheal instillation of pooled sputum from children with CF. Phase II clinical trial is underway in Europe.	[54-55]
ANTIBIOTICS			

Azithromycin	Oral	Significant improvement in FEV_1. Less exacerbations.	[57]
Clarithromycin	Oral	Reduction in TNF-α and IL-8 levels in plasma and sputum. Significant improvement of lung functions.	[58]
PRO-RESOLUTION STOP SIGNALS			
Lipoxins	Oral	Suppression of neutrophilic inflammation and reduction disease severity in a mouse model of CF.	[61]
Protectins		Potent anti-inflammatory activity in vitro and in animal models. No data available in CF.	[62]
Resolvins		Potent anti-inflammatory activity in vitro and in animal models. No data available in CF.	[62]
DHA	Oral	Increase in tissue DHA levels in CF patients. Significant improvement of liver disease in *Cftr*-/- mice.	[64-65]