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Proteases and Cystic Fibrosis

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Abstract

Cystic fibrosis is the most common, inherited fatal disease in Caucasians. The major cause of morbidity and mortality is chronic lung disease due to infection and inflammation in the airways leading to bronchiectasis and respiratory failure. The signature pathologic features of CF lung disease including abnormal mucus obstructing airways, chronic infection with *S. aureus*, *P. aeruginosa* and other gram negative bacteria, and a robust neutrophil-dominant airway inflammation, are exacerbated by unopposed proteases present at high concentrations in the ASL. There is strong evidence that proteases, particularly neutrophil elastase, contribute to the pathology of CF by impairing mucociliary clearance, interfering with innate immune functions, and perpetuating neutrophilic inflammation. The mechanisms employed by proteases to impact airway function in CF will be reviewed.

Keywords

cystic fibrosis; neutrophil elastase

Cystic Fibrosis, Pathogenic Mechanisms

Cystic fibrosis is an autosomal recessive disease which is the most common inherited disease causing mortality in caucasians. The disease is manifest by abnormalities in exocrine gland function resulting in altered ion composition and increased viscosity of epithelial secretions including the sweat duct and salivary glands, the small intestine, the pancreatic exocrine gland ducts, the biliary tract, the vas deferens, and the respiratory tract (Voynow, 2005). The disease is caused by loss of expression/loss of function mutations in the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR is an apical chloride channel but also regulates other ion channels including the epithelial sodium channel, the outwardly rectifying chloride channel and ROMK potassium channel (Schwiebert et al., 1999).

The organ system most severely affected is the respiratory tract; lung disease causes 95% of the morbidity and mortality in CF patients (Voynow, 2005). There is some controversy concerning how loss of CFTR function instigates the pathogenesis of the disease and several mechanisms have been proposed. Abnormal CFTR function may directly impact airway surface liquid (ASL) volume, ASL salt concentrations, and ASL bicarbonate levels/pH, ASL mucus viscosity, and submucosal gland duct secretion of innate immune molecules (Verkman, 2003). Mutant CFTR may also regulate the intracellular organellar pH affecting Golgi (Scanlin

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and Glick, 2001) or endosomal function (Bertrand and Frizzell, 2003) resulting in altered membrane trafficking and post-translational modification of proteins which would impact airway epithelial innate immune function.

Although there is controversy over the exact mechanisms by which mutant CFTR initiates lung pathogenesis, the pathologic features of CF lung disease are well-established. Obstruction of submucosal glands is an early pathologic feature on airway histology (Sturgess, 1982) and submucosal gland hyposalivation has recently been demonstrated in intact submucosal glands ex vivo (Joo et al., 2006). CF ASL has altered pH and increased viscosity (Verkman, 2003). CF airways become infected early with bacteria; first with *S. aureus* and *H. influenza*, then later with *P. aeruginosa* and other opportunistic gram negative organisms (Ramsey, 1996). Although organisms are predominantly located in mucus plugs in the airway lumen (Potts et al., 1995), they activate epithelial production and release of several cytokines including IL-8 (Sadikot et al., 2005). There is a vigorous inflammatory response with increased cytokine production including IL-8 and IL-6 (Noah et al., 1997), and a neutrophil-dominant inflammatory response in the airways associated with increased concentrations of neutrophil elastase in the airway surface liquid (ASL) that overwhelms antiprotease capacity (Goldstein and Doring, 1986, Konstan et al., 1994, Birrer et al., 1994, Khan et al., 1995, Armstrong, 1997). Chronic infection and inflammation are established in the airways and progressively destroy the normal architecture. Sputum biomarkers of IL-8, neutrophil counts, and neutrophil elastase correlate directly with disease severity (Mayer-Hamblett et al., 2007). Both bacterial exoproducts and neutrophil mediators upregulate production of mucin glycoproteins, the major macromolecular constituent of mucus (Rose and Voynow, 2006), and increase mucus secretion into the airway resulting in a favorable milieu for bacterial proliferation. Due to aberrant ion and water flux in the airway, mucus plaques become viscous which favors the development of biofilms (Matsui et al., 2006) and impedes neutrophil phagocytosis of bacteria (Matsui et al., 2005). Over time, airway secretory remodeling takes place with epithelial loss and heterogeneous zones of proliferation (Voynow et al., 2005), hypertrophy of goblet cells, and hyperplasia of submucosal gland cells (Hays and Fahy, 2006). Secretory cell hypertrophy, increased mucus secretions, and ciliary injury impair mucociliary clearance. Chronic mucus obstruction, infection, and neutrophil-driven inflammation lead to bronchiectasis and eventually respiratory failure.

CF lung disease results from a failure of pulmonary innate immune functions: inadequate mucociliary clearance, loss of airway antimicrobial activity, and failure of immune mechanisms to successfully opsonize and kill bacteria. The presence of large numbers of airway neutrophils and high concentrations of neutrophil proteases, particularly neutrophil elastase (NE), suggest that neutrophils and proteases disrupt normal pulmonary innate immune functions and significantly contribute to the pathogenesis of CF lung disease. NE content in ASL overwhelms normal antiprotease capacity due to serine leukoprotease inhibitor (SLPI) or α -1-antitrypsin (a1AT). Antiprotease capacity is further diminished by inactivation of a1AT due to oxidation of methionine 358 in its active site by neutrophil-derived oxygen radicals (Johnson and Travis, 1979). SLPI is fragmented and inactive in CF (Vogelmeier et al., 1991). Finally, neutrophils do not undergo apoptosis and are not successfully cleared by macrophage phagocytosis (Vandivier, 2002). Instead, neutrophils necrose in the airway resulting in release of intracellular contents (DNA and f-actin) that increase sputum viscosity, and release of granule contents including proteases and oxidants that further propagate airway injury.

In this review, we will present evidence to support the concept that in CF, exuberant, unopposed proteases participate in many of the pathogenic events leading to abnormal mucociliary clearance, impaired immune function, and airway remodeling. We will also review the strategies tested thus far to reduce neutrophilic inflammation or augment antiprotease defenses in the airway.

Proteases in the CF airway

Most proteases in the CF airway are released by neutrophils. The neutrophil serine proteases, NE, proteinase 3, and cathepsin G, are stored in azurophilic granules. Azurophilic granules usually release into phagosomes but there may be leakiness if neutrophils phagocytose large particles or if neutrophils undergo cell death (Lucey et al., 1997). Although concentrations of NE and NE activity levels are predominant in the ASL of CF patients, high levels of cathepsin G (Goldstein and Doring, 1986), and proteinase 3 are also present in bronchoalveolar lavage (BAL) fluid of CF patients (Witko-Sarsat et al., 1999). The neutrophil metalloproteases, collagenase (MMP-8) and gelatinase (MMP-9), stored in specific granules and tertiary granules respectively, are also released into the ASL of CF patients under inflammatory conditions and are found in high concentrations in the BAL (Ratjen et al., 2002, Gaggari et al., 2007).

The neutrophil-derived proteases have several well-established effects on airway biology. NE is present at the highest concentrations in the airway and is the best studied; the impact of this protease on airway biology will be discussed in more detail later in this review. Of the other neutrophil-derived proteases, both cathepsin G (Lundgren et al., 1994) and proteinase 3 (Witko-Sarsat et al., 1999) stimulate mucus secretion. MMP-9 cleaves IL-8 to produce a more active truncated form of IL-8 (Van den Steen et al., 2000). Furthermore, MMPs cleave α 1AT, further decreasing the anti-elastase capacity in the ASL (Liu et al., 2000).

In addition to neutrophil-derived proteases, other proteases contribute to the ASL protease load. Cysteine proteinases, Cathepsins B, L, and S, derived from several cellular sources as lysosomal cathepsins (Wolters and Chapman, 2000) are present in the CF BAL. *Pseudomonas aeruginosa* elastase and alkaline protease are also present in the ASL (Suter, 1994). Cathepsins B, L, and S interfere with antimicrobial defenses by degrading SLPI (Taggart et al., 2001), beta-defensins (Taggart et al., 2003), and lactoferrin (Rogan et al., 2004). *Pseudomonas aeruginosa* elastase stimulates mucus secretion (Adler et al., 1983), slows ciliary beat frequency (Amitani et al., 1991), and degrades surfactant proteins A and D (Mariencheck et al., 2003). These proteases diminish the innate immune capacity of the lung and directly injure the airways in CF. The mechanisms underlying protease-mediated chronic infection and inflammation are best understood for the most abundant protease in the CF airway, NE.

Neutrophil Elastase

NE is present in micromolar concentrations in CF ASL (Birrer et al., 1994, Konstan et al., 1994, Khan et al., 1995, Armstrong, 1997). NE is a positively charged 29 kD molecule that cleaves neutral, non-aromatic dipeptides (Hubbard, 1991). Because of its charge and size, it is restricted to the extracellular space, and cleaves cell surface structures and molecules. NE contributes to the altered ASL composition in CF by activating the apical epithelial sodium channel which increases sodium uptake from the ASL (Caldwell et al., 2005). NE also aggravates neutrophilic inflammation in CF by upregulating epithelial expression of a major neutrophil chemokine, IL-8 (Nakamura et al., 1992, Devaney et al., 2003). IL-8 increases NE release from CF neutrophils (Taggart et al., 2000) resulting in a self-perpetuating cycle of neutrophil inflammation and excessive NE in ASL. NE transcriptionally upregulates IL-8 by two mechanisms: activation of TLR4 resulting in signaling via MyD88 and IRAK (Devaney et al., 2003) and EGFR transactivation and signaling via p38 MAPK (Kuwahara et al., 2006). NE indirectly increases IL-8 by cleaving CXCR1, an IL-8 receptor on neutrophils, and the soluble CXCR1 fragment activates TLR2 on airway epithelia increasing the transcription of IL-8 (Hartl et al., 2007). NE sustains proteolytic inflammation by other mechanisms. NE degrades α 1AT, decreasing the anti-protease capacity of the lung (Cantin et al., 1989). NE activates pro-MMP-9 and degrades its natural inhibitor, tissue inhibitor of metalloprotease-1 (TIMP-1), further activating MMP-9 in the CF airway.

Proteolytic activity of NE at the cell surface of airway epithelia and immune cells has a profound impact on the CF airway (summarized in Table 1). NE impairs mucociliary clearance by inhibiting ciliary motility and by increasing mucin gene regulation, and mucin secretion. NE affects airway remodeling by regulating airway epithelial cell cycle progression and by regulating expression of ErbB receptor tyrosine kinases. Finally, although neutrophil-associated NE is required for gram negative bacterial killing, excessive NE in the ASL impairs airway immune functions and inhibits bacterial recognition and killing.

Neutrophil Elastase and Mucins, Mucus Secretion, and Mucociliary Clearance

NE regulates mucin MUC5AC gene expression by both transcriptional (Song et al., 2005, Shao and Nadel, 2005b, Kohri, 2002, Shao and Nadel, 2005a) and post-transcription mechanisms (Voynow, 1999). NE stimulates translocation of PKC δ to the plasma membrane, resulting in activation of dual oxidase 1 (DUOX1 (Shao and Nadel, 2005a) and subsequent activation of TNF α -converting enzyme (TACE) to cleave and release the EGFR ligand, TGF α , permitting activation of EGFR and down-stream signaling via RAS-Raf-MEK1/2-ERK1/2 and either NF- κ B (Song et al., 2005) or Sp1 (Perrais et al., 2002) activation. We and others have shown that NE also induces oxidant signaling in airway epithelial cells (Aoshiba et al., 2001, Fischer and Voynow, 2002, Zheng et al., 2007) and these oxidants are required for increased MUC5AC expression. We have recently reported that NE upregulates a cytoplasmic oxidoreductase, NADPH quinone oxidoreductase 1 that is required for NE-induced lipid peroxidation and for NE-regulated MUC5AC mRNA expression (Zheng et al., 2007). NE also upregulates two membrane-associated mucins, MUC4 (Fischer, 2003) and MUC1 (Kuwahara et al., 2005), in airway epithelial cells. MUC1 and MUC4 regulate intracellular signaling via the ErbB family of receptor tyrosine kinases in cancer cells (Hollingsworth and Swanson, 2004) and may have similar functions in the lung.

In addition to increasing mucin production, NE stimulates mucus secretion from human airway epithelial cells (Lundgren et al., 1994, Park et al., 2005) and releases cell surface mucins from hamster tracheal epithelial cells (Kim et al., 1987). NE induces hypersecretion of two major respiratory tract mucins, MUC5AC and MUC5B, by activation of protein kinase C δ , causing translocation of PKC δ from the cytoplasm to the plasma membrane and associated transient activation of myristoylated alanine-rich C-kinase substrate (MARCKS), a key secretory granule chaperone during stimulated exocytosis (Park et al., 2005). Finally, NE slows ciliary beat frequency (Amitani et al., 1991) and causes epithelial disruption (Venaille et al., 1998). Together, these airway epithelial changes result in abundant mucin production and failure of adequate ciliary clearance. These problems are compounded by the process of airway remodeling that is triggered in part by airway NE.

NE and Airway Remodeling

NE regulates several aspects of airway remodeling. NE has been reported to cause epithelial disruption, an effect that is concentration and time-dependent (Amitani et al., 1991, Venaille et al., 1998). Importantly, following NE exposure, epithelial cells enter a cell cycle arrest associated with increased expression of the cyclin kinase inhibitor, p27 (Fischer et al., 2007). NE also decreases airway epithelial cell surface ErbB2 expression with associated decrease in epithelial proliferation (Fischer et al., 2005). During the recovery period after NE exposure, ErbB2 levels increase at the cell surface, and epithelial proliferation also increases. In CF airways, basal-like cells that express EGFR have a high proliferative index, and they are present in a patchy distribution in the airway (Voynow et al., 2005). Both increased p27 expression and decreased ErbB2 expression possibly permit time for epithelial repair of proteolytic and oxidant injury prior to proliferation and differentiation.

NE has been reported to induce bronchial secretory metaplasia in hamsters (Breuer et al., 1985) and in mice (Voynow, 2004). Secretory cell metaplasia is due to proteolytic activity of NE as inhibition of proteolytic activity with inhibitors prevented the secretory cell remodeling. Goblet cell size is increased in CF although the number of goblet cells is unchanged (Voynow et al., 2005, Hays and Fahy, 2006); this is associated with increased neutrophil elastase at the superficial epithelial surface (Hays and Fahy, 2006). NE also contributes to destructive and fibrotic processes in the airways and parenchyma. Matrix degradation and elevated concentrations of glycosaminoglycans and elastin in the BAL and thickening of the reticular basement membrane are greater in CF than in control chronic respiratory disease patients and these profiles correlate positively with TGF- β 1 levels and with NE ASL concentrations (Hilliard et al., 2007). NE has been reported to release VEGF, PDGF-AA and -BB from the extracellular matrix of esophageal epithelial cells (Wada et al., 2007), and pancreatic elastase releases fibroblast growth factor-2 and TGF- β from murine lung into the BAL (Buczek-Thomas, 2004). These reports support the concept that protease degradation of the extracellular matrix releases growth factors important for epithelial repair and that possibly may also induce subepithelial fibrosis.

NE and Immune Modulation

NE is required in the neutrophil phagosome for gram negative bacterial killing (Belaouaj et al., 1998) and is required for neutrophil bacterial capture and killing by the neutrophil extracellular trap, a complex of chromatin and NE (Brinkmann et al., 2004). However, excess NE released into the airway lumen interferes with bacterial capture and killing. For example, NE cleaves *P. aeruginosa* flagella (Lopez-Boado et al., 2004) and also down-regulates flagellar production (Sonawane et al., 2006) resulting in blunting of *P. aeruginosa* activation of TLR signaling (Prince, 2006, Zhang et al., 2005) and decreased bacterial killing.

NE impairs innate and adaptive immune function (reviewed by (Taggart et al., 2005)) by degrading antimicrobial proteins in the ASL. NE cleaves lactoferrin, an important antimicrobial protein (Rogan et al., 2004) and cleaves the collectins, SP-D (Hirche et al., 2004) and SP-A (Rubio et al., 2004), which are important for pathogen opsonization. NE also degrades other proteins important for opsonization including C3bi on opsonized *Pseudomonas* and CR1 on neutrophils resulting in interference with neutrophil phagocytosis (Tosi et al., 1990, Berger et al., 1989). NE fragments IgG opsonins in the airway resulting in failure of *pseudomonas* phagocytosis and killing by neutrophils (Fick et al., 1984). NE also negatively impacts adaptive immune responses. NE cleaves T cell surface receptors (Doring et al., 1995) including CD2, CD4, CD8, and CD14 which impairs LPS-mediated monocyte activation (Le-Barillec et al., 1999). NE blocks dendritic cell maturation; NE downregulates CD40, CD80 and CD86 expression on dendritic cells and inhibits the process of dendritic cell antigen presentation (Roghani et al., 2006a). Together, the impact of NE protease activity on innate and adaptive immune functions sustains *pseudomonas* infection and inhibits neutrophil, lymphocyte and dendritic cell functions.

Anti-inflammatory and Antiprotease Therapies in CF

Given the relentless neutrophilic inflammation in CF airways, several anti-inflammatory therapies have been tested. Ibuprofen inhibits neutrophil migration and release of lysosomal enzymes. High dose ibuprofen was evaluated in a 4-year, prospective, double-blind, randomized, placebo-controlled trial and found to maintain pulmonary function; ASL or sputum inflammatory markers were not directly evaluated (Konstan et al., 1995). However the effect of Ibuprofen on neutrophil migration is concentration dependent; peak plasma concentrations > 50 μ g/ml are required to decrease neutrophil counts in oral mucosal washes while plasma concentrations <50 μ g/ml were associated with increased neutrophil counts

(Konstan et al., 2003). In addition to requiring pharmacokinetics for therapeutic benefit, ibuprofen therapy has not widely been accepted due to the risk of side effects (Oermann et al., 1999) including gastrointestinal bleeding and renal failure. Another anti-inflammatory therapy, oral glucocorticoids administered every other day, was evaluated in a 4 year prospective, double-blind, randomized, placebo controlled study of 285 patients. Glucocorticoids, 1 mg/kg every other day, improved pulmonary function in patients colonized with *P. aeruginosa* at baseline, and decreased IgG levels (Eigen et al., 1995). However, glucocorticoid therapy was not recommended as a routine therapy because persistent growth impairment was noted 6–7 years after this study was completed in boys 18 years of age or older (Lai et al., 2000). Other approaches to anti-inflammatory therapy including antioxidant therapies and immunosuppressive therapies are currently being investigated in preclinical and early clinical trials.

A rational approach to inhibit excess extracellular protease activity in the CF airway is to administer inhaled antiproteases to augment the antiprotease capacity in the airway. Interestingly, this approach may have some additional benefits as some antiproteases inherently have anti-microbial activity, and regulate adaptive immunity and inflammation (Table 2). Elafin, a low molecular weight inhibitor of NE that is secreted in the lung directly kills *Pseudomonas aeruginosa* and *Staphylococcus aureus* in vitro (Simpson et al., 1999). Elafin also increases the number and activation of dendritic cells after overexpression in vivo (Roghani et al., 2006b). Aerosol treatment of human monocyte/neutrophil elastase inhibitor, a serpin that inhibits NE, cathepsin G, and proteinase-3, to mice with chronic *Pseudomonas aeruginosa* lung infection, inhibits lung inflammatory injury and promotes *P. aeruginosa* clearance (Woods et al., 2005). SLPI inhibits NF- κ B activation by preventing lipopolysaccharide-induced degradation of I κ B α which sequesters NF- κ B in the cytosol and inhibits its release and translocation to the nucleus (Taggart et al., 2002). SLPI also impairs TLR2- and TLR4 mediated responses in monocytes, thus blocking NF- κ B activation (Greene et al., 2004). SLPI has another salutary effect by increasing ASL glutathione levels (Gillissen et al., 1993); there is a deficiency of glutathione, an important antioxidant, in CF and increased oxidant stress in the CF airway. However, the native airway antiproteases consisting of a1AT, SLPI, and elafin are insufficient to inhibit the enormous load of NE and other proteases in the CF airway. The task of NE inhibition is further complicated by NE binding/sequestration in polyanionic filaments such as DNA and mucin in the sputum. When these polyanionic filaments are disrupted, there is initially increased free NE in the sputum that decreases over time as sputum is cleared from the airway (Costello et al., 1996, Shah et al., 1996). Furthermore, as discussed above, a1AT is inactive due to degradation and/or oxidation of the active site; SLPI may be degraded or oxidized; and TIMP-1 may be degraded. Therefore trials of antiprotease inhibitors delivered directly to the airway have been evaluated in CF.

Two phase II trials of a1AT in CF have been completed (Martin et al., 2006, Griesse et al., 2007). Both studies were prospective but had different experimental designs, different doses of inhaled a1AT, different subject populations, and evaluated different outcomes (Table 3). In one prospective study, 52 CF patients received daily a1AT (25 mg) via inhalation for 4 weeks. Patients had moderately severe airway obstruction, and were colonized with *P. aeruginosa*. Induced sputums were obtained to determine a1AT levels, NE, cytokine and neutrophil measures, IgG and bacteriology. Results at 2 and 4 weeks were compared to baseline values for each subject. At 4 weeks, there was a significant increase in a1AT levels by ELISA and a significant decrease in free NE activity, the number of neutrophils, *Pseudomonas* colony forming units, IL-8 and TNF- α levels and intact IgG. There was no significant difference in pulmonary function (FEV1). In another prospective double-blinded, randomized, placebo-controlled phase II trial, 39 subjects with mild-moderate lung disease were randomized to receive nebulized treatment (125 mg, 250 mg, or 500 mg) daily for 4 weeks followed by 2–4 weeks with no study treatment and then a 2-week rechallenge phase. Although absolute sputum

NE levels were not significantly different, sputum NE/a1AT complex and myeloperoxidase levels were lower on a1AT therapy than on placebo. There was no significant change in pulmonary function, resting respiratory rate or quantitative microbiologic assessment. Although a1AT inhaled therapy was safe, there is insufficient evidence thus far to determine whether supplementation of a1AT by inhalation is sufficient to correct NE-mediated CF lung inflammation. Alternative approaches to therapy are currently being evaluated including modified SLPI, small molecular inhibitors of NE or dithiol reagents that reduce NE and decrease sputum viscosity (Lee et al., 2005).

Summary

Neutrophil predominant inflammation and unopposed proteases are important causes of CF airway pathology. Proteases including NE, proteinase-3, MMP-8, -9, cathepsins G, B, S, L, and *P. aeruginosa* proteases all are active in the ASL and contribute to impaired innate and adaptive immunity, pro-inflammatory signaling, airway epithelial injury and remodeling, and impaired mucociliary clearance. No therapies currently available adequately control this important component of CF pathogenesis. New approaches to administer inhaled medications to inhibit or modify proteases or to augment antiprotease concentrations in the airway are rational approaches to tackle this significant problem.

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Abbreviations

ASL	airway surface liquid
NE	Neutrophil elastase
TLR	Toll-like receptor

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Table 1

Actions of NE

Impaired Mucociliary Clearance	Increases MUC5AC expression Increases MUC1 and MUC4 expression Increases mucin secretion Decreases ciliary beat frequency Injures cilia
Airway Remodeling	Induces goblet/mucus cell metaplasia Increases p27 and cell cycle arrest Decreases cell surface ErbB2 Increases epithelial permeability Degrades extracellular matrix
Pro-inflammatory Effects	Increases IL-8 expression Activates TLR4 and EGFR Cleaves CXCR1 resulting in TLR2 activation Activates Pro-MMP-9 Degrades TIMP-1 Degrades a1AT
Impaired innate and adaptive immunity	Cleaves neutrophil phosphatidyl serine/Inhibits apoptotic neutrophil phagocytosis Cleaves opsonin and receptor pair: C3bi & CR1 Cleaves opsonins SP-A, SP-D, IgG Degrades lactoferrin Cleaves T cell surface markers with associated decreased LPS response Impairs dendritic cell maturation

Table 2

Antiprotease Activities

AIAT	Inhibits NE, cathepsin G
SLPI	Inhibits NE, cathepsin G
	Inhibits TLR activation
	Inhibits NF- κ B activation
	Increases ASL glutathione levels
Elafin	Inhibits NE
	Has antimicrobial activity
	Activates dendritic cells
Monocyte/neutrophil elastase inhibitor	Inhibits NE, cathepsin G, proteinase 3
	Has anti-inflammatory effect <i>in vivo</i>
	Promotes clearance of lung <i>P. aeruginosa in vivo</i>

Table 3
Clinical Trials of a1AT in CF

Study Design	Subjects	Results
(McElvaney et al., 1991) Open label aerosol a1AT (1.5–3 mg/kg)	12 CF subjects 12 healthy subjects (C) mean age: 28 yrs (CF); 29 yrs (C) PFTs: Moderate obstruction	↑ ASL a1AT ↑ ASL anti-NE capacity when every 12 h for 1 week a1AT ≥ 8 μM
(Martin et al., 2006) Prospective, randomized, Double-blind, placebo-controlled Aerosol a1AT: 125, 250 or 500 mg/day × 4 weeks Sputum measures compared to placebo (Griese et al., 2007) Prospective, randomized Peripheral vs. central airway deposition Aerosol a1AT: 25 mg/day × 4 weeks Sputum measures compared to baseline	39 CF (32 male) mean age: 27.5 yrs. PFT: Moderate obstruction	↓ NE/a1ATcomplex ↓ myeloperoxidase ↓ NE Good safety profile ↓ NE ↑ a1AT ↓ PMN ↓ <i>P. aer.</i> ↓ IL-8, IL-1β, TNFα, ↓ LTB4 ↑ full-length IgG No difference between peripheral and central deposition
	52 CF (26 male) mean age 25 yrs. PFT: Mild obstruction	