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ORIGINAL RESEARCH

Airway inflammatory markers in individuals with cystic fibrosis and non-cystic fibrosis bronchiectasis

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Abstract: Bronchiectasis is an airway disease characterized by thickening of the bronchial wall, chronic inflammation, and destruction of affected bronchi. Underlying etiologies include severe pulmonary infection and cystic fibrosis (CF); however, in a substantial number of patients with non-CF-related bronchiectasis (NCFB), no cause is found. The increasing armamentarium of therapies now available to combat disease in CF is in stark contrast to the limited tools employed in NCFB. Our study aimed to evaluate similarities and differences in airway inflammatory markers in patients with NCFB and CF, and to suggest potential common treatment options. The results of this study show that NCFB bronchoalveolar lavage fluid samples possessed significantly increased NE activity and elevated levels of matrix metalloproteinases 2 (MMP-2) and MMP-9 compared to healthy controls ($P < 0.01$); however, the levels detected were lower than in CF ($P < 0.01$). Interleukin-8 (IL-8) concentrations were significantly elevated in NCFB and CF compared to controls ($P < 0.05$), but in contrast, negligible levels of IL-18 were detected in both NCFB and CF. Analogous concentrations of IL-10 and IL-4 measured in NCFB and CF were statistically elevated above the healthy control values ($P < 0.05$ and $P < 0.01$, respectively). These results indicate high levels of important proinflammatory markers in both NCFB and CF and support the use of appropriate anti-inflammatory therapies already employed in the treatment of CF bronchiectasis in NCFB.

Keywords: bronchiectasis, cystic fibrosis, proteases, inflammation

Introduction

Bronchiectasis is a respiratory disease characterized by dilated thick-walled bronchi, periods of acute infective exacerbations, inflammation, and purulent sputum expectoration. The cycle of infection and inflammation results in permanent lung tissue damage and remodeling. Causes or genetic conditions leading to the development of bronchiectasis include immunodeficiency, primary ciliary dyskinesia or severe pulmonary infection, but the most common cause of bronchiectasis in children is cystic fibrosis (CF).[1]

In the United States, the prevalence of non-CF-related bronchiectasis (NCFB) in the older population (>65 years) now stands at 1106 cases/100,000 persons and is increasing at a rate of 8.7% per year from the year 2000 through to 2007 with consequent costs to health care delivery.[2] Indeed, the prevalence and severity of NCFB intensifies with age and when in association with other diseases or autoimmune symptoms, including chronic obstructive pulmonary disease (COPD) or rheumatoid arthritis respectively, the relative risk of death increases.[3,4] In COPD, bronchiectasis has been reported in 50% to 57% of patients.[5–7]

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On many levels, the pathogenesis of CF lung disease and the properties of CF airway secretions are comparable to those of NCFB. For example, CF is caused by mutations in the CF transmembrane conductance regulator chloride channel, which results in increased epithelial sodium channel (ENaC)-mediated ion absorption in the superficial airway epithelial.[8,9] In concurrence, it has been shown that some patients with idiopathic bronchiectasis carry a mutation in the ENaC β or γ genes, resulting in a defective ENaC protein.[10] Moreover, lower respiratory tract infections with Gram-negative bacteria including *Pseudomonas aeruginosa* are a major cause of morbidity and mortality among patients with CF and NCFB and are associated with worsening lung function and an excess loss in forced expiratory volume in 1 second (FEV_1).[11,12] Patients with CF and NCFB experience excessive neutrophilic infiltration resulting in delivery of high levels of enzymes such as neutrophil elastase (NE) to the airways, the latter playing a pivotal role in the destruction of lung tissue.[13,14] The inflammatory response within the airways is dependent upon the interplay between proinflammatory and anti-inflammatory cytokines. Proinflammatory mediators, which favor neutrophil trafficking to the bronchial lumen, include interleukin-8 (IL-8), IL-1 and tumor necrosis factor-α (TNF-α). IL-8 is markedly increased in CF bronchial samples[15,16] and in NCFB a significant correlation has been recorded between high-resolution computed tomography (HRCT) severity scores and sputum IL-8 and TNF-α levels.[17] In response to TNF-α, neutrophils also release matrix metalloproteinases (MMPs),[18] which have the potential to augment inflammation and cause extensive airway remodeling.[19,20] Increased levels of MMP-8 and MMP-9 have been reported in bronchoalveolar lavage (BAL) fluid of both CF and NCFB subjects.[21–24]

Despite the reported similarities, CF and NCFB are quite distinct and respond differently to clinical management. For example, as high concentrations of DNA contribute to the viscosity of CF airway secretions, treatment with recombinant human DNase (rhDNase), an enzyme which cleaves DNA polymers, results in a significant decrease in the viscosity of mucopurulent sputum in CF[25] and has demonstrated an improved in FEV_1.[26–28] In contrast, in adults with idiopathic bronchiectasis, treatment with rhDNase has been shown to have a negative effect on FEV_1 and has led to increased pulmonary exacerbations.[29,30]

Until recently, no guidelines existed to direct the clinician to the optimum diagnosis, investigation, and treatment options available for NCFB due to the relative paucity of well-controlled clinical trials and basic science research. Even now, the lack of well-designed clinical trials concerning the management of NCFB prevents the transfer of existing treatment options for CF bronchiectasis for use in patients with NCFB. To date, clinical reports have focused on differences between CF or NCFB airway inflammatory markers compared to healthy control individuals, but relatively few studies have directly compared CF to NCFB. Most importantly, there is now an urgency to understand the similarities and differences between these two diseases to identify the common or different treatment options. To this end, the present study was designed to directly compare airway inflammatory markers in patients with CF to NCFB.

Materials and methods

Chemicals

All chemicals and reagents were purchased from Sigma Aldrich Chemical Co, Ltd (Dublin, Ireland), unless indicated otherwise.

Patient recruitment and BAL fluid sample collection

Prior to recruitment, nonsmoking patients had to be exacerbation-free over the preceding 6-week period. There were 6/10 and 5/10 males recruited to the NCFB and CF study, respectively (Table 1). The median and interquartile range of the age was 65 (51.5–71.5) and 22.50 (20.50–26.5) years for the NCFB and CF groups, respectively. Pulmonary function tests revealed a median FEV_1 of 71% (range 47%–95%) in the NCFB group. In all study patients, the diagnosis of bronchiectasis was made by HRCT using well-established criteria.[31]

The underlying cause of NCFB was postinfectious in five patients (including pertussis infection), immunoglobulin G subclass 1 deficiency in one, and in the remaining patients no cause was identified (total n = 10 patients). No patients were identified as having alpha-1 antitrypsin deficiency. Exclusion criteria for NCFB patients were: exacerbation of bronchiectasis in the last 6-week period; current smokers; documented history of COPD, congestive cardiac failure, or neuromuscular disorder which could lead to altered spirometry; or any inflammatory disorder associated with abnormal inflammatory markers. CF was excluded by at least two negative results of sweat tests conducted in patients with a clinical presentation compatible with CF.[32,33] Microbiology results showed that two patients with NCFB were positive for *Moraxella catarrhalis* and two for *P. aeruginosa*.

Inclusion criteria for the CF cohort of patients required a confirmed diagnosis of CF by sweat chloride concentrations > 60 mmol/L on at least two separate occasions and subsequent confirmatory genotyping. CF patients (n = 10) were heterozygous for the ΔF508 mutation and illustrated a median FEV_1 of 39% (23%–53%). Patients with CF were

Table 1 Characteristics of patients included in BAL studies

	CF	NCFB	Control
No of subjects	10	10	10
Sex, % male/female	50/50	60/40	60/40
Age, years	22.5 (20.5–26.5)	65 (51.5–71.5)	25 (22.5–33)
FEV_1, % predicted	40 (25.5–49.5)	83.5 (53.5–95)	99 (91–110)
Genotype	ΔF508 heterozygous	–	–
Serum protein levels			
Alpha-1 antitrypsin (mg/mL)	1.26 (0.86–1.94)	1.42 (1.34–1.51)	–
IgG (6.0–15.0)	18.9 (13.45–18.9)	11 (8.56–13)	–
IgA (0.7–4.0)	2.73 (0.41–6.1)	2.63 (1.39–3.52)	–
IgM (0.5–2.3)	1.29 (0.53–2.29)	0.66 (0.16–1.21)	–
Bacteriology	AF (n = 3)	MC (n = 2)	–
	PA (n = 5)	PA (n = 2)	
	AF + PA (n = 1)		
	AF + SM + SA (n = 1)		
BAL cells (min–max)			
Total cells × 10^3/mL	1021 (196–3257)	243 (10–1086)	–
Neutrophils (%)	73 (60–80)	67 (56–88)	–
Macrophages (%)	20 (10–36)	27 (8–38)	–

Note: Results are expressed in medians and interquartile ranges unless otherwise stated.
Abbreviations: BAL, bronchoalveolar lavage; CF, cystic fibrosis; NCFB, non-CF bronchiectasis; FEV_1, forced expiratory volume in 1 second; Ig, immunoglobulin; AF, *Aspergillus fumigatus*; PA, *Pseudomonas aeruginosa*; SM, *Stenotrophomonas maltophilia*; MC, *Moraxella catarrhalis*; SA, *Staphylococcus aureus*.

colonized with *Aspergillus fumigatus* (n = 3) or *P. aeruginosa* (n = 5). One patient was cocolonized with *A. fumigatus* and *P. aeruginosa* and one patient with *A. fumigatus*, *Stenotrophomonas maltophilia*, and *Staphylococcus aureus.*

BAL fluid samples were collected from patients attending Beaumont Hospital. This study complied with the tenets of the Declaration of Helsinki and full informed consent was obtained preprocedure according to a protocol approved by the Beaumont Hospital Ethics Committee. BAL fluid was obtained from adult individuals with CF (n = 10), NCFB (n = 10), sarcoidosis (n = 10), or healthy control volunteers (n = 10). BAL fluid samples (10 mL) were obtained by wedging the bronchoscope into the most abnormal appearing lobe identified by reviewing the HRCT images and visual inspection during bronchoscopy. Sterile normal saline was instilled into this abnormal lobe and was suctioned immediately into a trap. The sample was then split and half was sent for microbiological examination, total cell count, and differential count. The remaining half was centrifuged at 1000 *g* for 10 minutes. The resulting cell-free supernatants were aliquoted and stored frozen at −80°C for subsequent analysis.

Cell counts and NE activity in CF BAL fluid

The total cell count and enumeration of neutrophil and macrophages in BAL fluid was as described previously.[34] NE activity was quantified using an NE-specific substrate, *N*-methoxysuccinyl-ala-ala-pro-val-p-nitroanilide (R&D Systems, Minneapolis, MN, USA) as described previously.[35] NE activity was quantified in 15 μL of BAL fluid at 405 nm. BAL fluid samples were measured for protein with Bio-Rad protein assay (Bio-Rad, Richmond, CA, USA) and NE activity among patient and healthy control samples expressed as Units (U)/μg of BAL fluid protein.

Gelatinase zymography

Gelatin zymography was performed on BAL fluid samples as described previously.[35] Protein concentration was determined and 500 ng of BAL fluid was incubated for 10 minutes in nonreducing sample buffer (0.25% [w/v] bromophenol blue, 50 mM Tris, pH 7.5, 40% [v/v] glycerol and 1% [w/v] sodium dodecyl sulfate [SDS]). Samples and molecular weight markers (BioRad Precision Plus Protein Standard; Bio Rad) were electrophoresed on a 7% SDS-polyacrylamide gel electrophoresis containing gelatin (1 mg/mL). After electrophoresis, the gels were incubated in 50 mM Tris (pH 7.5), 5 mM $CaCl_2$, 1 μM $ZnCl_2$ containing 2.5% (v/v) Triton X-100 for 30 minutes at room temperature. The gels were washed in the same buffer without the Triton X-100 for 5 minutes and then incubated at 37°C overnight in the same buffer supplemented with 1% (v/v) Triton X-100. To visualize gelatinases, gels were stained with Coomassie blue stain (0.2% [w/v] Coomassie brilliant blue, 45% [v/v] methanol, 10% [v/v] acetic acid) and then destained (25% [v/v] methanol, 10% [v/v] acetic acid). Gelatinases appeared as translucent bands on gels

ranging from approximately 175 kDa to 62 kDa. As previously described, bands at approximately 85 kDa and 72 kDa potentially representing MMP-9[36] and MMP-2,[37] respectively, were quantified by densitometry using the Syngene G:BOX Chemi XL gel documentation system (Syngene, Cambridge, UK).

Quantification of BAL fluid cytokine levels

Concentrations of IL-8, IL-18, IL-10, and IL-4 in BAL fluid samples were measured by ELISA (R&D Systems). Cytokine concentrations were expressed as pg/mg of BAL fluid protein.

Statistical analysis

The data were analyzed with GraphPad Prism (version 4.03 for Windows; GraphPad Software, San Diego, CA, USA) and results are expressed as median value. The groups were compared by two-tailed independent sample Student's *t*-test when normally distributed or by the nonparametric Mann–Whitney *U* test, as appropriate. A *P*-value < 0.05 was deemed statistically significant.

Results

Comparative quantification of NE activity in CF, NCFB, and healthy control BAL fluid samples

Quantification of NE activity by fluorometric analysis revealed a significant difference in NE activity between all three groups ($P < 0.01$), with the highest level detected in BAL fluid from patients with CF (110.2×10^4 U/µg BAL fluid) (Figure 1). BAL fluid samples from individuals with NCFB illustrated an intermediate level elevated above the healthy control values (9.312×10^4 and 1.5×10^4 U/µg BAL fluid respectively; $P < 0.01$), but also statistically lower than the CF cohort ($P < 0.01$). In line with these results, NCFB BAL fluid contained a significantly lower number of neutrophils compared to CF (162×10^3/mL compared to 745×10^3/mL, respectively; $P < 0.01$).

Increased MMP-2 and MMP-9 levels in CF and NCFB BAL fluid samples

Previous studies have demonstrated the impact of NE on MMP-2 expression[38] and a correlation exists between MMP-9 and NE activity.[23] This led us to evaluate MMP-2 and MMP-9 levels in bronchoalveolar samples by zymography. Representative SDS-PAGE zymograms indicating BAL fluid gelatinase levels in healthy control volunteers, NCFB or CF are shown in Figure 2A–C. Results of densitometry analysis of a 72 kDa gelatinase band, assumed to represent MMP-2 (Figure 2D) and an 85 kDa band, potentially MMP-9 (Figure 2E), are indicated in the respective scatter plots. Statistically significant differences in median relative BAL fluid MMP-2 levels were observed in CF and NCFB in comparison to healthy control volunteers (85.8, 16.8, and 0.40, respectively; $P < 0.01$). The difference in median relative BAL fluid MMP-2 levels between NCFB and CF was also statistically significant ($P < 0.01$). Median relative BAL fluid MMP-9 levels were higher in CF (101.5) and NCFB (65.78) in comparison to healthy control volunteers (0.74), and were also statistically different from each other ($P < 0.01$). Collectively these results provide evidence for dysregulated MMP-2 and MMP-9 levels in NCFB, albeit at a level lower to that detected in CF.

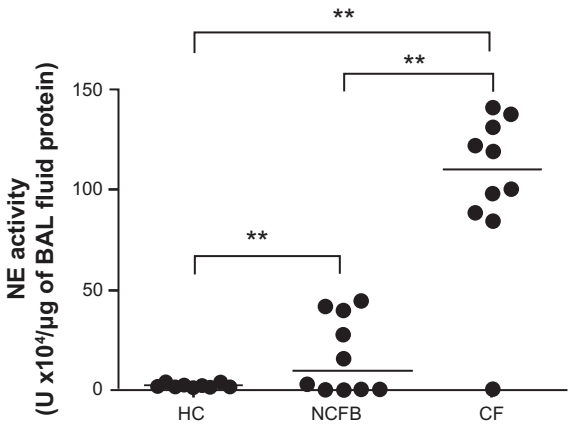


Figure 1 Comparative quantification of neutrophil elastase (NE) activity in CF and NCFB BAL fluid.

Notes: NE activity levels in BAL fluid of healthy control volunteers (n = 10), patients with CF (n = 10) and NCFB (n = 10) were compared. Individual points are from separate subjects (**$P < 0.01$) and horizontal bars represent median values. NE activity was significantly increased in CF and NCFB compared to healthy control samples.

Abbreviations: BAL, bronchoalveolar lavage; CF, cystic fibrosis; HC, healthy control volunteers; NCFB, non-CF bronchiectasis; NE, neutrophil elastase.

Comparative quantification of IL-8 and IL-18 in CF and NCFB BAL fluid samples

NE has been shown to upregulate proinflammatory cytokines including IL-8 expression and secretion from bronchial epithelial cells[39,40] and negatively impact IL-18 protein integrity.[16] For this reason we compared the levels of IL-8 and IL-18 in CF and NCFB BAL fluid. IL-8 measurements by ELISA revealed statistically significant differences in median BAL fluid IL-8 concentrations in CF (400.5 pg/mg of BAL fluid) and NCFB (282.3 pg/mg of BAL fluid) when compared to healthy control volunteers ($P < 0.01$ and

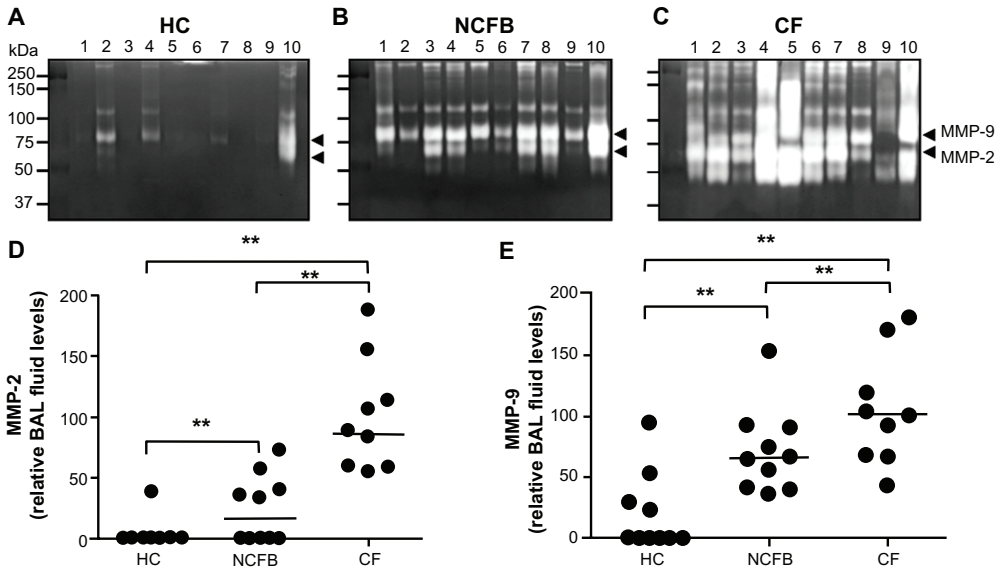


Figure 2 MMP-2 and MMP-9 levels in CF and NCFB BAL fluid. (**A**–**C**) Gelatinase levels in BAL fluid from healthy controls (**A**; HC, n = 10), NCFB (**B**, n = 10) and patients with CF (**C**, n = 10) were compared, with each lane representing BAL fluid from a separate subject. BioRad Precision Plus Protein Standard was electrophoresed and is indicated on the left. Putative MMP-9 (85 kDa) and MMP-2 (72 kDa) bands are indicated by the arrows. Results of densitometry analysis of MMP-2 (**D**) and MMP-9 (**E**) levels are indicated in the respective scatter plot (**$P < 0.01$).

Note: MMP-2 and MMP-9 were significantly increased in CF and NCFB compared to healthy control samples.

Abbreviations: BAL, bronchoalveolar lavage; CF, cystic fibrosis; HC, healthy control volunteers; MMP, matrix metalloproteinase; NCFB, non-CF bronchiectasis.

$P < 0.05$, respectively). The difference in median BAL fluid concentrations of IL-8 between NCFB and CF was also statistically significant ($P < 0.05$). This set of experiments demonstrates that IL-8 levels in NCFB are at a level intermediate to the healthy control and CF groups. In contrast, CF and NCFB BAL fluid IL-18 levels were consistently low and no significant difference was observed when compared to the healthy control volunteers (Figure 3B). As a positive control for this experiment BAL fluid from parents with sarcoidosis was employed, as this has previously been shown to contain high levels of IL-18.[41]

IL-10 and IL-4 protein levels are similar in CF and NCFB BAL fluid samples

Previous studies have revealed the prevalence of high levels of anti-inflammatory cytokines within the CF airways.[42,43] Consequently, we next compared the levels of IL-10 and IL-4 in CF BAL fluid to NCFB samples. Results of ELISA analysis of IL-10 and IL-4 are indicated in Figure 4. Statistically significant differences in BAL fluid IL-10 median levels were observed in CF and NCFB in comparison to healthy control volunteers (75.62, 151.3, and 0.1 pg/mg, respectively; $P < 0.05$) (Figure 4A). The difference in IL-10 levels

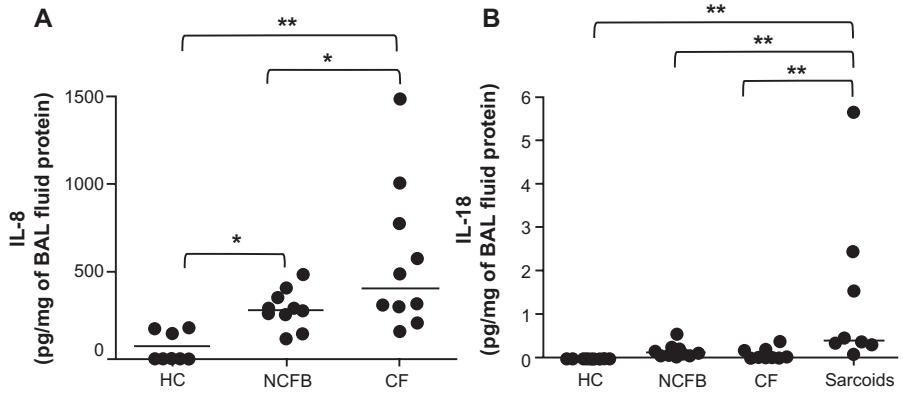


Figure 3 IL-8 and IL-18 levels in BAL fluid. BAL fluid proinflammatory IL-8 (**A**) and IL-18 (**B**) concentrations were determined by sandwich ELISA.

Notes: Patients with CF (n = 10), NCFB (n = 10), sarcoidosis (sarcoids, n = 8), and healthy control volunteers (HC, n = 10) were compared in the respective ELISAs. Sarcoidosis samples were used as a positive control for the IL-18 ELISA. All values points are from separate subjects (*$P < 0.05$; **$P < 0.01$) and presented as pg/mg of BAL fluid protein. Horizontal bars represent median values.

Abbreviations: BAL, bronchoalveolar lavage; CF, cystic fibrosis; HC, healthy control volunteers; IL, interleukin; NCFB, non-CF bronchiectasis.

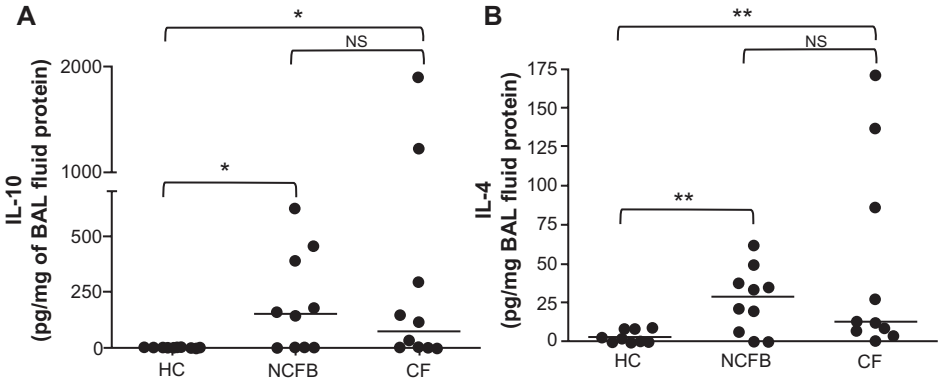


Figure 4 IL-10 and IL-4 levels in BAL fluid.
Notes: BAL fluid anti-inflammatory IL-10 and IL-4 concentrations were determined by sandwich ELISA. All value points from patients with CF (n = 10), NCFB (n = 10), and healthy control volunteers (n = 10) were compared. Data are presented as pg/mg of BAL fluid protein (*$P < 0.05$; **$P < 0.01$) and horizontal bars represent median values. Levels of IL-10 and IL-4 are similar in CF and NCFB.
Abbreviations: BAL, bronchoalveolar lavage; CF, cystic fibrosis; HC, healthy control volunteers; IL, interleukin; NCFB, non-CF bronchiectasis; NS, nonsignificant.

between NCFB and CF was not statistically significant ($P = 0.9$). Median BAL fluid IL-4 levels were higher in CF (12.55 pg/mg) and NCFB (28.38 pg/mg) compared to healthy control samples (2.12 pg/mg), but were not statistically different from each other ($P = 0.9$) (Figure 4B). Collectively these data indicate that in contrast to disproportionate levels of proteases and proinflammatory IL-8 levels (Figures 1–3), these results provide evidence for analogous concentrations of anti-inflammatory cytokines including IL-4 and IL-10 in NCFB and CF bronchial samples.

Correlation of NE and MMP-9 with FEV_1 in CF and NCFB BAL fluid samples

Many studies have shown reduced FEV_1 to be associated with increased inflammatory markers in respiratory diseases.[44–47] Our study compares two diseases with similar manifestations but with different pathogenesis and this dichotomy is evident when we look at the result of NE with respect to FEV_1 (Figure 5A). Individuals with NCFB clearly have higher median FEV_1 (83.5% versus 40% in CF) in association with greatly reduced median NE when compared with CF. In fact the median NE is reduced by a factor of approximately ten times in the NCFB compared to the CF group defining a separate region in the graphic (110.2×10^4 versus 9.312×10^4 U/μg BAL fluid). Nevertheless there is a poor, but statistically significant, correlation of NE with declining FEV_1 ($r^2 = 0.2660$; $P = 0.01$). This difference in NE may be explained by the difference in the total neutrophil number seen in NCFB BAL fluid compared to CF (Table 1). When we examine the relationship between FEV_1 and the neutrophil-derived MMP-9, a similar, but not as marked, separation is evident, with NCFB demonstrating higher FEV_1 with lower MMP-9 than CF BAL fluid (Figure 5B). A significant correlation between falling FEV_1 and increasing MMP-9 exists ($r^2 = 0.4646$; $P = 0.01$) which is likely a surrogate marker of the degree of severity of illness and frequency of exacerbation in CF over that seen in NCFB.

Discussion

In this study, we evaluated the levels of airway inflammatory markers in adults with CF and NCFB. The results indicate high levels of inflammatory markers in both CF and NCFB airway samples and although divergent activity of proteases and proinflammatory IL-8 levels were detected, which may be explained by neutrophil number and severity of disease, respectively, comparable levels of the anti-inflammatory cytokines IL-10 and IL-4 were present in CF and NCFB bronchial samples.

The airway microbiome within the CF and NCFB milieu involves recognized pathogens such as *P. aeruginosa*,[11,12,48] *Streptococcus pneumoniae*, *Haemophilus influenzae*,[49] *S. maltophilia*, and *S. aureus* and/or colonization by the filamentous fungi *A. fumigatus*.[50] Bacterial-released enzymes including *Pseudomonas* pseudolysin (elastase) and aeruginolysin (alkaline protease) have been shown to proteolytically cleave and inactivate the antiprotease elafin[51] and to completely degrade IL-18.[16] However, the presence of inflammation in the absence of detectable microbes in the CF[52] and NCFB[14] airways has also been described. Indeed, six of the 10 NCFB patients recruited to this study were free from microbial colonization, yet presented with high levels of NE-enhanced inflammation. Although lower levels of NE activity were detected in samples of BAL fluid from NCFB compared to CF individuals, within the airways the persistently dominant activity of NE can lead to degradation of structural matrix proteins including elastin,

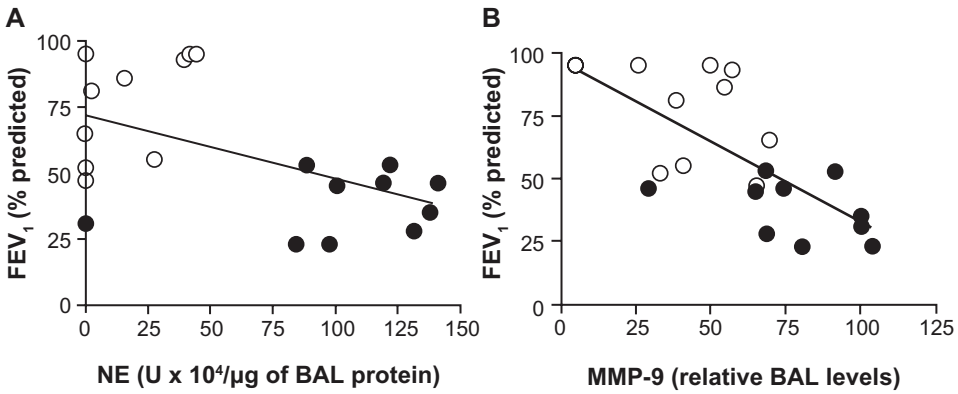


Figure 5 Correlation between NE activity and MMP-9 levels with FEV_1. Correlation between NE activity (**A**) or MMP-9 levels (**B**) and FEV_1 in CF (•) and NCFB (○). (**A**) A significant correlation of NE with declining FEV_1 ($r^2 = 0.2660$, $P = 0.01$) was observed. (**B**) A significant correlation between declining FEV_1 and MMP-9 was recorded ($r^2 = 0.4646$, $P = 0.01$).

Abbreviations: BAL, bronchoalveolar lavage; CF, cystic fibrosis; FEV_1, forced expiratory volume in 1 second; HC, healthy control volunteers; MMP, matrix metalloproteinase; NCFB, non-CF bronchiectasis; NE, neutrophil elastase.

collagen, and proteoglycans.[13] As demonstrated in this study (Figure 5A), NE concentrations in NCFB sputum have been shown to correlate positively with neutrophil cell numbers[14] and negatively with lung function in CF and NCFB.[53] Our study illustrates two distinct patterns of NE in NCFB and CF BAL fluid, which may be explained by the difference in total neutrophil numbers. The decreased neutrophil number in our NCFB BAL fluid samples could in turn lead to reduced neutrophil-derived DNA in the airway, in part clarifying the previously described lack of successful treatment of NCFB with rhDNase.[30]

In this paper, we also compared the presence of two MMPs in BAL samples, with MMP-9 and MMP-2 levels in NCFB illustrating an intermediate range between those detected in CF and healthy control samples. These data are in contrast to a previous study that found no significant difference in the level of MMP-9 in exhaled breath condensate of children with CF compared to NCFB.[54] This variance in results is possibly due to the zymographic detection of gelatinase levels in BAL fluid in our study, compared to the use of ELISA in the study by Karakoc et al.[54] Indeed, both MMP-9 and MMP-2 are secreted as an inactive proform and a role for neutrophil-derived serine proteases in their activation has been described.[23,55] A previous study has established a strong correlation between NE and MMP-9 activity in CF samples[23] and incubation of human HT-1080 cells with either NE, cathepsin G or proteinase-3 resulted in pro-MMP-2 activation, an effect reversed by inclusion of the serine protease inhibitor alpha-1 antitrypsin.[55] When we examined the relationship of FEV_1 to the neutrophil-derived MMP-9 (Figure 5B), a clear correlation of increasing MMP-9 levels with falling FEV_1 was observed. Moreover, MMP-2 has been implicated in the pathogenesis of a number of pulmonary disorders including COPD and asthma.[56] Recently we have demonstrated that NE can augment MMP-2 expression, potentially leading to an increased inflammatory response within the CF airways, with important implications for antiprotease therapeutic intervention.[38]

A number of comprehensive studies have evaluated the cytokine profile, including IL-8 levels in lungs of children and adults with CF.[42,57] Compared to healthy control volunteers, within the present study we found that IL-8 concentrations are elevated in both CF and NCFB airway samples, although a significant difference was apparent between the two patient groups with lower levels detected in NCFB. The levels of IL-8 detected in NCFB compared to healthy controls is in agreement with a previous report demonstrating a positive correlation between sputum IL-8 levels and NCFB symptom scores, and a negative correlation with lung function.[17] Of interest, a recent study found no significant difference in sputum IL-8 levels in NCFB patients who were either *Pseudomonas* colonized or noncolonized, suggesting IL-8 as a potential target for anticytokine therapy.[14] Moreover, excessive neutrophilic inflammation in CF is partially explained by the stability of IL-8 within the lung. The aforementioned stability has been attributed to the capacity of IL-8 to bind glycosaminoglycans, which are overexpressed within the CF airways,[58–62] although a similar phenomenon in NCFB has yet to be explored. Hypertonic saline is now a cornerstone of airway clearance therapy in CF and until recently has been considered primarily as an airway hydration therapy. However, a new mechanistic study has shown that hypertonic saline also posseses immunomodulatory properties as it disrupts the interaction between glycosaminoglycans and IL-8, rendering IL-8 more susceptible to proteolytic degradation, thus helping to resolve inflammation in the airway.[63] In this

light, the use of hypertonic saline in NCFB appears to hold great promise as the present study shows that the airways hold similar increased levels of IL-8 to CF bronchiectasis.

In contrast to IL-8, IL-18 binds poorly to glycosaminoglycans and is highly sensitive to proteolytic degradation.[16] Reduced IL-18 protein levels have previously been described in CF BAL fluid samples[16,64,65] and it has been reported that IL-18 degradation and clearance occurs within minutes after exposure to serine proteases including NE and cathepsin G.[16] The susceptibility of IL-18 to serine protease degradation may explain why no IL-18 was detected in NCFB BAL fluid. Reduced T_H1 response to microbial infection has been correlated with IL-18 deficiency[66] and hence this study suggests that a strategy leading to a reduction in levels of serine protease activity, such as nebulized antiproteases,[67] may positively affect IL-18 stability and improve the pulmonary outcome of microbial-infected CF and NCFB individuals. Indeed, the concept of strategies which decrease serine protease activity has had some initial success in CF bronchiectasis where inhalation of alpha-1 antitrypsin over 4 weeks has been shown not only to reduce free elastase, but also the proinflammatory mediators IL-8, TNF-α, IL-1β, and LTB_4.[68]

The biological activity of IL-4 and the T_H2 bias of CF lymphocytes have been comprehensively studied by a number of investigators.[43,69] Within the present study we observed that IL-4 is equally expressed at high levels in CF and NCFB BAL fluid compared to healthy control samples. As pulmonary protein levels of IL-4 have previously been shown to inversely correlate with lung function in CF,[43] these findings may have implications for patients with NCFB. IL-10 is also an important anti-inflammatory mediator that functions to inhibit production of cytokines by T lymphocytes, modulates IL-8 production by macrophages, and promotes resolution of lung inflammation by promoting neutrophil apoptosis. Within this study, we observed equal protein expression of IL-10 in CF and NCFB, with recorded levels statistically increased above the median of the healthy control. This result is in contrast to those demonstrating that IL-10 was significantly depressed and differed little from healthy controls.[43] However, our data are supported by two other studies which demonstrated significantly higher levels of IL-10 present in BAL fluid[42] and sera[65] from infected and uninfected patients with CF when compared to either pristine or disease control subjects.

A number of limitations to this study should be discussed. Firstly, the differences observed between CF and NCFB in this study may in part be explained by the greatly reduced FEV_1, extent or severity of bronchiectasis, and the presence of airway bacterial colonization including *P. aeruginosa* infection present in the CF lung. Secondly, the patient number recruited is small due to strict inclusion and exclusion criteria and single-hospital nature of this study and the patients are not well matched for age. The above factors may limit the generalizability of the study results and conclusions. Larger multicenter studies looking at airway inflammatory markers in the two diseases and randomized controlled trials of specific therapies are required. Thirdly, BAL samples were acquired from the most affected lobe identified by HRCT and visual inspection for both the CF and NCFB groups, given that it is well recognized that inflammation and severity of disease are heterogeneous in NCFB the results may reflect the worst case scenario for inflammation in NCFB rather than reflect the whole lung condition as seen in the more homogenous inflammation observed in CF bronchiectasis.

Despite these limitations however, many similarities in inflammatory markers exist, which suggests that the existing strategy of transferring therapies from the CF population to the NCFB population carries significant merit despite the obvious exception of rhDNase.[30] Further work is suggested by this study, for example a prospective assessment of inflammatory markers before and after *P. aeruginosa* eradication to elucidate the pathogenesis of this important pathogen. Azithromycin, a macrolide antibiotic and well-established treatment for patients with CF bronchiectasis who are colonized with *Pseudomonas* is now emerging as a treatment for COPD, asthma, and bronchiolitis obliterans where it is used as a novel anti-inflammatory.[70–74] Indeed a recently published study by Wong et al, does suggest that azithromycin treatment may prove beneficial in the prevention of exacerbations in individuals with NCFB.[75] In contrast to CF, FEV_1 in NCFB remains a poor indicator of disease response to treatment; therefore along with patient related outcomes, time to exacerbation and number of exacerbations, airway proinflammatory cytokine release may find a place as a surrogate marker of successful treatment. In conclusion, these data suggest that therapeutic strategies pertinent to CF bronchiectasis, including anti-inflammatory therapies, are of potential benefit in treatment of NCFB.

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Disclosure

None of the authors have a financial relationship with a commercial entity that has an interest in the subject of the presented manuscript.

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