Reduced neutrophil elastase inhibitor elafin and elevated transforming growth factor-β_1 are linked to inflammatory response in sputum of cystic fibrosis patients with *Pseudomonas aeruginosa*

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Shareable abstract (@ERSpublications)
P. aeruginosa infection is linked to an imbalance of NE and NE inhibitor elafin, and increased TGF-β_1 sputum levels. Inhibition of NE and TGF-β_1 are promising therapeutic strategies in future CF therapy. https://bit.ly/3emeI0u

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Abstract

Research question Pulmonary disease progression in patients with cystic fibrosis (CF) is characterised by inflammation and fibrosis and aggravated by *Pseudomonas aeruginosa* (Pa). We investigated the impact of Pa specifically on: 1) protease/antiprotease balance; 2) inflammation; and 3) the link of both parameters to clinical parameters of CF patients.

Methods Transforming growth factor-β_1 (TGF-β_1), interleukin (IL)-1β, IL-8, neutrophil elastase (NE) and elastase inhibitor elafin were measured (ELISA assays), and gene expression of the NF-κB pathway was assessed (reverse transcriptase PCR) in the sputum of 60 CF patients with a minimum age of 5 years. Spirometry was assessed according to American Thoracic Society guidelines.

Results Our results demonstrated the following: 1) NE was markedly increased in Pa-positive sputum, whereas elafin was significantly decreased; 2) increased IL-1β/IL-8 levels were associated with both Pa infection and reduced forced expiratory volume in 1 s, and sputum TGF-β_1 was elevated in Pa-infected CF patients and linked to an impaired lung function; and 3) gene expression of NF-κB signalling components was increased in sputum of Pa-infected patients, and these findings were positively correlated with IL-8.

Conclusion Our study links Pa infection to an imbalance of NE and NE inhibitor elafin and increased inflammatory mediators. Moreover, our data demonstrate an association between high TGF-β_1 sputum levels and a progress in chronic lung inflammation and pulmonary fibrosis in CF. Controlling the excessive airway inflammation by inhibition of NE and TGF-β_1 might be promising therapeutic strategies in future CF therapy and a possible complement to cystic fibrosis transmembrane conductance regulator (CFTR) modulators.

Introduction

Cystic fibrosis (CF) is the most common lethal autosomal recessive disease, caused by mutations and subsequent absence/dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR). While CF affects multiple organs, the majority of life-limiting sequelae are related to progressive lung disease caused by bronchial inflammation, bacterial infection and lung matrix remodelling resulting in continuous decline of lung function [1]. *Pseudomonas aeruginosa* (Pa) is one of the most prevalent microorganisms in CF, chronically infecting the lungs of 50–60% of adult CF patients already early in life [2–4]. Persistence

of Pa over years substantially contributes to rapid progression of lung disease and higher mortality and morbidity in CF patients [5, 6]. While Pa infection has been widely recognised as an adverse pulmonary outcome parameter, the mechanisms and potential biomarkers linking Pa to these devastating lung changes over time remain elusive.

Pa infection aggravates CF-related lung disease by adversely affecting the impaired mucociliary clearance and increasing influx of inflammatory cells. These pathophysiological changes result in a release of cytokines, growth factors and proteases, ultimately leading to a protease/antiprotease imbalance [7–9]. Various clinical and experimental studies have shown that protease activity is mechanistically important in CF-related lung matrix remodelling [10, 11]. In particular, neutrophil elastase (NE), released by activated neutrophils, the most prominent inflammatory cell type, is one of the main proteases inducing structural lung damage in CF by impairing mucociliary clearance and mediating proinflammatory activity by degrading elastic fibres [10–16]. Recent studies in CF confirmed a strong association between high NE activity in bronchoalveolar lavage (BAL) fluid and the onset and progression of structural abnormalities including early bronchiectasis and future lung function decline as well as reduced treatment response in pulmonary exacerbations [17, 18]. There is only one study, carried out in a limited number of CF patients, analysing NE and elafin concentrations in sputum which provides evidence that elafin is cleaved by its cognate enzyme NE [19].

There is a growing body of evidence that inflammatory pathways in CF are also extensively influenced by genetic modifiers, notable amongst these being transforming growth factor-β_1 (TGF-β_1). TGF-β_1 is not only a key regulator of bronchial inflammation, pulmonary fibrosis [20] and cell proliferation and cell differentiation [21], but has also been shown to inhibit CFTR expression [22]. Moreover, matrix remodelling and local hypoxia as a result of increased mucus deposition [23, 24] promote the release of proinflammatory cytokines, such as interleukin (IL)-1β, and activation of inflammatory pathways [11].

Reliable sputum biomarkers for therapeutic monitoring and/or predicting the clinical course of CF are gaining importance. While Pa has been shown to be closely related to the clinical outcome and survival of CF patients [25, 26], the variety and coherences of involved inflammatory mediators are poorly understood, and most studies have a limited number of patients. Therefore, we investigated the link between Pa infection and the protease/antiprotease imbalance, inflammatory cytokines, TGF-β_1 as a genetic modifier and the NF-κB signalling pathway in sputum inflammatory cells.

Material and methods

Study population

We investigated 60 patients with a confirmed diagnosis of CF according to the consensus guidelines of the Cystic Fibrosis Foundation [27]. Further inclusion criteria for this study were a minimum age of 5 years and the capability to produce and expectorate sputum. Patients with current pulmonal exacerbation or acute respiratory infection were excluded. Based on the Leeds criteria, Pa infection in our cohort is defined by three positive cultures over 12 months with at least a 1-month interval between the samples. Patients who underwent successful Pa eradication (three negative cultures in a row with at least a 1-month interval between the samples) were considered negative. All CF patients infected with Pa were being treated with cycled inhaled antibiotics.

Ethics, consent and permissions

Human guidelines of good clinical practice and the declaration of Helsinki (1964) and Edinburgh (2000) were followed in the conduct of the trial. Ethical approval was obtained from the Medical Ethical Committee of the University Hospital Cologne (approval number 12-168). All parents and all patients older than 8 years of age provided written informed consent.

Sputum analysis

Sputum was induced by inhalation of hypertonic saline during a routine physiotherapist session at regular outpatient visits. Sputum processing was performed according to the standard operating procedure of the TDN (Therapeutic Drug Development Network, USA). Sputum was processed within 1 h of collection and sputum plugs segregated from possible saliva. The sputum samples were diluted in 9:1 (weight to volume) phosphate-buffered saline (D-PBS), filtered through 100 µm and 40 µm mesh, and centrifuged for 10 min at 260×g at 6°C. Supernatants were stored at −80°C for further analysis; cell suspensions were concentrated by cytospin (1×10^6 cells·mL^{-1}) and stored at −20°C.

Elastase and elafin concentrations in sputum were determined by specific ELISA assays (EnzChek Elastase Assay Kit, Molecular Probes Europe, Leiden, Netherlands; Elafin/Skalp Human ELISA-kit, abcam,

Cambridge, UK). Proinflammatory cytokine concentrations in the sputum were assessed by using a human inflammatory cytokine ELISA-kit (BD Cytometric Bead Array Humane Inflammatory Cytokine Kit, San Jose, CA, USA). TGF-β_1 levels in sputum of all patients were determined using the TGF-specific ELISA-kit (Quantiki-neELISA Human TGF-β_1, R&D Systems, Minneapolis, MN, USA). All assays and kits were performed according to the manufacturer's protocol.

The different measurements of our sputum analysis have been done in succession with priorities given to the measurement of TGF-β_1, IL-1β and IL-8. For some patients the amount of sputum sample was inadequate to assess the levels of all the inflammatory mediators explored in this study; hence, the number of investigated samples varied among different measurements.

RNA isolation and quantitative reverse transcriptase PCR

Total RNA was isolated using Trizol reagent (Invitrogen, Paisley, Scotland, UK), and quantitative reverse transcriptase PCR was performed using the 7500 Real-time PCR system (Applied Biosystem, Foster City, CA, USA) [28]. The relative amount of the specific mRNA was normalised to β-actin. Primers were designed using Primer Express Software v3.0.1 (Thermo Fisher Scientific, Waltham, MA, USA); primer pairs are listed in supplementary table S1.

Spirometry

Spirometric measurements were assessed prior to any study intervention according to the American Thoracic Society guidelines [29] by the use of Master Screen Body (Jaeger, Heidelberg, Germany) and SentrySuite™ version 2.19 software (Carefusion, Becton Dickinson, Franklin Lakes, NJ, USA). For all spirometric measurements the Global Lung Function Initiative's reference equations were used (GLI-2012). Maximum values of forced expiratory volume in 1 s (FEV_1) % predicted were used for analysis, defined as FEV_1% of the patient divided by the average FEV_1% in the population for any person of similar age, sex and body composition.

Statistical analysis

Collected data in the text are reported as mean±SD. To compare datasets from two subgroups, we used an unpaired t-test for independent samples when the frequency distribution was normal, or the Mann–Whitney U-test when the distribution was not normal. Cytokine levels were correlated by Pearson or Spearman correlation depending on their distribution. The strength of correlation is defined depending on the correlation coefficient r (r=0.3–0.5=weak, r=0.5–0.7=moderate, r>0.7=strong). A p-value <0.05 was considered as statistically significant; statistical analysis was performed using Prism 7 software package (GraphPad 7, San Diego, CA, USA). All results were correlated to age, body mass index (BMI), FEV_1 values and status of Pa infection by using Pearson or Spearman correlation depending on their distribution.

Results

Clinical data of study population

A cohort of 60 CF patients was recruited based on our inclusion and exclusion criteria. As demonstrated in table 1, the mean±SD age was 21.2±12.2 years; 51.9% were male; 41 patients were Pa-negative (68.3%) whereas 19 patients were infected with Pa (31.7%). The FEV_1 values were reduced in the subgroup of Pa-positive (59.5±25.0% pred) in comparison to Pa-negative patients (79.8±22.7% pred), as well as in the subgroup of patients aged 18 years and older (63.8±23.8% pred). The average BMI of our cohort was 19.2 $kg \cdot m^{-2}$. Regarding age, BMI, sex and CFTR mutation, we detected no significant relation to the measured inflammatory mediators.

Increased NE and reduced elafin concentration in soluble CF sputum in Pa-positive CF patients

To determine if poor clinical outcome of Pa-colonised CF patients is linked to an imbalance of NE and its inhibitor elafin, we assessed both markers in soluble CF sputum. Concentrations of NE were significantly higher in Pa-positive CF sputa when compared with Pa-negative sputa (211.2±31.9 $ng \cdot mL^{-1}$ *versus* 359.1±65.8 $ng \cdot mL^{-1}$, p<0.05) (figure 1a). Elafin is primarily expressed by bronchial epithelial cells and inhibits NE. We found that elafin concentration was significantly lower in sputa of Pa-positive CF patients in comparison to sputa of Pa-negative patients (16 311±2184 $pg \cdot mL^{-1}$ *versus* 6975±943 $pg \cdot mL^{-1}$, p<0.001) (figure 1b), suggesting a NE/elafin imbalance in Pa-positive CF lungs, favouring elastic fibres degradation and fibrotic matrix remodelling.

Sputum IL-1β and IL-8 are linked to decline of lung function in in Pa-positive CF patients

To link Pa colonisation to the release of proinflammatory cytokines, IL-1β and IL-8 were determined in CF sputum samples. We detected a significant increase of IL-1β (+249.4%, p<0.001) and IL-8 concentrations (+218.4%, p<0.0001) in sputum of Pa-positive CF patients (IL-1β: 1278±314 *versus* 3187±407 $pg \cdot mL^{-1}$;

TABLE 1 Demographics of study population

Clinical parameters	Mean±SD or n (%)
Age years	21.2±12.2
<18 years	25 (41.7)
>18 years	35 (58.3)
Female	29 (48.3)
Male	31 (51.7)
***Pseudomonas aeruginosa* infection**	19 (31.6)
FEV_1 %	73.4±25.1
Pa infected	59.5±25.0
Pa negative	79.8±22.7
<18 years	86.8±20.7
⩾18 years	63.8±23.8
CFTR mutations	
F508del homozygous	32 (53.3)
F508del heterozygous	21 (35.0)
Other mutations	7 (11.7)

FEV_1: forced expiratory volume in 1 s; CFTR: cystic fibrosis transmembrane conductance regulator.

IL-8: 2804±465 *versus* 6124±483 pg·mL^{-1}) (figure 2). Sputum IL-1β and IL-8 concentrations were >3-fold and 1.8-fold, respectively, higher in patients with FEV_1 <80% when compared to patients with FEV_1 ⩾80% (IL-1β: FEV_1 ⩾80% *versus* FEV_1 <80%: 902±226 pg·mL^{-1} *versus* 2899±416 pg·mL^{-1}, p<0.0001; IL-8: FEV_1 ⩾80% *versus* FEV_1 <80%: 2811±553 pg·mL^{-1} *versus* 5021±548 pg·mL^{-1}, p<0.01) (figure 3). Finally, we determined a strong positive correlation between IL-1β and IL-8 levels in our cohort of Pa-positive and Pa-negative CF patients (r=0.7645; p<0.0001) (figure 4).

Linking concentrations of TGF-β_1 to clinical parameters and inflammatory cytokines in sputum of CF patients

Since TGF-β_1 has been identified as a genetic modifier for CF lung disease, we assessed TGF-β_1 concentrations in sputa of CF patients with or without Pa colonisation. High sputum TGF-β_1 was intimately linked to both Pa colonisation and to lower FEV_1 values at the timepoint of sample collection. Specifically, sputum TGF-β_1 was significantly higher in Pa-positive CF patients compared to Pa-negative CF patients (Pa-negative: 84.5±11.7 pg·mL^{-1}, Pa-positive: 173.8±24.0 pg·mL^{-1}, p<0.001) (figure 5a). Sputum TGF-β_1 levels were significantly higher in CF patients with reduced FEV_1 values <80% pred than in patients with FEV_1 values ⩾80% pred (FEV_1 ⩾80%: 85.7±14.2 pg·mL^{-1}, FEV_1 <80%: 139.7±18.9 pg·mL^{-1}, p<0.05) (figure 5b). Moreover, we tested the correlation between TGF-β_1 and the proinflammatory cytokines.

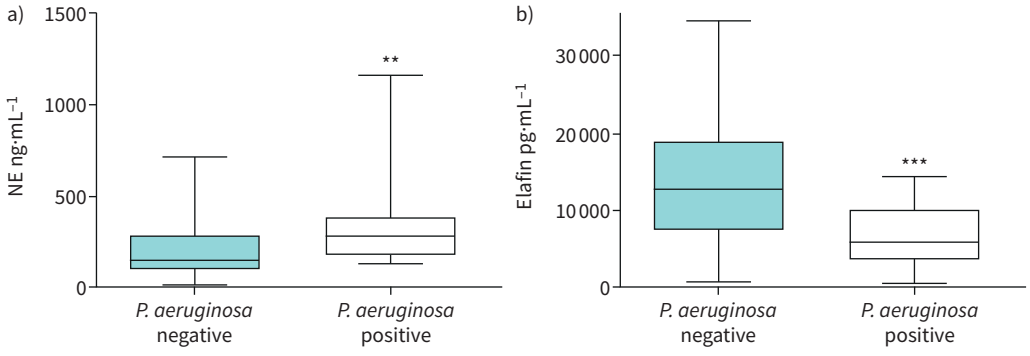

FIGURE 1 Assessment of a) neutrophil elastase (NE) concentrations (ng·mL^{-1}) and b) elafin concentrations (pg·mL^{-1}) in sputum of cystic fibrosis (CF) patients, related to *Pseudomonas* infection: *P. aeruginosa*-negative CF patients (n=32) and *P. aeruginosa*-positive CF patients (n=16). Data presented as median and interquartile range; Mann–Whitney U-test performed. **: p<0.01; ***: p<0.001.

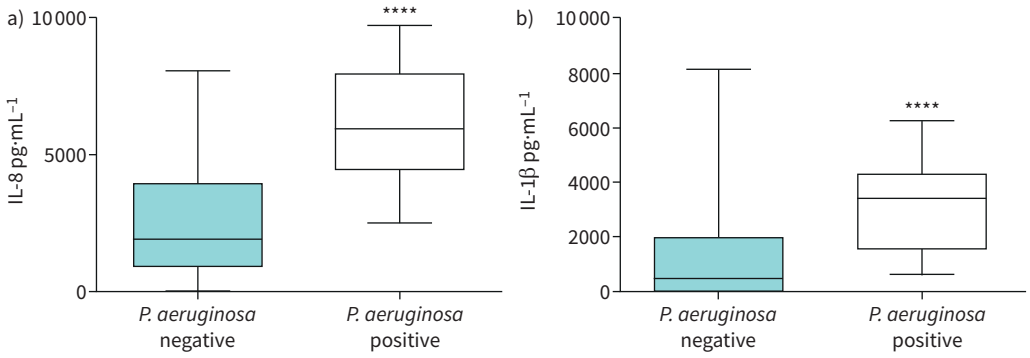

FIGURE 2 a) Interleukin (IL)-8 and b) IL-1β concentrations (pg·mL^{-1}) in sputum of cystic fibrosis (CF) patients, related to *Pseudomonas* infection: *P. aeruginosa*-negative CF patients (n=34) and *P. aeruginosa*-positive CF patients (n=18). Data presented as median and interquartile range; Mann–Whitney U-test performed. ****: p<0.0001.

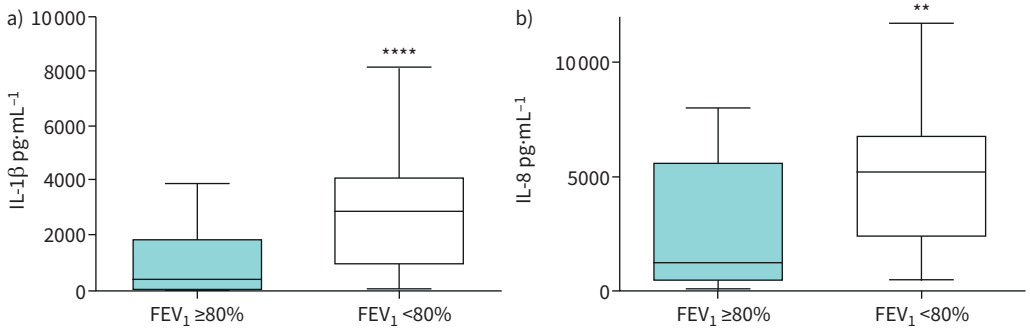

FIGURE 3 a) Interleukin (IL)-1β and b) IL-8 concentrations (pg·mL^{-1}) related to forced expiratory volume in 1 s (FEV_1) values: elevated IL-1β and IL-8 levels were detected in cystic fibrosis (CF) patients with FEV_1 <80% (n=27) compared to CF patients with FEV_1 values ⩾80% (n=25). Data presented as median and interquartile range; Mann–Whitney U-test performed. **: p<0.01; ****: p<0.0001.

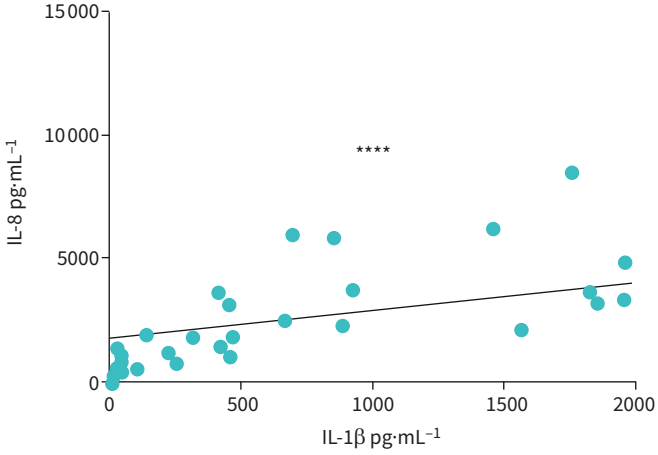

FIGURE 4 Pearson correlation between interleukin (IL)-1β and IL-8 concentrations (pg·mL^{-1}) in sputum of cystic fibrosis patients (n=52). A positive correlation between IL-1β and IL-8 levels was detected (r=0.763; ****: p<0.0001).

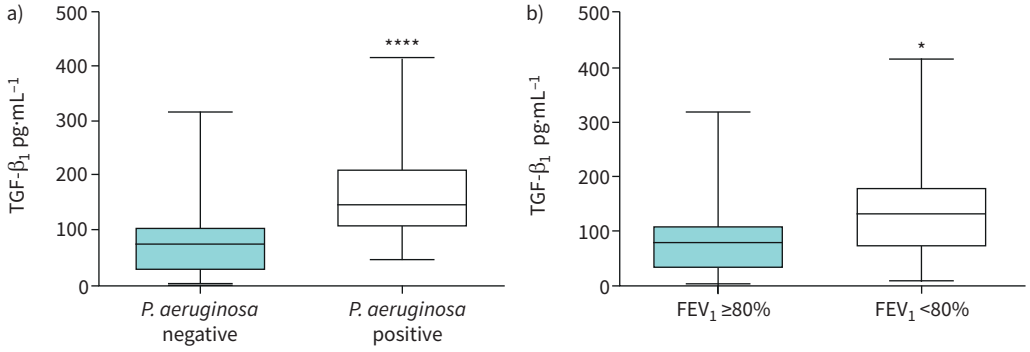

FIGURE 5 Assessment of sputum transforming growth factor-β_1 (TGF-β_1) concentrations (pg·mL^{-1}) in cystic fibrosis patients by specific TGF-β_1 ELISA. Correlation to a) *Pseudomonas aeruginosa* colonisation (*P. aeruginosa*-negative: n=33; *P. aeruginosa*-positive: n=17) and b) forced expiratory volume in 1 s (FEV_1) % predicted (FEV_1 ⩾80%: n=23; FEV_1 <80%: n=27). Data presented as median and interquartile range; Mann–Whitney U-test performed. *: p<0.05; ****: p<0.0001.

Indeed, both IL-1β and IL-8 showed a significant positive correlation to TGF-β_1 levels in sputum (IL-1β: r=0.707; p<0.0001; IL-8: r=0.670; p<0.0001) (figure 6a and b).

Expression of the NF-κB signalling cascade in sputum cells of CF patients is regulated by Pa

The above results linking Pa to increased inflammatory cytokines and higher concentrations of TGF-β_1 in lungs of CF patients led us to the question whether the expression of inflammatory signalling pathways in sputum cells is differentially regulated by Pa colonisation in CF lungs. To this end, we measured gene expression of mediators of the NF-κB signalling cascade and detected a significant increase in mRNA of IKKα, IL-6, p50 and p65 in sputum samples of patients with Pa infection (figure 7). Furthermore, we found a significant positive weak correlation between high IL-8 levels in sputum and the gene expression of p50 (r=0.402; p<0.01) and p65 (r=0.356; p<0.05) as markers of the NF-κB signalling cascade (figure 8).

Discussion

The present study shows that the reduction of lung function in Pa-positive CF lungs is intimately linked to an imbalance of proteases (sputum NE) and antiproteases (sputum elafin), and increased concentrations of sputum TGF-β_1 and proinflammatory cytokines (IL-1β, IL-8), which might adversely affect the inflammation and remodelling of CF lungs. An activation of NF-κB signalling in sputum cells, presumably neutrophils, might be triggering these processes.

Infections with Pa in CF patients are associated with significantly poorer outcomes [30]. While improvement of life expectancy has been mainly attributed to an early and aggressive treatment of Pa

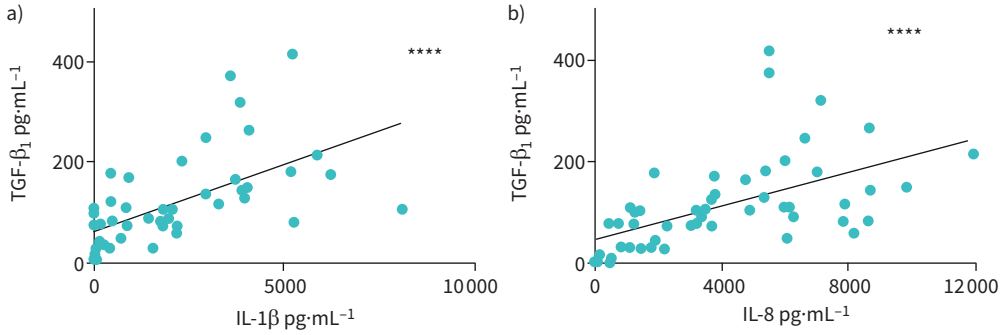

FIGURE 6 Spearman correlation between concentrations of sputum transforming growth factor-β_1 (TGF-β_1) (pg·mL^{-1}) and proinflammatory cytokines interleukin (IL)-1β and IL-8 in cystic fibrosis patients (n=50). Positive correlations between a) IL-1β and TGF-β_1 levels (r=0.707; ****: p<0.0001) and b) IL-8 and TGF-β_1 levels (r=0.670; ****: p<0.0001).

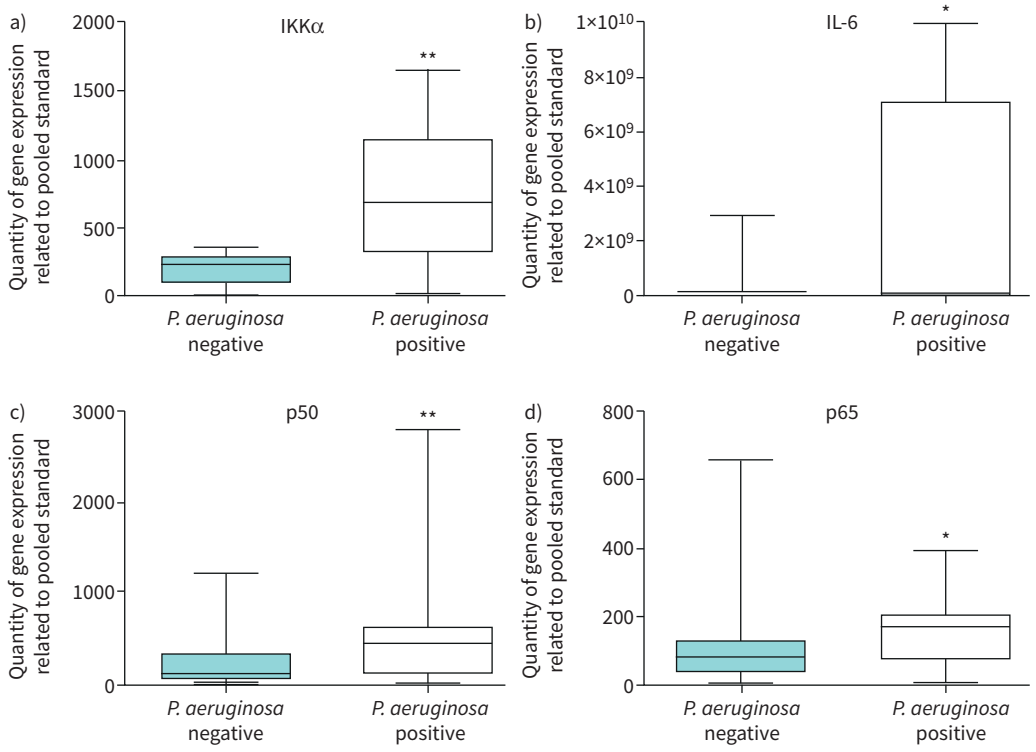

FIGURE 7 Gene expression of NF-κB signalling cascade mediators in cystic fibrosis sputum cells of 43 patients: p50, p65, IKKα and interleukin (IL)-6 levels were determined by quantitative reverse transcriptase PCR. Significantly elevated mRNA expression of mediators of the NF-κB signalling cascade: a) IKKα, b) IL-6, c) p50 and d) p65. Data presented as median and interquartile range; Mann–Whitney U-test performed. *P. aeruginosa*: *Pseudomonas aeruginosa*. *: p<0.05; **: p<0.01.

infections [31], the initial underlying processes triggering a persistent inflammation and leading to lung injury and destruction remain elusive. Several studies have investigated proinflammatory markers in sputum of CF patients [16, 32], but only a few reports addressed the impact of Pa colonisation on disease progression in CF [25, 26]. In our present study, correlation of inflammatory sputum markers with clinical parameters showed that colonisation with Pa was not only significantly related to higher inflammatory sputum markers, but also to reduced lung function. Specifically, we found that Pa is linked to higher concentrations of IL-1β and IL-8, which in turn were strongly correlated with increased sputum TGF-β_1. Both inflammatory cytokines IL-1β and IL-8 as well as TGF-β_1 induce inflammation and lung matrix remodelling favouring fibrosis, thereby contributing to irreversible structural lung changes and reduced

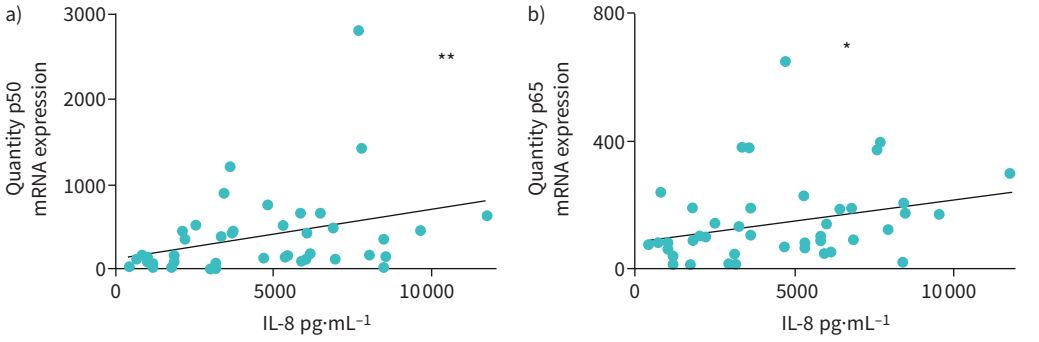

FIGURE 8 Spearman correlations between level of interleukin (IL)-8 and quantitative mRNA expression of p50 and p65. Significant positive correlations between IL-8 levels and quantity of mRNA expression of the NF-κB signalling proteins a) p50 (r=0.402; **: p<0.01) and b) p65 (r=0.356; *: p<0.05).

lung function [21]. Moreover, inflammatory markers have been identified as risk factors for lung function decline in CF or other chronic lung diseases independent of Pa [33].

Pa elicits massive neutrophil influx in part by release of pyocyanin [34] and modulates neutrophilic myeloid-derived suppressor cells (MDSCs) in CF lungs [35]. Here, we show a marked activation of gene expression of NF-κB signalling in inflammatory sputum cells of Pa-colonised CF patients, suggesting an activation of inflammatory cells, presumably neutrophils, promoting thereby the release of inflammatory cytokines and matrix-remodelling proteases. In parallel, increased sputum NE, a biomarker for monitoring CF lung disease, was significantly related to Pa. Elevated activity of NE is associated with bronchiectasis in CF [36], is predictive of future lung function decline [16] and is related to treatment response in pulmonary exacerbations [20]. Furthermore, recent studies in CF demonstrated a strong association between high NE activity in BAL fluid and the onset and progression of structural abnormalities including early bronchiectasis [18]. Previous *in vitro* experiments confirm this notion by demonstrating an inhibitory effect of inhaled anti-*Pseudomonas* antibiotic treatment on the activity of NE [37], indicating thereby an activating effect on NE by Pa. This enzyme is pivotal to lung damage because it releases growth factors, *e.g.* TGF-β_1, and degrades elastic fibres. Elastin fibre breakdown products are highly proinflammatory, promoting the recruitment of activated inflammatory cells [38]. Increased release of NE by recruited lung neutrophils and elastin peptide fragments are related to chronic lung diseases, such as pulmonary arterial hypertension [39] or pulmonary fibrosis [40].

Inhibition of elastase by lung endogenous elafin, which is primarily produced in bronchial epithelial cells, mitigates lung destructive processes [41, 42]. Measurement of elafin in our cohort showed a marked decrease of elafin in CF lungs colonised with Pa, suggesting a suppressive effect of Pa on elafin expression *in vivo*. Interestingly, Guyot *et al*. [19] demonstrated that elafin is proteolytically cleaved by its cognate enzyme NE in BAL fluid of CF patients infected with Pa. The confirmed elafin deficiency as seen in our cohort might be the result of an impaired bronchial epithelial cell homeostasis in Pa-positive lungs. Our present findings indicate a relevant imbalance of proteases and antiproteases in CF lungs. While elafin deficiency in CF lungs with Pa may relate to increased NE, other functions of elafin need to be considered. For example, prior studies demonstrated a marked inhibitory effect of elafin on NF-κB and TGF-β_1 activation in the lung [16, 42]. Thus, deficient release of elafin may promote activation of NF-κB and TGF-β_1 signalling and aggravate lung injury by triggering inflammatory response and lung matrix remodelling, respectively.

Pa and lung inflammation are important in the clinical course of CF [25, 26]. The present study shows a marked increased expression of components of the NF-κB pathway in sputum cells and elevated concentrations of sputum IL-1β and IL-8. These findings were supported by *in vitro* experiments showing that exposure of CF bronchial epithelial IB3-1 cells or CF nasal epithelial cells to Pa upregulates the gene expression of IL-1β, IL-8 or NF-κB activity [43]. Alternatively, the lack of inhibitory effect of elafin on NF-κB signalling could in part underly the activation of inflammatory NF-κB signalling and thereby enhance the expression of IL-1β and IL-8 [16]. Additionally, excessive NE as a result of elafin deficiency could promote IL-8 expression, neutrophil recruitment and a self-perpetuating cycle of neutrophil-mediated inflammation [11]. Interestingly, Carrabino *et al*. [44] demonstrated that stimulation of Pa-exposed CF nasal epithelial cells with IL-1β increased IL-8 expression. This strong link between IL-1β and IL-8 may explain the correlation of both cytokines in our cohort.

The intimate link between IL-1β/IL-8 and reduced lung function (lower FEV_1 values) does not only emphasise the important functional role of Pa in the clinical course of CF, but also the additional need for pharmacological approaches targeting specific inflammatory mediators. Initial investigations using IL-1β receptor inhibitor (Anakinra) demonstrated an amelioration of the inflammasome-dependent inflammation in human CF-mutated bronchial epithelial cells [45]. Previous studies demonstrated that IL-8 serves as first line of host defence against invading microorganisms [46] and as a potent chemoattractant for neutrophils. Moreover, NF-κB-mediated IL-8 and IL-1β chemokine secretion and neutrophil influx are prominent early in CF disease progression [47]. Our study identifies Pa colonisation as a possible aggravator for both activation of NF-κB-signalling and related increase of IL-1β and IL-8.

TGF-β_1 is a pleiotropic growth factor, involved in the regulation of cell differentiation and survival, inflammatory response and fibrotic processes of chronic lung diseases [19]. Furthermore, recent studies identified TGF-β_1 as an important genetic modifier in the lung pathobiology of CF. For example, inhibition of CFTR expression has been shown to be one mechanism by which TGF-β_1 modulates pathomechanisms in CF. However, it remains unclear if the changes in proteases/antiproteases and the increase of inflammatory cytokines in Pa-positive sputum samples are linked to impaired mucociliary clearance, or if

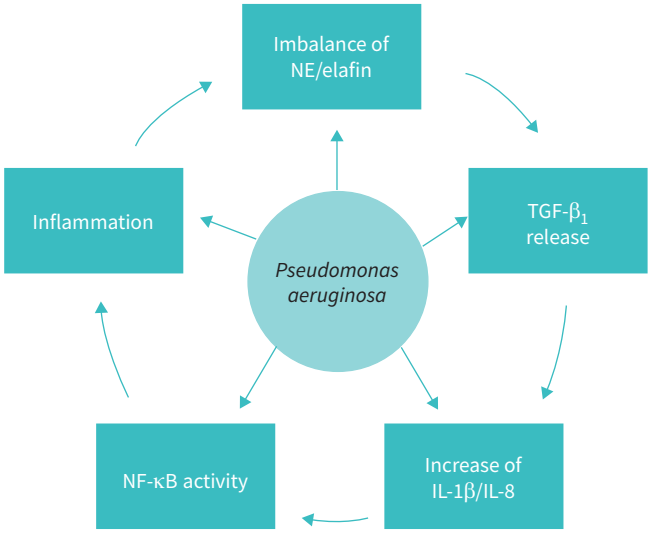

FIGURE 9 Simplified presentation of the vicious circle of chronic inflammation in cystic fibrosis (CF) as a result of high neutrophilic activity, disruption of the homeostatic protease/antiprotease balance and microbial infection with *P. aeruginosa* in the CF lung causing chronic inflammatory lung disease and pulmonary fibrosis. NE: neutrophil elastase; TGF-β_1: transforming growth factor-β_1; IL: interleukin.

an elevation in active TGF-β_1 aggravates these processes and therefore serves as a potential biomarker. Our results demonstrate a correlation between high sputum TGF-β_1 in CF patients and the degree of pulmonary inflammation, as well as an association to Pa colonisation and lower FEV_1 values. These findings indicate the possible role of TGF-β_1 as a sputum biomarker for disease progression in CF.

This study has some limitations. It is well known that longer duration of Pa colonisation is associated with CF lung disease progression [48], but our study did not evaluate the duration of Pa colonisation regarding measured mediators. Moreover, for some patients the amount of sputum sample was inadequate to assess the levels of all the inflammatory mediators explored in this study, and lack of associations between potential confounders may be related to the smaller sample size for some of the inflammatory mediators. In addition, investigating the influence of an inhibition of elafin and TGF-β_1 on the inflammatory response in the CF lung would be of interest. Therefore, further cell culture studies are needed and are planned by our working group for the future.

In conclusion, our results demonstrate a significant association between high inflammatory sputum mediators and Pa infection and confirm the importance of an early eradication therapy for newly colonised patients as well as an aggressive chronic treatment of Pa in already chronically infected CF patients. Our findings also demonstrate the important impact of Pa infection on NE/elafin imbalance and hyperinflammation by the release of TGF-β_1 and increase of IL-1β/IL-8 as well as NF-κB activity, all ultimately resulting in progress of chronic inflammatory lung disease and pulmonary fibrosis (figure 9). Reducing the excessive airway inflammation by inhibition of NE and TGF-β_1 might be a promising therapeutic strategy in future CF therapy and a promising complement to CFTR modulators.

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