Published in final edited form as:

Biomarkers. 2019 May ; 24(3): 232–239. doi:10.1080/1354750X.2018.1542458.

Inflammatory Signature in Lung Tissues in Patients with Combined Pulmonary Fibrosis and Emphysema

William D. Cornwell^{a,b}, Cynthia Kim, M.D.^b, Alejandra C. Lastra, M.D.^b, Chandra Dass, M.D.^c, Sudhir Bolla, M.D.^b, He Wang, M.D., Ph.D.^d, Huaqing Zhao, Ph.D.^e, Frederick V. Ramsey, Ph.D.^e, Nathaniel Marchetti, D.O.^{a,b}, Thomas J. Rogers, Ph.D.^{a,b}, and Gerard J. Criner, M.D.^{a,b}

^aCenter for Inflammation, Translational and Clinical Lung Research, Lewis Katz School of Medicine, Temple University, Philadelphia, Pennsylvania

^bDepartment of Thoracic Medicine and Surgery, Lewis Katz School of Medicine, Temple University, Philadelphia, Pennsylvania

^cDepartment of Radiology, Lewis Katz School of Medicine, Temple University, Philadelphia, Pennsylvania

^dDepartment of Pathology and Laboratory Medicine, Lewis Katz School of Medicine, Temple University, Philadelphia, Pennsylvania

^eDepartment of Clinical Sciences, Lewis Katz School of Medicine, Temple University, Philadelphia, Pennsylvania

Abstract

Background: The etiology and inflammatory profile of combined pulmonary fibrosis and emphysema (CPFE) remain uncertain currently.

Objective: We aimed to examine the levels of inflammatory proteins in lung tissue in a cohort of patients with emphysema, interstitial pulmonary fibrosis (IPF), and CPFE.

Materials and methods: Explanted lungs were obtained from subjects with emphysema, IPF, CPFE, (or normal subjects), and tissue extracts were prepared. Thirty-four inflammatory proteins were measured in each tissue section.

Results: The levels of all 34 proteins were virtually indistinguishable in IPF compared with CPFE tissues, and collectively the inflammatory profile in the emphysematous tissues were distinct from IPF and CPFE. Moreover, inflammatory protein levels were independent of the severity of the level of diseased tissue.

Corresponding Author: William D. Cornwell, Temple University Lewis Katz School of Medicine, 3500 North Broad St., Philadelphia, PA 19140; cornwell@temple.edu.

Declaration of interests

Authors WDC, CK, ACL, CD, SB, HW, FVR, NM, and TJR have no competing interests. GJC reports grants from Boehringer-Ingelheim, Novartis, Astra Zeneca, Respiroics, MedImmune, Actelion, Forest, Pearl, Ikaria, Aeris, PneumRx, and Pulmonx; Equity interest in HGE Health Care Solutions, Inc.; Consultation with Amirall, Boehringer-Ingelheim, and Holaira. All are outside the submitted work.

Conclusions: We find that emphysematous lung tissues have a distinct inflammatory profile compared with either IPF or CPFE. However, the inflammatory profile in CPFE lungs is essentially identical to lungs from patients with IPF. These data suggest that distinct inflammatory processes collectively contribute to the disease processes in patients with emphysema, when compared to IPF and CPFE.

Keywords

emphysema; IPF; CPFE; inflammatory protein; chronic inflammation

Introduction

The condition of combined pulmonary fibrosis and emphysema (CPFE) was first reported by Cottin et al. (Cottin et al., 2005), who described a cohort of patients with diffuse parenchymal fibrosis in the lower lung zones and emphysema in the upper lung zones. The frequency of this disease has been reported to range from 8 to 50% in patients with IPF, however, a strict definition has not been adopted (Ryerson et al., 2013). CPFE has gained increasing interest because these individuals exhibit an increased incidence of pulmonary hypertension and highly mortality when compared to patients with idiopathic pulmonary fibrosis alone (Mejia et al., 2009, Cottin et al., 2010, Jankowich and Rounds, 2010, Cottin and Cordier, 2012). CPFE has a strong correlation with tobacco smoking (approximately 98%), and a male: female predominance of approximately 9:1 (Cottin et al., 2005, Cottin and Cordier, 2012, Jankowich and Rounds, 2012). Patients with CPFE present with severe dyspnea, severely impaired transfer capacity for carbon monoxide (DL_{CO}), and hypoxemia with exercise (Cottin et al., 2011).

The pathogenesis of CPFE is poorly understood; it is unclear whether the emphysematous and the fibrotic lesions develop and progress independently, or if disorder promotes the development of the other. In many cases, CPFE is the result when fibrosis develops in a background of preexisting emphysema. Additional evidence suggests that emphysema alters the outcome of patients with IPF (Cottin, 2013).

Relatively little is known about the inflammatory processes responsible for the development of CPFE. Overexpression of either TNF α , platelet-derived growth factor (PDGF), or IL-13 in transgenic mice results in the development of lung tissue damage consistent with a combination of both emphysema and fibrosis, with generalized lung inflammation (Hoyle et al., 1999, Lundblad et al., 2005, Fulkerson et al., 2006). Additional experimental animal work suggests that neutrophil elastase contributes to the initial development of emphysematous lung tissue, and this evolves into fibrosis (Lucattelli et al., 2005). These studies support the notion that the development of CPFE is the result of production of multiple inflammatory mediators. The precise role of individual inflammatory cytokines and/or proteases in the pathogenesis of this disease remains uncertain.

To establish an inflammatory signature at the tissue level for emphysema vs IPF vs CPFE, we investigated the inflammatory mediator compositions of explanted lung tissues from subjects with IPF, emphysema, or CPFE. We also sought to determine whether the mediator

composition in less diseased regions differed from more diseased tissue within a lung from an individual patient.

Materials and Methods

Ethics approval and consent to participate

Lungs were obtained from lung transplant recipients and from the Gift of Life Donor Program (www.donors1.org). The Gift of Life Donor Program does not provide demographic data, and these donors typically suffered fatal trauma as a cause of death. Informed consent from all patients was obtained in compliance with the principles of the Declaration of Helsinki/International Conference on Harmonization Guidance for Good Clinical Practice and in accordance with Temple University Institutional Review Board.

Lung acquisition and processing

All selected lungs (Figure 1) were inflated to total lung capacity, frozen solid over a liquid nitrogen bath at total lung capacity, and then imaged with HRCT ex-vivo in a manner previously published (McDonough et al., 2011). The lungs were sliced in the same plane as the CT scan into approximately 2cm sections.

Selection of cores

A chest radiologist selected areas of the lungs to core based on radiographic evidence of disease activity on ex vivo HRCT images of the frozen inflated lung. HRCT of all selected explanted lungs (frozen, inflated to TLC) were obtained using the standard protocol (120kV, 120mAs, 1mm slice thickness and lung kernel). About 2 cm² areas of emphysema, fibrosis and CPFE were identified, visually graded and marked on the HRCT images. The explanted lungs were cut into 2cm thick slices, numbered from apex to base, and photographed in the same anatomical orientation as their corresponding HRCT images. The explanted lung slice photographic images and their paired HRCT images were placed side by side and 2 cm circular areas were marked on the tissue slice photographic images for core sampling using available local anatomical land marks (segmental location, bronchovascular bundles, pleura, fissure, blebs etc.). For emphysema and fibrosis, cores were obtained from regions in which there was more or less disease. For the CPFE lungs, cores were obtained from emphysematous, fibrotic, and a region of mixed disease. One area that appeared normal radiographically was chosen for each control lung. Once an area was identified on the HRCT image the area to core on the corresponding frozen lung slice was identified and cored with a 12mm diameter cork bore. Representative slices from an emphysematous lung, fibrotic lung and CPFE lung are presented here (Figure 2).

Lung tissue homogenization

Approximately 100mg from each tissue core was homogenized in a Wheaton 1ml tapered glass grinder on ice in a 500ul volume of PBS containing protease and phosphatase inhibitor cocktails (Cell Signaling Technology, Danvers, MA). The samples were centrifuged for 5 minutes at 12,000g to remove particulate matter, and the total protein concentration for each sample was determined.

Inflammatory protein measurements

Lung homogenates were assayed for the levels of 30 inflammatory proteins using multiplex analysis, obtained from either R&D systems (Minneapolis, MN) (TNF α , IL-1 β , IL-4, IL-5, IL-6, CXCL8, IL-10, MMP-1, MMP-2, MMP-8, ST2, SP-D, CXCL10, CCL2, CCL17, CCL20, CCL22, CCL24, CD62L, sCD163, IL-1RA, MPO, TNFR1I, and Lipocalin2) or EMD Millipore (Billerica, MA) (MMP-12, MMP-13, NTproBNP, IFN γ , IL-13, and IL-17A), and read on a Luminex 200 instrument (Luminex Corp, Austin, TX). Four inflammatory proteins, sCD14, CCL5, TGF β , and lysyl oxidase, were measured using ELISA (R&D Systems). In all cases, the assays were tested for cross-reactivity, and no detectable cross-reactivity was observed. The variability for intra-assay variations was tested for all assays and was below 0.1% in all cases. Homogenates were normalized based on total protein concentration. Most of these proteins have pro-inflammatory activity, while some are anti-inflammatory. They are simply referred to as inflammatory proteins herein.

MUC5B single-nucleotide polymorphism analysis

The MUC5B promoter polymorphism variant allele rs35705950 (G $\rightarrow$ T) primer set was obtained from ThermoFisher (Waltham, MA). Genomic DNA was isolated from both control and diseased lungs using the PureLink Genomic DNA kit (ThermoFisher). SNP analysis was performed using the MUC5B promoter polymorphism variant allele rs35705950 (G $\rightarrow$ T) primer set on a StepOnePlus thermocycler instrument (Life Technologies).

Statistical analysis

Marker concentrations were assessed by Kruskal-Wallis test with multiple comparisons based on four groups (normal, emphysema, fibrosis, and CPFE). Pairwise comparisons of concentrations were conducted (six pairwise comparisons), and p-values and q-values for these pairwise comparisons were determined. All statistical analyses were conducted using SAS® 9.4. Statistical significance was defined as $q > q(\alpha=0.05)$. All reported p-values are two-sided where applicable.

Results

Characteristics of study subjects and assessment of lung tissue

The patients with emphysema were slightly younger, had greater smoking exposure, were more obstructed, hyperinflated and air trapped and had lower diffusion capacity compared to patients with IPF or CPFE (Table 1).

All study subjects were analyzed for Muc5B SNP rs35705950, a genetic marker which has been associated with pulmonary fibrosis (Stock et al., 2013). The results show that all IPF subjects were heterozygous (G/T) for the Muc5B SNP, while all emphysema subjects (and normal control subjects) exhibited a homozygous G/G genotype. CPFE subjects varied with respect to this genetic marker (three wild-type, one heterozygous for the SNP, and one homozygous for the SNP).

Characteristic morphological changes were observed in the CT scans of the subjects (Figure 2(A-D)), and in the histological samples from the 3 groups of patients (Figure 2 (E-G)). In

the pulmonary tissues of the emphysematous group, air spaces were enlarged with loss of alveolar septa and fragments of alveolar walls that appeared unattached to adjacent structures. Both centriacinar and panacinar emphysema patterns were identified (Figure 2(F)). In the tissues from the IPF groups, variegated appearance with fibrosis and relatively 'normal' parenchyma was observed. Honeycombing changes were common, fibroblastic foci were randomly seen, and focal chronic inflammation was usually mild (Figure 2(E)). The tissues from CPFE patients revealed emphysema in the upper lobes and fibrosis, honeycombing and emphysema in the lower lobes. Increased vascular wall thickness were common findings (Awano et al., 2017), thick walled cystic lesions and fibroblastic foci were observed occasionally. In one case, acute alveolar damage was identified with eosinophilic hyaline membrane formation (Figure 2(G)).

Regional comparison of inflammatory proteins levels

Tissue cores were obtained from regions of the lungs from the emphysema and IPF groups, which were judged by CT analysis to be either more or less diseased. These cores were homogenized, and the levels of 34 inflammatory proteins were determined for each individual core. The data show (Supplementary Table S1) shows that the inflammatory protein regional levels were not significantly different for the emphysema lungs. Similarly, there was no significant regional difference in the lungs of IPF patients, except for macrophage-derived chemokine (CCL22 that had higher levels in the more diseased cores from IPF lungs (28.5 pg/mg vs 19.4 pg/mg; $p = 0.045$). The remaining 33 inflammatory proteins were not significantly different between the two tissue cores from the IPF lungs.

We employed a similar approach to determine how the levels of these inflammatory proteins compared between cores from emphysematous, fibrotic, and mixed regions in the CPFE lungs. We found (Supplementary Table S2) no significant difference in these inflammatory proteins levels when comparing the three distinct regions of CPFE lungs. The only exception was CCL22 that had significantly lower levels in the emphysematous regions of CPFE patients, compared with fibrotic regions (15.2 pg/mg vs 29.3 pg/mg; $p = 0.05$). Levels of the other 33 inflammatory proteins were not significantly different when comparing emphysematous, fibrotic and mixed regions. Because individual cores from various regions of the lungs were not significantly different, we averaged the values for the two cores from the emphysema and IPF groups, or the three cores for the CPFE group, to carry out additional analyses and comparisons between the subject groups.

Comparison of inflammatory proteins levels in lung tissues from patients with emphysema, IPF, and CPFE

We observed four distinct patterns of inflammatory proteins expression (Supplementary Table S3) in the emphysema, IPF and CPFE tissues in comparison to the normal control lung tissues. In group A, inflammatory proteins levels were increased in the emphysematous compared to normal lungs; in group B, the inflammatory proteins levels were decreased in emphysematous lungs relative to the IPF and CPFE lungs; in group C, the levels in the emphysema, IPF and CPFE were similar but different from normal lungs; and in group D there was a mixed pattern of expression.

In group A, there were five inflammatory proteins (IL-6, CXCL10, CCL2, IL-10, and IFN γ) that had notable increases in the lung tissues of emphysematous patients when compared to the normal control group (Figure 3; and Supplementary Table S3). Levels of IL-6, CCL2 and IL-10 (Figure 3(A,B,D,E)) were statistically increased in emphysematous tissue compared with normal lung. In contrast, there were no significant increases in these inflammatory proteins in the lung tissues of IPF or CPFE tissues when compared to the normal lung. For each of these inflammatory proteins, the levels in IPF lungs and CPFE lungs were not significantly different (Supplementary Table S3).

Inflammatory proteins in group B included IL-17A, IL-13, MMP1 and MMP13 in which there was an increase above the level in normal lung tissue in each of the diseased groups, but the levels in IPF and CPFE lungs were greater (Figure 4(A-D); Supplementary Table S3). In this group, the levels of MMP2, CCL24, and lysyl oxidase were decreased in emphysematous lungs, but not in lungs from IPF or CPFE subjects (Figure 4(E-G); Supplementary Table S3).

In contrast to groups A and B, inflammatory proteins in group C exhibited a pattern in which the levels were very similar in the three disease groups, but different from the levels in the normal lungs (Figure 5; Supplementary Table S3). The levels of sCD14, CCL5, TNFR1, SP-D, ST2, and IL-1 β were all lower in the diseased lungs than in the normal tissue (Figure 5(A-F)), and the levels of IL-4, IL-5, CXCL8 and TNF α in the diseased lungs were all similar, but higher than in the normal tissue (Figure 5(G-I)). Finally, the levels of the inflammatory proteins in group D did not fit within a discernable pattern, and the levels of the inflammatory proteins in this group did not resemble the patterns in groups A-C (Figure 6; Supplementary Table S3). However, in all the groups, there was no significant difference in the levels of the inflammatory proteins in the IPF and CPFE lungs.

Discussion

The first objective in our experiments was to evaluate the intra-organ variation in inflammatory protein levels in the emphysema, IPF, and CPFE lungs. We selected regions of the emphysema and IPF lungs that were more, or less, diseased based on a CT evaluation of the lungs. Our data showed that the levels of all the inflammatory proteins were similar in the different lung regions. These results suggest that the inflammatory status of the lung tissue in these patient groups was consistent throughout the lungs. This may be because these lung explants were obtained from patients with very advanced disease, and the inflammatory processes that were functioning in these lungs reached a point where even the 'less diseased' tissue represented an advanced inflammatory state.

We were somewhat surprised to find that the levels of the inflammatory proteins in the emphysematous, fibrotic, and mixed disease regions of the CPFE lungs were essentially indistinguishable. We expected that the tissue regions which were emphysematous would exhibit an emphysema-like inflammatory signature. However, the results showed that the emphysematous, fibrotic and mixed regions had very similar levels of all the inflammatory proteins in our study. Our results are consistent with published mRNA microarray results showing that, with the exception of MMP1, the levels of most inflammatory genes

(including the 34 inflammatory proteins studied in the present study) are not differentially expressed in fibrotic versus emphysematous regions of CPFE lungs (Hanaoka et al., 2012). This suggests that the inflammatory state in the very severe CPFE lungs is uniform throughout the lung, but that there may be a very different inflammatory pattern if one were able to examine regions in patients with milder forms of CPFE disease.

Similar inflammatory protein levels in distinct regions of lung tissue may possibly arise from diffusion of inflammatory proteins into adjacent lung tissues. With sufficient time and continuous accumulation of these proteins in the neighboring tissue, any differences in these regions may be lost. However, if this were the case, one would expect that the nature of the disease (i.e., fibrotic versus emphysematous) would become more homogeneous. Examination of the tissue histology, and the CT analysis, suggests that this is not the case. Moreover, there were distinct regions of emphysema in these lungs, and yet the inflammatory signature in these specific zones was indistinguishable from fibrotic regions, or with IPF lungs. It is possible that the emphysematous regions of the CPFE lungs develops early in the disease process when a more unique (emphysema-like) inflammatory signature is present. The inflammatory pattern may change over time as the inflammatory milieu becomes essentially fibrosis-like, but the emphysematous tissue damage may be retained.

There is relatively little known about the immunological basis for the development of CPFE. Previous studies have reported higher levels of CXCL8 in the bronchoalveolar lavage fluid of CPFE lungs relative to lungs of emphysema patients (Tasaka et al., 2012). Our results showed that this chemokine is modestly increased in subjects with CPFE relative to those with emphysema. Work carried out with experimental animals has shown that overexpression of either TNF α or IL-13 in transgenic mice can result in pathological lung tissue changes that show generalized inflammation and/or regions consistent with both fibrosis and emphysema (Lundblad et al., 2005, Fulkerson et al., 2006). Our results show that both cytokines are elevated in the lungs of patients with emphysema, IPF or CPFE, compared with the levels in normal lungs.

Our results show a higher level of several inflammatory proteins in the emphysematous lungs, when compared to the IPF or CPFE lung tissues. Most notably, levels of the chemokine CCL2 were elevated 4.1-fold relative to IPF lungs, and 4.4-fold ($p < 0.05$) relative to CPFE lung tissues (Supplementary Table S3). In addition, the levels of the chemokine CXCL10, were 5.9-fold higher than in IPF lungs, and 7.9-fold higher than in CPFE tissue. CCL2 has been reported to be elevated in the sputum, BAL fluid, and lungs of COPD patients (Capelli et al., 1999, de Boer et al., 2000, Traves et al., 2002), and is a potent chemoattractant for monocytes, T cells and epithelial cells. In addition, the expression of CXCL10 is reported to be elevated in the peripheral airways of smokers with COPD (Saetta et al., 2002). Interestingly, CXCL10 (and related CXCR3-selective chemokines) has been shown to inhibit the development of bleomycin-induced fibrosis by depressing fibroblast recruitment in laboratory animals, and by increasing the anti-fibrotic cytokine IFN γ (Jiang et al., 2004, Burdick et al., 2005, Jiang et al., 2010). This is consistent with our results which show that levels of IFN γ in the emphysema lung tissue were approximately 1.9- and 3.1-fold higher in IPF and CPFE lungs, respectively. This is also consistent with previous studies

with sputum from patients with CPFE which show a significant increase in the levels of CXCL10 (Zhao et al., 2012).

We identified several inflammatory proteins which were notably higher in IPF and CPFE lungs compared with the emphysema group. Within this category, MMP2 was 2.8-fold higher in IPF, and 2.4-fold higher in CPFE ($p < 0.05$) than in the emphysema group. This group of inflammatory proteins also included MMP1 (2.9-fold and 3.9-fold higher in IPF and CPFE, respectively), lysyl oxidase (6.1-fold and 6.8-fold higher in IPF and CPFE; $p < 0.05$), and MMP13 (7.6-fold and 16.4-fold higher in IPF than CPFE). These inflammatory proteins are all involved in the regulation of extracellular matrix processing and play an important role in tissue remodeling. Our results are consistent with previously reported gene expression analysis showing that MMP1 and MMP2 are among the most highly expressed genes in IPF (Zuo et al., 2002, Selman et al., 2006). Finally, recent studies suggest that fibrotic lung disease is driven in large part by epithelial cell damage or dysfunction (Buenda-Roldan et al., 2016, White et al., 2016, Selman et al., 2001), and the expression of these matrix metalloproteinases may be a component of the epithelial cell participation.

We found that the expression of several cytokines was significantly elevated in the lung tissues from all three of the patient groups. This included IL-6, CXCL10, TNF α , IL-13, and IL-17. These results are consistent with several studies conducted either with experimental animals, or ex vivo human cell cultures. This was particularly apparent for IL-17 in our study, which showed a 4.5-, 7.3-, and 7.1-fold increase in our tissue samples for emphysema, IPF, and CPFE lung tissues. This is consistent with recent reports which have shown a role for IL-17 in lung inflammation (Cai et al., 2018, Kang et al., 2015). In fact, a recent study with human lung explants, culture ex vivo, has shown that inflammatory activation (treatment with either IL-1 or LPS) results in the expression of most of the cytokines found in our study (Rimington et al., 2017). These results suggest that the inflammatory signature in the lung tissues for these diseases are likely to involve the combined influences of multiple cytokines, chemokines and matrix metalloproteinases.

The major limitation in these findings is the small number of subjects in each group. Statistically significant differences were difficult to achieve with the small sample size in our study. However, there were significant differences in the levels of these inflammatory proteins, particularly when compared to the normal lung tissues. Another limitation in the study involves the use of explanted tissue from patients with advanced disease. The levels of these inflammatory proteins may reflect the advanced stage of the disease, and the participation of these proteins during the progression of the disease at earlier stages may be distinct. Nevertheless, we were able to conclude from the data that differences in inflammatory protein levels in more versus less diseased sections of a given lung were minimal. The results also allowed us to identify patterns of inflammatory protein expression which were distinct in emphysema lungs, relative to the levels in either IPF or CPFE. Finally, and perhaps more importantly, our data showed that the levels of these 34 inflammatory proteins were virtually indistinguishable between IPF and CPFE. Our results support the notion that a potential therapeutic intervention for the development of IPF and CPFE may involve strategies to attenuate the expression of these inflammatory proteins in the tissue (Gioia et al., 2017, Wohlaue et al., 2012). One approach may involve gene

therapy in which selected inflammatory proteins may be targeted so that the specific lung disease may be ameliorated (Gioia et al. 2017).

Conclusion

The primary conclusion from these studies is that the inflammatory protein profile in the lung tissue of patients with CPFE is virtually indistinguishable from that of patients with IPF. In contrast, the levels of several proteins in the lungs of patients with emphysema were different from the CPFE and IPF lung tissues. In addition, the levels of inflammatory proteins were not different when comparing tissue from regions of more versus less severe disease. Finally, the levels of inflammatory proteins in the lungs of CPFE patients were indistinguishable in emphysematous regions, compared with regions with a high degree of fibrosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding

Supported by grants from the National Institutes of Health (DA040619, DA14230, DA25532, and P30DA13429).

References

- Awano N, et al., 2017 Histological analysis of vasculopathy associated with pulmonary hypertension in combined pulmonary fibrosis and emphysema: comparison with idiopathic pulmonary fibrosis or emphysema alone. *Histopathology*, 70, 896–905. [PubMed: 27992963]
- Buendia-Roldan I, et al., 2016 Increased expression of CC16 in patients with idiopathic pulmonary fibrosis. *Plos One*, 11, e0168552. [PubMed: 27977812]
- Burdick MD, et al., 2005 CXCL11 attenuates bleomycin-induced pulmonary fibrosis via inhibition of vascular remodeling. *Am J Respir Crit Care Med*, 171, 261–8. [PubMed: 15502109]
- Cai T, et al., 2018 IL-17-producing ST2+ group 2 innate lymphoid cells play a role in lung inflammation. *J Allergy Clin Immunol*, pii: S0091–6749(18)30453–6. doi: 10.1016/j.jaci.2018.03.007.
- Capelli A, et al., 1999 Increased MCP-1 and MIP-1beta in bronchoalveolar lavage fluid of chronic bronchitis. *European Respiratory Journal*, 14, 160–165. [PubMed: 10489845]
- Cottin V, 2013 The impact of emphysema in pulmonary fibrosis. *Eur Respir Rev*, 22, 153–7. [PubMed: 23728869]
- Cottin V & Cordier JF, 2012 Combined pulmonary fibrosis and emphysema in connective tissue disease. *Curr Opin Pulm Med*, 18, 418–27. [PubMed: 22781207]
- Cottin V, et al., 2010 Pulmonary hypertension in patients with combined pulmonary fibrosis and emphysema syndrome. *Eur Respir J*, 35, 105–11. [PubMed: 19643948]
- Cottin V, et al., 2005 Combined pulmonary fibrosis and emphysema: a distinct underrecognised entity. *Eur Respir J*, 26, 586–93. [PubMed: 16204587]
- Cottin V, et al., 2011 Combined pulmonary fibrosis and emphysema syndrome in connective tissue disease. *Arthritis Rheum*, 63, 295–304. [PubMed: 20936629]
- De Boer WI, et al., 2000 Monocyte chemoattractant protein 1, interleukin 8, and chronic airways inflammation in COPD. *J Pathol*, 190, 619–626. [PubMed: 10727989]
- Fulkerson PC, et al., 2006 Persistent effects induced by IL-13 in the lung. *Am J Respir Cell Mol Biol*, 35, 337–46. [PubMed: 16645178]

- Gioia SD, et al., 2017 From Genesis to Revelation: The Role of Inflammatory Mediators in Chronic Respiratory Diseases and their Control by Nucleic Acid-based Drugs. *Current Drug Delivery*, 14, 253–271. [PubMed: 27557670]
- Hanaoka M, et al., 2012 Comparison of gene expression profiling between lung fibrotic and emphysematous tissues sampled from patients with combined pulmonary fibrosis and emphysema. *Fibrogenesis Tissue Repair*, 5, 17. [PubMed: 23025845]
- Hoyle GW, et al., 1999 Emphysematous lesions, inflammation, and fibrosis in the lungs of transgenic mice overexpressing platelet-derived growth factor. *Am J Pathol*, 154, 1763–75. [PubMed: 10362801]
- Jankowich MD & Rounds S, 2010 Combined pulmonary fibrosis and emphysema alters physiology but has similar mortality to pulmonary fibrosis without emphysema. *Lung*, 188, 365–73. [PubMed: 20614219]
- Jankowich MD & Rounds SI, 2012 Combined pulmonary fibrosis and emphysema syndrome: a review. *Chest*, 141, 222–31. [PubMed: 22215830]
- Jiang D, et al., 2010 Inhibition of pulmonary fibrosis in mice by CXCL10 requires glycosaminoglycan binding and syndecan-4. *J Clin Invest*, 120, 2049–57. [PubMed: 20484822]
- Jiang D, et al., 2004 Regulation of pulmonary fibrosis by chemokine receptor CXCR3.[see comment]. *Journal of Clinical Investigation*, 114, 291–299. [PubMed: 15254596]
- Kang M-J, et al., 2015 Role of chitinase 3-like-1 in interleukin-18-induced pulmonary type 1, type 2, and type 17 inflammation; alveolar destruction; and airway fibrosis in the murine lung. *American Journal of Respiratory & Critical Care Medicine*, 191, 863–871.
- Lucattelli M, et al., 2005 Is neutrophil elastase the missing link between emphysema and fibrosis? Evidence from two mouse models. *Respir Res*, 6, 83. [PubMed: 16045796]
- Lundblad LK, et al., 2005 Tumor necrosis factor- α overexpression in lung disease: a single cause behind a complex phenotype. *Am J Respir Crit Care Med*, 171, 1363–70. [PubMed: 15805183]
- Mcdonough JE, et al., 2011 Small-airway obstruction and emphysema in chronic obstructive pulmonary disease. *N Engl J Med*, 365, 1567–75. [PubMed: 22029978]
- Mejia M, et al., 2009 Idiopathic pulmonary fibrosis and emphysema: decreased survival associated with severe pulmonary arterial hypertension. *Chest*, 136, 10–5. [PubMed: 19225068]
- Mitra S, et al., 2017 Hypertonic saline attenuates the cytokine-induced pro-inflammatory signature in primary human lung epithelia. *Plos One*, 12, e0189536. [PubMed: 29253007]
- Rimington TL, et al., 2017 Defining the inflammatory signature of human lung explant tissue in the presence and absence of glucocorticoid. *F1000Research*, 6, 460. [PubMed: 28721202]
- Ryerson CJ, et al., 2013 Clinical features and outcomes in combined pulmonary fibrosis and emphysema in idiopathic pulmonary fibrosis. *Chest*, 144, 234–40. [PubMed: 23370641]
- Saetta M, et al., 2002 Increased expression of the chemokine receptor CXCR3 and its ligand CXCL10 in peripheral airways of smokers with chronic obstructive pulmonary disease. *American Journal of Respiratory & Critical Care Medicine*, 165, 1404–1409. [PubMed: 12016104]
- Selman M, et al., 2001 Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. *Annals of Internal Medicine*, 134, 136–151. [PubMed: 11177318]
- Selman M, et al., 2006 Gene expression profiles distinguish idiopathic pulmonary fibrosis from hypersensitivity pneumonitis.[see comment]. *American Journal of Respiratory & Critical Care Medicine*, 173, 188–198. [PubMed: 16166619]
- Stock CJ, et al., 2013 Mucin 5B promoter polymorphism is associated with idiopathic pulmonary fibrosis but not with development of lung fibrosis in systemic sclerosis or sarcoidosis. *Thorax*, 68, 436–41. [PubMed: 23321605]
- Tasaka S, et al., 2012 Cytokine profile of bronchoalveolar lavage fluid in patients with combined pulmonary fibrosis and emphysema. *Respirology*, 17, 814–20. [PubMed: 22515655]
- Traves SL, et al., 2002 Increased levels of the chemokines GRO α and MCP-1 in sputum samples from patients with COPD. *Thorax*, 57, 590–595. [PubMed: 12096201]
- Wohlauer M, et al., 2012 Nebulized hypertonic saline attenuates acute lung injury following trauma and hemorrhagic shock via inhibition of matrix metalloproteinase-13. *Critical Care Medicine*, 40, 2647–2653. [PubMed: 22732292]

- White ES, et al., 2016 Plasma Surfactant Protein-D, Matrix Metalloproteinase-7, and Osteopontin Index Distinguishes Idiopathic Pulmonary Fibrosis from Other Idiopathic Interstitial Pneumonias. American Journal of Respiratory & Critical Care Medicine, 194, 1242–1251. [PubMed: 27149370]
- Zhao Y, et al., 2012 Characteristics of pulmonary inflammation in combined pulmonary fibrosis and emphysema. Chinese Medical Journal, 125, 3015–3021. [PubMed: 22932172]
- Zuo F, et al., 2002 Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. Proc Natl Acad Sci U S A, 99, 6292–7. [PubMed: 11983918]

Clinical Significance

- Little is known about the immunopathology of CPFE, or how it might differ from the inflammatory processes which take place during the development of either emphysema or IPF.
- Based on an assessment of the levels of 34 inflammatory and anti-inflammatory proteins, the inflammatory profiles of IPF and CPFE are indistinguishable, but differ significantly from emphysema.
- These results suggest that the immunopathological mechanisms which contribute to the development of CPFE are dominated by the pro-fibrotic inflammatory process(es).

Figure 1.
Selection of tissues used in the analysis.

Figure 2.

Characterization of normal, fibrosis, emphysema, and CPFE diseased lungs. Representative 2cm thick frozen tissue slices for each diseased lung are shown along with the corresponding CT scan of the respective slice (A-D, respectively). A tissue core sample was taken from each lung in a diseased region appropriate to each lung. The hole in each lung in the photos is the location from which the core was taken. Half of each core was used for inflammatory protein analysis and half was used for archiving and histological staining. Representative

H&E images are shown on the right for each disease lung (E-G, respectively). A magnification marker (500px) is shown (F).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 3.

Lung inflammatory protein expression with a higher level in emphysema relative to IPF or CPFE. Lung homogenate levels of IL-6 (A), CXCL10 (B), CCL2 (C), IL-10 (D), and IFN γ (E) are shown for the normal (N), emphysema (E), fibrosis (F), and CPFE lung samples. * = p value < 0.05 relative to normal lung. † = p value < 0.05 for emphysematous lung in comparison to CPFE lung.

Figure 4.

Lung inflammatory protein expression with a higher level in IPF and CPFE relative to emphysema. Lung homogenate levels of IL-17A (A), IL-13 (B), MMP1 (C), MMP13 (D), MMP2 (E), CCL24 (F), and Lysyl oxidase (G) are shown for the normal (N), emphysema (E), fibrosis (F), and CPFE lung samples. * = p value < 0.05 relative to normal lung. † = p value < 0.05 for emphysematous lung in comparison to CPFE lung. § = p value < 0.05 for emphysematous lung in comparison to fibrotic lung.

Figure 5.

Lung inflammatory protein expression with similar levels in emphysema, IPF, and CPFE.

Expression of inflammatory proteins sCD14 (A), CCL5 (B), TNFRII (C), SP-D (D), ST2 (E), IL-1 β (F), IL-4 (G), IL-5 (H), CXCL8 (I), and TNF α (J) are shown for the normal (N), emphysema (E), fibrosis (F), and CPFE lung samples. * = *p* value < 0.05 relative to normal lung.

Figure 6.

Heat map of the level of expression of all 34 proteins. Data are relative to the mean expression level of each protein in CPFE lungs. The key shows the range of values from those with the greatest increase relative to mean CPFE (red) versus the greatest decrease relative to CPFE (green). The proteins are organized in the same order as shown in Figs. 3, 4 and 5, respectively, starting at the top.

Table 1.Demographic data for the study subjects^a.

	Normal	Emphysema	Fibrosis	CPFE	<i>p</i> value ^b		
	(n=6)	(n = 5)	(n = 5)	(n = 5)	E vs F	E vs CP	F vs CP
Age (years)	ND	60.4 ± 3.8	68.6 ± 2.6	67.6 ± 1.5			
Race (C)	67%	66 %	75 %	80 %			
Gender (M)	ND	100 %	100 %	100 %			
MUC5B SNP	ND	5:G/G	5:G/T	3:G/G			
				1:G/T			
				1:T/T			
Pack-Years	ND	49.6 ± 13.2	15.4 ± 7.2	33.6 ± 9.2	p < 0.05	NS	p < 0.05
BMI	ND	26.8 ± 0.8	27.1 ± 3.3	27.2 ± 1.2	NS	NS	NS
FEV₁ (% pred.)	ND	16.2 ± 1.4	58.0 ± 9.0	52.8 ± 11.9	p < 0.05	p < 0.05	NS
FVC (% pred.)	ND	45.8 ± 4.3	47.8 ± 7.3	65.4 ± 11.0	NS	NS	NS
FEV₁/FVC	ND	35.6 ± 2.3	122 ± 4.5	79.7 ± 16.6	p < 0.05	p < 0.05	NS
TLC (% pred.)	ND	112.8 ± 9.9	44.6 ± 4.6	77.8 ± 13.5	p < 0.05	p < 0.05	p < 0.05
RV (% pred.)	ND	240 ± 30	36.4 ± 6.0	104 ± 44	p < 0.05	p < 0.05	NS
DLCO (% pred.)	ND	14.5 ± 0.9	27.8 ± 3.2	20.0 ± 4.8	p < 0.05	NS	NS

C = Caucasian; M = Male; E = Emphysema; F = Fibrosis; CP = CPFE; NS = Not significant; ND = Not determined.

MUC5B SNP: rs35705950. G = Wild-Type; T = SNP.

^aDemographic data are not available for the control subjects.^bComparisons between groups by Kruskal-Wallis test.