C-X-C Motif Chemokine 13 (CXCL13) Is a Prognostic Biomarker of Idiopathic Pulmonary Fibrosis

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Abstract

Rationale: C-X-C motif chemokine 13 (CXCL13) mediates B-cell trafficking and is increased, proportionately to disease activity, in many antibody-mediated syndromes. Dysregulated B cells have recently been implicated in idiopathic pulmonary fibrosis (IPF) pathogenesis.

Objectives: To determine if CXCL13 is associated with IPF progression.

Methods: CXCL13 was measured in lungs by DNA microarray and immunohistochemistry, and in plasma by ELISA.

Measurements and Main Results: CXCL13 mRNA was threefold and eightfold greater in IPF lungs ($n = 92$) compared with chronic obstructive pulmonary disease (COPD) ($n = 191$) and normal ($n = 108$) specimens, respectively ($P < 0.0001$). IPF lungs also showed increased CXCL13 staining. Plasma CXCL13 concentrations (pg/ml) were greater in 95 patients with IPF (94 ± 8) than in 128 subjects with COPD (53 ± 9) and 57 normal subjects (35 ± 3) ($P < 0.0001$). Circulating CXCL13 levels were highest in patients with IPF with

pulmonary artery hypertension ($P = 0.01$) or acute exacerbations ($P = 0.002$). Six-month survival of patients with IPF in the highest quartile of plasma CXCL13 was $65 \pm 10\%$ versus $93 \pm 10\%$ in the others (hazard ratio, 5.5; 95% confidence interval, 1.8–16.9; $P = 0.0008$). CXCL13 increases by more than 50% in IPF serial assays, irrespective of initial values, also presaged respiratory failure (hazard ratio, 7.2; 95% confidence interval, 1.3–40.0; $P = 0.008$). In contrast, CXCL13 clinical associations in subjects with COPD were limited to modest correlations with FEV₁ ($P = 0.05$) and progression of radiographic emphysema ($P = 0.05$).

Conclusions: CXCL13 is increased and is a prognostic biomarker in patients with IPF, and more so than in patients with COPD. This contrast indicates CXCL13 overexpressions are intrinsic to IPF, rather than an epiphenomenon of lung injury. The present data implicate CXCL13 and B cells in IPF pathogenesis, and support considerations for trials of specific B-cell-targeted therapies in patients with this intractable disease.

Keywords: adaptive immunity; autoimmunity; chronic obstructive pulmonary disease; CXCR5

Although the pathogenesis of idiopathic pulmonary fibrosis (IPF) is generally considered to be enigmatic (1), B-cell abnormalities that are recognized as pathognomonic and pathologic in other clinical syndromes are also present among

the patients with this lung disease. IPF lungs show overexpressions of B-cell antibody genes (2), and focal aggregates of these lymphocytes in proximity to fibroproliferative lesions (3–5). B-cell aggregates in disease tissues are

characteristic lesions of ongoing immune responses, and these lymphocytes have also been shown to directly exert numerous pathogenic effects (6–10). Complexes of antigens with the antibodies produced by B cells trigger cytotoxic and

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At a Glance Commentary

Scientific Knowledge on the

Subject: The biologic processes that cause progression of idiopathic pulmonary fibrosis (IPF) remain enigmatic, although several reports implicate a role for B cells in this process, including previous findings of B-cell aggregates in IPF lungs. The present findings show by various measures that C-X-C motif chemokine 13 (CXCL13), a critical and specific mediator of B-cell trafficking to inflammatory foci, is overexpressed in the lungs and circulation of patients with IPF. Concentrations of circulating CXCL13 were significantly associated with disease manifestations and prognoses of patients with IPF in cross-sectional and longitudinal analyses.

What This Study Adds to the

Field: These data indicate facile measurements of CXCL13 may be a useful biomarker for outcome prognostications of patients with IPF, and substantiate and extend prior observations of B-cell involvement in IPF progression. These cumulative findings raise possibilities that experimental treatments specifically targeted at B cells and/or the CXCL13 axis might be considered for patients with this morbid, medically refractory lung disease.

proinflammatory cascades (11), and these complexes are present in the circulation (12), bronchoalveolar lavage (13), and lung parenchyma of patients with IPF (5, 14). Circulating B-lymphocyte stimulating factor (BLyS), a trophic factor necessary for B-cell survival, maturation, and antibody production, is increased and correlated with the clinical features of patients who have recognized autoantibody-mediated disorders, such as systemic lupus erythematosus and rheumatoid arthritis. BLyS is similarly increased and correlated with important disease manifestations among patients with IPF, including their pulmonary artery (PA) pressures, predilections for acute exacerbations, and survival (5). Enhanced B-cell differentiation caused by repetitive antigen stimulation is another classic feature of systemic lupus

erythematosus, rheumatoid arthritis, and many other autoantibody syndromes, and is also present in and clinically associated with IPF (5). The production of autoantibodies with specificities for varied autoantigens is a common feature of immunologic diseases (15). Several self-reactive antibodies have also been identified in IPF cohorts (12, 14, 16–24). Moreover, some of these IPF autoantibodies have direct profibrotic, proinflammatory, or cytotoxic effects, and/or are highly associated with the clinical manifestations and outcomes of individual patients (14, 17–21).

A better understanding of the processes leading to the development and/or progression of IPF could justify trials of mechanistically focused therapies that have the potential to be more efficacious. As an example, antibody-mediated lung diseases are resistant to treatment with nonspecific immunosuppressants (e.g., glucocorticoids), as is IPF, whereas approaches that physically remove antibodies, or target the B cells that produce these immunoglobulins, more often have favorable clinical effects (25–29). If B cells play an important role in IPF pathogenesis, analogous, more mechanistically focused treatment regimens might also benefit patients with this otherwise inexorable syndrome (30).

C-X-C motif chemokine 13 (CXCL13) is a critical agent for B-cell homing to inflammatory foci that is implicated in the pathogenesis of several immunologic disorders (31–35). Circulating CXCL13 is increased in many classical autoimmune syndromes, and levels of this chemokine are often correlated with the clinical activity of these diseases (31–33). The B-cell ligand for CXCL13 that mediates lymphocyte chemotaxis is C-X-C chemokine receptor 5 (CXCR5) (36). The development of biologic response modulators that interrupt or interfere with the CXCL13-CXCR5 axis is an area of ongoing interest, given the therapeutic potential of these treatments (35).

We hypothesized that CXCL13 may be similarly increased and clinically associated in patients with IPF. If so, these findings would help to substantiate the roles of B cells in IPF, potentially identify a useful prognostic biomarker in this population, and could support the rationale for B cell-targeted treatments in these patients (30). Accordingly, we evaluated circulating and intrapulmonary CXCL13, and B-cell

surface expression of CXCR5, among subjects with IPF.

Methods

Subjects and Specimens

Heparinized peripheral blood was obtained from consecutive patients with IPF (5, 14, 16, 37), healthy volunteers, and subjects with cigarette smoking-attributable chronic obstructive pulmonary disease (COPD) and/or emphysema (henceforth collectively denoted as COPD) (38, 39).

Plasma was obtained by centrifugation of these specimens, aliquoted, and stored at -80° until used in ELISA. Peripheral blood mononuclear cells were isolated from phlebotomy specimens by density gradient centrifugation obtained prospectively from the most recent subjects with IPF and COPD, and used fresh in flow cytometry studies.

Disease diagnoses were established by expert clinicians, who analyzed all information, and were masked to the experimental laboratory tests. All subjects with IPF fulfilled American Thoracic Society and European Respiratory Society diagnostic criteria, and had negative conventional autoimmune serologic tests (5, 14, 16, 37). IPF acute exacerbations were defined by worsening hypoxemia and/or dyspnea during the preceding 30 days or less, with characteristic acute radiographic abnormalities, and no other attributable cause after thorough clinical evaluations (40). COPD was diagnosed by spirometry (38), and emphysema was detected and quantified by chest computed tomography scans (39). Subjects who were taking more than 10 mg prednisone per day, or other systemic immunosuppressants, were excluded from these analyses to avoid confounding effects of these medications on CXCL13 production (34). Subpopulations of subjects with IPF and COPD had PA pressures measured by right heart catheterizations during evaluations for lung transplantation or other clinical indications. These catheterizations were performed by cardiologists who were independent and unaware of this study.

Healthy volunteer control subjects were recruited from among hospital personnel or research registries. Those with tobacco smoking histories had normal spirometry and no evidence of emphysema on chest computed tomography scans.

All subjects gave written informed consent. This study was approved by the University of Pittsburgh Institutional Review Board.

Circulating CXCL13

CXCL13 was measured in plasma specimens by ELISA (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. Optical densities at 405 nm of replicate specimens were determined in a plate reader, blanked against untreated wells (5, 14).

CXCL13 Gene Expression

Log-normalized values for intrapulmonary CXCL13 mRNA expressions were abstracted from publicly assessable archives of the Gene Expression Omnibus (accession GSE47460) (41). The data analyzed here were limited to those generated by University of Pittsburgh investigators (41) in specimens that had been obtained from the Lung Tissue Research Consortium (42). DNA microarray methodologies and subsequent data processing are detailed online (41).

Immunohistochemistry

Lung explant specimens from patients with IPF undergoing therapeutic transplantations and normal lungs obtained during harvests of other organs (16) were fixed with neutral-buffer formalin, and embedded in paraffin. Goat antihuman CXCL13 treatments (R&D Systems) were followed by successive incubations with biotinylated anti-goat mAb and avidin-horseradish peroxidase, and imaged as described (5, 14, 38).

Lymphocyte CXCR5

The proportion of B cells ($CD45^{+}CD20^{+}$) among peripheral blood mononuclear cells that express surface CXCR5 were prospectively quantified by flow cytometry in fresh phlebotomy specimens from the most recently acquired lung disease subjects using methods detailed previously (5, 14, 37).

Statistical Analyses

Two- and three-group comparisons of continuous variables were made by Mann-Whitney or Kruskal-Wallis tests, respectively. Associations between continuous variables were established by Spearman correlations. Linear regression analysis was used to adjust analyses for confounding variables. Survival analyses were performed using Cox multivariate

Table 1: Demographic and Clinical Characteristics of the Lung Disease Subjects That Had Plasma CXCL13 Concentration Assays

	IPF	COPD
N	95	128
Age, yr	69 ± 1 (70, 51–84)	65 ± 1 (66, 47–81)*
Sex, % male	74	50*
FVC % predicted	66 ± 2 (65, 25–126)	88 ± 2 (89, 38–144)*
FEV ₁ % predicted	78 ± 2 (76, 31–133)	65 ± 2 (67, 12–130)*
FEV ₁ /FVC	0.84 ± 0.01 (0.84, 0.71–1.22)	0.55 ± 0.01 (0.59, 0.15–0.83)*
DL _{CO} %predicted	50 ± 2 (49, 14–109)	60 ± 2 (61, 17–122)*
%-910 HU	ND	27.8 ± 1.8 (23.4, 0.6–73.3)
Smoking history, %	53	100*

Definitions of abbreviations: %-910 HU = percentages of lung pixel values less than 910 Hounsfield Units; COPD = chronic obstructive pulmonary disease; CXCL13 = C-X-C motif chemokine 13; DL_{CO} = diffusing capacity of carbon monoxide; IPF = idiopathic pulmonary fibrosis; ND = not determined. Data are depicted as means ± SE, and in parenthesis (median, minimum-to-maximum ranges). Smoking history denotes subjects with ≥5 pack-years of cigarette smoking.

* $P \leq 0.001$ for comparisons between the IPF and COPD cohorts.

proportional hazards models to examine the effect of CXCL13 levels on patient survival, and are reported as hazard ratios (HR) and 95% confidence intervals (CI). A greater than 10-fold increase in the variance of HRs (i.e., variance inflation factor > 10) was interpreted as evidence of collinearity in the Cox model. Alpha values less than 0.05 were considered significant. Unless otherwise denoted, data are depicted as means ± SE.

Results

Subjects

Characteristics of the lung disease subjects who provided phlebotomy specimens for

CXCL13 plasma assays are detailed in Table 1. The ages of the 57 normal control subjects (63 ± 1 yr old) were near identical to those of subjects with COPD, and both of these were statistically less than IPF ages (Table 1) ($P = 0.003$). The proportion of males among the IPF cohort was greater ($P = 0.002$) than either COPD (Table 1) or normal subjects (61%). The proportion of subjects with smoking histories was similar among control subjects (64%) and IPF ($P = 0.11$), although both were significantly less than the COPD (Table 1).

Serial plasma specimens collected at yearly intervals were available for analyses from surviving subjects with IPF who were enrolled in a longitudinal study protocol. Forty-six of these subjects survived for at

Table 2: Demographic and Clinical Characteristics of the Lung Disease Cohorts for the Intrapulmonary CXCL13 mRNA Expression Studies

	IPF	COPD
N	92	191
Age, yr	65 ± 1 (65, 37–80)	64 ± 1 (65, 28–84)
Sex, % male	71*	55
FVC % predicted	65 ± 2 (65, 21–99)*	71 ± 1 (72, 13–121)*
FEV ₁ % predicted	73 ± 2 (74, 26–110)*	45 ± 1 (47, 11–79)*
DL _{CO} % predicted	50 ± 2 (51, 16–95)	53 ± 2 (49, 13–115)
Smoking history, %	64	100*

Definitions of abbreviations: COPD = chronic obstructive pulmonary disease; CXCL13 = C-X-C motif chemokine 13; DL_{CO} = diffusing capacity of carbon monoxide; IPF = idiopathic pulmonary fibrosis. The specimens used here were obtained from the Lung Tissue Research Consortium (42) and analyzed by DNA microarray at the University of Pittsburgh. The methods and results of these assays are archived in a publicly accessible online database (41).

Data are depicted as means ± SE, and in parenthesis (median, minimum-to-maximum ranges).

Smoking history denotes subjects with smoking histories >100 cigarettes.

* $P \leq 0.01$ for comparisons with the other cohort.

least 1 year after their initial evaluation and CXCL13 determinations. Twenty-eight subjects survived for at least 2 years after their study entry and initial CXCL13 measures. Repeated pulmonary function tests after intervals of 26.9 ± 0.4 months were available in 91 subjects with COPD.

Demographic and clinical characteristics of the disease subjects who provided lung specimens for CXCL13 gene expression assays are summarized in Table 2. Normal lung control specimens for these gene expression studies ($n = 108$) were obtained from subjects whose ages (64 ± 1 yr old) were near identical to subjects with IPF and COPD ($P = 0.90$). A lesser proportion of the normal control subjects were males (45%) compared with the diseased subject cohorts (Table 2) ($P = 0.0015$). The proportion of normal

control subjects with smoking histories (67%) was near identical to that of subjects with IPF (Table 2), and both were less than the subjects with COPD ($P < 0.001$).

Cross-Sectional Assays of Circulating CXCL13

Concentrations of CXCL13 in the plasma specimens obtained at initial subject enrollments were significantly greater among the IPF compared with COPD and normal cohorts (Figure 1A). Sex, smoking, and age had no discernable effects on plasma CXCL13 concentrations among normal control subjects or subjects with COPD (data not shown). Among subjects with IPF, CXCL13 concentrations (pg/ml) were similarly comparable among males (93 ± 10) and females (97 ± 15) ($P = 0.87$), and those with (95 ± 13) and without

(93 ± 10) smoking histories ($P = 0.72$). Plasma CXCL13 and age were uniquely correlated, albeit weakly, in the subjects with IPF ($r_s = 0.28$; $P = 0.006$) (see Figure E1 in the online supplement). Linear regression analysis showed these age-related effects did not explain the substantial difference of circulating CXCL13 levels between subjects with IPF and COPD (Figure 1A). Specifically, CXCL13 concentrations were on average 37.3 pg/ml greater in the IPF cohort compared with the subjects with COPD after adjusting for the older age of the patients with IPF ($P = 0.006$).

Intrapulmonary CXCL13

To substantiate the relevance of circulating CXCL13 levels, expression of this chemotactic mediator in lungs was assessed by two different techniques. CXCL13 mRNA expression in the lung tissues of the respective cohorts reflected their circulating levels of the chemokine (Figure 1B). The linear fold-increase of IPF mRNA expression levels was 8.3 compared with normal control subjects, and 3.0 compared with COPD. These mRNA expressions were independent of sex and smoking in all three subject cohorts. As an example, log-normalized intensities of CXCL13 mRNA in the subjects with IPF were near identical for males (7.4 ± 0.3) and females (7.2 ± 0.4) and for those with (7.2 ± 0.3) and without (7.3 ± 0.4) smoking histories. Among subjects with COPD (all of whom had smoking histories), there was no correlation between CXCL13 and pack-years smoking ($r_s = 0.005$; $P = 0.95$).

The extent of CXCL13 expression detected by immunohistochemistry was also much less in normal lungs than in the specimens from patients with IPF. These mediator expressions were localized to the intrapulmonary B-cell aggregates that are numerous in IPF lungs, and these in turn were typically proximate to fibroblastic foci (Figure 1C), as also reported by other investigators (3). Additional studies showed that macrophage lineage cells ($CD68^+$) were the predominant source of the CXCL13 within the IPF B-cell aggregates (see Figure E2). Immunohistochemical studies in COPD lungs have been recently detailed elsewhere (43, 44).

Lymphocyte CXCR5

These studies were intended to confirm that IPF B cells express the ligand responsible for CXCL13-mediated chemotaxis. CXCR5

Figure 1. (A) C-X-C motif chemokine 13 (CXCL13) in circulations of 57 normal control subjects (NI), 128 subjects with chronic obstructive pulmonary disease (COPD), and 95 subjects with idiopathic pulmonary fibrosis (IPF). Horizontal lines from bottom to top denote 10th, 25th, 50th, 75th, and 90th percentiles. Mean values are denoted by solid squares. (B) CXCL13 mRNA expression in 108 normal control, 191 COPD, and 92 IPF lung specimens. (C) Intrapulmonary CXCL13 immunostaining. Analyses were performed on normal (upper left, $n = 5$) and IPF (upper right, $n = 6$) lungs. Asterisks denote fibroblastic foci in IPF lungs. A human lymph node was used as a positive control (lower left) and shows CXCL13 staining in a germinal center. CXCL13 immunostaining was readily observed in the loosely formed lymphoid aggregates in IPF lungs but was rare in normal lungs. In the upper panels, magnification is $\times 40$, and the yellow inset bar represents 200 μ m. In the lower panels, magnification is $\times 100$, and the orange inset bar represents 100 μ m.

was expressed on most B cells in the circulation of subjects with IPF, as described in other populations (Figure 2) (33, 36, 43).

CXCL13 Clinical Associations

Given findings here that circulating and intrapulmonary CXCL13 are abnormally increased in the disease cohorts (Figure 1), and prior reports that levels of this mediator are correlated with disease manifestations in other populations (31–33), we examined plasma concentrations of this chemokine for associations with clinical features of the subjects with COPD and IPF.

COPD

There were no statistically significant associations between circulating CXCL13 levels and the cross-sectional demographic or physiologic characteristics denoted in Table 1 among subjects with COPD, except for a weak correlation of the mediator with a single measure of expiratory airflow (Figure 3A). CXCL13 levels similarly did not correlate with subsequent changes of pulmonary function over the next 2 years, except for a weak relationship with radiographic emphysema (Figure 3B). There were no associations of CXCL13 with PA pressures among subjects with COPD (data not shown), and levels of this mediator among the five patients with COPD who died during the next

Figure 2. Most peripheral blood CD20⁺ B cells among both subjects with chronic obstructive pulmonary disease (COPD) and subjects with idiopathic pulmonary fibrosis (IPF) expressed C-X-C chemokine receptor 5 (CXCR5). There were no significant differences between COPD and IPF values.

Figure 3. (A) The only statistically significant correlation among subjects with chronic obstructive pulmonary disease between circulating C-X-C motif chemokine 13 (CXCL13) levels and cross-sectional demographic or pulmonary function/radiographic characteristics at the time of specimen acquisitions (Table 1) was this inverse association of the chemokine concentrations with percent predicted values for FEV₁. (B) Repeated clinical assessments were available in 91 subjects with chronic obstructive pulmonary disease approximately 2 years after their initial evaluations and CXCL13 specimen acquisitions. The only significant correlation of CXCL13 with changes in pulmonary measures over the next 2 years was an association with interval changes (delta) in the percentages of lung pixel values less than 910 Hounsfield Units (HU).

approximately 2 years were 39 ± 3 pg/ml, compared with 54 ± 9 pg/ml among the survivors.

IPF

CXCL13 levels among the subjects with IPF at study entry were weakly associated with diffusing capacities of carbon monoxide (DL_{CO}) (Figure 4A). CXCL13 was similarly correlated, albeit even more weakly, with FVC of the subjects with IPF (Figure 4B).

CXCL13 concentrations in patients with IPF were highest in those who had PA hypertension, defined as PA mean pressure greater than 25 mm Hg with PA wedge pressure less than 15 mm Hg (Figure 4C).

A sizeable proportion of patients with IPF develop acute exacerbations that can result in very rapid progression of respiratory dysfunction and high mortality (40), and these sudden disease progressions have been recently linked to B cells (5, 14, 17, 18). The patients with IPF in this cohort who were having acute exacerbations at presentation, or would do so within the next 6 months, also had significantly greater concentrations of CXCL13 (Figure 4D).

Based on these findings, we hypothesized that levels of CXCL13 in subjects with IPF might have prognostic significance. Patients with IPF were stratified into the quartile with highest circulating CXCL13 concentrations versus the 75% of subjects with lower levels of this mediator. There were no clinical or demographic differences at the time of initial specimen acquisitions between those

patients in the lowest and highest CXCL13 subpopulations (Table 3). Nonetheless, the subsequent mortality rate in the highest CXCL13 quartile was more than fourfold greater than among the subjects with IPF with lower levels of this mediator (33% vs. 7%, respectively; $P = 0.005$). The proportion of subjects who had lung transplantations during this period was near equal (8.3% vs. 8.5%).

Actuarial analyses confirmed that major event-free survival (i.e., transplant-free survival [TFS]) was diminished in the quartile of subjects with IPF with the highest CXCL13 concentrations (Figure 4E). To eliminate the potential for biasing caused by vagaries of lung transplantation selections, *post hoc* analyses, in which transplantations were censored (end of observations), confirmed the subjects with IPF with the highest concentrations of CXCL13 had the greatest absolute mortality (Figure 4F). Adjustments for the clinical parameters described in Table 3 (i.e., age, sex, biopsy status, and percent predicted FVC) did not alter the relationship between CXCL13 levels and survival (HR, 23.2; 95% CI, 2.3–231.1; $P = 0.007$). Despite accumulating collinearities, the CXCL13-survival association also remained significant after the addition of percent predicted DL_{CO} to the other clinical parameters (HR, 14.9; 95% CI, 1.1–197.2; $P = 0.04$).

The survival disadvantage of the patients with IPF with highest CXCL13 levels persisted over at least the next 2 years.

Figure 4. (A) C-X-C motif chemokine 13 (CXCL13) concentrations were weakly correlated with diffusing capacities of carbon monoxide (DL_{CO}) (ml/min/mm Hg), and (B) FVC (L) of subjects with idiopathic pulmonary fibrosis (IPF). (C) Subjects with IPF with pulmonary artery hypertension (PH, $n = 15$) have greater circulating CXCL13 levels than patients with normal pulmonary artery pressures (noPH, $n = 23$). PH is defined as pulmonary artery mean greater than 25 mm Hg with pulmonary artery wedge pressures less than 15 mm Hg. (D) CXCL13 concentrations were greatest among patients with IPF who were having, or going to have, AE in the next 6 months ($n = 9$). (E) Transplant-free survival (TFS) was worse among the subjects with IPF with the highest quartile of CXCL13. Numbers in parentheses denote censored events (end of observations). (F) Actuarial analyses with transplantations censored confirmed the absolute mortality was greatest among the quartile of subjects with IPF with the highest CXCL13 levels. Cross hatches and numbers in parenthesis denote censored subjects. AE = acute exacerbations; CI = confidence interval; HR = hazard ratio.

Two-year TFS was $25 \pm 9\%$ among the highest CXCL13 quartile versus $62 \pm 6\%$ for subjects in the lowest three quartiles (HR, 2.9; 95% CI, 1.6–5.3; $P = 0.003$).

Serial analyses of plasma specimens collected at yearly intervals among the subjects with IPF who survived more than 1 year after study entry showed that CXCL13 increases in the latest available specimens, relative to their preceding assay results, were greatest among those individuals who subsequently died because of respiratory failure or required emergent lung

transplantation because of acute, severe (and rapidly progressive) IPF exacerbations (Figure 5A).

The longitudinal IPF study cohort was also stratified for actuarial analyses based on the magnitude of their changes in CXCL13 concentrations over time. Individuals in the highest quartile for interval CXCL13 increases (which happened to coincide with a relative CXCR13 increase of $>50\%$ in the serial measures) had lesser absolute 6-month TFS ($55 \pm 15\%$ vs. $86 \pm 6\%$; $P = 0.026$). The uncensored endpoints for

this analysis included deaths from all causes (e.g., cardiac events, malignancies, and so forth) and elective lung transplantations in stable patients (i.e., those not having acute exacerbations or in overt respiratory failure at the time of transplantation). The actuarial association between interval changes of serial CXCL13 levels and prognosis was even more significant when the uncensored outcome endpoints were limited to episodes of lethal respiratory failure or acute IPF exacerbations that required emergent transplantations during the exacerbation (Figure 5B).

The magnitude of subsequent changes of circulating CXCL13 concentrations among individual patients with IPF was not significantly correlated with absolute values of this chemokine in their initial plasma specimens obtained at study entry (Figure 5C).

Discussion

These studies show that CXCL13 is abnormally expressed in patients with IPF (Figure 1). Moreover, circulating concentrations of this chemokine are also highly associated with the clinical manifestations and disease progression of afflicted individuals (Figures 4 and 5). Other demonstrations here that CXCL13 expression is greater and more clinically correlated in patients with IPF than among lung disease control subjects with COPD further indicate that production of this chemokine is not a mere consequence of pulmonary injury *per se*, but instead is a disease-specific process of IPF.

The findings in these subjects with IPF parallel analogous observations in patients with other syndromes that are known to be mediated by pathologic B cells (31–33). Given the lack of progress in the treatment of IPF, which remains a highly morbid and almost invariably fatal disease (1), objective considerations of pathogenic paradigms that could illuminate new therapeutic approaches for this disease seem warranted.

Assays here show that CD68⁺ macrophage lineage cells within pulmonary B cell aggregates, which in turn are proximate to fibroproliferative lesions (3–5), are an origin of the CXCL13 among patients with IPF (Figures 1B and 1C; see Figure E2). In conjunction with demonstrations that the circulating B cells of patients with IPF are not uniquely

Table 3: Demographic and Clinical Characteristics of IPF Subjects Stratified by Plasma CXCL13 Levels

	Lowest CXCL13	Highest CXCL13
N	71	24
Age, yr	69 ± 1 (69, 53–84)	69 ± 2 (72, 51–82)
Sex, % male	76	67
Lung biopsy, %	56	50
FVC % predicted	67 ± 2 (67, 31–126)	61 ± 4 (59, 25–97)
DL _{CO} % predicted	51 ± 2 (50, 20–109)	46 ± 5 (44, 14–91)
Smoking history, %	55	48

Definitions of abbreviations: CXCL13 = C-X-C motif chemokine 13; DL_{CO} = diffusing capacity of carbon monoxide; IPF = idiopathic pulmonary fibrosis. Highest CXCL13 denotes those subjects with IPF with mediator values in the highest quartile. Data are depicted as means ± SE, and in parenthesis (median, minimum-to-maximum ranges). None of these characteristics differed significantly between the two subpopulations.

deficient in their expression of the chemotactic ligand CXCR5 (Figure 2), and numerous definitive reports in other populations (31–36), it is thus highly likely the CXCL13-CXCR5 axis is involved in the focal accumulations of nonproliferating B cells that were found in IPF lungs here and in other studies (3–5). Broadly comparable findings have been reported in COPD studies, in which those authors similarly concluded that CXCL13 plays an important role in the intrapulmonary B-cell accumulations of that lung disease (43, 44). B-cell aggregations in diseased tissues are

not only pathognomonic markers of adaptive immune responses (6), but these lymphocytes also exert directly injurious effects by their productions of antibodies and autoantibodies, cytokines, vasoactive substances, and other mediators, and by providing enhanced local antigen presentation (7). The roles of parenchymal B-cell aggregates and autoantibodies in the genesis of PA hypertension have been previously described in various other disease populations and animal models (8–10). Perivascular distributions of pathogenic immune complex and

complement depositions in IPF lungs have also been described, along with a strong association between another important mediator of B-cell function (BLyS) and PA hypertension (5).

A better understanding of the role B cells play in IPF progression could have several consequences. First, the course of IPF in individual patients is highly variable and often unpredictable. Accordingly, the use of biomarkers that accurately identify those patients destined for poor near-term prognoses could optimize timings of lung transplantations, and facilitate the selection of high-risk subjects for experimental clinical trials. The present data indicate that cross-sectional assays of CXCL13 may have prognostic use in patients with IPF (Figure 4). The longitudinal CXCL13 assays here (Figure 5) support the validity of these cross-sectional observations. Even more importantly, the serial assays show that relative increases of CXCL13 concentrations by 50% or more at later time-points (irrespective of their initial chemokine levels) also presage poor prognoses of individual patients with IPF and, in particular, the subsequent development of life-threatening respiratory failure (Figure 5). The longitudinal CXCL13 measures here are relatively unique among biomarker descriptions in patients with IPF (and many other disease populations).

In addition, a closer focus on B cells in IPF may provide a novel and relatively facile approach to target early (up-stream) processes that seem to be associated with the progression of this disease. Glucocorticoids do not favorably alter the natural history of IPF (1). However, these nonspecific agents are similarly ineffectual as primary (or sole) therapy of numerous (if not all) other antibody-mediated lung diseases, including granulomatosis with polyangiitis (Wegener's) (or antineutrophil cytoplasmic antibody-associated pulmonary vasculitides); Goodpasture syndrome; interstitial lung diseases (especially acute exacerbations) associated with polymyositis, scleroderma, and other conventional connective tissue diseases; or donor-specific antibodies in lung transplant recipients with chronic allograft rejection. Conversely, therapeutic modalities that deplete antibodies or target the B cells that produce these immunoglobulins often have beneficial clinical effects (25–29). A preliminary report indicates that autoantibody-targeted therapies may similarly benefit patients with

Figure 5. (A) The greatest increases in circulating C-X-C motif chemokine 13 (CXCL13) concentrations from previous measures (as a percentage change) were seen among the 16 subjects with idiopathic pulmonary fibrosis (IPF) who subsequently developed respiratory failure (RF) that resulted in their deaths ($n = 13$) or requirements for emergent lung transplantations necessitated by life-threatening acute exacerbations ($n = 3$) (Yes), compared with the other 30 patients with IPF in the longitudinal cohort (No). (B) subjects with IPF in the highest quartile for increases in serial CXCL13 measures ($n = 11$) were more likely than the other patients with IPF in the longitudinal cohort ($n = 35$) to develop RF resulting in death or require emergent lung transplantation because of severe acute exacerbations. (C) Relative changes of plasma CXCL13 in later assay determinations were not significantly correlated with concentrations of this chemokine in the initial specimens obtained from the subjects with IPF at their study enrollment. CXCL13 increases of more than 50% in serial assays (dotted line) were predictive of poor outcomes (Figures 5A and 5B), and often occurred among subjects with low initial chemokine concentrations. CI = confidence interval; HR = hazard ratio.

IPF with severe, life-threatening, acute exacerbations (30).

The present data are a further substantiation and extension of reports that implicate B cell-specific mediators and effector mechanisms (e.g., antibody productions), and other pathologic adaptive immune responses, in the development and/or progression of IPF (2–5,12–14,

16–24, 30,37,45–47). These cumulative findings could ultimately influence incremental investigations and approaches to treatment of this morbid and heretofore inexorable lung disease. In addition to currently available regimens for treatment of antibody-mediated syndromes (25–29, 48), the development of biologic response modifiers with specific actions at discrete,

critical steps of pathologic B-cell response pathways is a very active area of ongoing research, and there is reason to believe that even more efficacious therapies will be available in the not-too-distant future (7, 35, 49). ■

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