Targeting Interleukin-13 with Tralokinumab Attenuates Lung Fibrosis and Epithelial Damage in a Humanized SCID Idiopathic Pulmonary Fibrosis Model

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Abstract

The aberrant fibrotic and repair responses in the lung are major hallmarks of idiopathic pulmonary fibrosis (IPF). Numerous antifibrotic strategies have been used in the clinic with limited success, raising the possibility that an effective therapeutic strategy in this disease must inhibit fibrosis and promote appropriate lung repair mechanisms. IL-13 represents an attractive target in IPF, but its disease association and mechanism of action remains unknown. In the present study, an overexpression of IL-13 and IL-13 pathway markers was associated with IPF, particularly a rapidly progressive form of this disease. Targeting IL-13 in a humanized experimental model of pulmonary fibrosis using tralokinumab (CAT354) was found to therapeutically block aberrant lung remodeling in this model. However, targeting IL-13 was also found to promote lung

repair and to restore epithelial integrity. Thus, targeting IL-13 inhibits fibrotic processes and enhances repair processes in the lung.

Keywords: fibroblast; epithelium; fibrosis

Clinical Relevance

Numerous antifibrotic strategies have been used in the clinic with limited success. Here we show that IL-13 is an attractive target for idiopathic pulmonary fibrosis (IPF), being associated with rapidly progressive IPF. Moreover, blocking IL-13 in a humanized mouse model of IPF inhibits lung fibrosis and reduces epithelial apoptosis in the lung.

Idiopathic pulmonary fibrosis (IPF) is characterized by progressive scarring of the lung and ultimate decline in lung function (1, 2). One of the salient features of IPF is excess parenchymal deposition of extracellular matrix (ECM), which promotes the progressive decline in lung function observed in these patients (3). Whereas an increase in ECM components

serves to promote wound sterility, restore barrier protection, and maintain the intricate architecture and function of the alveolus, this process is clearly aberrant in IPF, leading to a scarred, ablated alveolar unit unable to facilitate gas exchange (4). The cellular mechanisms leading to IPF are not completely understood, but epithelial damage is commonly observed in the lungs

of these patients, and mediators from these cells, such as growth factors and cytokines, directly contribute to an increase in and the persistence of activated fibroblasts and myofibroblasts (5). IPF is highly heterogeneous, with a subset of patients showing rapid progression to death within 1 to 2 years of diagnosis and another subset of patients showing a more slowly

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progressive disease course over a 5-year period or longer (6). The clinical heterogeneity likely reflects biological heterogeneity in aberrant lung repair pathways.

Among the more widely implicated cytokines in lung fibrosis is IL-13 (7). This Th2-type cytokine promotes lung fibrosis in a number of experimental settings (8–13), leading to the hypothesis that targeting this key profibrotic mediator alone might prevent or slow the progression of pulmonary fibrosis (7, 14). During chronic lung remodeling, there are numerous immune cell types that can produce IL-13, including T lymphocytes and alternatively activated macrophages, but other putative cellular sources of IL-13, including structural cells, have not been extensively studied. Blocking IL-13 with tralokinumab, a human IL-13–neutralizing immunoglobulin G4 monoclonal antibody, in moderate to severe uncontrolled asthma improved FEV₁ in a recent phase 2 trial (15). Tralokinumab is currently being tested clinically. The same beneficial effect on FEV₁ was reported in patients with uncontrolled asthma in another anti-IL-13 clinical trial (16). In IPF, lung levels of IL-13 are elevated (17–19) and correlate inversely with FVC (19). Both IL-13 receptor subunits (IL-13R α 1 and IL-13R α 2) are highly expressed by IPF fibroblasts (20, 21), thereby enhancing the responsiveness of these cells to IL-13 compared with normal fibroblasts (18). Controversy surrounds the mechanism whereby IL-13 promotes pulmonary fibrosis, with studies showing that it does so in either a transforming growth factor (TGF)- β -dependent (22, 23) or a TGF- β -independent (11, 24) manner. IL-13 signals through its respective receptor subunits via a JAK2-STAT6–dependent (25) and AP1-dependent (23) pathways, but this cytokine can drive lung fibrosis in a STAT6-independent manner (13), and further controversy surrounds the signaling role of IL-13R α 2 in the lung (26). Thus, IL-13 and its receptor subunits contribute to the pathogenesis of pulmonary fibrosis through a poorly defined but targetable mechanism.

In this study, we further explored IL-13–directed mechanisms in pulmonary fibrosis via the use of tralokinumab, an investigational human IgG4 monoclonal antibody that selectively neutralizes human IL-13 (27–29). We observed that the IL-13 pathway was significantly enhanced in biopsy samples from patients who exhibited

a rapidly progressing form of IPF compared with patients with IPF with a slower rate of lung function loss (30), and inhibition of human IL-13 with tralokinumab attenuated established pulmonary fibrosis in a humanized SCID mouse model of IPF, notably with a reduction in lung epithelial cell damage. Together these data highlight that targeting IL-13 inhibits the fibrosis and promotes repair processes in the lung.

Materials and Methods

Additional information is provided in the online supplement.

Patient Recruitment

All studies were performed with human institutional care approval at Yale University School of Medicine, The University of Michigan School of Medicine, or UCSF. Patients with a diagnosis of IPF according to the ERS/ATS consensus statement (2) were recruited for involvement in these studies. Additional information is provided in the online supplement.

Humanized SCID Mouse Model of IPF

All studies were approved by the Institutional Animal Care and Use Committee at the University of Michigan Medical School. Female C.B-17-scid-beige (C.B-17SCID/bg) mice received single-cell preparations of IPF and normal fibroblasts (2×10^6 cells/ml) via tail vein injection. In IL-13 neutralization studies, mice were treated with PBS control, CAT251 (IgG4 mAb control, 3 mg/kg), or tralokinumab (anti-IL-13) (MedImmune, Cambridge, UK), a human recombinant IgG4 mAb that specifically binds IL-13 and blocks interactions with IL-13R α 1 and IL-13R α 2, 3 mg/kg, intraperitoneally every other day starting on Day 35. Mice were killed on Day 63 after the human pulmonary fibroblast transfer. Bronchoalveolar lavage (BAL) was taken by flushing the lungs with sterile PBS, and serum was prepared from a post mortem blood draw. Whole lung lobes were dissected for histological and biochemical analysis (*see below*).

Gene and Protein Analyses

The following samples were analyzed: single cell samples or for lung tissue analysis, lung biopsy tissue from patients with IPF/usual interstitial pneumonia, patients with nonspecific interstitial pneumonia (NSIP),

the normal margins of lung tumor resections, or lungs from mice and were processed for total RNA was obtained using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Gene expression levels were quantitated using real-time RT-PCR (Applied Biosystems, Foster City, CA) or branched-DNA-technology–based QuantiGene Reagent System (Panomics, Fremont, CA) according to the manufacturers' protocols. Transcript levels of genes of interest measured by quantitative RT-PCR were normalized to β -actin mRNA. For protein analysis, cytokine levels were measured by bead-based Luminex analysis or specific ELISA. Serum CC16 levels were measured via CC16 assay (Caltag Medsystems, Buckingham, UK) as per the manufacturer's instructions. BAL caspase 3 activity was measured using CaspaseGlo (Promega, Mannheim, Germany) as per the manufacturer's instructions.

In Vitro Fibroblast and Epithelial Cell Assays

All fibroblast cell lines were propagated via serial passaging, and purity was assessed using morphological and immunohistochemical staining as previously described (46). Fibroblasts were grown and assayed in DMEM media, normal bronchial epithelial cells (NHBEs) were grown, and assays were performed using bronchial epithelial growth media (Cambrex, Lonza, Slough, UK). Fibroblasts or NHBEs were plated into 96-well plates (Costar, Corning, NY) at 1×10^5 cells/well and allowed to adhere for 8 hours. The cells were washed with PBS and cultured overnight in serum-free media containing 1% penicillin and 1% streptomycin at 37°C in 5% CO₂/air. Cells were stimulated with or without recombinant human IL-13 (R&D Systems, Minneapolis, MN). At the designated time points, supernatants were removed, and gene expression levels were quantitated using branched DNA technology (Panomics) as per the manufacturer's instructions. Gene expression fold induction was determined after calibration of IL13R α 2 expression with GAPDH and normalized to corresponding unstimulated cells.

Histologic Analysis

Formalin-fixed and paraffin-embedded lung sections were stained with hematoxylin and

eosin to assess gross morphology or Mallory's trichrome stains to visualize collagen deposition. A modified Ashcroft histopathology scoring scale was used to quantify fibrotic alterations by a reviewer unaware of the treatment groups (32). Briefly, the entire left lung lobe from each animal was scanned and reviewed for fibrotic alterations in the alveolar septa. The alterations varied from none (i.e., normal) to confluent fibrotic masses in at least 50% of the visible lung structure (Grade 5). Five to eight whole lung sections were scanned from each group of mice. Formalin-fixed and paraffin-embedded lung sections were analyzed for immunohistochemical localization of IL-13R α 2. These assessments are described in the online supplement.

Statistics

Normally distributed data were expressed as means $\pm$ SEM and assessed for significance by Student's *t* test or ANOVA as appropriate. Data that were not normally distributed were assessed for significance using the Wilcoxon rank sum test or Mann-Whitney U test. Patient demographics were compared using Student's *t* test or Mann-Whitney analysis. Categorical variables were compared using Fisher's exact test. *P* values were determined for multiple comparisons using the Bonferroni correction. Data were clustered using the absolute value of correlation coefficients (distance measure) with hierarchical clustering, thereby identifying the transcripts that were strongly related to

each other using the R version 2.13.0 program (47). The "Ward" method, which derives spherical clusters, was used as the agglomeration algorithm for forming clusters. Values of $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.005$ were considered significant.

Results

Overexpression of IL-13 and Not IL-4 in IPF

IPF is characterized by excess ECM deposition in the lung due in part to an inappropriate IL-13-driven fibrotic process (3). Analysis of biopsied lung tissue from patients with IPF ($n = 8$) and patients with a histologically fibrotic-dominant form of NSIP ($n = 4$) or a histologically cellular-dominant form of NSIP ($n = 3$) indicated that *il-13* gene expression was more highly expressed in IPF lung tissue (Figure 1A) and that *il-4* was most up-regulated in fibrotic NSIP (Figure 1B), in comparison to nonfibrotic control tissue. To further extend this observation, we confirmed the expression of IL-13 at the protein level in lung sections using immunohistochemical approaches. Biopsies from the following histologically verified lung diseases were assembled on a tissue microarray and examined concomitantly for the presence of IL-13 protein: respiratory bronchiolitis interstitial lung disease (Figures 2A and 2C), IPF with acute exacerbation (Figure 2B), normal lung tissue (Figure 2D), fibrotic NSIP (Figure 2E), and IPF (Figure 2F). Under higher magnification

($\times 200$), it was apparent that IL-13 was most abundantly expressed in the interstitial areas of the biopsies obtained from patients with IPF who exhibited an acute exacerbation (Figure 2H) or rapid progression of disease in the first year of follow-up after disease diagnosis (Figure 2K). Substantially less IL-13 was detected in normal and stable IPF biopsy samples. Together, these data suggested that IL-13 was expressed in IPF, particularly in severe forms of this disease.

Increased IL-13 Pathway Activation in Patients with IPF Who Rapidly Progress from the Time of Diagnosis

Hierarchical cluster analysis of multiple IL-13-associated and profibrotic mediators in IPF biopsies revealed that there was a statistically significant clustering of many of these mediators; notably, *ccl26*, *ccl2*, and *cc11* were strongly associated with *cecam5*, *gata3*, and *mmp7* (Figure 3A). More importantly, *chi3l1*, *periostin*, *stat6*, *fibronectin*, *il-13ra2*, and *il-4ra* strongly correlated with the clinical parameter "percent predicted FVC" (FVCPRED), although the relationship between these transcripts and FVCPRED were either negative (i.e., negative values) or positive (Figure 3A). In another cohort of patients with IPF in which disease progression was known, 11 IL-13-associated transcripts, including *cst2*, *cecam5*, *cpa3*, *cnrip1*, *mfsd2a*, *cdh26*, *gsn*, *dnajc12*, *ptgs1*, *flg*, and *chi3l2*, more abundantly expressed in biopsy samples from patients that exhibited

Figure 1. Elevated IL-13 transcript levels in the lungs of patients with idiopathic pulmonary fibrosis (IPF). (A, B) mRNA expression measured by quantitative RT-PCR in whole lung biopsy tissue from patients with IPF ($n = 8$) and from nonspecific interstitial pneumonia (NSIP)-fibrotic ($n = 4$) and NSIP-cellular ($n = 3$) patients and normalized to normal lung control tissue for *il-13* (A) and *il-4* (B).

Figure 2. Immunolocalization of IL-13 in interstitial lung diseases. Lung biopsy sections, taken from patients with interstitial lung disease at the time of diagnosis, were stained with anti-IL-13 to visualize the expression of IL-13. Consecutive sections were stained with IgG control (see Figure E1 in the online supplement). Pathologist-identified diseased sections from respiratory bronchiolitis interstitial lung disease (A, C), patients with IPF who experienced an acute exacerbation within 1 year of diagnosis (B and H), normal lung tissue (D and G), fibrotic NSIP (E), or IPF (F, I–K). (G–K) Representative histological sections from normal patients and patients with IPF are at $\times 200$ magnification.

a rapidly progressive disease compared with those who exhibited more stable disease (Figure 3B). Analysis of airway epithelial brushing samples indicated that these cells were unlikely to be the source of IL-13 because we did not detect transcripts for this mediator in any of the brushing samples (data not shown), but a subset of epithelial cells from patients with IPF was found to express IL-13-associated targets, including *il-4ra*, *il-13ra1*, *gata3*, and *mmp7* (Figure 3C). In contrast, primary human fibroblasts cultured from patients with IPF, particularly those who showed a rapid course of disease, appeared to be one source of IL-13 in the lung (Figure 3D), whereas normal human lung fibroblasts did not spontaneously release IL-13 (data not

shown). Likewise, analysis of plasma from patients with IPF revealed that the IL-13-inducible chemokines CCL2 (Figure 3E) and CCL11 (Figure 3F) were significantly increased in the rapid form of IPF compared with age-matched healthy control subjects or stable patients with IPF. Finally, in this rapid cohort, an inverse correlation between plasma IL-13 levels and event time from diagnosis was observed (Figure 3G). The assessment of the relationship between lung *il-13ra2* and FVC indicated an inverse correlation where patients that had worse lung function had increased *il-13ra2* (Figure 3H). Moreover, lung *il-13ra2* positively correlated with lung *col3a1* expression, suggesting patients with greater matrix and fibrosis having elevated

il-13ra2 expression (Figure 3I). Together, these data confirmed that there is a strong IL-13 signal in IPF whole lung biopsies and plasma, particularly in patients who exhibited a rapidly progressive form of this disease.

IL-13 Directly Up-Regulates IL-13R α 2 in IPF Fibroblasts and Lung Epithelial Cells

To determine the direct effects of IL-13 on IL-13R α 2 expression, IPF fibroblasts or NHBEs were stimulated with IL-13, and *il-13ra2* gene expression and protein expression were assessed (Figure 4). Fibroblasts from patients with IPF were stimulated with increasing concentrations of IL-13 for 24 hours, and elevated *il-13ra2*

Figure 3. Elevated IL-13 pathway activation in the lungs and circulation, more closely associated with rapidly progressing IPF. (A) Correlations in levels of mRNA expression between IL-13 pathway genes and fibrosis markers in IPF lung biopsies ($n = 62$). Lighter colors represent greater correlations between genes. The larger value indicates a stronger the correlation. Negative values represent a negative correlation. (B) Increased IL-13 pathway gene expression, as measured by RT-PCR, with rapidly progressing IPF and stabling progressive IPF lung biopsies in comparison to normal healthy lung tissue. (C) Increased *il4r*, *il13ra1*, *gata3*, and *mmp7* in epithelial cell brushings from a subset of patients with IPF as measured by RT-PCR. (D) Spontaneous release of IL-13 from fibroblasts isolated from the lung biopsies of rapidly progressive patients with IPF. IL-13 was measured using specific ELISA. Data are mean $\pm$ SEM from four different patients with IPF. (E, F) Circulating CCL2/MCP-1 (E) and CCL11/eotaxin (F) were measured in the plasma of stable ($n = 19$) and rapid ($n = 14$) patients with IPF and healthy age-matched normal control subjects ($n = 20$). (G) Correlation of plasma IL-13 levels and the time of the first clinical event postdiagnosis in the rapid IPF patient subgroup. Correlation of lung tissue *il-13ra2* transcript expression and FVC % predicted (H) and collagen 3a1 (I) determined by RT-PCR. Each data point represents an individual patient. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.005$.

gene expression was detected by branched DNA technology (and presented as fold increase above unstimulated controls) (Figure 4A), which was confirmed and quantitated at the protein level via immunolocalization of IL13R α 2 (Figures 4B–4F). NHBEs were also stimulated with IL-13 for 24 hours, and *il-13ra2* gene induction was quantitated using branched DNA technology (Figure 4G). There were no direct effects of IL-13 on epithelial

cell viability or apoptosis observed in the *in vitro* cultures (data not shown).

Immunoneutralization of Human IL-13 with Talokinumab Therapeutically Inhibits the Pulmonary Fibrotic Response in a Humanized SCID Model

A humanized SCID mouse model in which intravenously injected IPF fibroblasts engraft in the lung and promote aberrant

ECM deposition (30) was used to explore the role of human IL-13 in the progression of pulmonary fibrosis. Lung-engrafted fibroblasts also increased mouse *il-13*, *il-13ra1*, and *il-13ra2* transcript levels in a time-dependent manner in SCID mouse whole lung samples (Figures 5A–5C). To determine whether the IL-13 generated by human fibroblasts contributed to the lung remodeling, SCID mice were treated with talokinumab from Days 35 to 63 after

Figure 4. IL-13 stimulation of IPF fibroblasts or epithelial cells results in an up-regulation of IL-13Rα2. (A–F) Fibroblasts from patients with IPF were stimulated with increasing concentrations of IL-13 for 24 hours. Supernatants were taken for ELISA analysis, whereas cell monolayers were lysed and assessed for changes in gene expression compared with unstimulated controls using branched DNA technology (Panomics; A) or IL-13Rα2 immunolocalized using anti-IL-13Rα2 staining and quantitated (Cellomics; B–F). Lung epithelial cells were stimulated with IL-13 for 24 hours, and *il-13ra2* expression was determined using branched DNA technology (G). For fibroblast studies, data shown are mean ± SEM from four different donors (gene) and are representative of one individual donor for IL-13Rα2 surface expression (lower graph, pictures). Data are mean ± SEM. **P* < 0.05 and ***P* < 0.01 significance in comparison to unstimulated controls.

the injection of the human fibroblasts from a patient who showed rapidly progressive disease (Figures 5D–5M). Although human IL-13 can signal via murine IL-13 receptors (31), tralokinumab only targets human IL-13 and has no neutralizing capacity on mouse IL-13 (31), indicating that these data are a consequence of inhibiting IPF fibroblast-derived human IL-13. At Day 63 after human fibroblast administration, the immunoneutralization of IL-13 reduced alveolar wall, airway epithelium, and basement membrane thickening, as observed in trichrome-stained lung sections (Figures 5D–5F). Moreover, fibrotic alterations were independently quantified using a modified Ashcroft histopathology scoring scale (32), and tralokinumab-treated mice exhibited significantly decreased fibrosis in comparison to PBS- or isotype control-treated SCID mice at Day 63 after the injection of human fibroblasts (Figure 5G). Tralokinumab also reduced serum CC16 (i.e., a marker of epithelial injury) (Figure 5H) and reduced BAL caspase 3 activity (Figure 5I) in this model. Next, the expression of IL-13Rα2 was determined via immunohistochemical analysis of whole lung samples from SCID mice at Day 63. This analysis demonstrated

that there was a marked reduction of the intensity of staining in the airway epithelium of tralokinumab-treated mice when compared with both control groups (Figures 5J–5M). Further examination of lung tissue by quantitative PCR analysis showed that there was no difference in human vimentin across the three SCID groups (Figure 6A), indicating that the beneficial effects induced by blocking IL-13 were not due to an alteration in the total number of fibroblasts in the lung. Moreover, there was no difference in the transcript levels of TGF-β₁ in SCID mice treated with tralokinumab, control IgG, or PBS (Figure 6B). In contrast, tralokinumab treatment enhanced the expression of epithelial-associated genes, including E-cadherin and all of the surfactants, compared with the PBS control-treated SCID mice (Figures 6C–6G). Together, these data demonstrate that human IL-13 promotes lung fibrosis in the humanized SCID model.

Discussion

IL-13 has emerged as a potent mediator of tissue fibrosis in experimental and clinical

fibrotic lung disease settings, indicating that it is a key regulator of ECM generation (14, 33). Controversy surrounds the activity of the second α subunit of the IL-13 receptor (whether stimulatory [23] or inhibitory [34]) in mediating cell activation and the TGF-β dependency of this cytokine during the fibrotic process (11, 22, 24, 26). Herein, we confirm published findings (19–21) that most extracellular and intracellular components of the IL-13 pathway show increased expression in lung biopsies from patients with IPF but present the novel finding that the strength of IL-13 signal in lung and blood samples correlates with rapidly progressive IPF. The testing of IL-13 targeting approaches in pulmonary fibrosis has been largely limited to experimental approaches targeting murine IL-13 (7), whereas the data presented herein are among the first to show that targeting fibroblast-derived human IL-13 with a novel human mAb, tralokinumab, attenuated lung fibrosis and the accumulation of ECM in a humanized SCID model of IPF. In this IPF model, the targeting of human IL-13 led to improvements in the expression of transcripts associated with healthy lung epithelium, as indicated by reduced lung

Figure 5. Neutralization of fibroblast-derived IL-13 inhibits lung fibrosis in an established fibroblast-driven disease setting in humanized SCID mice. (A–C) Isolated IPF fibroblasts ($n = 5$) or normal lung tissue fibroblasts ($n = 5$) were injected intravenously to SCID mice, and animals were killed at specific subsequent time points. Whole lung mRNA expression was measured by quantitative RT-PCR for *il-13* (A), *il13ra1* (B), and *il-13ra2* (C) and normalized to lung mRNA expression levels from mice that had received normal lung tissue-derived fibroblasts. (D–N) Thirty-five days after IPF fibroblast engraftment, mice were randomized and treated with PBS control, control IgG4, or tralokinumab (anti-IL-13 mAb) every other day until analysis at Day 63. Representative mouse lung sections stained with Masson's trichrome to depict the degree of fibrosis at Day 63 from SCID/IPF mice treated with PBS control (D), CAT251 IgG₄ isotype control (E), and tralokinumab anti-IL-13 antibody (F). Fibrosis was quantitated in all animals using a modified Ashcroft score of histological sections (G). Tralokinumab attenuated serum CC16 levels as measured by ELISA (H) and BAL caspase 3 activity (I). Data are mean $\pm$ SEM ($n = 5$). (J–M) Representative immunohistochemistry localizing IL-13Rα2 expression in the lungs of control SCID mice that have not received IPF fibroblast (J) or SCID/IPF mice treated with PBS control (K), isotype control (L), or tralokinumab (M). Original magnification: top panels, $\times 40$; bottom panels, $\times 200$. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.005$.

Figure 6. Enhanced epithelial markers in the lungs of SCID/IPF mice treated with tralokinumab. Whole lung mRNA expression from mice treated with isotype control or tralokinumab (anti-IL-13) was measured using quantitative RT-PCR. Levels of vimentin (A), TGF-β1 (B), surfactant protein a (C), surfactant protein b (D), surfactant protein c (E), surfactant protein d (F), and e-cadherin (G) were quantitated and normalized to lung mRNA expression levels from SCID/IPF mice that had received PBS control only. Data are mean ± SEM ($n = 5$). * $P < 0.05$.

apoptosis and markers of epithelial injury. Thus, the results from this study demonstrate that IL-13 is elevated in and contributes to IPF, perhaps leading to the rapid progression of this disease due to its inhibitory effects on lung repair mechanisms.

Patients with IPF experience a gradual worsening of symptoms (2, 6) or a rapid disease course punctuated by acute exacerbations (6), which highlights the heterogeneity in the clinical course of this disease (35, 36). When patients exhibit physiologic decline or a clinical event in the first year of follow-up, they are considered to have “rapid progression” (36). Although longitudinal changes in lung physiology (i.e., FVCPPRED) have been extensively studied as a surrogate of lung

fibrosis (37), these measurements provide no information about biological pathways that might be driving lung fibrosis in each patient. Recent studies have suggested that the IL-13 pathway might predict progression in IPF. For example, elevated levels of periostin, an IL-13-inducible matricellular protein, have been recently described to be associated with rapidly progressive forms of IPF (38). Experimentally, periostin promotes myofibroblast differentiation and contributes to bleomycin-induced lung fibrosis (39). From the present study, there was a strong inverse correlation between FVCPPRED and many IL-13-induced factors, including *chi3L1*, *stat6*, *fn1*, *il13ra2*, and *il4ra* in IPF. It was also evident that additional IL-13-related factors,

including *cst2*, *gsn*, and *cdh26*, were significantly enhanced in biopsy samples from patients who exhibited a rapidly progressing form of IPF compared with those patients with IPF with a slower rate of lung function loss. Specifically, gelsolin (*gsn*) is an actin-regulating protein that has been shown to contribute to cardiac remodeling after experimental myocardial infarction (40), but its role in pulmonary fibrosis requires further investigation. Other IL-13-inducible factors, including profibrotic CCL2 (18) and CCL11 (41), were detected at elevated levels in the circulation of patients with rapidly progressive disease compared with normal lung biopsies or with biopsies from patients who exhibited a more stable course of disease. Although freshly isolated

epithelial cells from patients with IPF did not appear to be a source of IL-13, these cells appeared to be affected by this cytokine on the basis of the increased *stat6* expression status in these cells compared with normal epithelial cells. Surprisingly, a prominent source of IL-13 appeared to be stromal fibroblasts on the basis of our survey of histological lung biopsy samples and cultured primary lung fibroblasts. Thus, IL-13 and several of its induced factors were prominently expressed in IPF, particularly in a subset of these patients who exhibited a rapid progression from the time of diagnosis.

The discovery that human IPF fibroblasts generate IL-13 allowed for an *in vivo* study directed at the role of this cytokine in a humanized SCID model of IPF (30, 42). An antibody-based approach was used in this model using a recombinant human IgG4, tralokinumab, which blocks human IL-13. The administration of tralokinumab at Day 35 after the injection of human IPF fibroblasts and the establishment of fibrosis in the lungs of SCID mice (42) led to a significant reduction in quantifiable histological (i.e., reduced alveolar wall thickening, airway epithelium thickness, and basement membrane thickness), circulating (i.e., serum CC16), and lung-associated

(i.e., reduced BAL caspase 3 activity) markers of lung fibrosis compared with PBS- or control IgG4-treated SCID mice that received the same IPF fibroblasts. Taken together, these data indicate that tralokinumab reduced lung fibrosis by inhibiting the profibrotic pathway evoked by IPF fibroblast-derived human IL-13 in a SCID IPF model, leading to improved lung repair. The improved epithelial appearance and reduced apoptosis observed in the tralokinumab-treated humanized SCID mice did not appear to be a direct consequence of IL-13 on the mouse airway epithelium because we observed no proapoptotic effects of IL-13 on the epithelium *in vitro*. However, fibroblasts isolated from fibrotic lung tissue have been shown to promote epithelial cell apoptosis (43), so it is plausible that IL-13 may be acting to modulate the levels of antiapoptotic factors *in vivo*. For example, prostaglandin E₂ has been shown to attenuate epithelial–fibroblast profibrotic responses (44), and dinoprostone protects epithelial cells from apoptosis while promoting fibroblast apoptosis (45). In addition, there may also be a wider role for IL-13 in patients with IPF because fibroblasts are not the only source of IL-13 in IPF, and other cells, including T and B cells, appear to be involved in the

pathogenesis of this disease. This is one of the limitations of using a SCID model, in that T and B cells are absent. However, on the basis of our immunolocalization studies in our *in vivo* model, fibroblasts appear to also be a source of this profibrotic cytokine, and blocking IL-13 with tralokinumab had a significant antifibrotic effect in this humanized SCID system. Thus, further assessment of the link between IL-13 and epithelial protective factors, including prostaglandins, in this model is warranted in future studies.

In conclusion, the present study demonstrates that IL-13 contributes to the progression of IPF via its profibrotic and antirepair effects in the lung. The strong correlation between the IL-13 pathway and rapidly progressing IPF is of considerable clinical importance given that predicting rapid progression should allow for timely referral of eligible patients for clinical therapeutic trials or for lung transplantation. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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