Effects of Interferon-γ 1b on Biomarker Expression in Patients with Idiopathic Pulmonary Fibrosis

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In a recent study of IFN-γ 1b in 330 patients with idiopathic pulmonary fibrosis (IPF), progression-free survival was unchanged; however, a trend toward lower mortality was seen in IFN-γ 1b–treated patients compared with placebo-treated patients (9.9 vs. 16.7%; $p = 0.08$). The purpose of this randomized, double-blind, placebo-controlled trial was to characterize molecular effects of subcutaneous IFN-γ 1b (200 μg) thrice weekly for 6 months versus placebo in 32 patients with IPF. Messenger RNA in transbronchial lung biopsies and bronchoalveolar lavage cell pellet and protein levels in bronchoalveolar lavage fluid (BALF) and plasma were evaluated. After IFN-γ 1b treatment, IFN-inducible T cell-α chemoattractant/CXCL11 (a chemokine with immunomodulatory, antiangiogenic, and defensin-like antimicrobial properties) increased in BALF ($p = 0.016$) and plasma ($p < 0.001$); BALF levels of epithelial neutrophil-activating protein-78/CXCL5 ($p = 0.054$), platelet-derived growth factor A ($p = 0.033$), and Type I procollagen ($p = 0.096$) were lower; and IFN-γ levels were higher ($p = 0.093$) versus placebo. For messenger RNA in transbronchial biopsies, trends ($p > 0.05$ and ≤ 0.10) associated with IFN-γ 1b treatment included an increase in IFN-inducible T cell-α chemoattractant/CXCL11, a decrease in elastin, and smaller increases for Type III procollagen and platelet-derived growth factor B. Changes in biomarkers of fibrosis, angiogenesis, proliferation, immunomodulation, and antimicrobial activity suggest that IFN-γ 1b may affect IPF through multiple pathways.

Keywords: IFN-γ 1b; proteins; pulmonary fibrosis; RNA

IFN-γ 1b, a pleiotropic cytokine highly homologous with natural IFN-γ, has antimicrobial, antifibrotic, antiproliferative, and immunomodulatory properties and has been shown to decrease the frequency and severity of infection in patients with chronic granulomatous disease (1) and to delay the progression of malignant osteopetrosis (2).

In a recent randomized, double-blind, placebo-controlled study of 330 patients with idiopathic pulmonary fibrosis (IPF), IFN-γ 1b had no effect on the primary endpoint of progression-free survival; however, there was a trend toward decreased mortality in patients treated with IFN-γ 1b (9.9 vs. 16.7%; $p = 0.08$) with a 41% relative reduction in the risk of death (hazard ratio = 0.6; 95% confidence interval = 0.3–1.1) (3). The mechanism by which survival may be prolonged is not clear.

IPF is characterized by insidious onset of dyspnea and abnormal pulmonary function (4, 5) and a median survival of only 2.5–3.5 years after diagnosis (6). The etiology is unknown, and no therapy has been proved to be beneficial. Histopathologic examination reveals temporal heterogeneity of alternating zones of interstitial fibrosis with fibroblastic foci (i.e., newer fibrosis), inflammation, honeycomb changes (i.e., older fibrosis), and normal lung architecture (4–7). Abnormal levels of angiogenic and angiostatic chemokines (8) and aberrant vascular remodeling are found in conjunction with the fibrotic process (9, 10). A possible pathogenic role for pulmonary infection related to viruses and atypical bacteria has been suggested (5, 11).

Molecular, cellular, and whole-animal studies have identified pathways involved in fibrosis and the production and deposition in the lung of extracellular matrix proteins (e.g., procollagens and elastin). These pathways include growth factors, such as transforming growth factor-β (TGF-β), connective tissue growth factor (CTGF), platelet-derived growth factors (PDGF), and cytokines and chemokines associated with inflammation, cellular trafficking, angiogenesis, and immunity (12, 13).

In preclinical studies, IFN-γ affects molecules associated with fibrosis and infection or their effects in the following ways: (*1*) downregulation of extracellular matrix proteins, (14–16) TGF-β (17), PDGF (18), epithelial neutrophil-activating protein (ENA-78/CXCL5) (19), interleukin (IL)-8 (IL-8/CXCL8) (19), IL-4 (20), and IL-13 (20); and (*2*) upregulation of defensins (21) and IFN-inducible CXC chemokines (monokine induced by IFN-γ [MIG/CXCL9] [22], IP-10[IFN-γ–inducible protein-10]/CXCL10 [22], and IFN-inducible T cell-α chemoattractant [ITAC/CXCL-11] [22, 23]).

We designed this randomized, double-blind, placebo-controlled, multicenter study to characterize the effects of IFN-γ 1b on biologic markers associated with fibrosis, aberrant vascular remodeling, and immunomodulatory and antimicrobial activity in the lungs and plasma of humans with IPF.

METHODS

Patients and Study Design

After giving written informed consent, 32 patients were enrolled at 15 centers in the United States and randomly assigned (1:1) to receive either 200 μg (100 μg for the first 2 weeks) subcutaneous IFN-γ 1b (n = 17)

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or matching placebo (n = 15) thrice weekly for 26 weeks. The principles of the Declaration of Helsinki as endorsed by the U.S. Food and Drug Administration were observed. Patients, age 20 through 79 years, were required to have an FVC of between 50% and less than 90% predicted, confirmation by high-resolution computed tomography and tissue diagnosis, and to be worsening. Patients were required to show lack of improvement after steroids and had to be able and willing to take prednisone 10 mg for at least 21 days before the first bronchoscopy and until the end of study treatment. A complete list of patient inclusion criteria is presented in the online supplement.

Bronchoalveolar lavage (BAL) and transbronchial biopsy (TBB) were performed at baseline and at 6 months (weeks 23–24) to determine messenger RNA (mRNA) transcription in TBB tissue (3 biopsies each from the right, middle, and lower lobes) and BAL cell pellet, as well as protein levels in BAL fluid (BALF) and plasma. Sample collection and initial processing techniques for all centers were standardized by training of site personnel and providing them written instructions for sample preparation, handling, and shipping to the central laboratory at the University of California at Los Angeles. The central laboratory followed standardized procedures for final processing and analysis of all specimens. Prospectively defined biologic markers of growth, fibrosis, angiogenesis, angiostasis, and antimicrobial activity anticipated to be responsive to IFN-γ 1b (*see* tables) were assessed in TBB tissue, BAL cell pellet, BALF, and plasma. Expression of mRNA was measured by TaqMan quantitative real-time polymerase chain reaction (PCR) using the ABI Prism Analyzer (Applied Biosystems, Foster City, CA). Proteins, except the defensins, which were measured by bioassay, were measured by specific ELISA; detail is provided in the online supplement. Other endpoints including FVC % predicted, resting arterial blood gas assessment (room air) of alveolar–arterial oxygen gradient, percent predicted diffusion of carbon monoxide, dyspnea, highest oxygen use, and the maximal oxygen flow rate during each month were measured at baseline and at 6 months, in the week before the second bronchoscopy (week 22–23), and at intervening visits (i.e., at weeks 2, 4, 8, 12, 16, and 20). Distance walked in the 6-minute walk test was an exploratory endpoint. Safety was assessed by adverse event monitoring and clinical laboratory tests (hematology, chemistry, liver function, thyroid function, and urinalysis).

Statistical Analysis

All patients who received at least 80% of the scheduled study drug doses were included in the endpoint analyses. The primary endpoint was the change from baseline to 6 months in the level of mRNA transcription for TGF-β and CTGF in TBB tissue (Table 1), adjusted by a stable, constitutively expressed gene, the housekeeping gene GAPDH. Samples that did not contain adequate levels of the housekeeping gene were excluded from the mRNA analyses. Change from baseline was represented by relative expression (RE) calculations as follows: $RE = 2^{(\Delta Ct[pre] - \Delta Ct[post])}$, where ΔCt = FAM – VIC. FAM represents the threshold cycle at which the target gene was detected and VIC represents the threshold cycle at which the housekeeping gene was detected. RE is expressed in reference to the baseline amount: a value of RE greater than 1 indicates that more of the target gene was expressed after treatment than before treatment; a value of RE less than 1 indicates that less of the target gene was expressed after treatment than before treatment; and an RE value of 1 indicates no change from baseline. The sample size of 15 patients per group provided more that 90% power to detect a difference of three levels of mRNA transcription for TGF-β and CTGF, adjusted for the housekeeping gene and represented by relative expression calculations, between the IFN-γ and the placebo groups, assuming a standard deviation of 1.5 levels.

Details of the statistical analysis are provided in the online supplement.

RESULTS

The first patient enrolled began treatment in January 2002 and the last finished the double-blinded treatment period in October 2002. The majority of patients were male (70.6% in the IFN-γ 1b group and 53.3% in the placebo group) and white (94.1 and 86.7% in the IFN-γ 1b the placebo groups, respectively). Mean age was 64.1 years (range, 51–78 years) and 63.0 years (range, 52–75 years) in the respective groups. Clinical and pulmonary function parameters were similar across the groups at the time of study entry except for longer distance walked in the 6-minute walk test in the IFN-γ 1b group (mean 423.1 m vs. 340.0 m, p = 0.035, *t* test) (Table 2). All patients received more than 80% of scheduled doses and were included in all analyses. Of 32 patients enrolled (17 IFN-γ 1b, 15 placebo), 2 (both placebo recipients) discontinued treatment prematurely for reasons unrelated to the study drug (fatal pneumonia, non–small cell carcinoma). IFN-γ 1b and placebo recipients took a mean 98.45% (SD, ± 2.020) and 98.09% (SD, ± 5.209) of planned study medication, respectively. At baseline, mRNA was found in the TBB tissue and BAL cell pellet of at least 80% of patients with adequate housekeeping gene for all biomarkers except IL-4 and IL-13 in TBB tissue (found in 30% and 37%, respectively), IL-4 and IL-13 in BAL cell pellet (found in 13% and 19%, respectively), and elastin in BAL cell pellet (found in 55%).

At 6 months, mRNA RE did not differ significantly between the study groups for all biomarkers measured in TBB tissue, including the primary endpoint markers of TGF-β and CTGF, or in BAL cell pellet (Table 1). However, a trend toward a statistically significant difference ($p > 0.05$ and ≤ 0.10) was seen in TBB tissue mRNA RE in the expected directions for elastin, ITAC/CXCL11, PDGF-B, and Type III procollagen (Table 1). Elastin mRNA RE decreased in the active treatment group but increased in the placebo group (median 0.680 vs. 1.535, p = 0.054, by analysis of covariance [ANCOVA]). Lesser increases occurred in the active group than in the placebo group in mRNA RE for PDGF-B (1.100 vs. 1.520, p = 0.085, ANCOVA) and Type III procollagen (1.530 vs. 1.800, p = 0.092, ANCOVA). Expression of ITAC/CXCL11 increased in the active group but decreased in the placebo group (median 1.560 vs. 0.610, p = 0.063, ANCOVA) (Figure 1).

In the categorical analyses of TBB tissue and BAL cell pellet, only two findings, both in TBB tissue, were statistically significant or trending toward significance. There was a significant difference between the treatment groups in the expression of IL-4 level, with more patients (45.5%) having an increase in the placebo group than in the active group (5.9%) (p = 0.049, Wilcoxon rank-sum test). A trend toward downregulation in SMAD-7 (p = 0.084, Wilcoxon rank-sum test) was contrary to the expected result: among IFN-γ 1b recipients, SMAD-7 decreased in 70.6% (12/17) and increased in 29.4% (5/17), whereas among placebo recipients, SMAD-7 decreased in 36.4% (4/11) and increased in 63.6% (7/11).

Examination of protein levels with the Wilcoxon rank-sum test revealed statistically significant differences between the treatment groups in ITAC/CXCL11 in both BALF (p = 0.016) and plasma ($p < 0.001$), and in PDGF-A in BALF (p = 0.033) (Table 3). In BALF, ITAC/CXCL11 increased from a baseline median of 0 pg/ml to 450 pg/ml at 6 months in the IFN-γ 1b group, but did not change from the baseline median of 0 pg/ml in the placebo group. In plasma, ITAC/CXCL11 rose from a median 0 pg/ml at baseline to 8,150 pg/ml at 6 months in the active drug group, and again the median did not change from the baseline level of 0 pg/ml in the placebo group. These changes were consistent with the upregulation of ITAC/CXCL11 anticipated in association with IFN-γ 1b treatment. In addition, levels of ENA-78/CXCL5 (p = 0.054) and Type I procollagen (p = 0.096) decreased, and levels of IFN-γ (p = 0.093) increased. These changes were consistent with the changes expected in association with IFN-γ 1b treatment. All other proteins measured in BALF and plasma did not differ significantly between treatment groups. All levels for IL-4 in BALF and plasma were below the level of assay detection.

TABLE 1. MEDIAN RELATIVE EXPRESSION OF MESSENGER RNA OF BIOLOGIC MARKERS IN TRANSBRONCHIAL BIOPSY TISSUE AND BROCHOALVEOLAR LAVAGE CELL PELLET FOR CHANGE FROM BASELINE TO THE 6-MONTH ENDPOINT

	Transbronchial Biopsy					BAL Cell Pellet				
	IFN-γ 1b		Placebo			IFN-γ 1b		Placebo		
Biomarker	n	RE*	n	RE*	p Value†	n	RE*	n	RE*	p Value‡
TGF-β	17	1.300	9	1.420	0.811	17	1.100	12	1.380	0.586
CTGF	17	1.250	11	0.620	0.433	17	1.040	12	0.595	0.371
Type I procollagen	17	0.740	11	1.790	0.754	17	0.760	12	0.340	0.705
Type III procollagen	15	1.530	11	1.800	**0.092**	16	1.495	12	3.360	0.763
Elastin	16	0.680	10	1.535	**0.054**	17	0.370	12	2.055	1.000
PDGF-A	17	0.730	11	1.000	0.109	17	2.880	12	0.695	0.877
PDGF-B	17	1.100	11	1.520	**0.085**	17	1.510	11	1.250	0.646
PDGF-D	17	0.910	11	0.870	0.354	17	0.900	11	1.830	0.940
IL-8/CXCL8	15	0.950	11	0.600	0.343	17	0.150	12	0.560	0.191
ENA-78/CXCL5	17	1.670	11	1.010	0.814	17	1.030	12	1.425	0.254
IP-10/CXCL10	17	1.240	11	0.820	0.903	17	0.250	12	0.585	0.735
MIG/CXCL9	16	1.040	11	0.970	0.522	17	0.480	12	0.525	0.934
ITAC/CXCL11	15	1.560	11	0.610	**0.063**	17	0.240	12	0.385	0.688
MDC/CCL22	17	0.870	11	1.030	0.190	17	0.950	12	1.485	0.401
MIP-1δ/CCL15	17	0.640	11	0.470	0.315	17	0.730	12	0.990	0.881
IL-4	17	2.200	11	1.870	0.202	17	0.970	12	4.420	0.186
IL-13	17	1.070	11	0.070	0.425	17	1.930	12	2.260	0.902
IFN-γ	14	0.610	10	1.670	0.164	17	0.090	12	0.635	0.401
SMAD-7	17	0.910	11	1.410	0.218	17	1.430	11	1.220	0.954

Definition of abbreviations: BAL = bronchoalveolar lavage; CTGF = connective tissue growth factor; ENA = epithelial neutrophil–activating protein; IL = interleukin; IP-10 = IFN-γ–inducible protein-10; ITAC = IFN-inducible T cell-α chemoattractant; MDC = macrophage-derived chemokine; MIG = monokine induced by IFN-γ; MIP-1δ = macrophage inflammatory protein-1δ; PDGF = platelet-derived growth factors; RE = relative expression; TGF = transforming growth factor.

The numbers (n) represent those samples with adequate levels of housekeeping gene.

p Values representing trends ($p \leq 0.1$) are shown in bold.

* $RE = 2^{(\Delta Ct[pre] - \Delta Ct[post])}$. A value of $RE > 1$ indicates that more target gene was expressed after treatment than before; a value of $RE < 1$ indicates that less target gene was expressed after treatment than before; and an RE value of 1 indicates no change from baseline.

† Wilcoxon rank-sum test for ENA-78/CXCL5; analysis of covariance (ANCOVA) applied to $\log_{10}$-transformed RE (with classification effect for treatment and baseline $\log_{10}$ ΔCt[pre] as covariate) for other markers.

‡ Wilcoxon rank-sum test for elastin and IL-8; analysis of variance with classification effect for treatment for CTGF, ENA-78/CXCL5, MDC/CCL22, and TGF-β; ANCOVA (*see* note above) for other markers.

For the clinical measures, statistical significance was approached in favor of the IFN-γ 1b group for alveolar–arterial gradient (mean change from baseline of −0.3 vs. 6.0 mm Hg, p = 0.054, ANCOVA) and for FEV_1 in liters (mean change from baseline of 0.014 vs. −0.128, p = 0.067, ANCOVA). No other changes from baseline to the 6-month endpoint were statistically significant or showed a trend toward significance for functional tests, dyspnea, oxygen use, or distance walked during the 6-minute walk test, and except for alveolar–arterial gradient and FEV_1, changes in the functional and pulmonary test results over this 6-month period were clinically minimal (Table 4).

Treatment-emergent adverse events were reported by most patients in both treatment groups (94.1% in the IFN-γ 1b group and 86.7% in the placebo group). More patients had constitutional symptoms in the IFN-γ 1b group than in the placebo group, including headache (47.1 vs. 20.0%), pyrexia (35.3 vs. 6.7%), and fatigue (35.3 vs. 13.3%). The number of patients with serious adverse events was lower in the IFN-γ 1b group (5.9%, 1 event in 1 patient) than in the placebo group (40.0%, 11 events in 6 patients). One placebo patient with pneumonia died.

No clinically significant differences between the treatment groups were seen in changes in total BAL cell count or changes in percentage of alveolar macrophages, lymphocytes, or neutrophils between baseline and 6 months (Table 5). Most treatment-emergent laboratory toxicities were mild or moderate in severity; no laboratory toxicity was cited as a serious adverse event and none required discontinuation of the study treatment.

DISCUSSION

This is the first study in humans to characterize the effects of IFN-γ 1b on blood and lung biomarkers, hypothesized to play a central role in the pathogenesis of IPF. Our results are consistent with extensive preclinical data indicating that IFN-γ downregulates molecules associated with fibrosis, proliferation, and inflammation, and upregulates molecules associated with antimicrobial defense and antiangiogenesis (14–24). These findings may provide a basis for the recent results from a large clinical trial in which a trend toward prolonged survival time was observed in patients treated with IFN-γ 1b (3).

Using the extremely sensitive TaqMan real-time PCR to measure mRNA expression, we found that most of the molecules we examined were expressed before treatment in these IPF patients. Only mRNA for IL-4 and IL-13 in lung tissue and BAL cell pellet and elastin in BAL cell pellet were not detected in most patients.

Based on the report by Ziesche and colleagues (25), the primary hypothesis for this study was that IFN-γ 1b would downregulate mRNA for TGF-β or CTGF. In contrast to the finding of Ziesche and colleagues (25), we did not detect a direct effect of IFN-γ 1b on mRNA for TGF-β or CTGF. To our knowledge, the Ziesche study is the only study to report an IFN-γ 1b–mediated downregulation of these molecules in humans. Lack of effect on transcription, however, does not negate preclinical data showing that IFN-γ modifies TGF-β effects through other pathways. Preclinical studies have provided considerable evidence of the antifibrotic actions of IFN-γ, including direct inhibition of

TABLE 2. BASELINE CHARACTERISTICS

Characteristic	IFN-γ 1b (*n = 17*)	Placebo (*n = 15*)	p Value†
Age, yr			
51–60	5 (29.4%)	7 (46.7%)	0.698
61–70	7 (41.2%)	5 (33.3%)	—
71–80	5 (29.4%)	3 (20.0%)	—
Mean (SD)	64.1 (7.54)	63.0 (8.60)	—
Median	64.0	63.0	—
Range	51–78	52–75	—
Sex			
Male	12 (70.6%)	8 (53.3%)	0.467
Female	5 (29.4%)	7 (46.7%)	—
Race			
White	16 (94.1%)	13 (86.7%)	0.212
Asian	1 (5.9%)	0	—
Hispanic/Latino	0	2 (13.3%)	—
Days from IPF diagnosis*			
Mean (SD)	340.1 (261.02)	377.1 (396.01)	0.680
Median	310.5	268.0	—
Range	97–1135	72–1482	—
A-a gradient (mm Hg)			
Mean (SD)	19.974 (9.4954)	20.814 (9.9562)	0.809
Median	17.770	20.520	—
Range	5.82–39.90	3.53–41.85	—
FVC % predicted			
Mean (SD)	67.0 (12.21)	67.6 (12.04)	0.890
Median	70.0	67.0	—
Range	51–85	51–93	—
D_{LCO} % predicted			
Mean (SD)	43.8 (9.16)	40.1 (10.80)	0.312
Median	43.0	39.0	—
Range	31–61	28–65	—
6-minute walk test, m			
Mean (SD)	423.1 (119.70)	340.0 (87.99)	0.035
Median	437.0	324.0	—
Range	227–690	199–511	—

Definition of abbreviations: A-a = alveolar–arterial; D_{LCO} = diffusing capacity of carbon monoxide; IPF = idiopathic pulmonary fibrosis.

All randomized patients, n = 32.

* n = 14 for IFN-γ 1b; n = 13 for placebo.

† *t*-Test for continuous data, Wilcoxon rank-sum test for time-to-event variables, and Fisher's exact test for categorical variables.

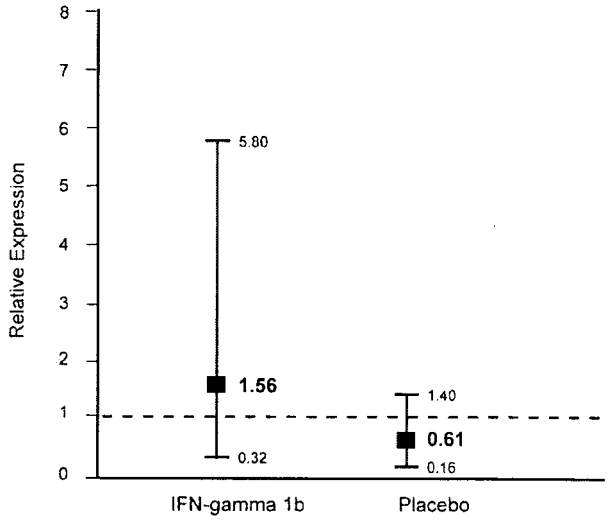

Figure 1. Relative expression (at 6 months compared with baseline) of CXCL11 messenger RNA in transbronchial biopsy tissue (medians, ± 25th and 75th percentile).

proliferation of fibroblasts (14); inhibition of collagen synthesis by cultured human lung fibroblasts *in vitro* (14, 15); reversal of stimulation of collagen gene expression by TGF-β in normal human fibroblasts (17); and inhibition of signaling, and thereby gene activation, by TGF-β (24). In the bleomycin model of pulmonary fibrosis, IFN-γ appeared to decrease the number of collagen fibers (16, 26) and significantly decreased both the total hydroxyproline content of the lungs (16, 26) and the number of fibroblasts (26). Thus, downregulation of the fibrotic process by IFN-γ 1b may occur both directly and indirectly by modification of the fibroblast response to TGF-β. It is also possible that lesser degrees of change may occur than were detectable in this study.

In this study, we found statistically significant differences ($p \leq 0.05$) or trends ($p > 0.05$ and ≤ 0.10) in changes in biomarkers of fibrosis consistently in the direction expected given the proposed effects of IFN-γ 1b based on preclinical data. These biomarker results were secondary endpoints, and the p values were not adjusted for multiplicity; thus, these results should be considered as information for generating hypotheses for future research rather than as definitive. For example, we demonstrated downregulation of mRNA for elastin, Type III procollagen, PDGF-B, and, in the categorical analysis, IL-4 and upregulation of mRNA for ITAC/CXCL11 in the lung versus placebo. In addition, we observed increased protein levels of ITAC/CXCL11 in BALF and plasma and decreased BALF protein levels of ENA-78/CXCL5, Type I procollagen, and PDGF-A. Only the categorical analysis of SMAD-7 mRNA in the lung trended opposite the expected direction, and the biological significance of this trend, if any, is unclear. These molecules are known to be important in fibrosis. Elastin mRNA was elevated 70- to 80-fold in a butylated hydroxytoluene and oxygen model of fibrosis (27). Type III procollagen predominates in early granulation tissue formation and is gradually replaced by Type I procollagen (28). Both PDGF-A and PDGF-B are elevated in alveolar macrophages from patients with IPF, and PDGF-B is increased 10-fold as much as PDGF-A (29). IL-4 promotes fibroblast synthesis of extracellular matrix proteins (30) and is a chemotactic factor for fibroblasts (31). The changes in expression of these molecules (less elastin, Types I and III procollagen, PDGF-A, PDGF-B, ENA-78/CXCL5, and IL-4) after treatment with IFN-γ 1b, as compared with changes after placebo treatment, suggest beneficial effects of IFN-γ 1b on the process of fibrosis.

Results of a recently completed Phase III study of 330 patients with IPF randomized to IFN-γ 1b or placebo for approximately 1 year, however, demonstrated little change on clinical measures of fibrosis (FVC and alveolar–arterial gradient) but did show a potentially large effect on mortality (3). This apparent paradox between a treatment effect on survival and the absence of any effect on conventional measures of disease progression could be explained either by the insensitivity of these functional outcomes to an IFN-γ 1b effect on fibrosis or by a different mechanism of action. In this study, we also assessed the impact of IFN-γ 1b on pathogenic mechanisms other than fibrosis, based on favorable preclinical evidence of an effect on host defense and angiogenesis.

Infectious agents have been postulated to play a role in the pathogenesis of IPF (4, 11). The strongest finding in this study was an upregulation of ITAC/CXCL11 mRNA in lung tissue, coupled with a significant increase in protein levels of ITAC/CXCL11 in BALF and plasma. In a recent radial diffusion assay study, ITAC/CXCL11, an IFN-inducible CXC chemokine, and the other CXC chemokines IP-10/CXCL10 and MIG/CXCL9, demonstrated defensin-like antimicrobial activity in potentially microbiocidal concentrations against *Escherichia coli* and *Listeria monocytogenes* (21). This finding expands previous observa-

TABLE 3. BASELINE MEAN (SD), AND MEAN CHANGE (SE) FROM BASELINE TO 6 MONTHS OF BIOLOGIC MARKERS* EXPRESSED IN BRONCHOALVEOLAR LAVAGE FLUID AND PLASMA†

		BALF			Plasma		
Biomarker	Time Point	IFN-γ 1b	Placebo	p Value‡	IFN-γ 1b	Placebo	p Value§
TGF-β	Baseline mean (SD)	0 (0)	6 (23)	—	10,195 (6,345)	11,057 (10,399)	—
	Mean change (SE)	0 (0)	5 (14)	1.000	3784 (6,834)	−755 (4,316)	0.834
Type I procollagen	Baseline mean (SD)	1,159 (1,192)	453 (774)	—	ND	ND	—
	Mean change (SE)	−534 (342)	519 (330)	**0.096**	ND	ND	ND
PDGF-A	Baseline mean (SD)	88 (177)	3 (10)	—	70 (250)	78 (174)	—
	Mean change (SE)	−71 (47)	51 (35)	**0.033**	−61 (62)	25 (97)	1.000
PDGF-B	Baseline mean (SD)	4 (10)	0 (0)	—	30 (124)	29 (56)	—
	Mean change (SE)	−4 (2)	3 (3)	0.108	−14 (35)	46 (65)	0.735
IL-8/ CXCL8	Baseline mean (SD)	26 (107)	170 (324)	—	68 (210)	5 (21)	—
	Mean change (SE)	1 (33)	−16 (40)	0.532	−68 (51)	−6 (6)	0.688
ENA-78/CXCL5	Baseline mean (SD)	179 (283)	231 (221)	—	299 (403)	267 (378)	—
	Mean change (SE)	−98 (72)	54 (61)	**0.054**	60 (113)	132 (169)	0.785
IP-10/CXCL10	Baseline mean (SD)	41 (167)	8 (22)	—	40 (138)	69 (172)	—
	Mean change (SE)	−41 (41)	3 (3)	0.108	67 (65)	−16 (40)	0.821
MIG/CXCL9	Baseline mean (SD)	279 (1,152)	347 (985)	—	319 (909)	87 (276)	—
	Mean change (SE)	−279 (279)	86 (93)	0.322	1219 (696)	140 (136)	0.133
ITAC/CXCL11	Baseline mean (SD)	62 (255)	0 (0)	—	217 (517)	563 (1,440)	—
	Mean change (SE)	346 (94)	60 (60)	**0.016**	10813 (1,924)	−489 (453)	< **0.001**
MDC	Baseline mean (SD)	9 (13)	105 (342)	—	1372 (2,281)	7,194 (23,757)	—
	Mean change (SE)	−1 (4)	77 (65)	0.115	96 (614)	503222 (501,417)	0.315
MIP-1δ	Baseline mean (SD)	114 (170)	160 (143)	—	2629 (1,261)	2,691 (774)	—
	Mean change (SE)	−7 (32)	18 (34)	0.475	−60 (153)	79 (174)	0.554
IL-4	Baseline mean (SD)	0 (0)	0 (0)	—	0 (0)	0 (0)	—
	Mean change (SE)	0 (0)	0 (0)	N/C	0 (0)	0 (0)	N/C
IL-13	Baseline mean (SD)	4 (9)	15 (40)	—	0 (0)	0 (0)	—
	Mean change (SE)	26 (12)	−12 (13)	0.145	2 (2)	0 (0)	0.420
IFN-γ	Baseline mean (SD)	43 (119)	52 (199)	—	0 (0)	49 (191)	—
	Mean change (SE)	37 (73)	20 (72)	**0.093**	90 (71)	115 (152)	0.788
β-defensin-2	Baseline mean (SD)	6,785 (2,977)	7,822 (4,628)	—	ND	ND	—
	Mean change (SE)	−824 (1,029)	−1232 (2,156)	0.706	ND	ND	ND
Neutrophil defensin	Baseline mean (SD)	829,531 (796,203)	1,855,977 (2,323,848)	—	ND	ND	—
	Mean change (SE)	−333,704 (197,974)	−873,078 (700,861)	0.738	ND	ND	ND
VEGF	Baseline mean (SD)	15 (42)	3 (10)	—	4 (12)	5 (21)	—
	Mean change (SE)	−10 (7)	−3 (3)	0.688	6 (9)	−6 (6)	0.520

Definition of abbreviations: BALF = bronchoalveolar lavage fluid; ENA = epithelial neutrophil–activating protein; IL = interleukin; IP-10 = IFN-γ–inducible protein-10; ITAC = IFN-inducible T cell-α chemoattractant; MDC = macrophage-derived chemokine; MIG = monokine induced by IFN-γ; MIP-1δ = macrophage inflammatory protein-1δ; N/C = not calculable (test cannot be done due to zero variability); ND = not done; PDGF = platelet-derived growth factors; TGF = transforming growth factor; VEGF = vascular endothelial growth factor.

* Laboratory results were reported in ng/ml and converted to pg/ml for this presentation.

† For IFN-γ 1b group, n = 17 at baseline and Month 6 for all biomarkers; for placebo group, n = 15 at baseline and n = 13 at Month 6 for all biomarkers.

‡ Wilcoxon rank-sum test. p values representing trends ($p \leq 0.1$) and significant differences ($p \leq 0.05$) are highlighted in bold text.

§ ANCOVA (see †, above) for MIP-1δ; Wilcoxon rank-sum test for other markers. P value representing significant difference ($p \leq 0.05$) is highlighted in bold text.

tions in humans of the antimicrobial actions of IFN-γ 1b that include increased Fcχ receptor expression on phagocytes, increased serum levels of opsonin LPS-binding protein, improvement in phagocytes' capacity to ingest microbes, and improvement in phagocyte trafficking (32). These characteristics of IFN-γ 1b support the clinical observation that IFN-γ 1b ameliorates infection in both chronic granulomatous disease and malignant osteopetrosis (2). Thus, IFN-γ 1b could potentially modulate infectious processes that fuel the pathogenesis of IPF or could reduce the incidence or severity of infectious sequelae of IPF.

In addition to its direct antimocrobial activity, ITAC/CXCL11 also inhibits angiogenesis, (22) which could favorably impact the pathogenesis of IPF through several mechanisms, including shunt formation or less aberrant vascular remodeling resulting in diminished fibrosis. Like IP-10/CXCL10 and MIG/CXCL9, ITAC/CXCL11 inhibits neovascularization in the corneal micropocket assay of angiogenesis in response to either of the CXC chemokines, vascular endothelial growth factor, or basic fibroblast growth factor, by ligation to their putative receptor (CXCR3) on endothelial cells (33, 34). We have previously demonstrated that the IFN-inducible CXC chemokine, IP-10/CXCL10, inhibits bleomycin-induced pulmonary fibrosis by suppressing angiogenesis (35), and, in a similar preclinical model, ITAC/CXCL11 treatment analogously inhibits bleomycin-induced pulmonary fibrosis (unpublished observation, R. Strieter, 1 May 2003).

We believe it is unlikely that the ITAC/CXCL11 levels in the BAL simply reflect the levels in plasma due to leakage into the alveolar airspace. The dilutional effect of the volume of BALF used in this study could lead to a 100- to 200-fold decrease in the measured levels of the cytokines. If this dilutional effect is taken into account, the 6-month level of ITAC/CXCL11 (450 pg/ml) measured in the BAL would represent approximately 45–90 ng/ml at the tissue level. These levels are clearly higher than the levels that were measured in the plasma at 6 months (8.15 ng/ml).

Importantly, we found no evidence of a proinflammatory effect of IFN-γ 1b. Examination of BAL cells revealed stability

TABLE 4. CLINICAL ENDPOINTS AT BASELINE AND 6-MONTH ENDPOINT*

Parameter	IFN-γ 1b (*n = 17*)†	Placebo (*n = 15*)‡	p value§
Secondary Efficacy Endpoints			
Mean % predicted FVC			
Baseline	67.0	67.6	—
6-Month endpoint	65.9	64.7	—
Mean change (SE)	−1.0 (1.44)	−3.0 (1.54)	0.374
Mean FVC, L			
Baseline	2.669	2.471	—
6-Month endpoint	2.641	2.366	—
Mean change (SE)	−0.036 (0.0519)	−0.096 (0.0552)	0.435
Mean A-a gradient, mm Hg			
Baseline	19.974	20.814	—
6-Month endpoint	19.699	26.783	—
Mean change (SE)	−0.272 (2.1274)	5.965 (2.2650)	0.054
Mean % D_{LCO}			
Baseline	43.8	40.1	—
6-Month endpoint	40.0	36.7	—
Mean change (SE)	−3.4 (1.75)	−3.8 (1.87)	0.881
Mean change in modified MRC scale¶			
Baseline to endpoint	0.13	0.29	0.512
Mean dyspnea indices (SD)			
BDI‖	6.4 (1.84)	6.1 (2.26)	0.490
TDI**	0.2 (2.30)	−1.4 (3.59)	0.216
UCSD Shortness of Breath Questionnaire††			
Baseline, Mean (SD)	35.3 (17.44)	47.1 (22.42)	—
Baseline to 6-month endpoint, mean change (SE)	6.2 (5.21)	8.3 (4.96)	0.573
Most severe O_2 use requirement			
At baseline			
None	12 (80%)	11 (78.6%)	—
With activity	1 (6.7%)	3 (24.1%)	—
At rest	2 (13.3%)	0	0.951
At 6-month endpoint			
None	10 (62.5%)	6 (42.9%)	—
With activity	4 (25.0%)	2 (14.3%)	—
At rest	2 (12.5%)	6 (42.9%)	0.149
Median weekly maximal flow rate, L/min			
At baseline	0.00	0.00	0.866
At 6-month endpoint	0.00	2.00	0.603
Other clinical measures			
Mean % predicted FEV_1			
At baseline	72.6	73.7	—
At 6-month endpoint	73.5	69.7	—
Mean change (SE)	0.9 (2.05)	−4.0 (2.19)	0.112
Mean % predicted TLC			
Baseline	62.3	61.8	—
6-month endpoint	63.3	60.8	—
Mean change (SE)	1.0 (1.78)	−1.0 (1.99)	0.465
Mean distance walked in 6-minute walk test, m‡‡			
Baseline mean	423.1	334.3	—
6-month endpoint	415.9	329.0	—
Mean change (SE)	−7.8 (28.18)	−4.6 (31.30)	0.942

Definition of abbreviations: A-a = alveolar–arterial; BDI = baseline dyspnea index; D_{LCO} = diffusing capacity of carbon monoxide; MRC = Medical Research Council; TDI = transition (endpoint) dyspnea index; TLC = total lung capacity; UCSD = University of California at San Diego.

* Six-month endpoint was the last postbaseline measurement before the second bronchoscopy.

† Except for TLC (n = 15), most severe O_2 use requirement (n = 15 at baseline, n = 16 at endpoint), weekly maximal flow rate (n = 16 at baseline, n = 17 at endpoint), and modified MRC scale (n = 16).

‡ Except for TLC (n = 12), 6-minute walk test (n = 14), transitional dyspnea index (n = 14), most severe O_2 use requirement (n = 14 at baseline and endpoint), weekly maximal flow rate (n = 14 at baseline and endpoint), UCSD Shortness of Breath Questionnaire (n = 14), and modified MRC scale (n = 14).

§ p Value derived from ANCOVA with classification effect for treatment and baseline value included as a covariate except for Wilcoxon rank-sum comparisons of dyspnea indices, most severe O_2 use requirement, and weekly maximal flow rate.

¶ Modified MRC scale: 0 = none; 1 = slight; 2 = moderate; 3 = severe; 4 = very severe.

‖ BDI is the sum of assessment in three areas: functional impairment, magnitude of task, and magnitude of effort, each rated on a scale of 0 (very severe) to 4 (no impairment).

** TDI is rated on a scale from −3 (major deterioration) to +3 (major improvement) for each of three areas (functional impairment, magnitude of task, and magnitude of effort), which are then summed.

†† Total scale ranged from 0–120 with higher values indicating increasing shortness of breath.

‡‡ Exploratory endpoint.

TABLE 5. MEAN CELL COUNT* AND PERCENTAGE OF ALVEOLAR MACROPHAGES, LYMPHOCYTES, AND NEUTROPHILS IN BRONCHOALVEOLAR LAVAGE: BASELINE AND CHANGE FROM BASELINE AT 6 MONTHS†

	IFN-γ 1b	Placebo
Mean cell count, ×1000		
Baseline	155	247
Change from baseline	17	22
Alveolar macrophages, %		
Baseline	70	71
Change from baseline	16	4
Lymphocytes, %		
Baseline	11	14
Change from baseline	−5	−5
Neutrophils, %		
Baseline	7	12
Change from baseline	−2	1

* Excluding red blood cells.

† n = 17 for IFN-γ 1b and n = 15 for placebo at baseline; n = 17 for IFN-γ 1b and n = 13 for placebo at 6 months.

of cell counts after 6 months of treatment, suggesting no increase in inflammatory activity in BALF. In addition, ENA-78/CXCL5 in BALF was decreased relative to baseline in patients treated with IFN-γ 1b. Like IL-8/CXCL8, ENA-78/CXCL5 is a potent chemoattractant and activator of neutrophils (19), as well as a promoter of angiogenesis (36) in IPF (37).

No changes were observed for the remaining molecules (i.e., IL-8/CXCL8, IP-10/CXCL10, MIG/CXCL9, MDC (macrophage-derived chemokine)/CCL22, IL-13, defensins, and vascular endothelial growth factor). Reasons for these observations might include, in addition to true lack of effect, effects of dose, timing of measurements, and sampling.

Significant differences between treatment groups were not observed in pulmonary function, dyspnea, or oxygen use; however, trends toward improvement in alveolar–arterial gradient and FEV_1 were observed. Prolonged treatment with IFN-γ 1b was well tolerated in patients with steroid-refractory IPF. No IFN-γ 1b recipient discontinued study drug treatment for any reason, and no deaths occurred in the IFN-γ 1b treatment group.

This study provides direct evidence in humans with IPF that IFN-γ 1b alters expression of certain molecules postulated, based on preclinical models, to play a central role in the pathogenesis of IPF. Important findings include a pronounced and consistent elevation in ITAC/CXCL11 levels across the lung and plasma compartments, with trends toward downregulation of fibrotic and angiogenic molecules. We suggest that mortality in patients with IPF could potentially be altered by IFN-γ 1b through antimicrobial, antiangiogenic, antifibrotic, and/or immunomodulatory effects. Thus, our findings support the potential utility of IFN-γ 1b in the treatment of IPF.

Conflict of Interest Statement: R.M.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; K.M.S. is a study sponsor employee of InterMune and has patents pending and stock options; R.I.E. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; I.N. received $3,000 in 2003 and $1,000 in 2002 for an advisory board and speaking engagement sponsored by InterMune; V.G.V. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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