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IFN-γ-Inducible Protein-10 Attenuates Bleomycin-Induced Pulmonary Fibrosis Via Inhibition of Angiogenesis

Michael P. Keane, John A. Belperio, Douglas A. Arenberg, Marie D. Burdick, Zuo J. Xu, Ying Y. Xue, and Robert M. Strieter

Few studies have addressed the importance of vascular remodeling in the lung during the development of bleomycin-induced pulmonary fibrosis (BPF). For fibroplasia and deposition of extracellular matrix to occur, there must be a geometric increase in neovascularization. We hypothesized that net angiogenesis during the pathogenesis of fibroplasia and deposition of extracellular matrix during BPF are dependent in part on a relative deficiency of the angiostatic CXC chemokine, IFN-γ-inducible protein-10 (IP-10). To test this hypothesis, we measured IP-10 by specific ELISA in whole lung homogenates in either bleomycin-treated or control mice and correlated these levels with lung hydroxyproline. We found that lung tissue from mice treated with bleomycin, compared with that from saline-treated controls, demonstrated a decrease in the presence of IP-10 that was correlated to a greater angiogenic response and total lung hydroxyproline content. Systemic administration of IP-10 significantly reduced BPF without any alteration in lung lymphocyte or NK cell populations. This was also paralleled by a reduction in angiogenesis. Furthermore, IP-10 had no direct effect on isolated pulmonary fibroblasts. These results demonstrate that the angiostatic CXC chemokine, IP-10, inhibits fibroplasia and deposition of extracellular matrix by regulating angiogenesis.


Diabetic pulmonary fibrosis (IPF) is a chronic and often fatal pulmonary disorder. A prevalence rate of 27–29 cases/100,000 has been reported that may even be as high as 250 cases/100,000 in individuals >75 yr of age (1). The incidence of IPF appears to be on the rise in certain parts of the world (United Kingdom, New Zealand, and Germany) (2). Conventional treatment with immunosuppressive therapy has been disappointing, with a median survival of 50%. The elucidation of mediators that orchestrate this aberrant tissue repair will allow the development of novel interventions to treat this disorder.

The pathology of IPF demonstrates features of dysregulated and abnormal repair with exaggerated angiogenesis, fibroproliferation, and deposition of extracellular matrix, leading to progressive fibrosis and loss of lung function. The contribution of neovascularization to the progression of fibrosis in IPF has been largely ignored. The existence of neovascularization in IPF was originally identified by Turner-Warwick (3), who examined the lungs of patients with widespread interstitial fibrosis (IPF) and demonstrated neovascularization leading to anastomoses between the systemic and pulmonary microvasculatures and evidence of extensive vascular remodeling in areas of fibrosis. Further evidence of neovascularization during the pathogenesis of pulmonary fibrosis has been seen in a rat model of bleomycin-induced pulmonary fibrosis (BPF) (4).

Our laboratory has shown that members of the CXC chemokine family exert disparate effects in mediating angiogenesis as a function of the presence or the absence of three amino acid residues (Glu-Leu-Arg; the ELR motif) that immediately precedes the first cysteine amino acid of the primary structure of these cytokines (5, 6). Although IL-8, an ELR CXC chemokine, was initially discovered on the basis of chemotaxis and activation of neutrophils, our laboratory and others have found that IL-8 in vitro has endothelial cell chemotactic activity and in vivo induces neovascularization in the cornea of rats and rabbits, without inflammation (6–8). In contrast, IP-10, a non-ELR CXC chemokine inhibits angiogenesis and endothelial cell chemotaxis (5, 9, 10).

We have previously shown that the CXC chemokines, IL-8 and IP-10, regulate angiogenic activity in IPF (11). To demonstrate proof of this principle, we extended these studies to a murine model of BPF, and we have recently shown that neutralization of MIP-2, a murine functional homologue of IL-8, attenuates BPF via inhibition of angiogenesis (12).

In the current study we hypothesized that net angiogenesis during the pathogenesis of fibroplasia and the deposition of extracellular matrix during BPF is dependent in part on a relative deficiency of the angiostatic CXC chemokine, IP-10. We found that lung tissue from mice treated with bleomycin, compared with that from saline-treated controls, demonstrated a significant decrease in the presence of the CXC chemokine, IP-10, that was inversely correlated to total lung collagen and a greater angiogenic response compared with control lung tissue. Furthermore, pulmonary fibrosis was significantly reduced when bleomycin-exposed animals were treated with recombinant IP-10 related to a reduction in pulmonary fibrosis. These results demonstrate that the angiostatic CXC chemokine, IP-10, is an important factor that regulates angiogenesis in BPF.
Materials and Methods

Reagents
Polyclonal anti-murine IP-10 Abs and murine IP-10 were purchased from R & D (R & D Systems, Minneapolis, MN). The specificity of the Ab was assessed by either Western blot analysis or ELISA against a panel of other recombiant cytokines. Abs were specific in our sandwich ELISA without cross-reactivity to a panel of cytokines, including IL-1 antagonist protein (IRAP), IL-1, IL-2, IL-4, IL-6, TNF-α, IFN-γ, MG, and other members of the CXC and CC chemokine families (10, 13). The anti-protease buffer for tissue homogenization consisted of 1× PBS with one Complete tablet (Boehringer Mannheim, Indianapolis, IN) per 50 ml. Rabbit anti-factor VIII-related Ag Abs were purchased from (Biomed, Foster City, CA).

Animal model of pulmonary fibrosis
Female C57Bl6 and CBA/J mice (6–8 wk old) were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were maintained in specific pathogen-free conditions and provided with food and water ad libitum. To induce pulmonary fibrosis, mice were treated with intratracheal bleomycin (Blenoxane, a gift from Bristol Myers, Evansville, IN; 0.15 U/kg) on day 0 as previously described (12, 14). Control animals received only sterile saline as previously described (12, 14). Briefly, mice were anesthetized with 250 µl of 12.5 µg/ml ketamine injected i.p. followed by intratracheal instillation of 0.025 U of bleomycin in 25 µl of sterile isotonic saline. At 2, 8, 12, and 20 days postinflammation, animals were euthanized, and both lungs were removed for homogenization as described below. In separate experiments bleomycin-treated mice were given daily i.m. injections of recombinant IP-10 (1 µg in 20 µl of 0.25% HSA) or HSA (20 µl 0.25% HSA) until day 12. Mice were sacrificed on day 12 for hydroxyproline assay.

Lung tissue preparation
Bleomycin- or saline (control)-treated lungs were homogenized and sonicated in anti-protease buffer using a method previously described (10, 15). Specimens were centrifuged at 900 × g for 15 min, filtered through 1.2-µm Sterile Acridosics (Gelman Sciences, Ann Arbor, MI), and frozen at −70°C until thawed for assay by specific IP-10 ELISA. A portion of the specimen was lyophilized (Speed-Vac, Savant, Farmingdale, NY), and used in the corneal micropocket model of neovascularization for analysis of angiogenic activity. Additionally, some lungs were fixed in 4% paraformaldehyde and embedded in paraffin for histologic and immunohistochemical analyses.

IP-10 ELISA
Antigenic murine IP-10 was quantitated using a modification of an ELISA as previously described (10, 13). The sensitivity of our ELISA is ≥50 pg/ml. Briefly, flat-bottom 96-well microtiter plates (Nunc, Copenhagen, Denmark) were coated with 50 µl/well of the polyclonal anti-murine IP-10 Ab (1 ng/µl in 0.6 M NaCl, 0.26 M HIB4, and 0.08 N NaOH, pH 9.6) for 24 h at 4°C and then washed with PBS, and 0.05% Tween-20 (wash buffer). Non-specific binding sites were blocked with 2% BSA. Plates were rinsed, and samples were added (50 µl/well), followed by incubation for 1 h at 37°C. Plates were then washed, and 50 µl of the appropriate biotinylated polyclonal Ab (3.5 µg/ml in wash buffer and 2% FCS) was added for 45 min at 37°C. Plates were washed three times, streptavidin-peroxidase conjugate (Bio-Rad, Richmond, CA) was then added, and the plates were incubated at room temperature to the desired extinction. Plates were read at 490 nm in an automated microplate reader (Bio-Tek Instruments, Winooski, VT). Standards were half-log dilutions of recombinant IP-10 from 100 ng to 1 pg/ml (50 µl/well).

Hydroxyproline assay
Total lung collagen was determined by analysis of hydroxyproline as previously described (12, 14). Briefly, lungs were harvested on days 2, 8, 12, and 20 postbleomycin administration and homogenized in 2 ml of PBS, pH 7.4, with a Tissue Tearor (PRO-Scientific, Monroe, CT). One-half milliliter of each sample (both lungs) was then digested in 1 ml of 6 N HCl for 8 h at 120°C. Five microliters of citrate/acetal buffer (5% citric acid, 7.24% sodium acetate, 3.4% sodium hydroxide, and 1.2% glacial acetic acid, pH 6.0) and 100 µl of chloramine-T solution (282 mg of chloramine-T, 2 ml of H2O, and 16 ml of citrate/acetal buffer) were added to 5 µl of sample, and the samples were left at room temperature for 20 min. Next, 100 µl of Ehrlich’s solution (2.5 g of 4-(dimethylamino) benzaldehyde (Aldrich, Milwaukee, WI), 9.3 ml of n-propanol, and 3.9 ml of 70% perchloric acid (Eastman Kodak, Rochester, NY) were added to each sample, and the samples were incubated for 15 min at 65°C. Samples were cooled for 10 min and read at 550 nm on a Beckman DU 640 spectrophotometer (Fullerton, CA). Hydroxyproline (Sigma, St. Louis, MO) concentrations from 0–10 µg/ml were used to construct a standard curve.

Histology
Lungs were perfused in situ through the right ventricle with saline and then inflated under a constant pressure of 30 cm H2O with 1 ml of 4% paraformaldehyde. Lungs were ligated at the trachea, removed en bloc, and immersed in 4% paraformaldehyde for 24 h at which time they were changed to 70% alcohol before paraffin embedding, followed by sectioning and hematoxylin and eosin staining. Briefly, sections were dewaxed, rehydrated, and placed in hematoxylin solution (Sigma) for 5–10 min. Sections were washed in running water and differented in 1% acid-alcohol (using microscopic control) to ensure that only the nuclei were stained, followed by washing in water. Sections were then rinsed in ammonia water for 1 min, followed by brief rinsing in distilled water. Slides were then counterstained in eosin (Sigma) for 2–5 min, washed well in water, dehydrated, and mounted. Ten fields of random hematoxylin- and eosin-stained lung sections (n = 4) from three lungs of mice treated with each IP-10 or HSA were examined for the presence of fibrosis using a modification of the method described by Ashcroft (16). Ten fields of random sections (n = 4) from three lungs per condition were examined using an Olympus BH-2 microscope (New Hyde Park, NY) coupled to a Sony 3CCD camera (Tokyo, Japan) and a Macintosh IIfx computer. The total area of fibrosis was quantitated using NIH Image 1.55 software as previously described (10). For the purpose of quantitation, fibrosis was defined as areas that had Ashcroft grade 7 or 8 (16). These grades represent severe distortion of structure and large fibrous areas, including honeycomb lung, or total fibrous obliteration of the field. Results were expressed as square pixels at ×400 magnification.

Fibroblast proliferation
Murine lung fibroblasts were cultured as previously described (11, 12). Briefly, murine pulmonary fibroblasts were grown to 80% confluence and passaged. At the time of the fourth passage, pulmonary fibroblasts were >99% positive for vimentin, laminin, and fibronectin, and were >90% negative for α-smooth muscle actin and desmin. This technique allowed the establishment of murine pulmonary fibroblast cell lines. On the day of use, pulmonary fibroblasts were plated out in 96-well plates at a concentration of 0.00001 cells/well and allowed to adhere overnight. Fibroblasts were washed free of serum and cultured for 24 h under serum-free conditions. At 24 h serum was again added along with varying concentrations of IP-10 and/or PDGF, and fibroblasts were cultured for an additional 24, 48, and 72 h. [3H]Thymidine was added 12 h before harvesting using a cell harvester (Brandel, Gaithersburg, MD). [3H]Thymidine incorporation was assayed using a Beckman LS 1801 scintillation counter (Schaumburg, IL) and was expressed as counts per minute.

Conveal micropocket (CMP) assay of angiogenesis
Angiogenic activity of lung homogenates was assayed in vivo in the avascular cornea of hooded Long-Evans rat eyes as previously described (5, 10–13, 15). Briefly, equal volumes of lyophilized lung tissue specimens normalized to total protein were combined with sterile Hydron (Interferon Sciences, New Brunswick, NJ) casting solution. Five-microliter aliquots were pipetted onto the flat surface of an inverted sterile polypropylene strip (250 µg/kg) i.p. Rat corneas were anesthetized with 0.5% proparacaine hydrochloride ophthalmic solution followed by implantation of the Hydron pellet into an intracorneal pocket (1–2 mm from the limbus). Six days after implantation, animals received 1000 U of heparin and ketamine (150 mg/kg) i.p. Each cornea was observed or treated with an occasional sprout or hairpin loop displaying no evidence of sustained growth was detected. All animals were handled in accordance with the University of Michigan unit for laboratory animal medicine.
FACS analysis of CD31, factor VIII-related Ag, and leukocyte populations

Lung single-cell suspension preparations were made using a method previously described (12, 17). Briefly, lungs were harvested on day 12 from bleomycin-treated animals who had been treated with either IP-10 or HSA. Lungs were minced with scissors to a fine slurry in 15 ml of digestion buffer (RPMI, 5% FCS, 1 mg/ml collagenase (Boehringer Mannheim), and 30 μg/ml DNase (Sigma)). Lung slurry was enzymatically digested for 45 min at 37°C. Any undigested fragments were further dispersed by drawing the solution up and down through the bore of a 10-ml syringe. The total lung cell suspension was pelleted, resuspended, and spun through a 20% Percoll gradient. Cell counts and viability were determined using trypan blue exclusion on a hemocytometer. Single-cell suspensions were stained with anti CD45-Tricolor (Caltag, South San Francisco, CA) to allow live gating on either CD45-positive cells for analysis of leukocytes or CD45-negative cells for analysis of the nonleukocyte cell populations. CD31 Abs directly conjugated to PE (PharMingen, San Diego, CA) and primary rabbit anti-rabbit Abs (PharMingen) were added for further analysis of the nonleukocyte cellular populations. We have demonstrated that our digestion buffer does not alter the expression of either CD31 or factor VIII-related Ag from endothelial cells in culture. For analysis of lung lymphocyte or NK cell populations the following Abs directly conjugated to FITC or PE (PharMingen, San Diego, CA) were used: CD4, CD8, CD19, and NK1.1.

Statistical analysis

Data were analyzed on a Power Macintosh 7500 computer (Apple Computer, Cupertino, CA) using the StatView 4.5 statistical package (Abacus Concepts, Berkeley, CA). Comparisons were made using the unpaired t test. Data were considered statistically significant if \( p \leq 0.05 \). All values represent the mean ± SEM.

Results

Lung tissue from mice treated with bleomycin express lower levels of IP-10

We obtained lung tissue from bleomycin-treated mice (\( n = 6 \)) or from saline-treated controls (\( n = 6 \)) at each time point and measured IP-10 by specific ELISA standardized per lung on days 2, 8, 12, and 20. We chose these time points because fibrosis does not occur before day 7 in this model (18–23). Lung tissue from bleomycin-treated animals demonstrated lower levels of IP-10 compared with saline-treated controls on days 12 and 20 (\( p < 0.05 \); Fig. 1). Furthermore, the levels of IP-10 were inversely correlated with pulmonary fibrosis as determined by total lung hydroxyproline on days 2, 8, 12, and 20 (Fig. 1). These results suggest a temporal relationship between decreased levels of IP-10 and the development of pulmonary fibrosis.

Lung tissue from mice treated with bleomycin induces greater angiogenic activity

To substantiate that this CXC chemokine may be modulating lung tissue-derived angiogenic activity, we next assessed the in vivo angiogenic activity of six random pooled samples of either bleomycin-treated lung tissue or saline control lung tissue at 12 days using the rat CMP assay of neovascularization (Fig. 2). We found that lung tissue from bleomycin-treated mice (Fig. 2, 1A; \( n = 6 \) for each manipulation) induced a greater angiogenic response than lung tissue from saline-treated controls (Fig. 2, 1A; \( n = 6 \) for each manipulation). Taken together with our previous findings of vascular remodeling as evidenced by the immunolocalization of factor VIII-related Ag in the bleomycin model (12), these results are further evidence of a significant role for neovascularization in the development of the pulmonary fibrotic response to bleomycin.

IP-10 reduces bleomycin-induced pulmonary fibrosis

Based on our findings of increased angiogenic activity from bleomycin-treated lung tissue, which was directly and inversely correlated to the development of fibrosis and IP-10 levels, respectively, we next assessed whether administration of exogenous IP-10, by repeated i.m. injection, during BPF would attenuate the fibrotic response. Administration of IP-10 on days 1–12 led to reduced total lung hydroxyproline on day 12 compared with that in controls (Fig. 3A). Furthermore, these findings correlated with histopathologic findings of reduced fibrosis. For the purpose of quantitation, fibrosis was defined as areas that had Ashcroft grade 7 or 8 (16). These grades represent severe distortion of structure and large fibrous areas, including honeycomb lung, or total fibrous obliteration of the field (Figs. 3B and 4).

IP-10 reduces in vivo angiogenesis

Having shown that administration of IP-10 led to a reduction in bleomycin-induced fibrosis we next assessed the effect of administration of IP-10 on in vivo angiogenesis. Lungs from mice treated...
with IP-10 demonstrated decreased angiogenic activity in the CMP assay (Fig. 2, II B; zero of six corneas (0%) positive) compared with lungs from HSA-treated control mice (Fig. 2, IIA; five of six corneas (83%) positive). Furthermore, IP-10 led to a reduction in the total number of endothelial cells in the lung as assessed by dual expression of CD31 and factor VIII-related Ag using FACS analysis (Fig. 3C).

Systemic administration of IP-10 does not alter lung lymphocyte populations

IP-10 is a potent T cell and NK cell chemoattractant, and BPF has been associated with T cell infiltration. To exclude the possibility that the beneficial effect of systemic (i.m.) administration of IP-10 was due to the creation of a gradient preventing lymphocytes and NK cells from entering the lung, we assessed lymphocyte populations in the lungs of mice treated with either IP-10 or HSA. We found no difference in the presence of CD45-, CD4-, CD8-, and NK1.1-positive leukocytes between the two groups (Table I).

IP-10 has no effect on fibroblast proliferation

Having shown that IP-10 reduces BPF, we were next interested to determine whether IP-10 had any direct effect on pulmonary fibroblasts. Pulmonary fibroblasts were isolated from mouse lungs and stimulated with various concentrations of IP-10 in combination with PDGF, whereas PDGF alone was used as a positive control. Proliferation was measured using the incorporation of [3H]thymidine. As shown in Fig. 5, IP-10 had no effect on fibroblast proliferation, nor did it inhibit PDGF-induced proliferation. The same concentrations of IP-10 alone had no effect on proliferation compared with that in controls (data not shown).

Discussion

Our findings support the idea that a proangiogenic environment exists during the pathogenesis of BPF, and that IP-10 is an important factor in the regulation of this angiogenic environment. Studies directed at understanding the pathogenesis of IPF have primarily focused on mechanisms related to fibroplasia and deposition of extracellular matrix. However, these investigations have often been based on the evaluation of IPF in a “snap-shot” manner, not in a temporal fashion, and usually at end-stage fibrosis when extracellular matrix and nonviable scar have replaced the cellular phase of IPF. Although a number of eloquent studies have delineated mechanisms of fibroplasia and deposition of extracellular matrix in IPF, few studies have addressed the importance of angiogenesis in the lung during injury and subsequent fibrosis.

Although angiogenesis has been shown to play a role in the evolution of tissue repair and fibroplasia associated with acute lung injury and sarcoidosis (24, 25), the contribution of neovascularization to the pathogenesis of fibrosis in IPF has until recently been largely ignored. The existence of morphological neovascularization in IPF was originally identified by Turner-Warwick (3), who performed postmortem studies on the lungs of patients with widespread IPF and demonstrated neovascularization/vascular remodeling that was often associated with anastomoses between the systemic and pulmonary microvasculatures.

Further evidence of neovascularization during the pathogenesis of pulmonary fibrosis has been seen in a rat model of BPF (4). Peao and associates perfused the vascular tree of rat lungs with methacrylate resin at a time of maximal pulmonary fibrosis (4). Using scanning electron microscopy, these investigators demonstrated major vascular modifications that included neovascularization of an elaborate network of microvasculature located in the peribronchial regions of the lungs and distortion of the architecture of the alveolar capillaries. The location of neovascularization was closely associated with regions of pulmonary fibrosis, similar to the findings for human lung (3), and this neovascularization appeared to lead to the formation of systemic-pulmonary anastomoses (4).

We have shown that the CXC chemokines, IL-8 and IP-10, are important factors that regulate angiogenic activity in IPF and that an imbalance exists in their expression, which favors net angiogenesis in this disease (11). We found that levels of IL-8 were greater from tissue specimens of IPF patients compared with those in control tissue (11). In contrast, IP-10 levels were higher from tissue specimens obtained from control subjects compared with those from IPF patients (11). When IL-8 or IP-10 were depleted from IPF tissue specimens, tissue-derived angiogenic activity was markedly reduced or enhanced, respectively (11). These findings support the idea that IL-8 and IP-10 are important factors that regulate angiogenic activity in IPF.
To effectively assess the relevance of these mechanisms during the pathogenesis of pulmonary fibrosis in vivo, we have recently extended our studies to an animal model of fibrotic lung disease (12). Bleomycin sulfate has been used in rodents to initiate fibrotic lung lesions, which have many of the histologic components of IPF (18, 19). Bleomycin administration results in a route-, dose-, and strain-dependent pulmonary inflammatory response characterized by increases in leukocyte accumulation, fibroblast proliferation, and collagen content (18 –23). Although these pathologic changes occur in a more rapid fashion than human IPF, the rodent pulmonary inflammatory response to intratracheal bleomycin challenge constitutes a representative model of human IPF. We found that lung tissue from mice treated with bleomycin, compared with that from saline-treated controls, demonstrated a significant increase in the presence of MIP-2 that was correlated to a greater angiogenic response and total lung hydroxyproline content (12). When MIP-2...
was depleted in vivo by passive immunization, fibrosis was significantly reduced without a change in the presence of pulmonary neutrophils, fibroblast proliferation, or increases in collagen mRNA levels in isolated fibroblast cultures (12). This was also paralleled by a reduction in angiogenesis (12). These results demonstrate that the angiogenic CXC chemokine, MIP-2, is an important factor that regulates angiogenesis/fibrosis in pulmonary fibrosis (12).

In the current study we have demonstrated that during the pathogenesis of BPF there is a relative deficiency of the CXC chemokine, IP-10, which favors augmented net angiogenic activity. Lung tissue from bleomycin-treated animals had decreased levels of IP-10 compared with controls and demonstrated in vivo angiogenic activity that could be significantly attenuated in the presence of IP-10. These findings support our previous observations of increased angiogenic activity in IPF lung tissue, which was associated with a deficiency of IP-10 in the presence of elevated levels of IL-8, and our recent demonstration that neutralization of the angiogenic CXC chemokine, MIP-2, attenuates the fibrotic response to bleomycin via inhibition of angiogenesis (11, 12).

Systemic administration of recombinant IP-10 attenuated the fibrotic response to bleomycin. The magnitude of reduction in hydroxyproline was similar to that previously shown in cytokine neutralization studies (12, 14, 26–28). These findings correlated with histopathologic findings. Furthermore, systemic administration of IP-10 in our in vivo model led to a significant reduction in lung-derived angiogenic activity. Further indirect support for the role of IP-10 as an angiostatic factor during the pathogenesis of BPF is the lack of a direct effect of IP-10 on fibroblast proliferation. This supports the contention that IP-10 has no direct effect on pulmonary fibroblasts, suggesting that the beneficial effects of systemic IP-10 are mediated through the regulation of angiogenesis. Moreover, IP-10 transgenic mice have impaired wound healing, with abnormal blood vessel formation (29). This demonstrates an important role for IP-10 in modulating the repair process through its angiostatic properties. Interestingly, angiogenesis has been implicated in the pathogenesis of other chronic inflammatory disorders, such as psoriasis and rheumatoid arthritis, and the inhibition of angiogenesis improves Ag-induced arthritis in rabbits (30–33).

These findings are similar to our previous studies of IP-10 in the regulation of angiogenesis in association with tumorigenesis of human non-small cell lung carcinoma and its potential to inhibit tumor growth and metastases via its angiostatic activity (10). Interestingly, IFN-γ, a major inducer of IP-10 from a number of cells (34–38), is a known inhibitor of wound repair, in part due to its angiostatic properties (39), and has been shown to attenuate fibrosis in BPF (40). Moreover, the anti-tumor effect of IL-12 appears to be mediated via a cytokine cascade involving IFN-γ, and hence IP-10 and MIG through their inhibitory effects on tumor vasculature (41, 42). This supports the idea that the distal mediator of the effects of IFN-γ is in part related to IP-10.

IP-10 is a potent T cell and NK cell chemotactant (38), and BPF is associated with T cell infiltration, in which CD4 or CD8 lymphocytes modulate production of profibrotic mediators from mononuclear phagocytes (43, 44). Furthermore, the fibrotic response to bleomycin is significantly reduced in athymic mice (43). Depletion of CD4 or CD8 lymphocytes attenuates BPF (44). Interestingly, combined depletion of CD4 and CD8 lymphocytes has a greater than additive benefit over depletion of either subset alone (44). Systemic administration of IP-10 did not lead to any alteration in lung lymphocyte populations or NK cells. These studies suggest an alternative biological role for IP-10 in BPF other than lymphocyte recruitment, specifically in the regulation of angiogenesis. We cannot exclude that other chemokines or chemotactants may be recruiting T cells and NK cells in this model. Moreover, recent work calls into question the role of the adaptive immune response in BPF and supports the idea that IP-10 is working in a T cell-independent manner (45).

In conclusion, we have shown that in the context of BPF, a proangiogenic environment exists. This proangiogenic environment may be important in supporting fibroplasia and deposition of extracellular matrix during the pathogenesis of BPF. This persistent proangiogenic environment contrasts with the normal process of tissue repair, in which angiogenesis is usually rapidly expressed, transient, and tightly regulated (46–48). In addition, administration of IP-10 attenuates the fibrotic response to bleomycin through a mechanism that is independent of fibroblast proliferation or alteration of lung lymphocyte or NK cell populations. Our findings support the idea that IP-10 inhibits fibroplasia and deposition of extracellular matrix by regulating angiogenesis. These results suggest that targeting the regulation of angiogenesis may represent a viable therapeutic option for the treatment of pulmonary fibrosis.

References


