Impaired synthesis of prostaglandin E\textsubscript{2} by lung fibroblasts and alveolar epithelial cells from GM-CSF\textsuperscript{−/−} mice: implications for fibroproliferation

Ryan P. Charbeneau,\textsuperscript{1} Paul J. Christensen,\textsuperscript{1} Cara J. Chrisman,\textsuperscript{1} Robert Paine III,\textsuperscript{1,2} Galen B. Toews,\textsuperscript{1,2} Marc Peters-Golden,\textsuperscript{1} and Bethany B. Moore\textsuperscript{1}

\textsuperscript{1}Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor 48109; and \textsuperscript{2}Pulmonary Section of the Department of Veterans Affairs Medical Center, Ann Arbor, Michigan 48108

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IDIOPATHIC PULMONARY FIBROSIS (IPF) is a disease associated with high morbidity and mortality for which standard treatment, namely glucocorticoids and immunosuppressive therapy, is largely ineffective (1). This is consistent with the emerging view that a dysfunctional fibroproliferative response to some inciting form of lung injury is more important in the pathogenesis of IPF than is inflammation (31). The lung injury that is incurred in IPF results in significant damage to alveolar epithelial cells (AECs), disruption of basement membranes, fibroproliferation, extracellular matrix deposition, and the genesis of fibroblastic foci comprising phenotypically altered fibroblasts (1, 31). Consequently, the normal cell-cell interactions that are critical for mitigating fibrogenesis are disrupted.

Though best known for its ability to regulate immune and inflammatory cells, granulocyte-macrophage colony-stimulating factor (GM-CSF) also plays a key role in modulating wound repair and fibrosis. Experimental studies of wound healing demonstrate that GM-CSF facilitates wound contraction, recruits inflammatory cells, induces keratinocyte proliferation, and promotes reepithelialization (14). Furthermore, clinical studies indicate that recombinant human GM-CSF can promote the healing of refractory wounds of various sorts (12, 22, 28).

In initial studies examining the role of GM-CSF in bleomycin-induced pulmonary fibrosis in mice, the intraperitoneal injection of GM-CSF resulted in reduced collagen deposition in whole lung homogenates and decreased fibrosing alveolitis histologically, whereas administration of anti-GM-CSF antibody had the opposite result (29). We have confirmed that anti-GM-CSF antibody worsens pulmonary fibrosis (7); moreover, we have reported that GM-CSF expression is diminished in whole lung and in AECs isolated from bleomycin-treated rats (7). We subsequently reported that bleomycin-induced pulmonary fibrosis was exacerbated in mice with a targeted deletion of the GM-CSF gene (GM-CSF\textsuperscript{−/−} mice), as determined by both quantitative and histologic analysis (23). Interestingly, levels of the antifibrotic eicosanoid prostaglandin E\textsubscript{2}...
(PGE$_2$) were significantly diminished in whole lung homogenates and alveolar macrophage cultures from GM-CSF$^{-/-}$ mice treated with bleomycin relative to those of wild-type (GM-CSF$^{+/+}$) mice (23). Addition of exogenous GM-CSF to alveolar macrophage cultures from both GM-CSF$^{-/-}$ and GM-CSF$^{+/+}$ mice resulted in significant increases in PGE$_2$ production (23).

As IPF is increasingly being perceived as an “epithelial-fibroblastic” disease (31), we wished to further investigate the exaggerated susceptibility of GM-CSF$^{-/-}$ mice to bleomycin-induced pulmonary fibrosis by specifically examining the function of their fibroblasts and AECs. Because our previous work (23) suggested that regulation of macrophage PGE$_2$ synthesis represents one potential mechanism by which GM-CSF modulates fibrosis, we hypothesized that a similar defect in PGE$_2$ synthesis exists in fibroblasts and AECs from GM-CSF$^{-/-}$ mice and that key prostanoid synthetic enzymes may be deficient. In this report we provide further compelling evidence that GM-CSF plays an important role in regulating fibrogenesis by its ability to affect the production of the potent antifibrotic eicosanoid PGE$_2$.

**MATERIALS AND METHODS**

**Mice.** Breeding pairs of GM-CSF$^{-/-}$ and GM-CSF$^{+/+}$ mice with transgenic expression of GM-CSF under the SpC promoter (SpC-GM mice) backcrossed eight generations onto the C57BL/6 background were obtained from G. Dranoff and J. A. Whitsett (Cincinnati, OH) and have been previously described (10, 15, 16). The mice were bred in the University of Michigan Laboratory Animal Medicine facilities under specific pathogen-free conditions. Control C57BL/6 mice were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were used at 4–6 wk of age. All experiments were approved by the University of Michigan Committee on the Use and Care of Animals.

**Fibroblast purification.** Murine lungs were perfused with 5 ml of normal saline and removed under aseptic conditions. Lungs were minced with scissors in DMEM complete media containing 10% fetal calf serum. Lungs from a single animal were minced with scissors in DMEM complete media containing 10% fetal calf serum at 37°C in 5% CO$_2$. The final adherent population included only 4% nonepithelial cells at day 2 in culture as determined by intermediate filament staining.

**Enzyme immunoassay.** Cell-free fibrinoplastic and AEC supernatants were collected following overnight culture and were analyzed by enzyme immunoassay for the predominant cyclooxygenase (COX) product PGE$_2$, using a commercially available kit from Cayman Chemicals (Ann Arbor, MI).

**Immunoblot analysis.** Fibroblasts or AECs, after having been stimulated for 24 h with 10 ng/ml LPS, were detached by scraping into ice-cold lysis buffer (50 mM Tris-HCl, 25 mM KCl, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 1 mM leupeptin, pH 7.4), and lysates were sonicated by homogenization in 1% SDS-polyacrylamide gel electrophoresis under reducing conditions. Rainbow molecular weight markers as well as standards for cytosolic phospholipase A2 (cPLA$_2$), COX-1, and COX-2 were run in parallel. After transfer to nitrocellulose membranes, membranes were incubated overnight with anti-cPLA$_2$ (1:5,000 dilution; Genetics Institute, Cambridge, MA), anti-COX-1 (1:5,000 dilution; kind gift from Dr. W. Smith, Michigan State University) (32), or anti-COX-2 antisera (1: 10,000 dilution; Cayman Chemical), followed by peroxidase-conjugated goat anti-rabbit IgG (1:5,000). Proteins of interest were detected by the enhanced chemiluminescence method (Amersham Pharmacia Biotech, Piscataway, NJ).

**Fibroblast proliferation assays.** Fibroblasts purified from lungs of GM-CSF$^{-/-}$ and GM-CSF$^{+/+}$ mice were cultured at 5,000 cells/well in 96-well flat-bottomed tissue culture plates in complete media (DMEM, 10% fetal calf serum, and 1% penicillin-streptomycin). Cells were allowed to grow for 48 h in the presence or absence of PGE$_2$, after which time 10 µCi of [³H]thymidine was added to each well for a final 16 h. Cells were harvested onto glass fiber filters using a cell harvester, and incorporated radioactivity was determined by beta-scintillation counting.

**AEC-fibroblast proliferation assays.** For fibroblast-AEC cocultures, AECs were purified as described and plated onto fibronectin-coated plates (50,000 cells/well) on day 2 postincubation. AECs were allowed to adhere for 24 h before being washed three times with 1× PBS. Fresh medium (DMEM, 10% fetal calf serum, and 1% penicillin-streptomycin) containing fibroblasts was added (5,000 cells/well), and fibroblasts were allowed to grow in the presence or absence of AECs for 24–48 h. [³H]thymidine was added (10 µCi/well, Amersham) during the final 16 h of culture, and incorporated radioactivity was determined with a beta-scintillation counter. As purified AECs grow poorly in culture and incorporate only low levels of [³H]thymidine, proliferation counts reflect primarily fibroblast numbers (21, 37). Control cultures of AECs alone consistently incorporated <5% of the total counts measured in coculture.

**Fluorescein isothiocyanate-induced pulmonary fibrosis.** Pulmonary fibrosis was induced experimentally by the intratracheal injection of fluorescein isothiocyanate (FITC; Sigma, St. Louis, MO) (8, 24). Mice were anesthetized with pento-barbital sodium. The trachea was exposed and entered with
a needle under direct visualization. FITC was dissolved in saline (21 mg of FITC in 10 ml of saline), vortexed extensively, and sonicated for 30 s before the slurry was transferred to multiuse vials. The vials were vortexed extensively before 50-µl aliquots were removed for intratracheal injection via a 26-gauge needle. Mice of each genotype were injected with either FITC or saline. On day 21 postinjection, mice were euthanized by CO₂ asphyxiation and perfused via the heart with 5 ml of normal saline. Individual lung lobes were removed, and all five lobes from a single mouse were homogenized in 1 ml of saline and hydrolyzed by the addition of 1 ml of 12 N HCl. Samples were then baked at 100°C for 12 h. We then assayed aliquots (5 µl) by adding chloramine-T solution for 20 min followed by development with Erlich’s reagent at 65°C for 15 min as previously described (33). Absorbance was measured at 550 nm, and the amount of hydroxyproline was determined against a standard curve generated using a known concentration of hydroxyproline standard. Results are presented as the values obtained in FITC-treated mice normalized to the values obtained in saline-treated mice.

**Data analysis.** Statistical significance was analyzed with the InStat 2.01 program (GraphPad Software) on a Power Macintosh G3. We used Student’s t-tests to determine P values when comparing two groups. When comparing three or more groups, we performed ANOVA with a post hoc Bonferroni test to determine which groups showed significant differences. P < 0.05 was considered significant.

**RESULTS**

**Impaired production of PGE₂ by GM-CSF⁻/⁻ fibroblasts and AECs.** We first examined the ability of cultured fibroblasts from GM-CSF⁺/⁺ and GM-CSF⁻/⁻ mice to elaborate PGE₂. Cell-free supernatants were collected from fibroblasts after 48 h and analyzed for PGE₂ by specific immunoassay. Basal levels of PGE₂ produced by GM-CSF⁻/⁻ fibroblasts were approximately threefold lower than those from GM-CSF⁺/⁺ fibroblasts (Fig. 1A, P = 0.006).

Next, we examined the ability of AECs from GM-CSF⁺/⁺ and GM-CSF⁻/⁻ mice to produce PGE₂. Cell-free supernatants were collected and analyzed for PGE₂ by specific immunoassay. The capacity for PGE₂ synthesis by GM-CSF⁻/⁻ AECs was significantly less (P = 0.02) than that by GM-CSF⁺/⁺ AECs (Fig. 1B).

**Reduced expression of the key prostaglandin synthetic enzymes cPLA₂, COX-1, and COX-2.** Given the impaired production of PGE₂ by GM-CSF⁻/⁻ pulmonary fibroblasts and AECs, we sought to determine whether there was diminished expression of one or more of the key prostaglandin synthetic enzymes. In this analysis, we considered cPLA₂, the key enzyme responsible for hydrolysis of arachidonic acid from membrane phospholipids, and both isoforms of cyclooxygenase (COX-1 and COX-2), which initiate prostaglandin synthesis from arachidonate. Immunoblot analysis of GM-CSF⁺/⁺ pulmonary fibroblast lysates revealed (Fig. 2A) low-level expression of all three enzymes. Stimulation with LPS resulted in a modest increase in expression of cPLA₂ and a dramatic increase in COX-2. LPS did not affect COX-1 levels. Analysis of GM-CSF⁻/⁻ fibroblasts revealed relatively reduced basal levels of COX-1 and COX-2 and slightly higher levels of cPLA₂. LPS augmented expression of cPLA₂ and COX-2, but to a far lesser extent than in GM-CSF⁺/⁺ fibroblasts. COX-1 levels were again not affected by LPS in the GM-CSF⁻/⁻ fibroblasts. Immunoblot analysis of GM-CSF⁺/⁺ AEC lysates (Fig. 2B) revealed expression of cPLA₂ and COX-1, but low basal levels of COX-2. Stimulation with LPS resulted in a significant increase in COX-2 levels but did not increase cPLA₂ or COX-1 expression. Analysis of GM-CSF⁻/⁻ AEC lysates revealed relatively reduced basal levels of cPLA₂ and COX-1. LPS stimulation increased cPLA₂ and COX-2 levels only minimally and had no effect on COX-1 levels. Thus GM-CSF deficiency results in diminished basal and/or induced expression of key prostaglandin synthetic enzymes in both cell types.

**Fibroblasts from GM-CSF⁻/⁻ mice exhibit increased proliferation.** Because pulmonary fibroblasts from GM-CSF⁻/⁻ mice exhibited a reduced capacity for synthesis of PGE₂, which can act in an autocrine fashion to downregulate fibroproliferation, we hypothesized that GM-CSF⁻/⁻ fibroblasts would proliferate to a greater extent than GM-CSF⁺/⁺ fibroblasts. Accordingly, we isolated pulmonary fibroblasts from mice of both genotypes and examined their proliferation in response to 10% serum. As estimated by incorporation of [³H]thymidine, proliferation of GM-CSF⁻/⁻ fibroblasts was greater (P = 0.0046) than that of GM-CSF⁺/⁺ fibroblasts (Fig. 3).
ished responsiveness to the suppressive effects of endogenous PGE2. Fibroblasts were isolated from GM-CSF−/− and GM-CSF+/+ mice. Cells were then cultured for 48 h in the presence of 0.1 μM PGE2, after which time proliferation was estimated by incorporation of [3H]thymidine. The dose of 0.1 μM PGE2 was chosen because this is a physiological concentration that represents an optimal concentration for PGE2 receptor (EP) binding and signaling. Indeed, dose response experiments (not shown) demonstrated that this was the optimal dose for fibroblast inhibition assays in wild-type cells. Fibroblasts of both genotypes were inhibited by PGE2 (Fig. 4). There was no significant difference in the degree to which GM-CSF−/− fibroblasts were inhibited compared with GM-CSF+/+ fibroblasts. This lack of differential inhibition of fibroblast proliferation by exogenous PGE2 is consistent with our finding that cells from the two genotypes had similar levels of mRNA expression for PGE2 receptors EP1–4 (data not shown). Thus enhanced proliferation of GM-CSF−/− fibroblasts is not due to defects in PGE2 responsiveness but, rather, reflects reduced production of PGE2 by these cells.

AECs are another important source of PGE2 that might affect fibroblast proliferation. To examine functional consequences of diminished PGE2 production by GM-CSF+/− AECs, we studied proliferation of GM-CSF+/− fibroblasts in coculture with AECs from both genotypes. AECs from the respective genotypes were grown in coculture with GM-CSF+/− pulmonary fibroblasts for 48 h, and fibroblast proliferation was then measured by incorporation of [3H]thymidine. As expected, fibroblast proliferation was inhibited by AECs of both genotypes, but to a lesser degree (P < 0.04) by GM-CSF+/− AECs (Fig. 5). This is consistent with the observation that GM-CSF−/− AECs produce less PGE2 and thus are less able to inhibit fibroblast proliferation.

Effect of GM-CSF add-back to fibroblasts and AECs. We next wanted to determine whether the addition of GM-CSF to cultures of GM-CSF−/− fibroblasts and
GM-CSF−/− AECs could increase the production of PGE2 in these cells. To test the effects of exogenous GM-CSF administration on fibroblasts, we added exogenous GM-CSF to GM-CSF−/− fibroblast cultures in vitro. The results are shown in Fig. 6A and demonstrate that exogenous GM-CSF administration increases the production of PGE2 in these fibroblast cultures. This results in decreased proliferative capacity in fibroblast cultures as well (Fig. 6B).

To examine the effect of GM-CSF addition to AECs, we utilized a genetically altered mouse that expresses the murine GM-CSF gene as a transgene under the control of the surfactant protein C promoter within GM-CSF−/− mice (SpC-GM mice) as previously described (16). These mice specifically overexpress GM-CSF in the alveolar epithelial cells within the lung but do not express GM-CSF in any other cell within the body. The AECs in these animals produced approximately seven times more PGE2 than did AECs from normal mice (Fig. 6C). Thus the genetic addition of GM-CSF to AECs augments the production of PGE2 by these cells.

Transgenic overexpression of GM-CSF exclusively in AECs protects from experimental pulmonary fibrosis. We have previously shown that GM-CSF−/− mice develop a more exuberant fibrotic response to bleomycin than do wild-type controls. Fibrosis can be induced experimentally by many agents, and we have previously documented that the particulate antigen FITC induces acute lung injury followed by pulmonary fibrosis (8, 24). We now report that GM-CSF−/− mice are more susceptible to FITC-induced fibrotic responses (Fig. 7) compared with wild-type mice. Interestingly, however, SpC-GM mice are protected from FITC-induced pulmonary fibrosis compared with wild-type animals (Fig. 7). This protection is likely related, at least in part, to the increased production of PGE2 in these mice, as levels of PGE2 in lung homogenates from SpC-GM mice (20.6 ± 6.1 ng/ml) were approximately four times higher than levels in wild-type mice (5.1 ± 1.6 ng/ml) when measured during the fibroproliferative period (day 10, P = 0.06). Thus overproduction of PGE2 in vivo in the SpC-GM mice can be attributed to its greater elaboration by AECs (Fig. 6C).

**DISCUSSION**

In this study, we found that pulmonary fibroblasts and alveolar epithelial cells isolated from mice with a targeted deletion of the GM-CSF gene elaborate less of the antiﬁbrotic prostanooid PGE2. This deficiency was associated with decreased basal and LPS-stimulated levels of COX-1, COX-2 and, in the case of GM-CSF−/− AECs, cPLA2 key enzymes in the biosynthesis of PGE2. GM-CSF−/− pulmonary fibroblasts exhibited greater proliferation than did GM-CSF+/+ fibroblasts. GM-CSF−/− AECs were less able to inhibit proliferation of pulmonary fibroblasts in culture than were GM-CSF+/+ AECs. Furthermore, we demonstrated that the addition of GM-CSF back to AECs and fibroblasts from GM-CSF−/− mice can restore PGE2 production and decrease fibroproliferation. Finally, we demonstrate that overexpression of GM-CSF exclusively in AECs, resulting in pulmonary overproduction of PGE2 in vivo, is sufficient to protect mice from experimental pulmonary fibrosis.

There is abundant evidence in the literature that GM-CSF is important in wound repair. Experimental studies of wound healing have found that GM-CSF facilitates wound contraction, recruits inflammatory cells, induces keratinocyte proliferation, and promotes reepithelialization (14). GM-CSF has been used clinically to facilitate the healing of incisional wounds in animal models (6), as well as postsurgical and radiation-induced wounds (12). Additionally, randomized placebo-controlled trials have confirmed the utility of GM-CSF in chronic leg ulcers (22). As fibrotic lung diseases may be viewed as a manifestation of dysfunctional wound repair, a pathogenic role for GM-CSF is not surprising.

Nearly 10 years ago, Piquet et al. (29) found that bleomycin-induced pulmonary fibrosis in mice was associated with initially increased levels of GM-CSF mRNA followed by diminished levels at a later time point. They demonstrated that administration of GM-CSF at days 7–15 protected mice from bleomycin-induced pulmonary fibrosis, whereas anti-GM-CSF antibody exacerbated collagen deposition. Recently our laboratory provided additional data (7) supporting the important role of GM-CSF in pulmonary fibrosis. Be-
were indeed reduced in rat lung homogenates postbleomycin administration, with levels beginning to recover on day 14. Type II AECs isolated from bleomycin-injured animals also exhibited diminished capacity for GM-CSF synthesis, both basally and on stimulation with LPS. Administration of anti-GM-CSF antibody to rats at days 0, 4, and 8 postbleomycin resulted in increased lung hydroxyproline levels, findings consistent with those of Piquet et al. (29). In addition, we have demonstrated that GM-CSF−/− mice develop more severe pulmonary fibrosis following bleomycin injury (23). These data collectively suggest that GM-CSF is beneficial in the setting of pulmonary fibrosis.

However, some investigators have raised the possibility that GM-CSF might, in fact, contribute to pulmonary fibrosis. This suggestion is based on experiments in which very high level murine GM-CSF expression was induced in rat lungs by adenovirally mediated gene transfer (35, 36). These investigators found that adenoviral transfer of GM-CSF to the lungs of rats led to the accumulation of eosinophils and macrophages, tissue injury, and histological evidence of fibrosis (35). They also found that adenoviral delivery of murine GM-CSF to rat lungs resulted in an increase in granuloma formation, fibroblast accumulation, and expression of the fibrogenic cytokine transforming growth factor-β1 (36). In contrast to these findings, several pieces of information suggest that GM-CSF itself is not directly profibrotic in the lung. First, transgenic overexpression of GM-CSF in the lung under control of the surfactant protein C promoter for the life of an animal does not result in pulmonary fibrosis (15, 16). Likewise, adenovirally mediated transfer of murine GM-CSF into mice (38) or aerosol treatment with GM-CSF in mice for extended periods (30) does not induce pulmonary fibrosis. There are several potential explanations for these apparently contradictory findings that are related to species, timing, and dose. In the studies by Xing et al. (35, 36), adenovirally transferred murine GM-CSF was expressed at very high levels in the lungs of rats transiently and at early time points. It is therefore possible that the species divergence between cytokine and receptor or a host response to the murine protein may have had some effect in these experiments. Furthermore, the extremely high levels of GM-CSF used in these studies suggest that the beneficial effects of GM-CSF may be realized only at lower concentrations. Our previous studies have examined lung injury induced by the fibrogenic agent bleomycin. It is possible that the injury induced by adenoviral administration results in a different pathological set of events. Finally, the timing of the GM-CSF administration may be critical. The adenoviral gene transfer experiments delivered GM-CSF simultaneously with the adenoviral administration and induced only transient expression. This could be considered an early time point postinjury, whereas previous experiments have shown that exogenous GM-CSF was effective when given during the fibroproliferative phase of bleomycin injury (days 7–15), a relatively later time point. Finally, our experiments using

cause AECs are a prominent source of GM-CSF in the lung, we hypothesized that bleomycin-induced lung injury, which damages AECs, would result in diminished levels of GM-CSF. We found that GM-CSF levels

Fig. 6. GM-CSF addition restores PGE2 production and limits proliferation in fibroblasts from GM-CSF−/− mice. A: fibroblasts were purified from GM-CSF−/− mice and cultured at 5,000 cells/well in 96-well plates in the presence of media alone or media supplemented with 10 ng/ml GM-CSF. After 24 h of culture, supernatants were collected and PGE2 was analyzed by specific immunoassay. Exogenous GM-CSF increases PGE2 production in GM-CSF−/− fibroblasts (n = 6 representative of 2 independent experiments, P = 0.01). B: fibroblasts were purified and cultured as in A. After 24 h of incubation with or without GM-CSF, [3H]thymidine was added for 16 h, and proliferation was assessed. The addition of GM-CSF to the GM-CSF−/− fibroblasts significantly reduced proliferation consistent with the increased production of PGE2 noted in A (n = 6 representative of 2 independent experiments, P = 0.0015). C: GM-CSF addition restores PGE2 production in AECs from GM-CSF−/− mice. AECs were purified from wild-type GM-CSF−/− mice or mice with transgenic expression of GM-CSF under the SpC promoter (SpC-GM mice). Cells were then cultured at 50,000 cells/well for 48 h before medium was changed. Cell-free supernatants were collected after 24 h and were analyzed for PGE2 by specific immunoassay (n = 4, representative of 2 independent experiments). SpC-GM AECs produce ~7 times the amount of PGE2 made by wild-type cells (P < 0.0001).
SpC-GM mice demonstrate clearly that these mice are protected from FITC-induced pulmonary fibrosis and that this protection correlates with increased production of PGE2. Thus we believe that, together, these data support the hypothesis that exogenous GM-CSF may be beneficial in the treatment of pulmonary fibrosis if given in appropriate doses and at appropriate time points postinjury.

The antifibrotic properties of PGE2 have been well documented. PGE2 is known to inhibit fibroblast proliferation in response to numerous mitogens (3, 11), as well as to inhibit collagen synthesis (13, 19) and promote its degradation (2). Bleomycin-induced pulmonary fibrosis is exacerbated in COX-2−/− mice (18) and in normal mice treated with the COX inhibitor indomethacin (23). Fibroblasts isolated from the lungs of bleomycin-treated fibrotic rats have been shown to exhibit reduced capacity for PGE2 synthesis (25). Furthermore, reduced levels of PGE2 have been found in bronchoalveolar lavage fluid (4), as well as conditioned medium obtained from alveolar macrophages (26) and lung fibroblasts isolated from patients with IPF (34).

A link between GM-CSF and PGE2 has been previously established in vitro in macrophages, where GM-CSF was shown to increase the synthesis of a range of eicosanoids, including PGE2 (5). Utilizing GM-CSF−/− mice, we performed additional experiments to elucidate this relationship in vivo in the context of fibrotic lung disease (23). We found that GM-CSF−/− mice manifested an increased susceptibility to bleomycin-induced pulmonary fibrosis as measured by both hydroxyproline deposition and histological analysis. Furthermore, alveolar macrophages isolated from GM-CSF−/− mice exhibited a diminished capacity for the synthesis of PGE2, which was ameliorated by the administration of exogenous GM-CSF. To our knowledge, this was the first data linking GM-CSF and PGE2 within the context of pulmonary fibrosis.

As pulmonary fibroblasts and AECs produce significant quantities of PGE2 and are central effector cells in fibrotic lung responses, the present study addressed PGE2 synthesis in these cell types from GM-CSF−/− mice. As previously observed for lung macrophages, we found that GM-CSF−/− pulmonary fibroblasts elaborate less PGE2 than do GM-CSF+/+ fibroblasts. This was associated with a relative basal deficiency of COX-1 in the GM-CSF−/− fibroblasts. Increased levels of COX-1 were not induced with LPS in either genotype, consistent with the constitutive nature of COX-1. The inducible isoenzyme, COX-2, was upregulated by LPS in both genotypes, but to a lesser degree in the GM-CSF−/− fibroblasts.

Cytosolic PLA2 levels were not deficient in GM-CSF−/− fibroblasts. This was of interest given that previous work from our laboratory (5) demonstrated increased expression of cPLA2 by alveolar macrophages treated with exogenous GM-CSF. However, this effect was not observed in peritoneal macrophages or peripheral blood monocytes, suggesting that regulation of cPLA2 by GM-CSF is likely a cell-specific phenomenon. Similarly, AECs from GM-CSF−/− mice demonstrated reduced basal or LPS-induced expression of all three key prostanoid synthetic enzymes.

The effects of PGE2 are mediated by a family of G protein-coupled receptors, EP1–4. As there is precedent for EP receptor expression being regulated by PGE2 levels (17), it is possible that increased proliferation of GM-CSF−/− fibroblasts reflected altered EP expression and, therefore, diminished responsiveness to this suppressive prostanoid. Our data do not support this hypothesis. Pulmonary fibroblasts from both genotypes were inhibited to the same degree by exogenous PGE2, and their EP receptors were expressed similarly.

Although cultured AECs are known to produce PGE2 (7, 10, 19, 25), Pan et al. (27) recently reported that rat AECs inhibit human pulmonary fibroblast proliferation in coculture primarily by stimulating fibroblast PGE2 synthesis. We have recently verified the importance of AEC-derived PGE2 with respect to pulmonary fibroblast inhibition through the use of COX-deficient mice (20). Our present studies utilizing GM-CSF−/− AECs in coculture with GM-CSF+/+ fibroblasts (Fig 5) and the in vivo protection from FITC-induced fibrosis seen in SpC-GM mice further emphasize the impor-
tance of AECs as a source of the antifibrotic prostanooid PGE2.

In sum, the previous and current studies suggest that GM-CSF production is crucial in limiting fibrotic responses. Furthermore, the kinetic results that have been reported support the hypothesis that GM-CSF expression drives the production of PGE2 in vivo. Neutralization of GM-CSF from days 0 to 8 postbleomycin is detrimental (29). The administration of GM-CSF from days 7 to 15 is beneficial (29), thus GM-CSF likely needs to be present at least by day 7 postbleomycin to be effective. This time point correlates well with the onset of fibroproliferation following bleomycin. We have demonstrated that the neutralization of PGE2 starting at day 10 postbleomycin during the fibroproliferative phase is also detrimental (23). Therefore, we suggest that GM-CSF expression by day 7 likely drives PGE2 production during the fibroproliferative phase (days 7–15), and thus this is the time point when the antifibrotic effects of PGE2 would be most beneficial.

In conclusion, pulmonary fibroblasts and AECs from GM-CSF−/− mice exhibit a reduced capacity for synthesis of the antifibrotic prostanooid PGE2 with functional consequences for fibrogenesis. These results extend our understanding of the cells and mechanisms that are responsible for the antifibrotic effects of GM-CSF in models of pulmonary fibrosis. Further elucidation of the interplay between cytokines and eicosanoids may have important implications for treatment of pulmonary fibrosis.

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