ORIGINAL RESEARCH

Role of Platelet-Derived Growth Factor/Platelet-Derived Growth Factor Receptor Axis in the Trafficking of Circulating Fibrocytes in Pulmonary Fibrosis

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Abstract

Circulating fibrocytes have been reported to migrate into the injured lungs, and contribute to fibrogenesis via CXCL12–CXCR4 axis. In contrast, we report that imatinib mesylate prevented bleomycin (BLM)-induced pulmonary fibrosis in mice by inhibiting platelet-derived growth factor receptor (PDGFR), even when it was administered only in the early phase. The goal of this study was to test the hypothesis that platelet-derived growth factor (PDGF) might directly contribute to the migration of fibrocytes to the injured lungs. PDGFR expression in fibrocytes was examined by flow cytometry and RT-PCR. The migration of fibrocytes was evaluated by using a chemotaxis assay for human fibrocytes isolated from peripheral blood. The numbers of fibrocytes triple-stained for CD45, collagen-I, and CXCR4 were also examined in lung digests of BLM-treated mice. PDGFR mRNA levels in fibrocytes isolated from patients with idiopathic pulmonary fibrosis were investigated by real-time PCR. Fibrocytes expressed both PDGFR-α and -β, and migrated in response to PDGFs. PDGFR inhibitors (imatinib, PDGFR-blocking antibodies) suppressed fibrocyte migration *in vitro*, and reduced the number of fibrocytes in the lungs of BLM-treated mice. PDGF-BB was a stronger chemoattractant than the other PDGFs *in vitro*, and anti–PDGFR-β–blocking antibody decreased the numbers of fibrocytes in the lungs compared with anti–PDGFR-α antibody *in vivo*. Marked expression of PDGFR-β was observed in fibrocytes from patients with idiopathic pulmonary fibrosis compared with healthy subjects. These results suggest that PDGF directly functions as a strong chemoattractant for fibrocytes. In particular, the PDGF-BB–PDGFR-β biological axis might play a critical role in fibrocyte migration into the fibrotic lungs.

Keywords: fibrocyte; platelet-derived growth factor; lung fibrosis

Clinical Relevance

This research shows that platelet-derived growth factor (PDGF) directly functions as a strong chemoattractant for fibrocytes, and that PDGF inhibitors, including imatinib, reduce fibrocyte pool size in the lungs. In addition, the phase II study with nintedanib (BIBF1120), which is an inhibitor for multikinases (three kinases), including PDGF receptor (PDGFR), clearly shows the efficacy for idiopathic pulmonary fibrosis (IPF). These results suggest the clinical efficacy of PDGFR inhibitors for treatment of patients with IPF.

Idiopathic pulmonary fibrosis (IPF) is a progressive and lethal disease of the lungs, characterized by the proliferation of fibroblasts and deposition of extracellular matrix (1, 2). IPF is a consequence of loss of alveolar–capillary barrier function, disruption of basement membrane integrity by epithelial injury/inflammation, subsequent failure of re-epithelialization, and dysregulation of tissue repair driven by profibrotic mediators, including growth factors (2, 3). Moreover,

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epithelial–mesenchymal transition and a bone marrow–derived progenitor cell (the fibrocyte) are cellular modulators of fibrosis, as is the resident tissue fibroblast (4–7). A number of studies have demonstrated the potential for bone marrow–derived circulating fibrocytes to enter tissues after injury and contribute to wound healing and fibrosis (8–11). Fibrocytes appear to be derived from the differentiation of $CD14^+$ peripheral blood mononuclear cells. They express markers of hematopoietic cells (CD34), leukocytes (CD11b, CD13, and CD45), as well as fibroblast products (collagens I and III and fibronectin) (12). Tanjore and colleagues (7) reported that bone marrow–derived cells migrate to the lungs and contribute to the fibroblast pool, accounting for roughly 1/5 of $S100A4^+$ lung fibroblasts at 2 weeks after bleomycin (BLM) in mice. The marked expansion of circulating fibrocyte pool in patients with interstitial pneumonia, and the positive correlation between the abundance of fibroblastic foci and the amount of lung fibrocytes in patients with IPF, have been reported (13, 14), suggesting that fibrocytes are involved in the pathogenesis of human pulmonary fibrosis. Moeller and colleagues (15) also found that the percentages of CD45/collagen-1–positive fibrocytes were increased in peripheral blood of patients with IPF, and especially high circulating fibrocyte percentages were predictive of their poor clinical outcome.

Imatinib mesylate (gleevec in the United States and glivec in Europe) has been demonstrated to be highly active against chronic myeloid leukemia and gastrointestinal stromal tumors (16–19), which is a potent and specific tyrosine kinase inhibitor acting against bcr-abl, c-kit, and platelet-derived growth factor receptor (PDGFR) (20). Platelet-derived growth factor (PDGF), which plays roles in fibroblast growth and migration, is an important growth factor in the development of pulmonary fibrosis (21, 22). We and others have demonstrated that imatinib treatment attenuated the development of pulmonary fibrosis using a BLM or radiation model in mice via inhibiting fibroblast growth (23–25). However, we found that the early treatment of imatinib (Days 0–14) significantly prevented the development of pulmonary fibrosis in the BLM model in mice (24). Neef and colleagues (26) also reported that early (Days 0–21) imatinib treatment was effective in inhibiting liver fibrosis using a bile duct ligation model. These results raise the critical question of how imatinib plays a role during the early phase (inflammatory phase).

Therefore, we focused on fibrocytes and examined the expression of PDGFRs in fibrocytes, the migration of fibocytes in response to PDGF, and fibrocyte pool size in the lungs of BLM-treated mice with or without PDGFR inhibitors (imatinib, PDGFR-blocking antibodies) to test the hypothesis that PDGF might directly contribute to the migration of fibrocytes into the injured lungs.

Materials and Methods

Detailed methods are described in the online supplement.

Mice and Materials

C57BL/6 female mice (7 wk old) were purchased from Charles River Japan, Inc. (Yokohama, Japan). Mice were maintained in the animal facility of the University of Tokushima under specific pathogen-free conditions according to the guidelines of our university (24). Imatinib mesylate was kindly provided by Novartis Pharma AG (Basel, Switzerland). The anti–mouse PDGFR-blocking antibodies, termed APA5 (PDGFR-α) and APB5 (PDGFR-β), were kindly provided by S. I. Nishikawa (Kyoto University) (27). BLM was purchased from Nippon Kayaku Co. (Tokyo, Japan). PDGF-AA, -BB, -AB, -CC, and CXCL12 were obtained from Sigma-Aldrich (St. Louis, MO).

BLM Treatment

Osmotic minipumps (model 2001; Alza Pharmaceuticals, Palo Alto, CA), containing 200 μl of saline with or without BLM (125 mg/kg), were implanted subcutaneously (28). Each experiment was performed in at least three mice per group.

Administration of Imatinib and Anti-PDGFR Antibodies

Imatinib powder was dissolved in distilled water (Otsuka Pharmaceutical Co., Tokushima, Japan) at concentrations of 5 mg/ml. Imatinib (50 mg/kg/d) or distilled water was injected intraperitoneally from Days 0 to 21. The anti–mouse PDGFR–blocking antibodies (APA5 and APB5) and control rat IgG were dissolved in PBS at a concentration of 2.5 mg/ml. APA5, APB5, or control rat IgG (0.5 mg/mouse/every other day) was injected intraperitoneally from Days 0 to 10.

Bronchoalveolar Lavage

Bronchoalveolar lavage (BAL) was performed five times with saline (1 ml) using a soft cannula (24). After counting cell number in the BAL fluid, cells were cytospun onto glass slides and stained with Diff-Quick (Baxter, Miami, FL) for cell classification.

Chemotaxis

Migration of fibrocytes was assayed as previously described (29). Recombinant human PDGF-AA, -AB, -BB, -CC, or CXCL12 (100 ng/ml) was used as chemoattractant in Boyden chambers coated with collagen-1. The migration induced by PDGF-AA or -BB was examined in the presence of imatinib (0.1–3 μM), anti–PDGFR-α antibody (Ab; 1–10 μg/ml), or anti–PDGFR-β Ab (0.3–3 μg/ml). All assays were performed in triplicate. Migration was assessed by counting the number of cells in four high-power fields with a light microscope.

FACS Analysis

The minced lungs were digested, and the harvested cells were stained with phycoerythrin-cyanine 5–labeled anti–mouse CD45 Ab, phycoerythrin-labeled anti–mouse CXCR4 Ab, and biotin-conjugated anti–mouse collagen-1 Ab, followed by streptavidin–FITC. The stained cells were analyzed by a FACScan flow cytometer (BD Biosciences-Pharmingen, San Diego, CA) (29).

Immunohistochemical Staining

Paraffin-embedded lung sections were stained with primary antibodies at 4°C overnight and subsequently stained with fluorescence-conjugated secondary antibodies and 4′,6-diamidino-2-phenylindole at room temperature for 1 hour. Fluorescence images were captured with a confocal laser scanning microscope at ×20 magnification (Leica TCS NT; Leica, Heidelberg, Germany) (30).

Statistical Analysis

Comparisons among multiple groups were made using one-way ANOVA with Newman-Keuls *post hoc* correction (Prism, version 5.0; GraphPad Software Inc.,

San Diego, CA). Differences were considered statistically significant if P values were less than 0.05.

Results

Anti-Inflammatory Effect of Imatinib Is Limited in BLM-Induced Pulmonary Fibrosis

To test whether imatinib has anti-inflammatory effect in BLM-induced pulmonary fibrosis, we measured proinflammatory cytokines in lung homogenates of BLM-treated mice by ELISA. The levels of inflammatory cytokines had peaks on Day 7 after BLM treatment. Imatinib treatment did not affect IL-1 and IL-6 levels in lung homogenates on Days 3, 7, and 14 (Figures 1A and 1B), whereas TNF-α level was attenuated by imatinib treatment on Day 7 (Figure 1C). Next, we examined BAL fluid to test whether imatinib is involved in inflammatory cell migration to the lungs in BLM-treated mice. BLM treatment resulted in increased numbers of total BAL cell, macrophage, lymphocyte, and neutrophil in BAL fluid on Days 7 and 14. Differential cell counts revealed that imatinib treatment did not affect the number of inflammatory cells in BAL fluid of BLM-treated mice on Days 7 and 14 (Figure 1D).

Elevated PDGF Levels in BLM-Treated Mice at Early Phase

The levels of PDGF-AA, -AB, and -BB in BAL fluid of BLM-treated mice were measured by ELISA (Figures 2A and 2B). There were elevated levels of PDGF-AB and -BB in BAL fluid, which seems to have had two peaks—at the early phase (Day 7) and the late phase (Days 21 and 28)—whereas PDGF-AA was not detected.

Isolation of Human and Murine Fibrocytes and the Expression of PDGFRs

Next, we examined whether fibrocytes express PDGFRs using both human and murine fibrocytes. Immunochemical double-staining for collagen-1 (*green*) and CD45 or CXCR4 (*red*) confirmed that cells isolated from human peripheral blood or murine lungs were fibrocytes (Figure E1 in the online supplement). All of the cells had fibroblast-like spindle-shape morphology, and were stained positively for collagen-1 intracellularly, and CD45 or CXCR4 on the cell membrane. Flow cytometric analysis also confirmed that fibrocytes used in the present study were negative for CD3, CD19, CD14, and positive for CD45, CXCR4, CCR7, and collagen-1, which is consistent with the phenotype of fibrocytes (Figure 3A). When we examined the expression of PDGFR-α and -β in human and murine fibrocytes, flow cytometric analysis clearly demonstrated the positive expressions of PDGFR-α and -β in both human and murine fibrocytes (Figure 3B), which were also confirmed by RT-PCR (Figure 3C).

PDGF Is a Strong Chemoattractant for Fibrocytes

We performed migration assay for fibrocytes to test whether PDGF functions as a chemoattractant for fibrocytes. Human fibrocytes migrated in response to PDGF-AA, -BB, -AB, or -CC (100 ng/ml) compared with negative control (medium alone). Interestingly, the numbers of migrating cells in response to PDGF-BB were higher than those in response to CXCL12 (100 ng/ml), which is one of the

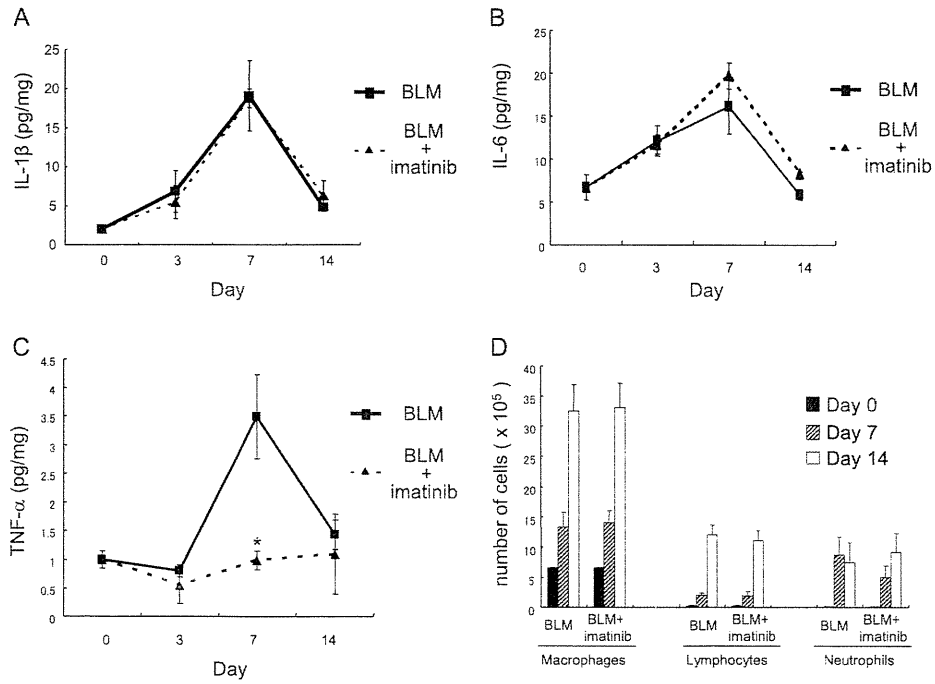

Figure 1. Anti-inflammatory effects of imatinib in bleomycin (BLM)-induced pulmonary fibrosis. The levels of (*A*) IL-1β, (*B*) IL-6, and (*C*) TNF-α in lung homogenates from BLM-treated mice with or without imatinib (50 mg/kg/d) on Days 0, 3, 7, and 14 were measured by ELISA. (*D*) Bronchoalveolar lavage (BAL) fluid was collected from BLM-treated mice with or without imatinib on Days 0, 7, and 14, and differential cell counts were performed. Data are presented as mean ± SD (n = 4 in each group). *P < 0.05 versus the value in the group treated with BLM alone.

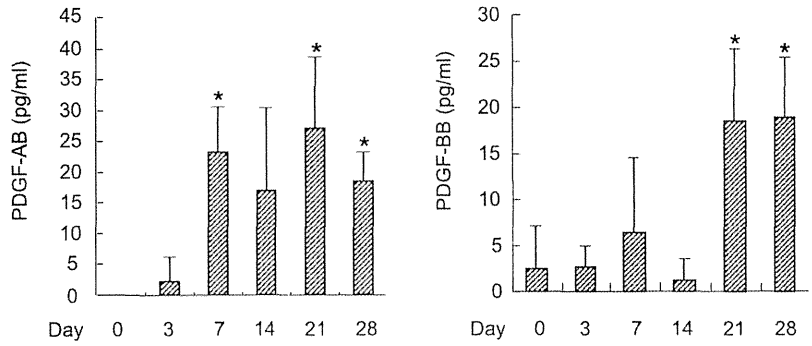

Figure 2. Elevation of platelet-derived growth factor (PDGF)-AB and -BB in BLM-treated mice. BAL fluid was collected from BLM-treated mice on Days 0, 3, 7, 14, 21, and 28. The levels of (*A*) PDGF-AB and (*B*) PDGF-BB in BAL fluid were measured by ELISA. Data are presented as mean ± SD ($n = 4$ in each group). *$P < 0.05$ versus the value on Day 0.

most important chemoattractants in fibrocyte migration into the lung (Figure 4A). Human fibrocytes migrated in response to PDGF-BB in a dose-dependent manner (Figure 4B). Imatinib (1 μM) significantly inhibited the migration of human fibrocytes in response to both PDGF-AA and -BB (Figures 4C and 4D). In addition, anti–PDGFR-α– and -β–blocking Abs dose-dependently inhibited the migration of fibrocytes in response to PDGF-AA and PDGF-BB, respectively (Figures 4E and 4F).

PDGF Inhibitors Reduce Fibrocyte Pool Size in the Lungs of BLM-Treated Mice

We examined the number of fibrocytes in the lungs of BLM-treated mice with or without imatinib treatment by using three-color FACS analysis (CXCR4, collagen-1, CD45) for lung digests of the lungs. The percentage of fibrocytes ($CXCR4^+Col1^+CD45^+$ cells) in whole-lung cells of BLM-treated mice was elevated to a peak on Day 10 after BLM treatment. Imatinib administration significantly reduced the number of fibrocytes in the lungs on Day 10 (Figures 5A and 5C). APA5 (anti–PDGFR-α Ab) and APB5 (anti–PDGFR-β Ab) also significantly reduced the number of fibrocytes in the lungs on Day 10 after BLM treatment (Figure 5B). The inhibitory effect by APB5 was stronger than that of APA5 (Figure 5D). These results suggest that the inhibition of PDGF signaling by imatinib, APA5, or APB5 suppresses fibrocyte migration into the lung after BLM-induced injury.

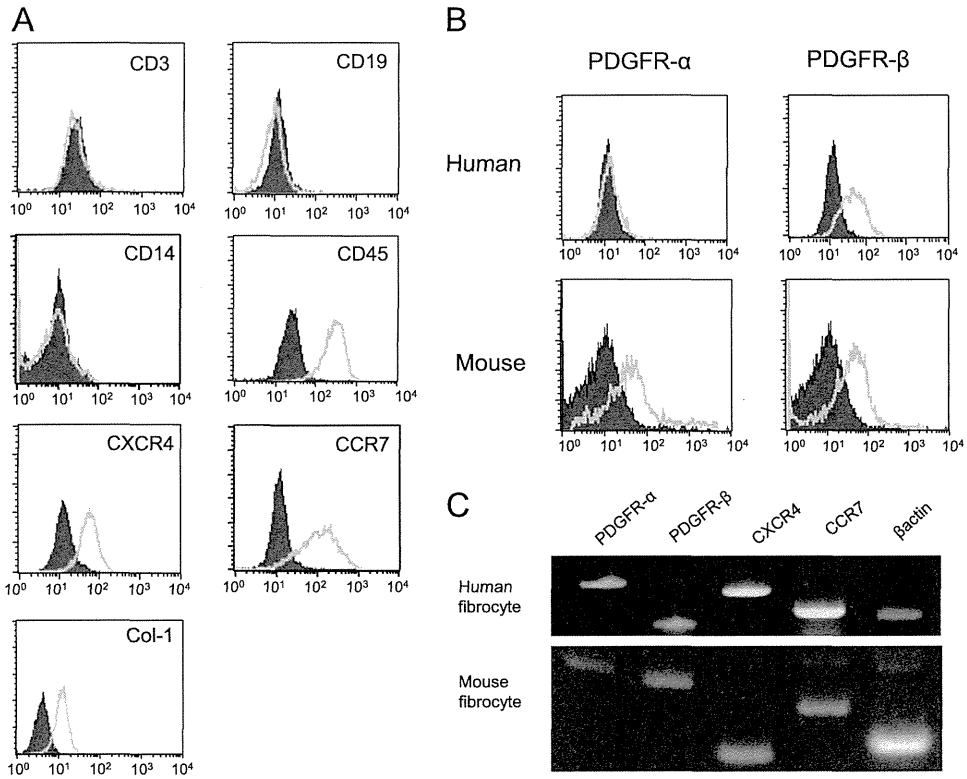

Figure 3. Expressions of PDGF receptor (PDGFR) -α and -β in human and murine fibrocytes. (*A*) The expressions of CD3, CD19, CD14, CD45, CXCR4, CCR7, and collagen-1 in human fibrocytes from peripheral blood were examined by flow cytometry. (*B*) The expression of PDGFR-α and -β in human fibrocytes from peripheral blood and murine fibrocytes from the lungs were examined by FACS. (*C*) The expression of PDGFR-α, -β, CXCR4, and CCR7 in human fibrocytes from peripheral blood and murine fibrocytes from the lungs were examined by RT-PCR.

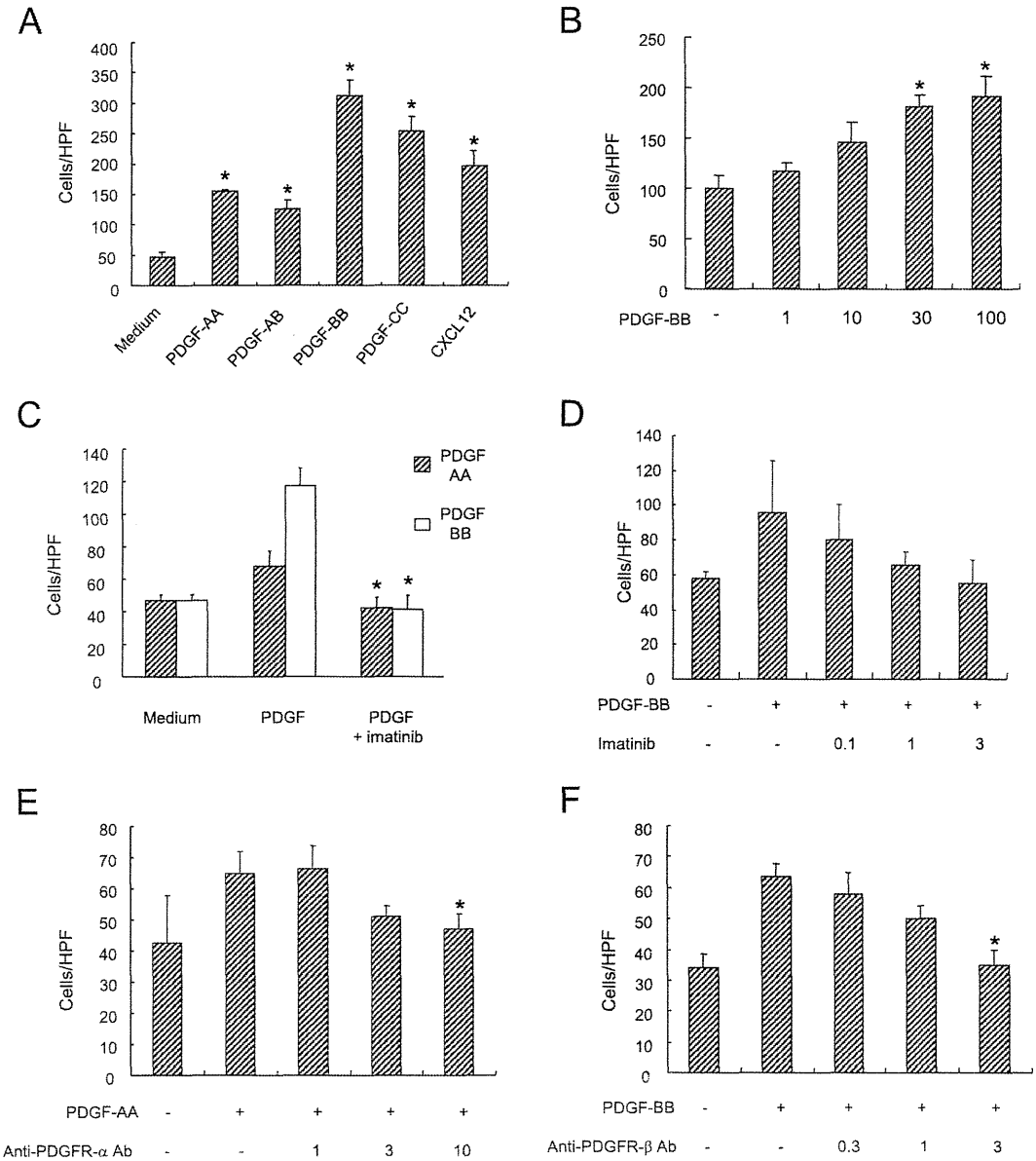

Figure 4. PDGF is a strong chemoattractant for fibrocytes. Human fibrocytes (1×10^4/well) from peripheral blood were added to the upper chambers, and medium containing PDGF-AA, -AB, -BB, -CC, or CXCL12 was added to the lower chambers. Fibrocytes were tested for chemotaxis to (*A*) PDGF-AA, -AB, -BB, -CC (100 ng/ml), and CXCL12 (100 ng/ml), or (*B*) PDGF-BB at various concentrations (1–100 ng/ml). *$P < 0.05$ versus the value in the group treated with medium alone. (*C*) Human fibrocytes treated with PDGF-AA (20 ng/ml) or PDGF-BB (20 ng/ml) in the presence or absence of imatinib (1 μM) were examined for chemotaxis. Fibrocytes were also treated with (*E*) PDGF-AA (20 ng/ml) or (*D* and *F*) PDGF-BB (20 ng/ml) in the presence of various concentrations of (*D*) imatinib (0.1–3 μM), (*E*) anti-PDGFR-α antibody (Ab; 1–10 μg/ml), or (*F*) anti-PDGFR-β Ab (0.3–3 μg/ml) were tested for chemotaxis to PDGF-AA or -BB. Data are presented as mean ± SD ($n = 4$ in each group). *$P < 0.05$ versus the value in the group treated with PDGF alone. HPF, high-power fields.

Detection of Fibrocytes in the Lungs of BLM-Treated Mice Using Immunohistochemistry

Paraffin-embedded lung sections of BLM-treated mice with or without imatinib on Day 10 were stained for S100A4/fibroblast-specific antigen-1 (FSP-1; *green*) and CD45 (*red*). Double-positive cells for S100A4/FSP-1 and CD45 observed in the lung sections are defined as fibrocytes, and single-positive cells for S100A4/FSP-1 are fibroblasts (Figure 6A). Imatinib treatment significantly reduced the number of fibrocytes per high-power field in the lungs of BLM-treated mice (Figure 6B).

Marked Expression of PDGFR-β in Fibrocytes from Patients with IPF

To test which receptor is important in fibrocyte recruitment into the lungs in patients with IPF, we examined the expression levels of PDGFR-α, -β, and CXCR4 mRNAs in fibrocytes from healthy volunteers and patients with IPF by

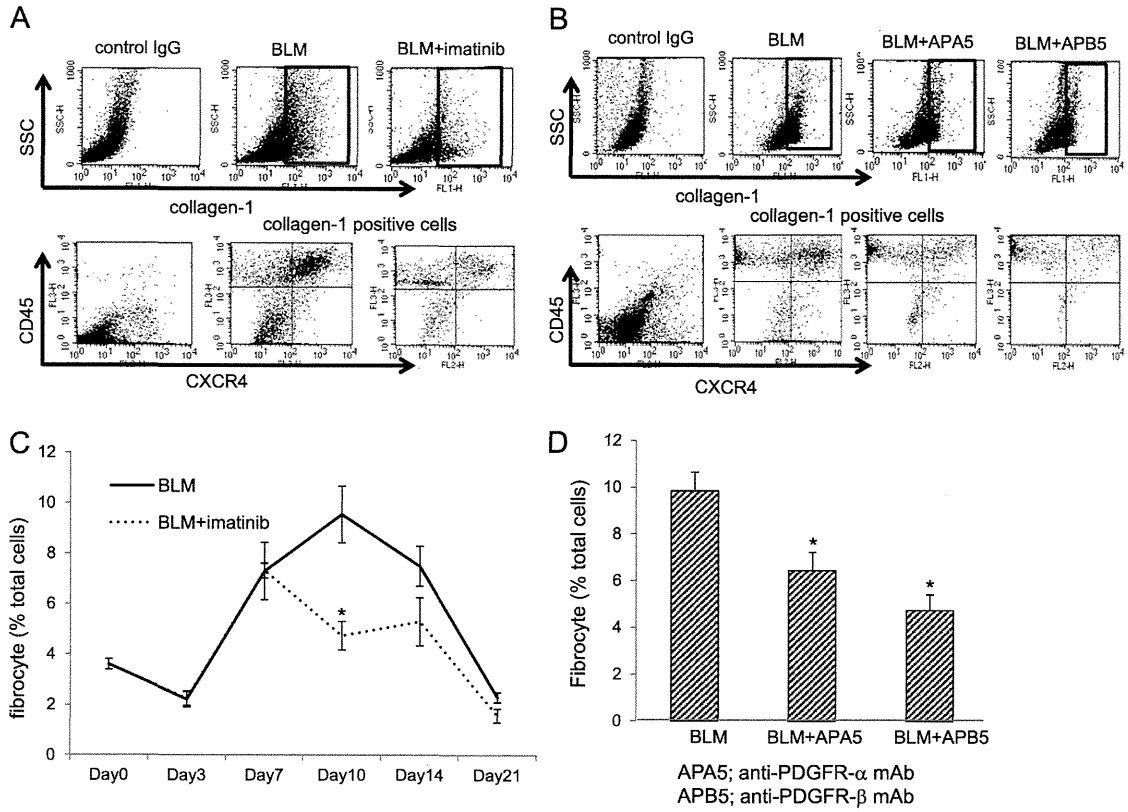

Figure 5. PDGFR inhibitors regulate fibrocyte pool size in the lungs in BLM-treated mice. Imatinib (50 mg/kg/d), APA5 (anti–PDGFR-α Ab), or APB5 (anti–PDGFR-β Ab) (0.5 mg/mouse/each other day) was intraperitoneally injected into BLM-treated mice. Lung digests stained for collagen-1, CD45, and CXCR4 were examined by flow cytometry. Lung digests were also stained for isotype control IgG labeled with FITC, phycoerythrin, or phycoerythrin-cyanine. Collagen-1$^+$ fibrocytes were examined for dual expression of CD45 and CXCR4 using the logical gates depicted. Collagen-1$^+$CD45$^+$CXCR4$^+$ fibrocytes in lung digests from (*A*) the mice with or without imatinib treatment on Days 0, 3, 7, 10, 14, and 21, or (*B*) the mice with or without PDGFR-blocking antibodies (APA5 or APB5) treatment on Day 10 were examined. The percent fibrocytes relative to total cells of lung digests ± SEM in (*C*) the mice with or without imatinib treatment, or (*D*) the mice with or without PDGFR-blocking antibodies (APA5 or APB5) treatment (n = 4 in each group). *P < 0.05 versus the value in the group treated with BLM alone.

real-time PCR. There were no significant differences in either CXCR4 or PDGFR-α mRNA levels between healthy volunteers and patients with IPF. On the other hand, PDGFR-β mRNA level was significantly higher in fibrocytes from patients with IPF than healthy volunteers (Figure 7).

Discussion

In the present study, we demonstrate the role of PDGF in fibrocyte migration into the injured lung in pulmonary fibrosis. PDGF significantly enhanced the migration of human fibrocytes. PDGFR inhibitors and anti–PDGFR-α– and -β–blocking Abs attenuated fibrocyte migration in response to PDGF *in vitro*, and reduced fibrocyte pool size in the lungs in BLM-treated mice.

In our previous study, we reported that imatinib treatment attenuated the development of pulmonary fibrosis using a BLM model in mice via inhibiting PDGF-induced fibroblast growth. Although imatinib had been expected to be an antifibrotic drug for IPF, Daniels and colleagues (31) reported that, in a randomized, placebo-controlled trial of patients with mild to moderate IPF, imatinib did not affect survival or lung function. On the other hand, we have already demonstrated that imatinib's failure to prevent pulmonary fibrosis in the BLM model in mice was caused by elevated α1-acid glycoprotein (AGP), which can bind imatinib with high affinity and blocks its biological activity, in serum and lung tissue, and that half of patients with IPF had levels of AGP high enough to prevent the effects of imatinib *in vitro* (32). Daniels and colleagues also described that the failure of the clinical trial with imatinib might have been due to AGP in addition to the lower number of patients enrolled, making it difficult to detect differences, as well as the doses of imatinib used. Imatinib might still be useful in combination with erythromycin or clarithromycin, which displace imatinib from AGP via the competition with imatinib, as we reported previously in the mouse model (32). Furthermore, in a double-blind, placebo-controlled, randomized, phase II trial, the tyrosine kinase inhibitor, BIBF1120, targeting PDGFR, vascular endothelial growth factor receptors, and fibroblast growth factor receptors, was associated with a trend toward a reduction in the decline in lung function in patients with IPF (33). Therefore, the inhibition of PDGF signaling still remains a target of antifibrotic therapy for IPF.

A study by Chaudhary and colleagues (34) demonstrated that imatinib inhibited the levels of inflammatory cytokines

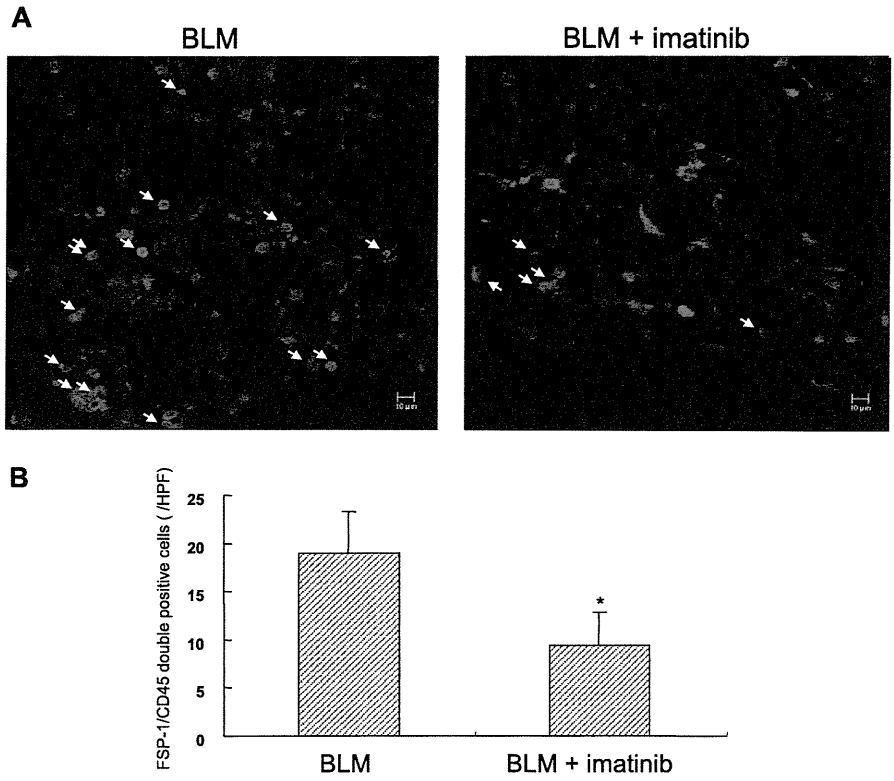

Figure 6. Murine fibrocytes in the lungs of BLM-treated mice using immunohistochemical staining for S100A4/fibroblast-specific protein (FSP) -1 and CD45. BLM-treated mice with or without imatinib (50 mg/kg/d) were killed on Day 10, and paraffin-embedded lung sections were stained with rabbit anti-S100A4/FSP-1 antibody (*green*) and rat anti-CD45 antibody (*red*). (*A*) Representative images are shown. *Arrows* indicate fibrocytes double stained for S100A4/FSP-1 and CD45. *Scale bar*, 10 μm. (*B*) S100A4/FSP-1 and CD45 double-positive cells were counted from 20 random fields per section at 40× magnification. Data are presented as mean ± SD (n = 4–5 in each group). *$P < 0.05$ versus the value in the group treated with BLM alone.

(IL-1α, -1β, and -6) in lung homogenates in BLM-treated rats. In contrast, in this study, imatinib treatment reduced the TNF-α level, but did not affect IL-1β and -6 levels, and did not change the number of inflammatory cells in BAL fluid in BLM-treated mice during early phase. The reason for the discrepancy is not clear, but might be due to the different species and delivery systems of BLM used in both studies.

Chaudhary and colleagues performed intratracheal injection in rats, but we used systemic infusion with a minipump in mice, which induces more chronic and mild model of pulmonary fibrosis in mice. We believe that the anti-inflammatory effects of imatinib were limited in our model, although imatinib partly had anti-inflammatory function.

PDGF-AB and -BB levels in BAL fluid were elevated not only at late phase, but also at early phase. These results suggest that PDGF may also play roles in the development of pulmonary fibrosis during both early and late phase, during which PDGF might function to induce fibroblast growth and migration, in BLM-induced pulmonary fibrosis in mice. Because PDGF could be a strong chemoattractant for cells of mesenchymal origin, we hypothesized that PDGF is also a chemoattractant for fibrocytes, and plays an important role in fibrocyte recruitment into injured lungs during the early phase of pulmonary fibrosis. In fact, fibrocytes were increased in the lungs of BLM-treated mice at the peak on Day 10, around which time PDGFs were elevated in BAL fluid, supporting our hypothesis.

Some chemokines (CXCL12, CCL12, CCL21) and chemokine receptors (CXCR4, CCR2, CCR7) have been identified as being correlated with fibrocyte recruitement into the tissues and the development of pulmonary fibrosis in BLM-treated mice in recent papers (6, 35–39). In particular, the CXCL12–CXCR4 biological axis plays an important role in the migration of fibrocytes in BLM-induced lung fibrosis (6). The marked expression of CXCL12 in both the lungs and plasma of patients with interstitial pneumonia, and the negative correlation between plasma levels of

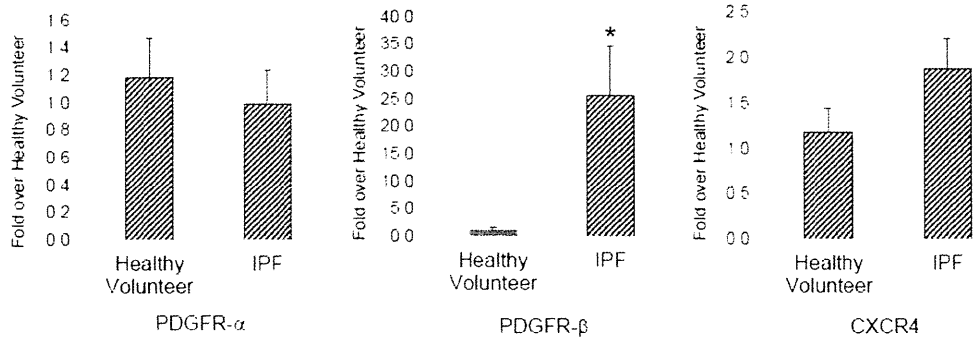

Figure 7. Marked expression of PDGFR-β in fibrocytes from patients with idiopathic pulmonary fibrosis (IPF). The expression of PDGFR-α, -β, and CXCR4 in fibrocytes from peripheral blood of healthy volunteers and patients with IPF were examined by real-time PCR. Data are presented as mean ± SEM (n = 4–5 in each group). *$P < 0.05$ versus the value in healthy volunteers.

CXCL12 and oxygen saturation on exercise in patients with IPF have been reported previously (13, 14). On the other hand, we demonstrated that fibrocytes expressed PDGFR-α and -β, and migrated in response to PDGF-AA, -AB, -BB, and -CC *in vitro*. Interestingly, the number of migrating fibrocytes in response to PDGF-BB was roughly 1.6-fold higher than that in response to CXCL12, suggesting that PDGF-BB is an important chemoattractant for fibrocytes, as is CXCL12. Imatinib and anti-PDGFR–blocking Abs decreased fibrocyte migration *in vitro* and reduced fibrocyte pool size in the lungs *in vivo*. The decrease associated with APB5 (anti–PDGFR-β Ab) was higher than that associated with APA5 (anti–PDGFR-α Ab) *in vivo*, which is consistent with the finding that PDGF-BB was the strongest chemoattractant among PDGFs used *in vitro*. In addition, a marked expression of PDGFR-β was observed in fibrocytes from patients with IPF compared with healthy volunteers, although there were no significant differences in PDGFR-α and CXCR4 expression levels between these groups. This suggests that the elevated expression of PDGFR-β in fibrocytes may induce the increased numbers of fibrocytes in lungs of patients with IPF, which may be involved in the development of the disease.

Our results extend those of a previous study by García-de-Alba and colleagues (40), who reported that human fibrocytes migrated in response to PDGF-BB *in vitro*. In this study, we have revealed the role of PDGF in fibrocyte migration into the lungs, not only *in vitro*, but also *in vivo*, and the importance of the PDGF-BB–PDGFR-β biological axis in many combinations of PDGFs and PDGFRs in fibrocyte migration.

Our findings also suggest that the antifibrotic effect of the early treatment with imatinib in BLM-induced pulmonary fibrosis may depend, at least in part, on the inhibition of fibrocyte recruitment by inhibiting PDGF signaling. When we measured CXCL12 in the BLM-treated lungs, treatment with imatinib did not affect the level of CXCL12 (Figure E2). However, Mehrad and colleagues (41) reported that PDGF up-regulated CXCR4 expression in fibrocytes, and PDGF-induced CXCR4 expression was attenuated by specific inhibition of phosphatidyl inositol 3-kinase, which is a major signal transduction pathway downstream of the PDGF receptor. Therefore, decreased numbers of fibrocytes in the lungs associated with the treatment with PDGF inhibitors may depend on both the direct inhibition of PDGF-induced migration of fibrocytes and the indirect inhibition of CXCL12-induced migration of fibrocytes through attenuation of CXCR4 expression. In addition, not only migration, but also proliferation of fibrocytes should be examined for a complete assessment of the effects of PDGF on fibrocytes. Our preliminary study showed poor growth ability of fibrocytes, especially in patients with IPF, compared with lung fibroblasts (Figure E3), suggesting that PDGF could play a role as a chemoattractant for fibrocytes.

In summary, fibrocytes expressed PDGFR and migrated in response to PDGF. PDGF inhibitors (imatinib and anti-PDGFR Abs) reduced the number of fibrocytes in the lungs in BLM-treated mice. PDGF is a strong chemoattractant for fibrocytes, as is CXCL12. PDGF may also contribute to the development of pulmonary fibrosis by inducing fibrocyte migration into the lung. In particular, the PDGF-BB–PDGFR-β biological axis may be more important in fibrocyte migration into the lungs in pulmonary fibrosis compared with the other PDGFs and PDGFR. We speculate that the inhibition of PDGF signaling will continue to be a target of antifibrotic therapy for IPF. ■

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

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フルエンザワクチン，肺炎球菌ワクチン」．

・肺癌の発生率が10－30％と比較的高率であることを説明する．

・遺伝性は明らかではないが，時に家族性の場合があることを説明する．

好酸球性肺炎

eosinophilic pneumonia

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病態と診断

・肺組織の好酸球増多により引き起こされると考えられる疾患群の総称を好酸球性肺疾患とよぶが，そのなかで好酸球が炎症細胞の主要な細胞であり，かつ原因不明の場合に（特発性）好酸球性肺炎と呼称される．

・発症様式の時間経過により，急性好酸球性肺炎（acute eosinophilic pneumonia：AEP）と慢性好酸球性肺炎（chronic eosinophilic pneumonia：CEP）に分けられ，疾患病態も異なると考えられている．

・肺の好酸球増多は，気管支肺胞洗浄（broncho－alveolar lavage：BAL）液や生検肺組織を用いて判定されるが，通常BAL液中の好酸球比率が25％以上を占める場合とされている．

Ⓐ 急性好酸球性肺炎（AEP）

・発症年齢は比較的若年者（平均30歳）に多い．数日－数週（1か月以内）の臨床経過で，発熱，乾性咳嗽，呼吸困難，胸痛などの自覚症状とともに発症する．呼吸困難の程度は強いことが多く，1週間以内に呼吸不全に進展することも多い．

・喫煙や薬剤，粉じん，ガスなどの吸入が誘因として報告されていることから，詳細な病歴聴取は重要である．通常，末梢血の好酸球増多は伴わないことが多い．胸部聴診上，fine cracklesを聴取することが多い．胸部画像所見では，両側肺野のびまん性浸潤影あるいはすりガラス影が認められる．

・診断基準として，① 1か月以内の発熱を伴う急性発症，② 両側肺野のびまん性の浸潤影，③ 低酸素血症（室内気で PaO_2 60 Torr未満，SpO_2 90％未満），④ 肺好酸球増多（BAL液で25％以上か組織学的診断），⑤ 原因不明，の5項目があげられている．また，通常再燃がないのも特徴的な所見である．

Ⓑ 慢性好酸球性肺炎（CEP）

・発症年齢は平均45歳で女性に多い（男女比は2：1）．2/3の症例で喘息の合併を認め，アレルギー性鼻炎，薬剤アレルギー，鼻ポリープの合併も多い．

・発症は亜急性で，診断まで数か月の経過をとることもある．一般的な臨床症状は，咳嗽，発熱，喀痰，呼吸困難である．

・AEPと異なり，ほとんどの症例で末梢血好酸球数の増多を認める一方，呼吸不全を呈する症例はまれである．また，約半数でIgEの上昇を認める．

・胸部画像所見では，両側肺野に末梢優位の非区域性浸潤影－すりガラス影を認め，しばしば移動性を示す．陰影は外側優位になることから，肺水腫のネガ像ともよばれる．

治療方針

AEP，CEPともにステロイド治療への反応性がよい．

Ⓐ AEP

AEPには自然軽快症例も存在する．重症度によりステロイド投与量が異なるが，一般に投与開始後48時間以内に改善が認められる．

1. 軽症・中等症例

℞ 処方例

プレドニゾロン錠（5 mg）　1回0.5 mg/kg　1日1回　朝食後．治療反応性をみながら漸減，中止

2. 重症例　人工呼吸管理を必要とする症例もある．

℞ 処方例

ソル・メドロール注　1回1,000 mg　1日1回　点滴静注　3日間

以後，プレドニゾロン錠（5 mg）　1回0.5－1.0 mg/kg　1日1回　朝食後．治療反応性をみながら漸減，中止

ステロイドの投与期間は定まってはいないが，3か月以内が多い．治療反応はよく，ほとんど再発がないといわれている．

Ⓑ CEP

℞ 処方例

プレドニゾロン錠（5 mg）　1回0.5 mg/kg　1日1回　朝食後．治療反応性をみながら漸減

漸減中に再燃をきたすことが多く，約半数の症例で再燃をきたしたという報告がある．治療は比較的長期になりやすく，6か月以上が必要である（平均6－12か月）．しかし，再燃時は初回治療時に比較してステロイドの必要量は少ない．喘息を合併した患者のなかで，吸入ステロイド使用症例については再燃が少ないことが知られている．

最新医学・別冊　新しい診断と治療のABC 85　(別刷)

全身性疾患の肺病変
第1章　膠原病と肺病変

強皮症

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第1章　膠原病と肺病変

強皮症

要旨

強皮症に合併する間質性肺疾患（SSc-ILD）の多くは予後良好であるが，進行性にILDが悪化し，肺機能の低下から呼吸不全となり，死亡に至る症例も存在する．進行性の症例には免疫抑制療法を行うが，治療適応となる症例の選択，治療内容については，標準的なものは確立されていない．最近になって大規模で質の高いRCTが複数発表され，それらに基づいた治療が行われるようになっている．本稿では，SSc-ILDの診断と治療の現状について説明する．

● キーワード

強皮症に合併する間質性肺疾患
肺活量
高分解能コンピューター断層撮影
シクロホスファミド

はじめに

全身性強皮症（SSc）または強皮症（scleroderma）は，皮膚を病変の主座とし，皮膚を含めた全身の諸臓器の線維化と微小血管障害による末梢循環不全を来す疾患である．診断基準としては，1980年のアメリカ・リウマチ学会の分類予備基準を改訂した『全身性強皮症・診断基準2003』（表1）が用いられる．SScは皮膚病変の範囲によって，びまん性（dcSSc）と限局型（lcSSc）に分類（表2）され，両者は臨床的に異なった特徴を持ち，肺病変を含めた臓器合併症の内容も異なる[1]．SScは高頻度に肺病変を合併するが，中でも間質性肺疾患（ILD），肺高血圧症などは進行性の病態で，SScの予後規定因子とされている．SScの肺病変には直接的な肺病変と間接的な肺病変があり，これらが合併する症例も存在する（表3）[2]．また，近年SScの肺病変も特発性肺線維症（IPF）と同様に急性増悪（AE）を来す症例が報告されている[3]．本稿では，SScに合併するILD（SSc-ILD）の診断と治療について説明する．

表1 全身性強皮症・診断基準 2003

大基準

手指あるいは足趾を超える皮膚硬化*

小基準

1）手指あるいは足趾に限局する皮膚硬化

2）手指尖端の陥凹性瘢痕，あるいは手指の委縮**

3）両側性肺基底部の線維症

4）抗トポイソメラーゼⅠ（Scl-70）抗体またはセントロメア抗体陽性

大基準，あるいは小基準1）および2）～4）の1項目以上を満たせば全身性強皮症と診断

*：限局性強皮症（いわゆるモルフェア）を除外する

**：手指の循環障害によるもので，外傷などによるものを除く

表2 全身性強皮症（SSc）病型分類（文献[1]より引用改変）

	びまん性（dcSSc）	限局型（lcSSc）
皮膚硬化	肘関節より近位皮膚硬化	肘関節より遠位皮膚硬化
進行	急速（皮膚硬化出現2年以内）	緩徐（皮膚硬化出現5年以上）
Raynaud 現象と皮膚硬化	皮膚硬化が先行するか，ほぼ同時	Raynoaud 現象が先行
毛細管顕微鏡所見	毛細血管の脱落	毛細血管の蛇行，拡張
爪上皮内出血点	進行期には消失	多数
腱摩擦音	腱摩擦音（+）（ただし日本人では少ない）	腱摩擦音（−）
関節拘縮	高度	軽度
石灰沈着	まれ	多い
主要臓器病変	肺，腎（日本人ではまれ），心，食道	肺高血圧症（日本人でまれ），食道
主要抗核抗体	抗トポイソメラーゼⅠ（Scl-70）抗体 抗 RNA ポリメラーゼ抗体	抗セントロメア抗体

合併頻度と予後

SSc は ILD を高率に合併するとされているが，その頻度は報告により，40～90％と差がある．剖検による検討では100％，高分解能コンピューター断層撮影（HRCT）では90％，肺機能検査では40～75％で ILD の所見を認めたとの報告がある[4)5)]．SSc-ILD は比較的緩徐で無治療でも肺機能は保たれ，生命予後良好な症例が多数を占める．

表3　全身性強皮症（SSc）の肺病変（文献[2]より引用改変）

Direct pulmonary involvement
間質性肺疾患（ILD）
ILDと肺高血圧症の合併
肺高血圧症
気道病変
胸膜病変
Indirect pulmonary complications
胃食道逆流による誤嚥
感染症
薬剤性肺障害
悪性腫瘍
呼吸筋低下
皮膚硬化による拘束性障害
Combination of direct and indirect pulmonary involvement

しかし，進行性に肺機能の低下を認め，呼吸不全から死亡に至る症例も存在し，25％の症例でSSc-ILDの診断後3年以内に重篤な呼吸不全を来すとの報告がある[6]．かつては難治性であった腎クリーゼがアンジオテンシン変換酵素（ACE）阻害薬による治療により予後が改善されたことから，現在ではILDがSScの最大の死因となっている．ILDの発症が先行し，その後SScと診断される症例も存在するため，特発性ILDと診断されている症例で膠原病の合併を示唆される症状を有する場合は，注意が必要である．

血液検査

SScは多くの自己抗体の発現が認められており，主なものとして，抗トポイソメラーゼⅠ（Scl-70）抗体，抗セントロメア抗体，抗RNAポリメラーゼⅢ抗体，抗U1-RNP抗体がある．自己抗体とSSc-ILDの関係では，抗Scl-70抗体陽性症例でILDの合併率が高く，抗セントロメア抗体陽性で低いとされている．Hamaguchiらの報告によると，SSc-ILDの合併率は抗Scl-70抗体陽性84％，抗セントロメア抗体陽性7％，抗U1-RNP抗体30％との結果であった[7]．自己抗体とSSc-ILDの重症度には有意な相関関係はなく，SSc-ILDの進行速度，予後の予測に有用なバイオマーカーは，現在までのところ同定されていない．

画像検査

1．胸部単純X線写真

胸部単純X線写真は，両下肺優位の網状影と肺の容積減少を認める．陰影の広がりや部位によっては異常所見を指摘できないこともあり，SSc-ILDのスクリーニング検査としては不十分である．

2．胸部 HRCT

胸部 HRCT は SSc-ILD の非侵襲的な検査として有用であり，胸部単純X線写真では指摘できない下葉背側の限局的な間質性陰影も指摘することが可能である．HRCT 上は大部分が非特異性間質性肺炎（NSIP）パターンであるが，通常型間質性肺炎（UIP）パターンをとる症例も存在する．所見としては，すりガラス影と網状影が中心であるが，蜂巣肺を伴うこともある[8]．すりガラス影は胸膜に接した末梢性の淡い陰影であり，主に炎症性細胞浸潤と浮腫を反映すると考えられている．網状影は肺野に不均等な分布を示す濃度上昇で，膠原線維や細胞成分の増加による線維化を反映する[9]．すりガラス陰影は SSc-ILD の進行を予測する画像所見と考えられていたが，網状粒状陰影を主体とする線維化陰影の存在が，1年後における肺機能悪化と有意な関連を示したとの報告があり[8]，今後さらなる検討が必要である．

肺機能検査

拘束性障害のパターンを示し，％肺活量（%VC）と％一酸化炭素肺拡散能（%DLco）の低下が主な所見である．%VC と %DLco の経時的な低下が予後不良と相関するとされている．現状で，SSc-ILD の進行度と生命予後を予測する最も確実な指標は，経時的な %VC の測定である[10]．胸部 HRCT での病変の広がりや %VC の低下と比較して，%DLco の低下がより高度の場合には，肺高血圧症の合併を考える．また，%VC は SSc による皮膚硬化や筋力低下でも低下するため，注意が必要である．

気管支肺胞洗浄（BAL）液検査

胸部 HRCT で異常所見のある症例の 38 ～ 72％で BAL での細胞数の増加（好中球，好酸球）を認める一方で，HRCT で異常所見を認めない症例の約半数で BAL の異常を認めたとの報告がある[11]．BAL の異常所見が胸部 HRCT 所見の悪化や，肺機能検査での %VC，%DLco の低下と相関するとの報告があったが，最近の報告で病変進行の予測や治療反応性の予測には有用でないことが示された[12]．気管支鏡は侵襲的な検査であり，現状での実地臨床上の適応は感染症などの鑑別目的に限られると言える．

肺生検（経気管支，胸腔鏡下）

SSc-ILDにおいて，胸部HRCT，BALで肺胞炎がないにもかかわらず肺機能の低下が認められる症例では，組織学的に肺胞炎の有無を確認することが有用な症例もある．まずは，経気管支肺生検を行うが，十分な情報が得られないことが多く，正確な診断のためには胸腔鏡下での肺生検が必要である．侵襲的な検査であり，現状では組織学的な分類に応じた治療は確立しておらず，肺生検の意義は限定的と考えられている．

治療

SSc-ILDは本邦SSc患者の死因として最も多く，進行性に肺機能が低下する症例は予後不良である．しかし，全く進行を認めない症例も多く存在し，呼吸不全へと進行する症例は半数以下である．前述したように，%VC，%DLcoの経時的な低下は予後不良と相関するため，積極的な治療が必要である[13]．また，胸部HRCTですりガラス様陰影を認める場合は，進行性の肺胞炎が存在すると考え，治療開始を考慮する．厚生労働省強皮症調査研究班によるSSc-ILDの治療指針を示す（図1）．具体的な治療法として確立したものはないが，現時点で有効性に関して高いエビエビデンスのある治療は，シクロホスファミド（CPA）の経口投与のみである．12ヵ月間の投与後にほかの免疫抑制薬に切り替えるが，いずれの薬剤を選択すべきかに関しては明確なエビデンスはない．CPA，副腎皮質ステロイドを含めた各種免疫抑制薬の具体的な用量用法を示す（表4）．

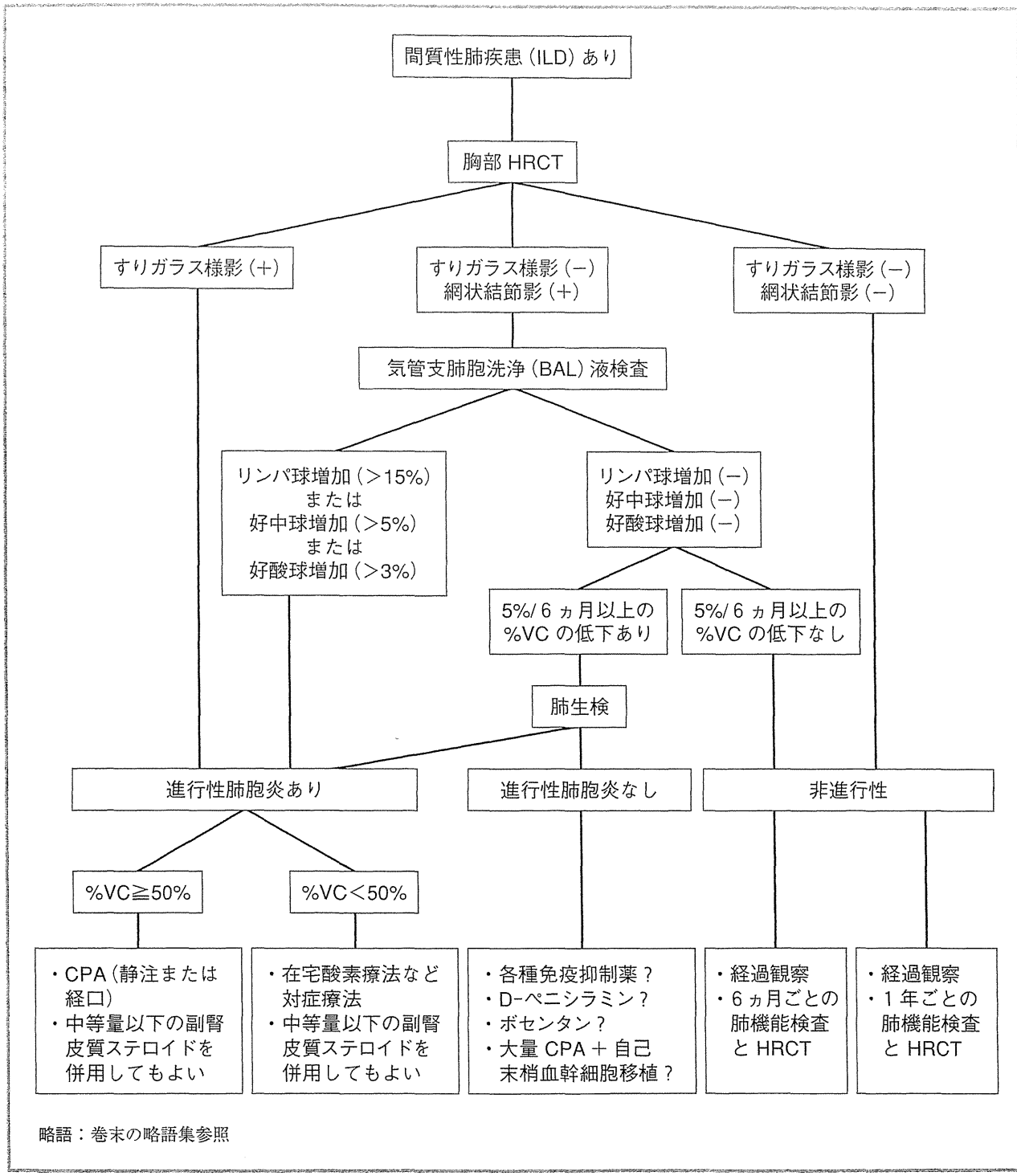
1．副腎皮質ステロイド

ほかの膠原病の治療で行われる大量副腎皮質ステロイドについては，単独使用の有効性に関しては，否定的な報告がほとんどである．また，大量副腎皮質ステロイドは腎クリーゼの危険因子とされており，使用は避けるべきである[14]．ただし，中等量以下の副腎皮質ステロイドの併用は，CPAの効果を増強する可能性が示唆されている．

2．CPA

2006年に大規模なRCTが2種類発表されており，これらの結果を受けて，現状最も使用されている薬剤である．Scleroderma Lung Study

図1 強皮症の治療指針 ―間質性肺病変― (文献[12]より引用改変)



略語:巻末の略語集参照

ではCPA経口投与(2 mg/kg/日)で1年後の努力肺活量(FVC),全肺気量(TLC)の改善を認めたと報告している[15]. また, Fibrosing Alveolitis in Scleroderma trialでは, CPAパルス療法と少量プレドニゾロンで, 6ヵ月治療後にアザチオプリン(AZP)で維持療法を行い, 1年後のFVCが改善したと報告している[16]. これらの

表4　全身性強皮症（SSc）の薬物療法（文献[12]より引用改変）

薬剤名	商品名	投与法
シクロホスファミド（CPA）	エンドキサン®	経口 1～2 mg/kg/日を 12ヵ月間 間欠静注療法 500～750 mg/m^2 1～3ヵ月に1回を計6回
副腎皮質ステロイド	プレドニゾロン®	経口 0.2～0.5 mg/kg/日
D-ペニシラミン	メタルカプターゼ®	経口 50～100 mg/日
ボセンタン	トラクリア®	経口 125～250 mg/日
アザチオプリン（AZP）	イムラン®, アザニン®	経口 1～2 mg/kg/日
シクロスポリン	ネオーラル®	経口 100～250 mg/日
タクロリムス	プログラフ®	経口 1～5 mg/日

RCTでは長期予後に関するデータは示されておらず，今後の課題と考えられる．

呼吸機能低下症例に対する治療

重症度分類4にあたる%VCが50%以下，あるいは在宅酸素療法を要する症例の死因の多くは肺感染症で，次いで気胸，肺がん，二次性肺高血圧症を伴った心肺機能不全である．このような症例では病変の可逆性は望めず，免疫抑制薬の使用は控えるべきである．

おわりに

ILDはSScに高率に合併し，予後規定因子となっている．呼吸不全から死亡に至る症例を早期に発見し，適切な治療を開始することが重要である．

岸　　潤・河野　弘・西岡安彦

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学術講演会

COPD診療の現状と徳島県での取り組み

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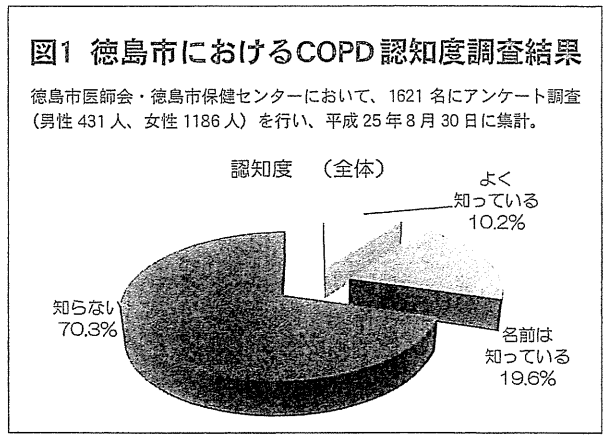
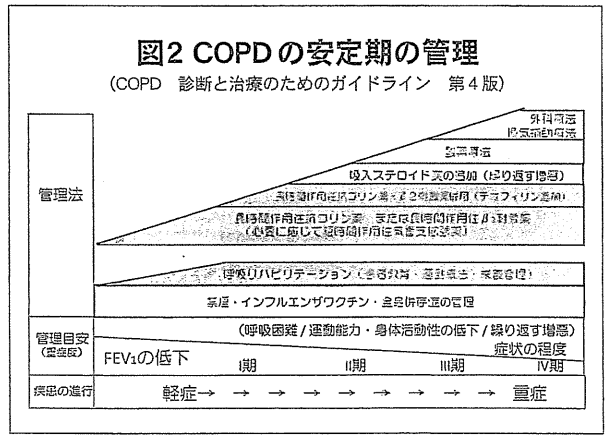
厚生労働省が主幹となり進められている啓発活動「健康日本21」の第2次プロジェクトが平成25年4月より開始されるとともに，新たにCOPDが重点疾患に追加された。それに伴い「健康徳島21」の重点目標にもCOPDが追加され，徳島県のホームページに掲載されている。COPDは「肺の生活習慣病」とも呼ばれ，WHOによると世界的に死亡者数は増加傾向にあり，2020年には世界の死亡原因の第3位になると推定されている。本邦においても患者数が500万人を超えると推定されているものの，治療を受けている患者数は約22万人と少なく，国民の認知度も低い状態にある。平成23年度の全国的な調査によるとCOPDの認知度は25%であり，平成34年に認知度を80%に上げることが「健康日本21」の目標とされている。一方，徳島県における現状でのCOPD認知度は不明であった。昨年8月に徳島市医師会，徳島市保健センターの協力により，徳島市で1621人に対してアンケート調査が行われCOPD認知度について調査した結果，29.8%という数値が得られた（図1）。徳島市においてもCOPDの認知度は全国的な数値とほぼ同じであると考えられる。そのため昨年度より徳島市，徳島県におけるCOPD認知度の向上に向けた活動が開始されている。





このような社会的な取り組みの中，COPDに対する診療も大きく変わろうとしている（図2）。長い間，治療法のない疾患と考えられていたCOPDであるが，最新のGOLDガイドラインには「予防と治療が可能な疾患」と記載されている。特に最近の薬物療法の進歩は著しく，吸入気管支拡張薬の新薬が次々と上市されているのが現状である。薬剤は，抗コリン薬とβ2刺激薬の2剤が基本であることには変わりがないが，個々の薬剤の固有活性が高く有害事象が少ない薬剤が開発されてきた。特に，上記2つの薬剤の合剤には強力な気管支拡張作用が確認されている。進行期のCOPDであっても，大幅な1秒量の改善が得られ，自覚症状の改善や運動耐容能の向上を患者自身が自覚できることが多い。さらに最近の大規模臨床試験の結果から，これらの気管支拡張薬にはCOPDの増