Mast Cell Basic Fibroblast Growth Factor in Silicosis

HIRONOBU HAMADA, VAL VALLYATHAN, CARLYNE D. COOL, ELIZABETH BARKER, YOSHIKAZU INOUE, and LEE S. NEWMAN

Division of Environmental and Occupational Health Sciences and Pulmonary Division and Department of Medicine, National Jewish Medical and Research Center, and Division of Pulmonary Science and Critical Care Medicine and Departments of Pathology, Medicine, and Preventive Medicine and Biometrics, University of Colorado Health Sciences Center, Denver, Colorado; National Institute for Occupational Safety and Health, Morgantown, West Virginia; and National Kinki-Chuo Hospital for Chest Diseases, Osaka, Japan

To investigate the role of mast cells (MC) and their fibrogenic growth factors in silicosis, we performed quantitative immunohistochemistry for MC tryptase and for basic fibroblast growth factor (bFGF) in lung tissue from silicotic and control subjects. Anti-bFGF antibody was bound to lung MC, basement membrane, endothelial cells, and smooth-muscle cells. Morphometric analysis revealed that the volume density (V_v) of MC was increased in silicotic lung and that the V_v of bFGF-positive ($bFGF^+$) cells was significantly higher than normal in silicotic lung. Most MC contained bFGF ($\rho = 0.88$, $p < 0.001$). The V_v of collagen/reticulin fibers was increased in silicosis and correlated with the V_v of $bFGF^+$ cells ($\rho = 0.81$, $p < 0.001$). Immature silicotic nodules contained $bFGF^+$ MC throughout the loose array of collagen/reticulin fibers. In large, mature nodules, the density of collagen/reticulin fibers was higher, and $bFGF^+$ MC were found only in the nodule periphery. Because of this circumferential MC alignment in silicotic nodules, we observed a negative correlation between the V_v of $bFGF^+$ MC and the density of collagen/reticulin fibers in silicotic nodules ($\rho = -0.80$, $p < 0.001$) and between the V_v of all other nodule-associated cells and the density of collagen/reticulin fibers in the hypocellular nodule centers ($\rho = -0.84$, $p < 0.001$). We conclude that MC that produce bFGF may play an important role in the development of silicosis.

The chronic inhalation of inorganic dust produces sustained pulmonary inflammation, resulting in the development of interstitial lung disease (1). Silicosis, caused by the inhalation of crystalline silica particles, is the most common inorganic dust disease. Histologically, this disease is characterized by hyalinized and fibrotic nodules, thickening of alveolar interstitium, and accumulation of inflammatory cells such as alveolar macrophages (AM) and lymphocytes (2). The pathogenesis of silicosis has been related to the accumulation of inflammatory cells that produce fibrogenic and inflammatory cytokines and growth factors, including tumor necrosis factor (TNF)-α (3–9), interleukin (IL)-1 (9, 10), transforming growth factor (TGF)-β (11, 12), macrophage inflammatory protein (MIP)-1 and MIP-2 (13, 14), platelet derived growth factor, insulinlike growth factor, and fibroblast growth factor (15, 16). AM are thought to be key inflammatory cells in silicosis, since they produce most of these fibrogenic factors in silicotic lung (3–16). The contributions of other immune cells to the pathogenesis of silicosis have been less well studied.

Recent reports suggest that mast cells (MC) are abundant in human fibrotic lung diseases (17–21) and may contribute to pulmonary fibrosis (20–22). Increased numbers of lung mast cells have been reported in animal models of bleomycin-, radiation-, and dust-induced fibrotic lung disease (23–28). Several studies have shown that MC produce mediators and growth factors such as tryptase (29), TNF-α (30–32), IL-1, interferon (IFN)-γ (33), and TGF-β (32), which play important roles in inflammation and fibrogenesis. In particular, basic fibroblast growth factor (bFGF; FGF-2) is made by human MC. Only one isoform (17.8 kD) of bFGF was found to be abundant in MC that accumulated in the fibrotic lungs of patients with idiopathic pulmonary fibrosis (IPF), sarcoidosis, and chronic beryllium disease (CBD) (34). Several reports have confirmed that human and murine MC produce and release bFGF in fibrotic diseases (35–37). However, we are aware of only a few reports of studies showing that MC may be related to the pathogenesis of silica-induced pulmonary fibrosis in rats and mice (27, 28). Saffiotti and colleagues found that the number and the degranulation rate of MC increased markedly in silicotic rat lungs as compared with those of controls (27). Furthermore, Suzuki and associates demonstrated that silica-induced pulmonary inflammation with fibrosis was attenuated in MC-deficient mice ($WBB6F_1$-W/W^v) (28). Although they hypothesized that histamine, serotoninlike substances, and several cytokines released from MC might be important inflammatory products, the pathophysiologic role of MC in silica-induced lung injury has not been examined. We find no reported examinations of MC in human subjects with silicosis, although one previous study described increased levels of histamine in the blood of silicotic subjects (38).

In conducting the present study we hypothesized that MC are present in increased numbers in areas of extracellular matrix deposition in silicosis, and that they produce bFGF. To test this hypothesis, we performed quantitative immunohistochemistry for MC tryptase and bFGF in lung tissue sections from human subjects with silicosis and from control subjects. Furthermore, we compared bFGF immunopositive ($bFGF^+$) cells with collagen/reticulin fibers as a marker of fibrotic severity, and examined the physical location of $bFGF^+$ MC in early and late stages of silicotic nodule formation.

METHODS

Study Population

The study population with silicosis consisted of 14 autopsied individuals drawn from a large number of cases of coal worker's pneumoconiosis submitted to the National Coal Workers' Autopsy Service of the National Institute for Occupational Safety and Health, Morgantown, WV. Selection of cases for the study was based on the presence of two or more of the following criteria: (*1*) an occupational history of crystalline silica exposure in certain job categories; (*2*) small opacities on chest radiography with a profusion score of 1/0 or more according to the International Labour Organization radiographic classification system for pneumoconioses (39); and (*3*) pathologic changes in the lungs consistent with silicosis (i.e., silicotic nodules).

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Correspondence and requests for reprints should be addressed to Lee S. Newman, M.D., National Jewish Medical and Research Center, Division of Environmental and Occupational Health Sciences, 1400 Jackson Street, Denver, CO 80206.

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Lung tissues from 14 control subjects included tissues from eight autopsied subjects who had noninterstitial lung diseases and from six lung allograft donors.

Table 1 summarizes the demographic data for all of the study subjects. There was no significant difference in the median age of patients with silicosis and that of control subjects. All of the coal miners with silicosis included in the study were white males and never-smokers. We observed no significant difference between the race and sex distribution and the smoking status of patients with silicosis and those of control subjects. Table 2 summarizes the occupational histories and pulmonary silicosis grade (40) of all subjects with silicosis. The mean duration of silica exposure was 40.5 ± 3.8 yr.

Sample Collection

Lung tissue was fixed with 10% buffered formalin and embedded in paraffin. One paraffin block was used for each subject. Each block contained one or two specimens obtained at autopsy or lung transplantation. Each block was cut into 2-μm-thick serial sections and laid on Superfrost/Plus slides (Fisher Scientific, Pittsburgh, PA). All samples were stained with hematoxylin and eosin (H&E), Movat's pentachrome (41), and immunohistochemical methods described subsequently.

Immunohistochemical Staining of Tryptase, CD68, and bFGF

To examine the relationship among MC, macrophages, bFGF, and fibrosis, we performed immunohistochemistry with methods using avidin–biotin complex (ABC) formation and alkaline phosphatase in a Vectastain ABC-AP kit (Vector Laboratories, Burlingame, CA) according to procedures described previously (34). Monoclonal antihuman MC tryptase antibody (1 μg/ml, $IgG_1\kappa$; Dako, Carpinteria, CA) and monoclonal antihuman CD68 antibody (3.6 μg/ml, PG-M1, $IgG_3\kappa$; Dako) were used to identify MC and macrophages, respectively. Bovine bFGF, type II (10 μg/ml, $IgG_1\kappa$; nonneutralizing antibody) from Upstate Biotechnology (Lake Placid, NY) was used in the study. This antibody is cross-reactive for bovine, human, rat, and mouse bFGF (34, 42), but is highly specific for bFGF.

In brief, sections were treated with 1 mg/ml hyaluronidase (Sigma Chemical Co., St. Louis, MO) at 37° C for 30 min. After blocking them with 1.5% normal horse serum for 30 min, we incubated the sections for 60 min with primary antibody at the appropriate dilution in 1.5% normal horse serum. To inhibit nonimmunologic binding of monoclonal antibody, we acidified both the buffers for dilution of bFGF and tryptase antibody and the washing solution to pH 6.0, using 2-(*N*-morpholino) ethanesulfonic acid (Sigma) (43). After washing, sections were incubated for 30 min at room temperature in biotinylated horse antimouse IgG antibody diluted 200-fold. After being washed in Tris-buffered saline, tissue sections were incubated in alkaline phosphatase-conjugated ABC for 30 min. We used Vector Red (Vector Laboratories) as the substrate for alkaline phosphatase. Levamisole (1.25 mmol/L; Vector Laboratories) was applied to the alkaline phosphatase substrate. Counterstaining was performed with either hematoxylin or Movat's pentachrome. With pentachrome staining, collagen/reticulin appears yellow and elastin appears purple/black. In some experiments, we performed double immunohistochemistry, for bFGF and tryptase, using methods previously reported (34).

Morphometric Analysis

We performed quantitative morphometric analysis of all lung histology specimens with computer-assisted video microscopy as described previously (34, 44, 45). This method allows determination of the volume of each cell type or tissue component of interest relative to the total volume of lung tissue examined. In brief, to determine the volume density (V_v) of interest (e.g., tryptase-immunopositive cells), we stored in the computer microscopic images of each histology section. A grid (42 or 125 points) was superimposed on each image, points of interest intersecting the grid were counted, and V_v for each point was calculated. To avoid false estimates of the V_v of interest because of silica dust-bound and -blocked tissue, we subtracted the V_v of dust particles from tissues of patients with silicosis. We counted points of interest in 12 random fields in each tissue section, from which we calculated a mean value for each section. To normalize for potential artifacts created by compression or expansion of lung specimens during fixation, the V_v of each component was corrected for the V_v of parenchymal lung tissue measured on H&E-stained sections at a magnification of ×100 with a 42-point grid according to the following formula: Normalized V_v = (V_v of the component of interest ×100)/V_v of lung tissue.

The V_v values of tryptase-positive cells and the V_v of $bFGF^+$ cells were calculated by counting the grid points that hit immunopositive cells (histiocyte-like cells, described subsequently) in the interstitium and the alveoli at a magnification of ×400 with a 125-point grid. The V_v of all immunopositive cells was calculated by summing the V_v of interstitial cells and the V_v of alveolar immunopositive cells. To determine the degree of lung fibrosis, we quantified the V_v of collagen/reticulin fibers and the V_v of elastic fibers at a magnification of ×400 with a 42-point grid in sections stained with Movat's pentachrome. After the random analysis and in order to clarify the pathophysiologic roles of $bFGF^+$ cells in the development of silicotic nodules, we chose 20 nodules (10 mature and 10 immature) and performed quantitative morphometric analysis of these nodules at a magnification of ×400 with a 125-point grid. We calculated the V_v of $bFGF^+$ cells, the V_v of all other nodule-associated cells, and the V_v of collagen/reticulin fibers.

Statistical Analysis

Data for duration of silica exposure are expressed as mean ± SD. Differences between two groups in race and sex distribution and in smok-

TABLE 1

DEMOGRAPHICS OF STUDY SUBJECTS

	Silicosis (*n* = 14)	Control (*n* = 14)	p Value
Age, yr*	71 (69, 77)	65 (39, 75)	n.s.
Race, n (%)			
White	14 (100)	12 (85.8)	n.s.
African American	0 (0)	2 (14.2)	
Sex, n (%)			
Male	14 (100)	11 (78.6)	n.s.
Female	0 (0)	3 (21.4)	
Smoking status, n (%)			
Ever-smoker	0 (0)	3 (21.4)	n.s.
Never-smoker	14 (100)	11 (78.6)	

Definition of abbreviation: n.s. = not significant.

*Age data are expressed as median with 25% to 75% interquartile ranges in parentheses.

TABLE 2

OCCUPATIONAL HISTORIES AND PULMONARY SILICOSIS GRADE OF PATIENTS WITH SILICOSIS

Patient No.	Age (*yr*)	Occupation	Duration of Silica Exposure (*yr*)	Pulmonary Silicosis Grade*
1	73	Mechanic	46	2
2	70	Laborer	45	2
3	67	Motorman	40	3
4	73	Motorman	42	3
5	78	Mine Operator	43	3
6	76	Digger	36	3
7	69	Roof Bolter	41	2
8	67	Motorman	38	2
9	70	Foreman	44	3
10	80	Loader	39	2
11	80	Miner	35	3
12	69	Machine Operator	37	2
13	61	Cutter, Loader	45	2
14	72	Mine Operator	36	3

* Determined as previously described (40). Grade 2 corresponds to nodular silicosis without conglomerate masses. Grade 3 corresponds to nodular silicosis with evidence of conglomerate masses.

Figure 1. Immunohistochemistry of tryptase (*red*) in control subject's lung (*A*) and silicosis subject's lung (*B*); immature silicotic nodule (*C*) and mature silicotic nodule (*D*). MC immunopositive with antitryptase antibody (*arrows*) were found circumferentially in the periphery of mature silicotic nodules, whereas small numbers of MC are seen in the central portion of immature nodules. The margins of the stained MC were irregular, and cytosolic granules were stained. Extracellular matrix, especially around these cells, also stained positively. Original magnifications: ×400 (*A*); ×400 (*B*); ×400 (*C*); ×100 (*D*) (*inset* ×400).

ing status were evaluated with Fisher's exact probability test. All data are expressed as medians, with 25% to 75% interquartile ranges reported in parentheses. Wilcoxon's nonparametric rank-sum procedure for ranked data was used to compare differences between two groups. For tests of association, we calculated Spearman's correlation coefficient (ρ). Statistical significance was accepted at $p < 0.05$.

RESULTS

Immunohistochemical Localization and Quantification of MC in Lung Tissue

Figure 1 illustrates the immunohistochemistry of tryptase. MC immunopositive for tryptase were found in the interstitium in almost all cases of silicosis, and infrequently also in control subjects (Figures 1A and 1B). Furthermore, in patients with silicosis, MC were aligned circumferentially in the periphery of large mature silicotic nodules, with few positive cells in the central portion of the nodules. Small, immature nodules contained small numbers of MC throughout their central portions (Figures 1C and 1D). As shown in Figure 2, the ratio of the V_v of all MC (tryptase-positive cells) to the V_v of lung tissue was significantly increased in patients with silicosis [1.90 (1.42 to 3.00)] over that of control subjects [0.78 (0.57 to 0.95)] ($p < 0.001$). Interestingly, in three cases of silicosis, the ratio of the V_v of all mast cells to the V_v of lung tissue overlapped with control values. One of these cases showed very early, mild silicosis with little collagen/reticulin deposition. The other two cases had advanced silicosis in which the normal lung architecture was completely replaced by dense collagen/reticulin.

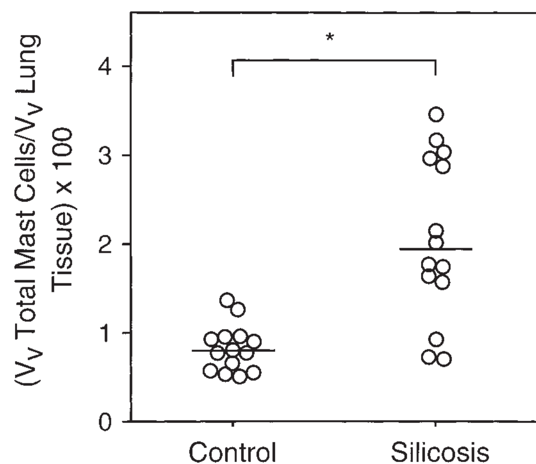

Figure 2. V_v of total MC (tryptase-positive cells) in silicosis and control subjects. *Horizontal bars* represent medians. *$p < 0.001$.

Immunohistochemical Localization of bFGF

Having identified MC in silicosis, we next tested these cells for the presence of bFGF, on the basis of previous observations (34). We observed that anti-bFGF antibody became bound to lung MC, basement membrane, endothelial cells, and smooth-muscle cells, with no immunostaining of lymphocytes, epithelial cells, or macrophages. The most strongly immunopositive cells were interstitial MC (Figures 3A and 3B). Antibody to bFGF reacted with the cytosolic granules of MC. These immunopositive MC were found in the interstitium in almost all cases of silicosis, and infrequently in control subjects. Furthermore, as with MC in general, these $bFGF^+$ cells were found circumferentially in the periphery of mature nodules, although a few such $bFGF^+$ cells could be found throughout the central portion of immature silicotic nodules. Double immunohistochemistry showed that MC-specific antitryptase antibody, and not macrophage-specific anti-CD68 antibody, became bound to these $bFGF^+$ (data not shown). In addition, the majority (91.5%) of tryptase-positive cells coexpressed bFGF, and 93.7% of $bFGF^+$ cells were also stained for MC tryptase. These results were consistent with our previous data in IPF, sarcoidosis, and CBD (34).

Quantification of $bFGF^+$ Cells in Lung Tissue

Figure 4 shows values of the ratio of V_v for all $bFGF^+$ cells to the V_v of lung tissue in patients with silicosis and in control

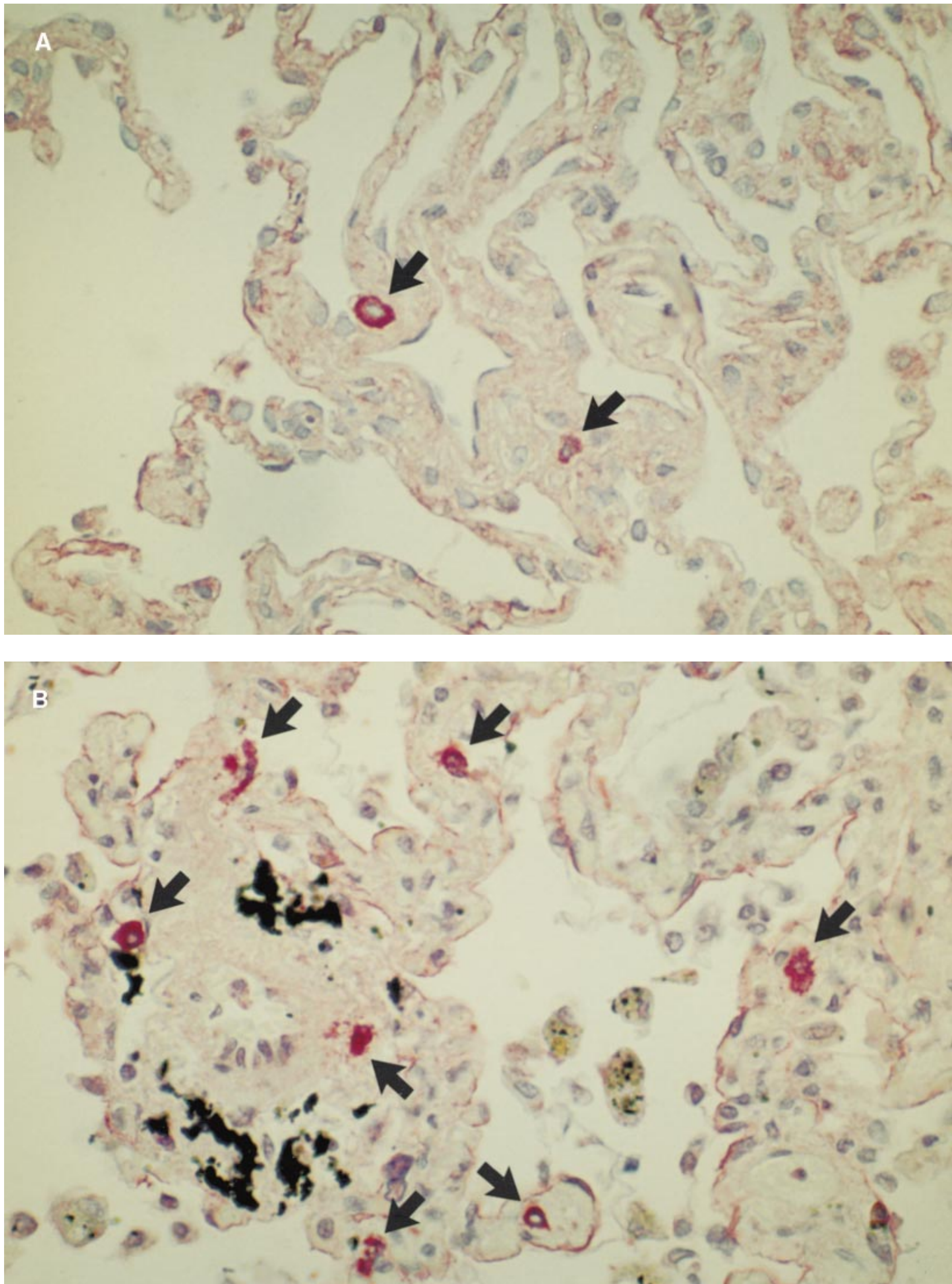

Figure 3. Immunohistochemistry of bFGF (*red*) in control subject's lung (*A*) and silicosis subject's lung (*B*). bFGF was strongly expressed on MC (*arrows*) and basement membrane, and weakly expressed in endothelial cells and smooth-muscle cells. The margins of the stained MC were irregular, and cytosolic granules were stained. Extracellular matrix, especially around these cells, also stained weakly positive. Original magnification: ×400.

subjects. The V_v of all bFGF$^+$ cells in silicosis [2.37 (1.77 to 2.91)] was significantly greater than that of control subjects [0.77 (0.65 to 0.86)] ($p < 0.001$). There was a significant correlation between the ratio of the V_v of total bFGF$^+$ cells to the V_v of lung tissue and the ratio of the V_v of total mast cells to the V_v of lung tissue ($\rho = 0.88$, $p < 0.001$) (Figure 5).

Distribution of Collagen/Reticulin Fibers and Elastic Fibers

Pentachrome staining showed that collagen/reticulin fibers dominated the central portion of mature silicotic nodules, the circumferences of immature nodules, and both vessels and thickening alveolar walls in patients with silicosis. We observed very few collagen/reticulin fibers in alveolar walls or in the circumferences of vessels in control subjects. Elastic fibers were distributed throughout alveolar walls and in the circumferences of vessels both in patients with silicosis and in control subjects. We found few elastic fibers in the central portion of silicotic nodules.

Quantification of Collagen/Reticulin Fibers and Elastic Fibers, and Correlation with bFGF$^+$ MC

Figure 6 shows the V_v of collagen/reticulin fibers and the V_v of elastic fibers in patients with silicosis and in control subjects. As expected, the ratio of the V_v of collagen/reticulin fibers to the V_v of lung tissue was significantly increased in patients with silicosis [59.82 (32.86 to 92.48)] as compared with control subjects [6.85 (5.93 to 8.56)] ($p < 0.001$). However, the ratio of the V_v of elastic fibers to the V_v of lung tissue did not differ in these two groups [6.86 (4.05 to 10.25) and 7.57 (5.91 to 9.15), respectively, p = ns]. There was a significant correlation between the ratio of the V_v of collagen/reticulin fibers to the V_v of lung tissue and the ratio of the V_v of all bFGF$^+$ cells to the V_v of lung tissue in patients with silicosis and control subjects ($\rho = 0.81$; $p < 0.001$) (Figure 7). However, we observed no association between the ratio of the V_v of elastic fibers to the V_v of lung tissue or the ratio of the Vv of total bFGF$^+$ cells to the V_v of lung tissue ($\rho = 0.10$; p = ns).

Association between Collagen/Reticulin Fibers and bFGF$^+$ MC in Silicotic Nodules

To clarify the pathophysiologic role of bFGF$^+$ MC in the development of granulomatous lesions, we examined the association between collagen/reticulin fibers and bFGF$^+$ cells. Small, immature nodules contained a few bFGF$^+$ cells scattered among the many other cells in a loose collagen/reticulin matrix in the central portion (Figure 8A). In contrast, large, mature nodules, composed of more dense collagen/reticulin fibers, had few cells of any type at their center. bFGF$^+$ MC and other cells such as fibroblasts, macrophages, and lymphocytes aligned circumferentially around the dense fibrotic cores of mature nodules (Figure 8B). We observed a significant negative correlation between the V_v of bFGF$^+$ MC and the V_v of collagen/reticulin fibers in the central portions of silicotic nodules ($\rho = -0.80$; $p < 0.001$, $n = 20$). Furthermore, a significant negative correlation was noted between the V_v of all other nodule-associated cells and the V_v of collagen/reticulin fibers ($\rho = -0.84$; $p < 0.001$, $n = 20$).

DISCUSSION

The present study is the first to observe involvement of MC in human silicosis. These cells appear to be located in areas of extracellular-matrix deposition in early and late silicotic nodules. Several reports have suggested that MC may contribute to pulmonary fibrosis in various types of fibrotic lung disease and in animal models of fibrosis (20–28). However, we are aware of only a few reports that MC may be related to the pathogenesis of silica-induced pulmonary fibrosis in rats and mice (27, 28). In studies of human silicosis, we find no reports describing MC, although one previous study found high concentrations of histamine in the blood of patients with this disease (38).

To explore the possible role of MC in silicosis, we tested for the presence of bFGF in MC, using immunohistochemistry, on the basis of a previous observation that MC bFGF is abundant in other fibrotic disorders (34). Our quantitative morphometric analysis, based on immunohistochemistry, showed that the V_v of bFGF$^+$ cells was significantly greater in patients with silicosis than in control subjects, and that it correlated significantly with the V_v of tryptase positive MC. Furthermore, our double immunohistochemical data confirmed that the main source of bFGF in human silicotic lungs is the interstitial MC. These findings are consistent with previous reports of MC bFGF in other forms of pulmonary and skin fibrosis (34–36).

In the present study, we used the V_v of collagen/reticulin fibers as a marker of pulmonary fibrosis and of disease severity, as in previous studies (34, 46). As expected, the quantity of

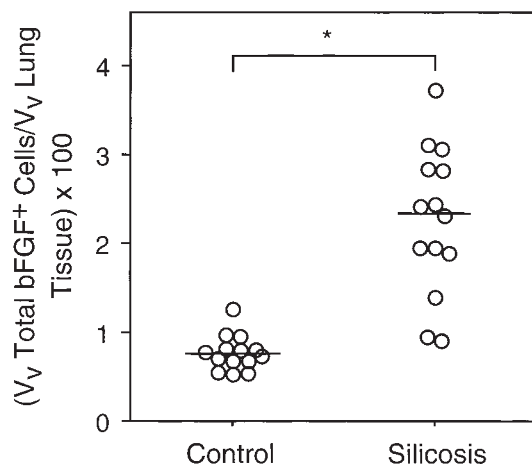

Figure 4. V_v of total bFGF$^+$ cells in silicosis and control subjects. *Horizontal bars* represent medians. *$p < 0.001$.

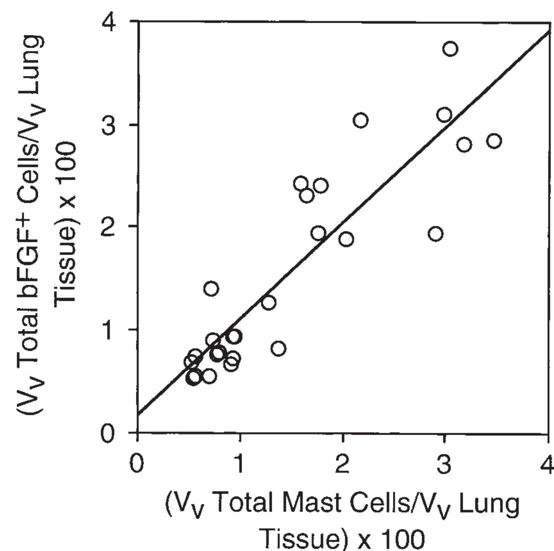

Figure 5. Correlation between V_v of total bFGF$^+$ cells and V_v of total mast cells ($\rho = 0.88$; $p < 0.001$, $n = 28$).

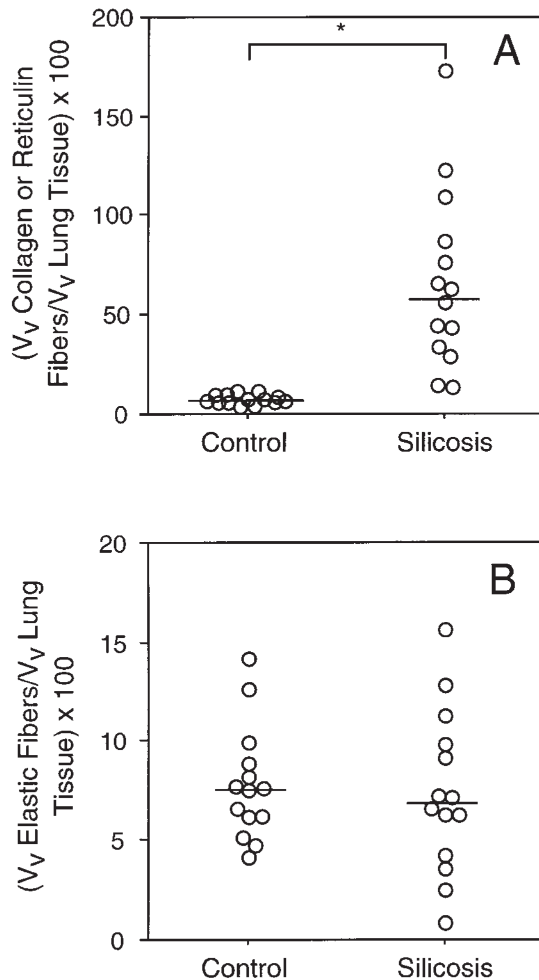

Figure 6. (*A*) V_v of collagen/reticulin fibers in silicosis and control subjects. *Horizontal bars* represent medians. *$p < 0.001$. (*B*) V_v of elastic fibers in silicosis and control subjects. *Horizontal bars* represent medians (p = NS).

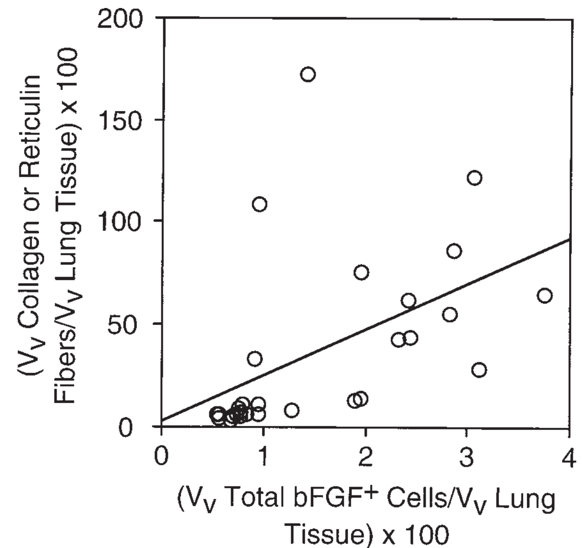

Figure 7. Correlation between V_v of collagen/reticulin fibers and V_v of total $bFGF^+$ cells ($\rho = 0.81$; $p < 0.001$, $n = 28$).

collagen/reticulin fibers in patients with silicosis was significantly greater than in control subjects. Furthermore, there was a significant correlation between the V_v of collagen/reticulin fibers and the V_v of all $bFGF^+$ MC both in patients with silicosis and in control subjects. These findings suggest that the presence of total $bFGF^+$ MC correlates with the pathologic severity of pulmonary fibrosis in silicosis, as has been reported in IPF, sarcoidosis, and CBD (34). Interestingly, we did not observe an increase in elastin related to silicosis. This may be explained by the absence of elastin from the central portions of silicotic nodules. Furthermore, the nodules, by distorting and destroying alveolar walls, may fragment normal elastin deposits.

To clarify the pathophysiologic role of $bFGF^+$ MC in the development of silicotic nodules, we examined both immature and more hyalinized mature nodules. In general, the cells found in early, developing silicotic nodules included numerous large macrophages containing particulate material at the centers of nodules and smaller macrophages and lymphocytes toward the periphery, in accord with previous descriptions (2, 47). After fibroblast proliferation and extracellular-matrix deposition, silicotic nodules become composed of whorled collagen and reticulin centrally, ringed by dust-laden macrophages, fibroblasts, and lymphocytes. The centers of the nodules become avascular and acellular (2, 47). Our studies of MC showed that the central portions of small, immature granulomas also contain small numbers of $bFGF^+$ MC among many other cells, and loose, dense collagen/reticulin fibers. In fact, our mildest case of silicosis had normal numbers of $bFGF^+$ MC (Figures 2 and 7). In the circumference of larger, mature nodules, we found abundant $bFGF^+$ MC among the fibroblasts, macrophages, and lymphocytes circumferentially present in the periphery. Interestingly, in the most fibrotic cases of silicosis, we observed few $bFGF^+$ MC. Lung specimens from these cases were largely composed of collagen/reticulin, and contained few cells of any type. These findings, although correlational, suggest that $bFGF^+$ MC may interact with proliferating fibroblasts in silicosis, and that these fibroblasts may in turn induce collagen deposition in a pattern that extends from the centers of nodules outward. Knowing that bFGF induces fibroblast proliferation (48), we speculate that bFGF is produced and released by MC, and that it may act in concert with macrophage-derived factors such as TGF-β in promoting silicotic nodule formation (11, 12). For example, bFGF may be most important in promoting fibroblast and smooth-muscle cell proliferation, whereas other growth factors, such as TGF-β, trigger collagen production.

In both chronic and acute pulmonary inflammation, $bFGF^+$ MC may help direct cell proliferation. For example, Liebler and colleagues recently demonstrated that MC produce bFGF in the rat bleomycin-induced model of pulmonary fibrosis, with the number of $bFGF^+$ MC rising in rat lungs 14 d after bleomycin inhalation (37). Additionally, Suzuki and colleagues reported that MC had already infiltrated the lungs of mice at 14 d after the tracheal instillation of silica particles (28). These data are consistent with our observation of $bFGF^+$ MC in immature silicotic nodules in humans, and suggest that MC may appear early in the course of lung injury. It should be noted that although our study subjects with silicosis were coal miners, their pathology was typical of silicotic nodules, not coal macules.

An interesting parallel can be seen between the MC found in silicotic nodules and those found in granulomatous lung diseases (34). In both conditions, MC containing bFGF align circumferentially in the rim of lesions, around granulomas and around silicotic nodules. Several studies have shown that bFGF plays an important role in the development of granula-

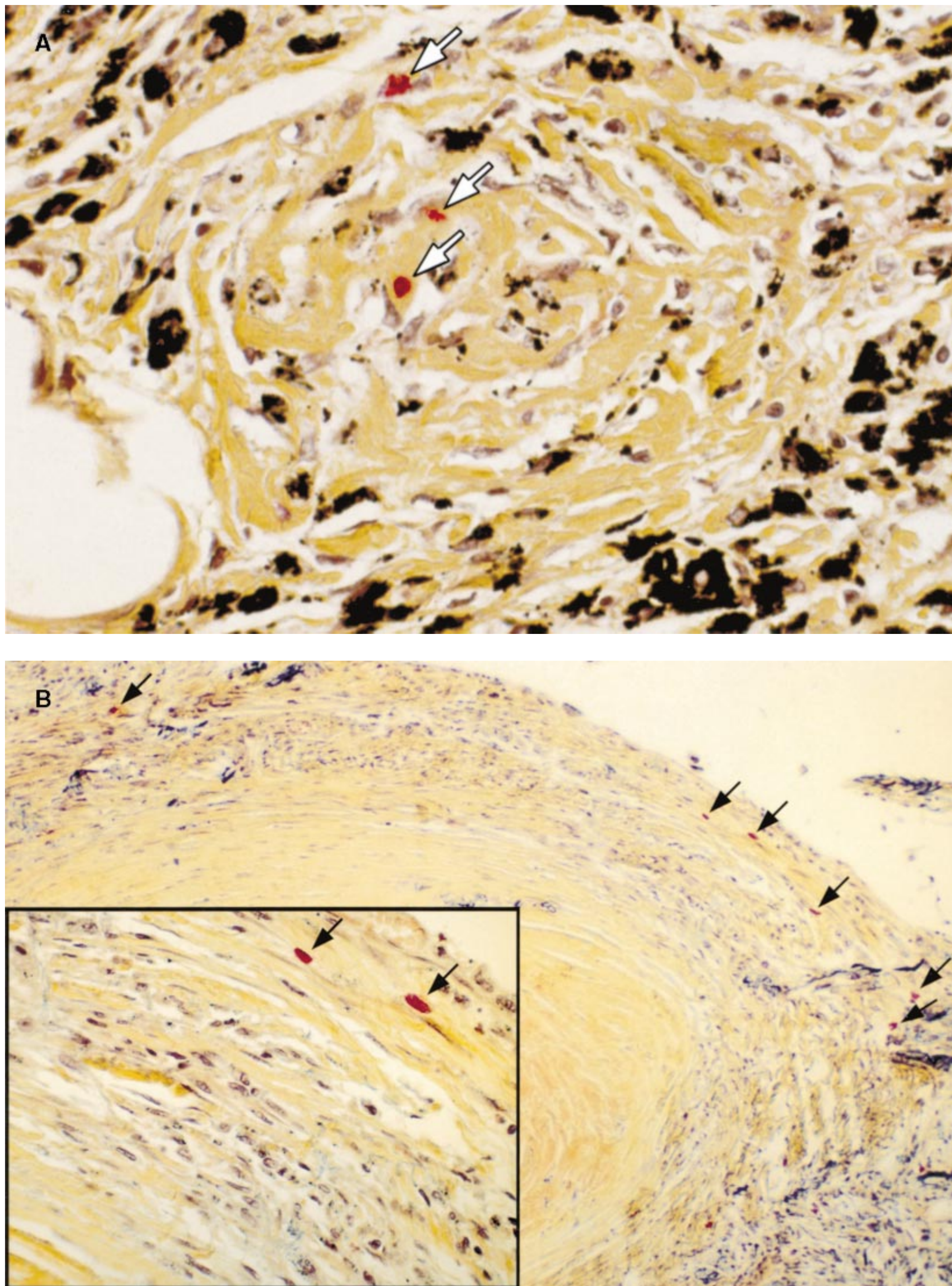

Figure 8. Immunohistochemistry of bFGF and pentachrome staining of nodular lesions in silicosis. (*A*) bFGF^{+} cells (*arrows*) are found in the central portion of immature nodules, along with many other inflammatory cells and with a loose matrix of collagen/reticulin fibers (*yellow*). Original magnification: ×400. (*B*) bFGF^{+} cells (*arrows*) are found circumferentially in the periphery of mature silicotic nodules, with dense collagen/reticulin fibers in the central portion. Original magnification: ×100 (*inset* ×400).

tion tissue (49, 50). Thus, we hypothesize that bFGF may help modulate the process by which the body tries to contain foreign particles, such as silica, in addition to limiting the cellular immune response to inhaled antigens, such as beryllium. Future studies will be needed to examine the interaction of bFGF produced by MC with other fibrosis-regulatory growth factors, and to explain the role of MC bFGF in the pathogenesis of silica-induced pulmonary fibrosis.

Other mediators produced by MC may also be important in the fibrogenic response. It has been shown that both histamine and tryptase stimulate fibroblast proliferation (51, 29), and that several proinflammatory factors, such as TNF-α, IL-1, IFN-γ, and TGF-β can be produced by MC (30–33). However, the pathophysiologic role of other MC mediators and cytokines in silica-induced lung injury has not been examined. Future studies will be required to explore the importance of other mediators and cytokines released from MC in the development of human and experimental silicosis, and the relative importance of MC bFGF.

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References

1. Rom, W. N., P. B. Bitterman, S. I. Rennard, A. Cantin, and R. G. Crys-

tal. 1987. Characterization of the lower respiratory tract inflammation of nonsmoking individuals with interstitial lung disease associated with chronic inhalation of inorganic dusts. *Am. Rev. Respir. Dis.* 136: 1429–1434.

2. Weill, H., R. N. Jones, and W. R. Parkes. 1994. Silicosis and related diseases. *In* W. R. Parks, editor. Occupational Lung Disorders, 3rd ed. Butterworth-Heinemann, Oxford. 285–339.
3. Vanhée, D., P. Gosset, A. Boitelle, B. Wallaert, and A. B. Tonnel. 1995. Cytokines and cytokine network in silicosis and coal workers' pneumoconiosis. *Eur. Respir. J.* 8:834–842.
4. Borm, P. J. A., N. Palmen, J. J. M. Engelen, and W. A. Buurman. 1988. Spontaneous and stimulated release of tumor necrosis factor-alpha (TNF) from blood monocytes of miners with coal workers' pneumoconiosis. *Am. Rev. Respir. Dis.* 138:1589–1594.
5. Gosset, P., P. Lassalle, D. Vanhée, B. Wallaert, C. Aerts, C. Voisin, and A.-B. Tonnel. 1991. Production of tumor necrosis factor-α and interleukin-6 by human alveolar macrophages exposed *in vivo* to coal mine dust. *Am. J. Respir. Cell Mol. Biol.* 5:431–436.
6. Dubois, C. M., E. Bissonnette, and M. Rola-Pleszczynski. 1989. Asbestos fibers and silica particles stimulate rat alveolar macrophages to release tumor necrosis factor: autoregulatory role of leukotriene B_4. *Am. Rev. Respir. Dis.* 139:1257–1264.
7. Gossart, S., C. Cambon, C. Orfila, M.-H. Séguélas, J.-C. Lepert, J. Rami, P. Carré, and B. Pipy. 1996. Reactive oxygen intermediates as regulators of TNF-α production in rat lung inflammation induced by silica. *J. Immunol.* 156:1540–1548.
8. Ohtsuka, Y., M. Munakata, H. Ukita, T. Takahashi, A. Satoh, Y. Homma, and Y. Kawakami. 1995. Increased susceptibility to silicosis and TNF-α production in C57BL/6J mice. *Am. J. Respir. Crit. Care Med.* 152:2144–2149.
9. Driscoll, K. E., R. C. Lindenschmidt, J. K. Maurer, J. M. Higgins, and G. Ridder. 1990. Pulmonary response to silica or titanium dioxide: inflammatory cells, alveolar macrophage-derived cytokines, and histopathology. *Am. J. Respir. Cell Mol. Biol.* 2:381–390.
10. Schmidt, J. A., C. N. Oliver, J. L. Lepe-Zuniga, I. Green, and I. Gery. 1984. Silica-stimulated monocytes release fibroblast proliferation factors identical to interleukin 1: a potential role for interleukin 1 in the pathogenesis of silicosis. *J. Clin. Invest.* 73:1462–1472.
11. Jagirdar, J., R. Bégin, A. Dufresne, S. Goswami, T. C. Lee, and W. N. Rom. 1996. Transforming growth factor-β (TGF-β) in silicosis. *Am. J. Respir. Crit. Care Med.* 154:1076–1081.
12. Williams, A. O., K. C. Flanders, and U. Saffiotti. 1993. Immunohistochemical localization of transforming growth factor-β_1 in rats with experimental silicosis, alveolar type II hyperplasia, and lung cancer. *Am. J. Pathol.* 142:1831–1840.
13. Driscoll, K. E., D. G. Hassenbein, J. Carter, J. Poynter, T. N. Asquith, R. A. Grant, J. Whitten, M. P. Purdon, and R. Takigiku. 1993. Macrophage inflammatory proteins 1 and 2: expression by rat alveolar macrophages, fibroblasts, and epithelial cells and in rat lung after mineral dust exposure. *Am. J. Respir. Cell Mol. Biol.* 8:311–318.
14. Yuen, I. S., M. A. Hartsky, S. I. Snajdr, and D. B. Warheit. 1996. Time course of chemotactic factor generation and neutrophil recruitment in the lungs of dust-exposed rats. *Am. J. Respir. Cell Mol. Biol.* 15:268–274.
15. Melloni, B., O. Lesur, T. Bouhadiba, A. Cantin, and R. Bégin. 1994. Partial characterization of the proliferative activity for fetal lung epithelial cells produced by silica-exposed alveolar macrophages. *J. Leukoc. Biol.* 55:574–580.
16. Melloni, B., O. Lesur, T. Bouhadiba, A. Cantin, M. Martel, and R. Bégin. 1996. Effect of exposure to silica on human alveolar macrophages in supporting growth activity in type II epithelial cells. *Thorax* 51: 781–786.
17. Haslam, P. L., A. Dewar, P. Butchers, Z. S. Primett, A. Newman-Taylor, and M. Turner-Warwick. 1987. Mast cells, atypical lymphocytes, and neutrophils in bronchoalveolar lavage in extrinsic allergic alveolitis: comparison with other interstitial lung diseases. *Am. Rev. Respir. Dis.* 135:35–47.
18. Walls, A. F., J. A. Roberts, R. C. Godfrey, M. K. Church, and S. T. Holgate. 1990. Histochemical heterogeneity of human mast cells: disease-related differences in mast cell subsets recovered by bronchoalveolar lavage. *Int. Arch. Allergy Appl. Immunol.* 92:233–241.
19. Hunt, L. W., T. V. Colby, D. A. Weiler, S. Sur, and J. H. Butterfield. 1992. Immunofluorescent staining for mast cells in idiopathic pulmonary fibrosis: quantification and evidence for extracellular release of mast cell tryptase. *Mayo Clin. Proc.* 67:941–948.
20. Pesci, A., G. Bertorelli, M. Gabrielli, and D. Olivieri. 1993. Mast cells in fibrotic lung disorders. *Chest* 103:989–996.
21. Bjermer, L., A. Engström-Laurent, M. Thunell, and R. Hällgren. 1987. The mast cell and signs of pulmonary fibroblast activation in sarcoidosis. *Int. Arch. Allergy Appl. Immunol.* 82:298–301.
22. Jordana, M. 1993. Mast cells and fibrosis: who's on first? *Am J. Respir. Cell Mol. Biol.* 8:7–8.
23. Goto, T., D. Befus, R. Low, and J. Bienenstock. 1984. Mast cell heterogeneity and hyperplasia in bleomycin-induced pulmonary fibrosis in rats. *Am. Rev. Respir. Dis.* 130:797–802.
24. Aldenborg, F., K. Nilsson, B. Jarlshammer, L. Bjermer, and L. Enerbäck. 1993. Mast cells and biogenic amines in radiation-induced pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* 8:112–117.
25. Wagner, M. M. F., R. E. Edwards, C. B. Moncrieff, and J. C. Wagner. 1984. Mast cells and inhalation of asbestos in rats. *Thorax* 39:539–544.
26. Keith, I., R. Day, S. Lemaire, and I. Lemaire. 1987. Asbestos-induced fibrosis in rats: increase in lung mast cells and autacoid contents. *Exp. Lung Res.* 13:311–327.
27. Saffiotti, U., A. O. Williams, L. N. Daniel, M. E. Kaighn, Y. Mao, and X. Shi. 1995. Carcinogenesis by crystalline silica: animal, cellular, and molecular studies. *In* V. Castranova, V. Vallyathan, and W. E. Wallace, editors. Silica and Silica-induced Lung Diseases. CRC Press, Boca Raton, FL. 345–381.
28. Suzuki, N., T. Horiuchi, K. Ohta, M. Yamaguchi, T. Ueda, H. Takizawa, K. Hirai, J. Shiga, K. Ito, and T. Miyamoto. 1993. Mast cells are essential for the full development of silica-induced pulmonary inflammation: a study with mast cell-deficient mice. *Am. J. Respir. Cell Mol. Biol.* 9:475–483.
29. Ruoss, S. J., T. Hartmann, and G. H. Caughey. 1991. Mast cell tryptase is a mitogen for cultured fibroblasts. *J. Clin. Invest.* 88:493–499.
30. Gordon, J. R., and S. J. Galli. 1990. Mast cells as a source of both preformed and immunologically inducible TNF-α/cachectin. *Nature* 346:274– 276.
31. Bradding, P., J. A. Roberts, K. M. Britten, S. Montefort, R. Djukanovic, R. Mueller, C. H. Heusser, P. H. Howarth, and S. T. Holgate. 1994. Interleukin-4, -5, and -6 and tumor necrosis factor-α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am. J. Respir. Cell Mol. Biol.* 10:471–480.
32. Gordon, J. R., and S. J. Galli. 1994. Promotion of mouse fibroblast collagen gene expression by mast cells stimulated via the Fc RI: role for mast cell-derived transforming growth factor β and tumor necrosis factor α. *J. Exp. Med.* 180:2027–2037.
33. Burd, P. R., H. W. Rogers, J. R. Gordon, C. A. Martin, S. Jayaraman, S. D. Wilson, A. M. Dvorak, S. J. Galli, and M. E. Dorf. 1989. Interleukin 3-dependent and -independent mast cells stimulated with IgE and antigen express multiple cytokines. *J. Exp. Med.* 170:245–257.
34. Inoue, Y., T. E. King, Jr., S. S. Tinkle, K. Dockstader, and L. S. Newman. 1996. Human mast cell basic fibroblast growth factor in pulmonary fibrotic disorders. *Am. J. Pathol.* 149:2037–2054.
35. Reed, J. A., A. P. Albino, and N. S. McNutt. 1995. Human cutaneous mast cells express basic fibroblast growth factor. *Lab. Invest.* 72:215–222.
36. Qu, Z., J. M. Liebler, M. R. Powers, T. Galey, P. Ahmadi, X.-N. Huang, J. C. Ansel, J. H. Butterfield, S. R. Planck, and J. T. Rosenbaum. 1995. Mast cells are a major source of basic fibroblast growth factor in chronic inflammation and cutaneous hemangioma. *Am. J. Pathol.* 147:564–573.
37. Liebler, J. M., M. A. Picou, Z. Qu, M. R. Powers, and J. T. Rosenbaum. 1997. Altered immunohistochemical localization of basic fibroblast growth factor after bleomycin-induced lung injury. *Growth Factors* 14:25–38.
38. Bezrodnykh, A. A., T. A. Kochetkova, T. A. Oganesyan, and N. V. Sidorova. 1972. Distinctive features of histamine metabolism in silicosis. *Sov. Med.* 35:17–21.
39. International Labour Organization. 1980. Guidelines for the use of ILO international classification of radiographs of pneumoconioses. Occupational Safety and Health Series, No. 22. International Labour Office, Geneva, Switzerland.
40. Hu, S.-N., V. Vallyathan, F. H. Y. Green, K. C. Weber, and W. Laqueur. 1990. Pulmonary arteriolar muscularization in coal workers' pneumoconiosis and its correlation with right ventricular hypertrophy. *Arch. Pathol. Lab. Med.* 114:1063–1070.
41. Movat, H. Z. 1955. Demonstration of all connective tissue elements in a single section. *Arch. Pathol.* 60:289–295.
42. Matsuzaki, K., Y. Yoshitake, Y. Matsuo, H. Sasaki, and K. Nishikawa. 1989. Monoclonal antibodies against heparin-binding growth factor II/basic fibroblast growth factor that block its biological activity: invalidity of the antibodies for tumor angiogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 86:9911–9915.
43. Schiltz, P. M., J. Lieber, R. C. Giorno, and H. N. Claman. 1993. Mast cell immunohistochemistry: non-immunological immunostaining mediated by non-specific $F(ab')_2$-mast cell secretory granule interaction. *Histochem. J.* 25:642–647.

44. Hyde, D. M., T. E. King, Jr., T. McDermott, J. A. Waldron, Jr., T. V. Colby, W. M. Thurlbeck, A. Flint, L. Ackerson, and R. M. Cherniack. 1992. Idiopathic pulmonary fibrosis: quantitative assessment of lung pathology. *Am. Rev. Respir. Dis.* 146:1042–1047.
45. Hirose, N., D. A. Lynch, R. M. Cherniack, and D. E. Doherty. 1993. Correlation between high resolution computed tomography and tissue morphometry of the lung in bleomycin-induced pulmonary fibrosis in the rabbit. *Am. Rev. Respir. Dis.* 147:730–738.
46. Saldiva, P. H. N., V. C. Delmonte, C. R. R. de Carvalho, R. A. Kairalla, and J. O. C. Auler, Jr. 1989. Histochemical evaluation of lung collagen content in acute and chronic interstitial diseases. *Chest* 95:953–957.
47. Davis, G. S., and W. J. Calhoun. 1993. Occupational and environmental causes of interstitial lung disease. *In* M. I. Schwarz and T. E. King, Jr., editors. Interstitial Lung Disease, 2nd ed. Mosby Year Book, St. Louis. 179–230.
48. Gospodarowicz, D. 1974. Localization of a fibroblast growth factor and its effect alone and with hydrocortisone on 3T3 cell growth. *Nature* 249:123–127.
49. Lynch, S. E. 1991. Interactions of growth factors in tissue repair. *Prog. Clin. Biol. Res.* 365:341–357.
50. Pierce, G. F., J. E. Tarpley, D. Yanagihara, T. A. Mustoe, G. M. Fox, and A. Thomason. 1992. Platelet-derived growth factor (BB homodimer), transforming growth factor-β1, and basic fibroblast growth factor in dermal wound healing: neovessel and matrix formation and cessation of repair. *Am. J. Pathol.* 140:1375–1388.
51. Russel, J. D., S. B. Russel, and K. M. Trupin. 1977. The effect of histamine on the growth of cultured fibroblasts isolated from normal and keloid tissue. *J. Cell Physiol.* 93:389–393.