Fibroblast chemotactic response elicited by native bronchoalveolar lavage fluid from patients with fibrosing alveolitis

J Behr, B C Adelmann-Grill, F Krombach, T Beinert, M Schwaiblmair, G Fruhmann

Abstract

Background—In fibrosing alveolitis activation of lung fibroblasts is the decisive event in the pathogenetic sequence leading to pulmonary fibrosis. Fibroblast stimulating activity was measured in bronchoalveolar lavage (BAL) fluid to assess its relationship to the activity of fibrosing alveolitis.

Methods—Nine control subjects and 40 patients with fibrosing alveolitis caused by idiopathic pulmonary fibrosis (n = 22) or pulmonary involvement in systemic sclerosis (n = 18) were studied. All patients were followed up by lung function testing for a minimum of six months (mean (SE) 13.3 (1.4) months). Twenty five patients received immunosuppressive therapy and 15 refused. At the beginning of follow up BAL was performed and, as a possible indicator of fibroblast stimulating mediators within the lungs, chemotactic migration of cultured human fibroblasts elicited by native BAL fluid was measured in Boyden-type chambers and expressed as a percentage of the chemoattractant effect of 25 ng/ml platelet derived growth factor. The procollagen III peptide level in BAL fluid served as a marker for collagen synthesis.

Results—Chemotactant activity was elevated in the patients with idiopathic pulmonary fibrosis and systemic sclerosis compared with the control group, (mean (SE) 56.4% (8.5%) and 72.3% (16.3%) v 12.6% (4.0%). Chemoattractant activity was inversely correlated with total lung capacity (TLC) (r = -0.45) and with vital capacity (VC) (r = -0.33). Procollagen III peptide concentrations in BAL fluid and chemoattractant activity were not significantly correlated. For further evaluation chemoattractant activity of 36% (mean value of controls + 2 SD) was used to separate normal (<36%) from elevated (≥36%) activity. At the end of follow up, untreated patients with high chemoattractant activity (≥36%) showed a significant reduction of VC, TLC, and exercise arterial oxygen tension (PaO₂) and a small decrease in carbon monoxide transfer factor (TLCO), whereas a significant improvement in VC, TLC, and TLCO and a small increase of exercise PaO₂ occurred in treated patients with high chemoattractant activity. Patients with low chemoattractant activity (<36%) showed no consistent change in lung function measurements, irrespective of treatment. In contrast, lung function results and differential cell counts in BAL fluid failed to identify progressive disease.

Conclusions—In patients with fibrosing alveolitis the chemoattractant activity of BAL fluid seems to be an independent indicator of lung fibroblast stimulating activity providing relevant information about disease activity, and may help to improve the clinical management of these patients.

(Thorax 1993;48:736–742)

Proliferation of fibroblasts and excessive deposition of extracellular connective tissue matrix are essential features of the fibrotic process which, in turn, is usually preceded by and closely linked to an inflammatory reaction. This pattern is clearly seen in fibrosing alveolitis, a heterogeneous group of diseases of the lower respiratory tract where inflammatory cells accumulate within the alveolar structures and progressive interstitial fibrosis ensues. There are several reasons for believing that fibroblasts play a crucial part in this process: they produce the collagen which represents the mass of the fibrotic tissue; cytokines released by inflammatory cells are known to modulate fibroblast functions such as proliferation, collagen synthesis, and directed migration. On the other hand, fibroblasts are capable of producing cytokines which may perpetuate the inflammatory and fibrotic processes, or both. This point of view strongly implicates the fibroblast as the focus of the pathophysiological processes leading to pulmonary fibrosis. It is therefore reasonable to measure the total effect of fibroblast activating mediators within the lungs to assess the activity of fibrosing alveolitis.

Several authors have sought more information about fibroblast activity in the alveolar tissue by examining bronchoalveolar lavage (BAL) fluid for its content of procollagen III peptide (as an indicator of collagen synthesis).
or for fibroblast growth inducing activities.\textsuperscript{11-14} We hoped that a better measure of the contribution of fibroblasts to the development of fibrosing alveolitis may improve the understanding and management of this process. We therefore measured the chemotactic activity of native BAL fluid for cultured human fibroblasts as a possible indicator of profibrotic activity within the lungs. The data were compared with the cytological finding of the BAL fluid, procollagen III peptide levels, lung function values, and clinical appearance of the patients.

**Methods**

**STUDY POPULATION**

**Control group**

The control group consisted of nine non-smoking volunteers (two women, seven men; mean age 43.3 (4-6) years) without pulmonary disease who underwent fibreoptic bronchoscopy and BAL for the purposes of this study (Table 1). The bronchial anatomy and mucosa were macroscopically normal. Differential cell counts in the BAL fluid\textsuperscript{14} and results of lung function tests were within the normal range in all control subjects.

**Patients**

Forty patients presenting with clinical, functional, and radiological signs of fibrosing alveolitis were investigated.\textsuperscript{14 17 18} There was no evidence of hypersensitivity pneumonitis and no history of exposure to organic or inorganic dusts. Idiopathic pulmonary fibrosis was diagnosed from previously reported clinical, radiological, and functional criteria\textsuperscript{14 17 18} in 22 patients (12 women, 10 men; mean age 56 (2.6) years) with no evidence of collagen vascular disease. The diagnosis was histologically confirmed by transbronchial biopsy in 17 patients, and by open lung biopsy in five in whom the transbronchial specimen was inconclusive. Eighteen patients (12 women, six men, mean age 48.9 (2.6) years) had pulmonary disease from systemic sclerosis which was diagnosed from the clinical picture and from symptoms in agreement with previously proposed criteria.\textsuperscript{19 20} All were non-smokers or had given up smoking for at least two years (n = 3). The study protocol was approved by the local ethical committee. Informed consent was obtained from the control subjects and patients.

**Table 1 Mean (SE) lung function characteristics of control subjects and patients before treatment**

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 9)</th>
<th>IPF (n = 22)</th>
<th>SSC (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VC (% predicted)</td>
<td>102.6 (7.1)</td>
<td>74.1 (5.1)†</td>
<td>74.6 (4.7)†</td>
</tr>
<tr>
<td>FEV\textsubscript{1} (% VC)</td>
<td>74.0 (5.2)</td>
<td>75.1 (1.9)</td>
<td>78.3 (2.0)</td>
</tr>
<tr>
<td>TLC (% predicted)</td>
<td>93.9 (6.3)</td>
<td>67.7 (4.5)†</td>
<td>69.8 (4.1)†</td>
</tr>
<tr>
<td>TLCO (% predicted)</td>
<td>87.5 (6.5)</td>
<td>55.3 (4.3)†</td>
<td>54.6 (4.5)†</td>
</tr>
<tr>
<td>Resting Pa\textsubscript{O\textsubscript{2}} (mm Hg)*</td>
<td>77.4 (4.9)</td>
<td>70.6 (1.9)†</td>
<td>77.1 (1.9)†</td>
</tr>
<tr>
<td>Exercise Pa\textsubscript{O\textsubscript{2}} (mm Hg)*</td>
<td>75.1 (5.6)</td>
<td>57.5 (2.6)†</td>
<td>68.8 (3.1)†</td>
</tr>
</tbody>
</table>

VC—vital capacity; FEV\textsubscript{1}—forced expiratory volume in one second; TLC—total lung capacity; TLCO—carbon monoxide transfer factor; Pa\textsubscript{O\textsubscript{2}}—oxygen partial pressure in arterialized capillary blood; IPF—idiopathic pulmonary fibrosis; SSC—systemic sclerosis.

*L 5 mm Hg = 1 kPa; †p < 0.05 v control; ‡p < 0.05 v idiopathic pulmonary fibrosis.

**LUNG FUNCTION TESTS**

Vital capacity (VC) was determined with a spirometer (Jaeger, Würzburg, Germany) and the carbon monoxide transfer factor (TLCO) and residual volume (RV) were measured by a single breath method using a gas mixture containing 0.2% carbon monoxide and 8% helium. Total lung capacity (TLC) was calculated from VC and RV values. Blood gas analysis (paired values) for Pa\textsubscript{O\textsubscript{2}}, Pa\textsubscript{CO\textsubscript{2}}, and pH was performed on arterialized capillary blood from the ear lobe at rest and during steady state exercise. Blood gas reference values were calculated individually according to Ulmer et al.\textsuperscript{21} Lung volumes were compared with European Community for Coal and Steel normal values.\textsuperscript{22} The TLCO was expressed as a percentage of our laboratory reference values.

**FOLLOW UP AND TREATMENT**

All patients were followed up for at least six months (mean (SE) 13.2 (1.5) months). Complete lung function tests, as described above, were performed at the end of the follow up period in each patient.

Immunosuppressive treatment was offered to all patients. After discussion of the possible benefits and side effects of this therapy 15 patients refused treatment but agreed to have control investigations to follow the course of their disease. In 25 patients immunosuppressive treatment was started with 1.0–1.5 mg/kg prednisolone daily for 8–12 weeks. Response to treatment was assessed by pulmonary function tests. If improvement or stabilisation occurred the corticosteroid dose was tapered by 10 mg/week to a maintenance dose of 10–20 mg/day. If deterioration occurred despite the high dose of prednisolone used (decline of VC or TLCO by more than 10%, or decrease of exercise Pa\textsubscript{O\textsubscript{2}} by 5 mm Hg or more, or both), cyclophosphamide (2–3 mg/kg) was added. The dose of cyclophosphamide was slowly reduced to a maintenance dose of 0.5–1.0 mg/kg after 4–6 months. The prednisolone maintenance dose remained at 10–20 mg daily, irrespective of additional cyclophosphamide treatment. At the end of the study 13 patients received prednisolone only and 12 patients were on treatment with prednisolone plus cyclophosphamide.

**BRONCHOALVEOLAR LAVAGE (BAL)**

BAL was performed in all patients at their first presentation as previously described.\textsuperscript{14} They had been off immunosuppressive drugs for at least three months.

A fibreoptic bronchoscope was wedged in a subsegmental bronchus of the middle lobe or lingula and five serial infusions and aspirations were performed, each of 20 ml sterile saline (0.9% NaCl). The recovered fluid (mean (SE) 45.8 (2.7) ml) was pooled, filtered through sterile gauze, centrifuged at 300 g for 10 minutes, and the supernatants stored at −70°C. Total cell counts were measured by a Coulter counter. Mean percentages of alveolar macrophages, neutrophils,
fibroblasts, lymphocytes, and mast cells were determined from slide preparations stained with the May–Grünewald–Giemsa stain, counting 600 cells.

**PROCOLLAGEN III PEPTIDE MEASUREMENTS**

Procollagen III amino terminal peptide related antigen (P-III-P) was determined in native BAL fluid using a radioimmunoassay kit (RIA-ghost P III P, Behringwerke AG, Marburg, Germany), based on the method of Rohde et al. The detection limit of the assay was 0-1 units/ml P-III-P. The antibody employed detects the whole P-III-P proteins but not P-III-P fragments and does not cross react with type I collagen, type I procollagen, 7S collagen, laminin, or fibronectin according to the manufacturer. The value of the detection limit was used for the calculations when procollagen III peptide was undetectable in the BAL fluid.

Results were expressed as units of procollagen III peptide per ml native BAL fluid since no satisfactory reference component for normalisation of BAL fluid proteins is available.

**PROTEIN MEASUREMENTS**

Protein concentration in BAL fluid was measured with a laser nephelometer (Behring-Nephelometer, Germany) after precipitating the proteins in BAL samples with 3% trichloroacetic acid. Protein standards were purchased from Behringwerke (Germany).

**FIBROBLAST CULTURE**

Normal human dermal fibroblasts were routinely maintained in Dulbecco’s minimal essential medium supplemented with 400 units/ml penicillin, 50 μg/ml streptomycin, and 10% fetal calf serum in a moist atmosphere of 5% CO₂ in air. The cells were routinely monitored for absence of contamination by bacteria, yeasts, fungi, and mycoplasma, for growth rate, and for limited population doublings.

**CHEMOTAXIS ASSAY**

Fibroblasts in logarithmic growth phase (10th–20th passage number) were detached with 0-05% trypsin, 0-02% EDTA in phosphate buffered saline, suspended in Dulbecco’s minimal essential medium without fetal calf serum, and introduced into the upper compartment of blind well Boyden chambers. The lower compartment contained BAL fluid and was separated from the upper compartment by polycarbonate filters (diameter 13 mm, pore size 8 μm, Nuclepore, Tübingen, Germany). Cells were allowed to migrate for four hours; those that had migrated to the lower face of the filters were stained (Diff-Quick, Merz and Dade, Düdingen, Switzerland) and counted in a microscope at 160× magnification. Results were recorded as the arithmetic mean (SE) of cell numbers in 20 fields on two filters. Details of this procedure have been published previously. Platelet derived growth factor (PDGF, recombinant PDGF-C-sis, B/B dimer, Amersham, Braunischweig, Germany) at 25 ng/ml was used as a reference attractant and was always assayed simultaneously with the study samples. PDGF at 25 ng/ml usually attracted 150–200 cells/field on the lower face of the membrane; BAL fluid from control subjects induced only minor effects (usually 5–40 cells/field) and near Dulbecco’s minimal essential medium or saline did not induce migration (5–10 cells/field). Raw data of responses to BAL fluid were calculated as a percentage of the PDGF response to compensate for day to day variability of chemotactic cell responsiveness.

**STATISTICS**

Data were expressed as the arithmetic mean and standard error of the mean (SE). The normal range of the chemotactic response was defined as the mean chemotaxis value of the controls plus 2 × standard deviation (SD). For statistical analysis the Mann-Whitney test (in dependent samples) and the Wilcoxon test (dependent samples) were employed when suitable. Correlation coefficients were determined by Pearson’s test. For differences between groups and for correlation coefficients between variables, a p value of <0.05 was considered significant with the two tailed test. The statistical computations were performed with the aid of SPSS/PC+ software.

**Results**

**FUNCTIONAL CHARACTERISTICS OF PATIENTS WITH FIBROSING ALVEOLITIS**

The results of lung function tests in the control group and in patients with idiopathic pulmonary fibrosis and systemic sclerosis are shown in table 1. FEV₁, VC, and TLC values were within the normal range in all three groups. There was a significant reduction in TLC, VC, and TLCO values in both groups of patients compared with the control group; there was no significant difference between the two patient groups with respect to these measurements. PaO₂ values at rest and during steady state exercise were significantly reduced only in patients with idiopathic pulmonary fibrosis.

**FIBROBLAST CHEMOTAXIS**

The chemotactic effect of native BAL fluid on cultured human fibroblasts was expressed as a percentage of the effect of 25 ng/ml PDGF. As depicted in fig 1, fibroblast chemotaxis was significantly increased (p < 0.01) in both the patient groups compared

---

*Figure 1. Chemoattractant activity of BAL fluid for fibroblasts in patients with idiopathic pulmonary fibrosis (IPF) or systemic sclerosis (SSC) compared with the control group. *p < 0.01 v control; closed squares, means (SE).*
Figure 2  Inverse correlation between chemoattractant activity of BAL fluid for fibroblasts and total lung capacity (%) predicted in patients with idiopathic pulmonary fibrosis or systemic sclerosis (see also table 2). n = 40; r = -0.45; p < 0.05.

Figure 3  Positive correlation between chemoattractant activity of BAL fluid for fibroblasts and BAL total cell count in patients with idiopathic pulmonary fibrosis or systemic sclerosis (see also table 3). n = 40; r = 0.60; p < 0.01.

with the control group (control, 12.6% (4.0%); idiopathic pulmonary fibrosis, 56.4% (8.5%); systemic sclerosis, 72.3% (16.3%)).

CORRELATIONS OF FIBROBLAST CHEMOTAXIS WITH CELL COUNTS OF BAL FLUID AND WITH LUNG FUNCTION MEASUREMENTS
In the combined patient group a significant inverse correlation (r = -0.45; p < 0.05) existed between fibroblast attracting activity of BAL fluid and TLC (fig 2) and between fibroblast attracting activity of BAL fluid and VC (r = -0.33, p < 0.05); correlations with all measurements of lung function in table 1 were not significant. A positive correlation was found between fibroblast chemotactic activity and total cell count in BAL fluid (fig 3); no significant correlations with other BAL fluid measurements including total protein and procollagen III peptide concentrations were observed.

FOLLOW UP
For further evaluation all patients were assigned, irrespective of their diagnosis, to two groups according to whether chemoattractant activity was lower or higher than 36% of the PDGF response of the indicator cells (mean value of the control group ± 2SD). These two groups were further subdivided according to treatment (with or without immunosuppressive therapy). The distribution of patients with idiopathic pulmonary fibrosis and systemic sclerosis to the four subgroups is shown in fig 4. The four subgroups A–D did not differ significantly with respect to duration of follow up, initial results of lung function tests, or differential cell counts in BAL fluid, although those patients who had refused treatment (subgroups A and C) tended to have less severe lung function impairment (table 2). Total cell count was significantly reduced in group A (low chemoattractant activity, no therapy) compared with the other three groups. Total protein and P-III-P concentrations did not differ significantly between the four groups.

Comparison of the results of the initial lung function tests (T1) with those performed at the end of the follow up period (T2) revealed that there were no consistent changes in the TLC, VC, TLCO, and exercise PaO2 in patients with low chemoattractant activity, irrespective of the treatment given (groups A and B, table 3). There was, however, a significant reduction of VC, TLC, and exercise PaO2 and a small decrease of TLCO in untreated patients with high chemoattractant activity (group C), whereas a significant improvement of VC, TLC, and TLCO and a small increase of exercise PaO2 was observed in patients with high chemoattractant activity receiving immunosuppressive therapy (group D), whereas a significant improvement of VC, TLC, and TLCO and a small increase of exercise PaO2 was observed in patients with high chemoattractant activity receiving immunosuppressive therapy (group D), whereas a significant improvement of VC, TLC, and TLCO and a small increase of exercise PaO2 was observed in patients with high chemoattractant activity receiving immunosuppressive therapy (group D), whereas a significant improvement of VC, TLC, and TLCO and a small increase of exercise PaO2 was observed in patients with high chemoattractant activity (group D) with the other three groups. Total protein and P-III-P concentrations did not differ significantly between the four groups.

Comparison of the results of the initial lung function tests (T1) with those performed at the end of the follow up period (T2) revealed that there were no consistent changes in the TLC, VC, TLCO, and exercise PaO2 in patients with low chemoattractant activity, irrespective of the treatment given (groups A and B, table 3). There was, however, a significant reduction of VC, TLC, and exercise PaO2 and a small decrease of TLCO in untreated patients with high chemoattractant activity (group C), whereas a significant improvement of VC, TLC, and TLCO and a small increase of exercise PaO2 was observed in patients with high chemoattractant activity receiving immunosuppressive therapy (group D), whereas a significant improvement of VC, TLC, and TLCO and a small increase of exercise PaO2 was observed in patients with high chemoattractant activity receiving immunosuppressive therapy (group D), whereas a significant improvement of VC, TLC, and TLCO and a small increase of exercise PaO2 was observed in patients with high chemoattractant activity receiving immunosuppressive therapy (group D), whereas a significant improvement of VC, TLC, and TLCO and a small increase of exercise PaO2 was observed in patients with high chemoattractant activity receiving immunosuppressive therapy (group D), whereas a significant improvement of VC, TLC, and TLCO and a small increase of exercise PaO2 was observed in patients with high chemoattractant activity (group D) with the other three groups. Total protein and P-III-P concentrations did not differ significantly between the four groups.

Discussion
The crucial role of fibroblasts in the pathogenesis of interstitial lung disease has long been recognised. Their activity is regulated by several soluble substances including cytokines, matrix proteins or their fragments, eicosanoids, and reactive oxygen species. At present no satisfactory method exists to assess, at any given time, the activity of these cells in vivo. In this study we measured fibroblast attracting activity in native BAL fluid as a possible indicator of profibrotic activity within the lungs. This approach is based upon the assumption that there is an equilibrium of soluble substances
between the tissue compartment and the epithelial lining fluid. The latter is accessible by BAL and contains at least some of the regulating substances. The net effect of the many fibroblast regulating factors should be one of the critical determinants of the course of pulmonary fibrosis, and the effect of BAL fluid on fibroblasts in vitro may thus provide relevant information about the activation potential for lung fibroblasts in vivo. Following this reasoning, Cantin et al.3 examined BAL fluid for fibroblast growth promoting activity. They found stimulatory activity in BAL fluid from patients with idiopathic pulmonary fibrosis but not in fluid from controls or patients with sarcoidosis. Adolf et al.2 reported preliminary data showing reduced fibroblast stimulating activity in BAL fluid from patients with interstitial lung disease who had responded to corticosteroid therapy. This may indicate that accelerated fibroblast growth elicited by BAL fluid is associated with active pulmonary fibrosis.

Table 2  Mean (SE) results in patients with low and high chemoattractant activity (CAA).

<table>
<thead>
<tr>
<th></th>
<th>Without therapy (Group A, n = 6)</th>
<th>With therapy (Group B, n = 7)</th>
<th>Without therapy (Group C, n = 9)</th>
<th>With therapy (Group D, n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CAA (% PDGF)</strong></td>
<td>20.7 (3.3)</td>
<td>11.7 (4.0)</td>
<td>106.1 (16.9)</td>
<td>87.2 (12.5)</td>
</tr>
<tr>
<td>Follow up (months)</td>
<td>16.2 (5.2)</td>
<td>10.0 (2.1)</td>
<td>13.2 (3.7)</td>
<td>13.4 (2.3)</td>
</tr>
<tr>
<td><strong>Lung function tests</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VC (% predicted)</td>
<td>80.2 (6.9)</td>
<td>71.9 (9.1)</td>
<td>83.6 (8.4)</td>
<td>68.7 (4.9)</td>
</tr>
<tr>
<td>TLCO (% predicted)</td>
<td>76.0 (6.3)</td>
<td>67.3 (8.2)</td>
<td>75.4 (8.3)</td>
<td>63.3 (3.8)</td>
</tr>
<tr>
<td>Exercise PaO2 (mm Hg)*</td>
<td>73.3 (5.5)</td>
<td>59.0 (6.3)</td>
<td>61.1 (8.0)</td>
<td>57.6 (4.3)</td>
</tr>
<tr>
<td><strong>Bronchoalveolar lavage (BAL) fluid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCC (× 10⁸)</td>
<td>4.0 (2.0)</td>
<td>13.9 (5.7)</td>
<td>17.7 (3.0)</td>
<td>8.8 (2.0)</td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>69.2 (8.3)</td>
<td>75.3 (5.9)</td>
<td>77.4 (8.6)</td>
<td>74.0 (4.4)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>17.7 (8.6)</td>
<td>8.9 (2.4)</td>
<td>5.8 (1.9)</td>
<td>11.3 (2.9)</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>1.3 (0.6)</td>
<td>1.6 (1.1)</td>
<td>2.3 (0.9)</td>
<td>3.3 (1.2)</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>11.3 (5.9)</td>
<td>14.3 (5.8)</td>
<td>14.0 (9.5)</td>
<td>10.7 (2.3)</td>
</tr>
<tr>
<td>T₄/T₈ ratio</td>
<td>0.9 (0.3)</td>
<td>2.1 (1.1)</td>
<td>0.5 (0.2)</td>
<td>0.9 (0.2)</td>
</tr>
<tr>
<td>Protein (mg/l)</td>
<td>19.8 (2.9)</td>
<td>14.8 (1.5)</td>
<td>17.9 (1.2)</td>
<td>18.7 (1.3)</td>
</tr>
<tr>
<td>P-III-P (units/ml)</td>
<td>0.1 (0.0)</td>
<td>0.2 (0.1)</td>
<td>0.2 (0.1)</td>
<td>0.4 (0.1)</td>
</tr>
</tbody>
</table>

CAA—chemoattractant activity; PDGF—platelet derived growth factor; VC—vital capacity; TLC—total lung capacity; TLCO—carbon monoxide transfer factor; TCC—total cell count; T₄/T₈ ratio—quotient of T helper to T suppressor lymphocytes; protein—total protein concentration in BAL fluid; P-III-P—amino terminal procollagen-III-peptide related antigen; *7.5 mm Hg = 1 kPa; p ≤ 0.05 v Groups B, C, and D.

Table 3  Mean (SE) results of lung function tests in patients with low and high chemoattractant activity at initial investigation and at follow up.

<table>
<thead>
<tr>
<th></th>
<th>Without therapy (Group A, n = 6)</th>
<th>With therapy (Group B, n = 7)</th>
<th>Without therapy (Group C, n = 9)</th>
<th>With therapy (Group D, n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow up (months)</td>
<td>16.2 (5.2)</td>
<td>10.0 (2.1)</td>
<td>13.2 (3.7)</td>
<td>13.4 (2.3)</td>
</tr>
<tr>
<td>VC (% predicted)</td>
<td>80.2 (6.9)</td>
<td>71.9 (9.1)</td>
<td>83.6 (8.4)</td>
<td>68.7 (4.9)</td>
</tr>
<tr>
<td>TLC (%) predicted</td>
<td>76.0 (6.3)</td>
<td>67.3 (8.2)</td>
<td>75.4 (8.3)</td>
<td>63.3 (3.8)</td>
</tr>
<tr>
<td>T₄CO (% predicted)</td>
<td>73.3 (5.5)</td>
<td>59.0 (6.3)</td>
<td>61.1 (8.0)</td>
<td>57.6 (4.3)</td>
</tr>
<tr>
<td>Exercise PaO₂ (mm Hg)*</td>
<td>71.3 (5.5)</td>
<td>59.0 (6.3)</td>
<td>61.7 (5.5)</td>
<td>64.9 (2.4)</td>
</tr>
</tbody>
</table>

CAA—chemoattractant activity; PDGF—platelet derived growth factor; VC—vital capacity; TLC—total lung capacity; T₄CO—carbon monoxide transfer factor; T₄—time of first investigation; T₈—time of follow up investigation; *7.5 mm Hg = 1 kPa; p ≤ 0.05 v T₀; pp ≤ 0.01 v T₈.

Figure 5  Initial (T₀) and follow up (T₈) measurement of the total lung capacity (TLC) in patients with low (< 36% PDGF) or high (> 36% PDGF) chemoattractant activity, without or with immunosuppressive therapy, respectively. Patient subsets are identical to groups A-D in Fig 4. A significant decrease of TLC was seen in group C and a significant increase of TLC in group D (see also Table 3).
In this study we have shown for the first time that there is elevated fibroblast attracting activity in BAL fluid in patients with fibrosing alveolitis. We do not know which or how many factors within the BAL fluid contributed to this observation. Growth factors such as PDGF are known to be released from activated alveolar macrophages and are believed to play an important part in the activation of fibroblasts.4,5 It is unhelpful, however, to search for effects of a single mediator within this highly complex network of cytokines, matrix proteins (fragments) and enzymes, as it is the net effect of all mediators which determines the activities of the lung fibroblasts. The results showing increased chemoattractant activity in BAL fluid from patients with fibrosing alveolitis, the inverse correlations of chemoattractant activity with TLC and VC, and the disease progression in untreated patients with high chemoattractant activity suggest that the fibroblast chemotactic response elicited by native BAL fluid in vitro represents, in part, the activation potential for lung fibroblasts in vivo. We suggest that chemotactic migration of fibroblasts towards inflammatory sites may be a relevant component of the fibrotic process within the lungs as some chemoattractants for fibroblasts are known to be released from activated inflammatory cells—for example, PDGF from macrophages—or liberated from the connective tissue matrix by lysosomal enzymes from activated phagocytes—for example, fragments of collagen, elastin, fibronectin.7 Histologically the distribution of the fibrosis in fibrosing alveolitis is homogeneous and often patchy.8,9 In the presence of chemoattractants directed migration may contribute considerably to this homogeneous accumulation of fibroblasts. Chemotactic activity was inversely correlated with TLC and VC, suggesting that fibroblast activation is linked both to the presence and the severity of the pulmonary disease. Lung volumes are more closely related to the extent of fibrosis than to the alveolitis; this is in accordance with the interpretation that chemoattractant activity is a marker of the fibrotic process rather than the alveolitis. Similarly the chemoattractant effect of BAL fluid was significantly related only to the total cell count and not to any of the individual cell types in the BAL fluid. This supports our interpretation that the chemotactic activity of fibroblasts elicited by BAL fluid represents the net effect of numerous factors regulating fibroblast activity, and is not attributable to a single cell type or mediator.

The result of the follow up study suggests a possible prognostic significance for the chemoattractant activity in at least one subgroup of patients (those with high chemoattractant activity of their BAL fluid). Figure 5 and table 3 show that patients with high chemotactic activity improved with immunosuppressive therapy and deteriorated if untreated. This is in agreement with others who found a decrease of fibroblast proliferation elicited by BAL fluid after successful immunosuppressive therapy.27 The data presented here, however, are not sufficiently secure to draw prognostic conclusions from measurements of chemoattractant activity.

Our data therefore support the underlying hypothesis that the qualitative and quantitative characterisation of fibroblast stimulating activity within the epithelial lining fluid may provide a new and potentially more clinically relevant information on the fibrotic process, and may ultimately help to improve the clinical management of patients with fibrosing alveolitis.

We gratefully acknowledge the expert technical assistance of Mrs Cornelia Plate, Mrs Anne-Marie Allmeling, Mrs Christine Kunior, Mrs Elisabeth Becker, and Mrs Anne Wirzlah. This study was supported in part by a grant to BCA from the German Ministry of Research and Technology. It was presented in part at the Joint Meeting SEP-SIPCR in September 1991 in Brussels, Belgium.

20 Arbeitsgruppe Sklerodermie der Arbeitsgemeinschaft


Fibroblast chemotactic response elicited by native bronchoalveolar lavage fluid from patients with fibrosing alveolitis.

J Behr, B C Adelmann-Grill, F Krombach, T Beinert, M Schwaiblmair and G Fruhmann

Thorax 1993 48: 736-742
doi: 10.1136/thx.48.7.736

Updated information and services can be found at:
http://thorax.bmj.com/content/48/7/736

Email alerting service

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/