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CCL18 as an Indicator of Pulmonary Fibrotic Activity in Idiopathic Interstitial Pneumonias and Systemic Sclerosis

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Objective. **In diffuse parenchymal lung diseases, the evolution of pulmonary fibrosis is often devastating and may result in death. In this study the role of CCL18 as a biomarker of disease activity in idiopathic interstitial pneumonias (IIPs) and systemic sclerosis (SSc) with lung involvement was evaluated.**

Methods. **CCL18 was assessed in supernatants of cultured bronchoalveolar lavage (BAL) cells as well as BAL fluid and serum samples from 43 patients with IIPs, 12 patients with SSc, and 23 healthy control subjects. Concentrations of CCL18 were measured by enzyme-linked immunosorbent assay, and expression of CCL18 was assessed by flow cytometry.**

Results. **CCL18 concentrations were statistically significantly increased in all patients with fibrotic lung diseases. Spontaneous CCL18 production by BAL cells was negatively correlated with total lung capacity and the diffusion capacity for carbon monoxide, whereas there was a positive correlation of CCL18 concentrations with BAL neutrophil and eosinophil cell counts. Flow cytometry revealed an increase in the percentage of CCL18-positive alveolar macrophages and an increase in the CCL18 fluorescence intensity per cell in patients with fibrotic lung diseases. In a cohort of patients who were followed up for at least 6 months (n = 40), a close negative correlation was observed between changes in the predicted total lung capacity and changes in CCL18 serum concentrations.**

Conclusion. **These findings suggest that CCL18 production by BAL cells and serum CCL18 concentrations reflect pulmonary fibrotic activity in patients with IIPs and those with SSc. Monitoring changes in CCL18 production might be an extraordinarily useful tool in clinical practice and in studies aimed at evaluating new approaches for treatment of fibrotic lung diseases.**

Fibrotic lung diseases have recently been given a new classification as idiopathic interstitial pneumonias (IIPs) and have been categorized into different subtypes, such as usual (UIP), nonspecific (NSIP), and desquamative (DIP) interstitial pneumonias as well as cryptogenic organizing pneumonia (COP) (1). However, fibrotic remodeling of lung parenchyma is also a main feature of lung involvement in patients with rheumatic diseases. Approximately half of all diagnosed cases with the typical histologic pattern of IIPs are associated with a rheumatic disease (2). Therefore, lung involvement in patients with rheumatic diseases can be identified by the same histologic and radiologic patterns as described in patients with IIPs, and is not distinguishable from the idiopathic variants. For example, in patients with systemic sclerosis (SSc), lung involvement may occur in a pattern typical of NSIP, but could also resemble that of UIP (2).

In patients whose disease is associated with fibrotic lung remodeling, including patients with SSc as well as patients with radiologic types III and IV sarcoidosis, hypersensitivity pneumonitis, or idiopathic pulmonary fibrosis (IPF) (UIP pattern), we recently found that a common feature is shared: the phenotype of the alveolar macrophages in these patients is characterized by alternative activation with exaggerated production of the CC chemokine ligand CCL18 (3). In addition,

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increased expression of CCL18 messenger RNA (mRNA) has been described in bronchoalveolar lavage (BAL) cells from patients with SSc (4). Recently, Kodera et al (5) observed increasing CCL18 concentrations in the serum of patients with SSc, indicating the presence of pulmonary fibrotic remodeling. Furthermore, their findings suggest that CCL18 might serve as an indicator of disease activity, since the authors observed decreasing CCL18 concentrations in 20 patients with SSc after treatment with immunosuppressive agents.

Our previous studies, along with those by Atamas et al (6), have shown that the increased production of CCL18 by alternatively activated alveolar macrophages in patients with pulmonary fibrosis promotes the production of collagen by lung fibroblasts (3). In these patients, we observed a vicious circle in which the interaction of alternatively activated alveolar macrophages and lung fibroblasts perpetuates the fibrotic process (3). CCL18 is a chemokine that has thus far been described only in primates and is abundantly produced within the lung (7–9). Its receptor is not known, and only myeloid-derived cells have been found to produce this mediator, which was previously termed pulmonary and activation-regulated chemokine (7–9).

Following the revised classification of the IIPs, several large studies exploring new therapeutic strategies for IPF and SSc have recently been published (10,11) or are in progress. Very often, the results of the various studies have been contradictory with regard to the influence of the tested treatment schedule on pulmonary function, mortality, or the 6-minute walk test (12,13). At present there is no established biomarker that can adequately reflect pulmonary fibrotic activity.

Given this background, we wondered whether CCL18 can serve as a biomarker of disease activity in patients with pulmonary fibrosis. We examined a total of 43 patients with either IPF, DIP, NSIP, or COP and 12 patients with SSc, as well as 23 healthy control subjects. Our results reveal that CCL18 is abundantly expressed in the culture supernatants of BAL cells and the BAL fluid and sera from patients whose pulmonary fibrosis is histologically proven. Our results also show that concentrations of CCL18 in BAL fluid are highly correlated with known markers of disease severity. In addition, in the course of disease in 40 patients with different IIPs or SSc, we observed a close negative correlation between changes in the total lung capacity and changes in the serum CCL18 concentrations. It therefore appears that the concentration of CCL18 in the serum may serve as an indicator of pulmonary disease activity in patients with pulmonary fibrosis, in both its idiopathic and its rheumatic forms.

PATIENTS AND METHODS

Subjects. Sixteen patients with IPF (UIP pattern), 12 patients with SSc and proven lung involvement, 7 patients with NSIP, 10 patients with DIP, and 10 patients with COP were studied. All patients displayed a typical histologic pattern of pulmonary fibrosis, as determined by transbronchial or video-assisted thoracoscopic biopsy. The diagnosis in all patients was confirmed as being in accordance with the American Thoracic Society/European Respiratory Society (ATS/ERS) criteria for IIPs (1) and the American College of Rheumatology (formerly, the American Rheumatism Association) criteria for SSc (14), by clinical evaluation, high-resolution computed tomography (HRCT), histologic assessment, and laboratory tests. None of the patients with IIPs had rheumatic diseases. All patients with SSc tested positive for anti–DNA topoisomerase I autoantibodies and were found to have a histologic pattern typical of NSIP on HRCT and histologic examination of the lung biopsy tissue. Twenty-three healthy volunteers served as the control group. Patients' characteristics, including age, sex, and pulmonary function data, as well as the distribution of the BAL cell subsets are shown in Table 1.

The study was approved by the local ethics committee.

Table 1. Characteristics and distribution of BAL cells in the study subjects*

Characteristic	Control	IPF	NSIP	COP	DIP	SSc
Age, years	36.0 ± 16.9	67.1 ± 14.1	60.1 ± 15.3	61.0 ± 14.4	54.8 ± 8.7	66.2 ± 13.4
Sex, no. male/no. female	13/10	13/3	4/3	7/3	8/2	3/9
TLC, % of predicted	98.8 ± 10.0	61.2 ± 11.1	58.1 ± 12.6	80.7 ± 18.1	91.2 ± 7.9	72.0 ± 20.5
DLCO, % of predicted	90.0 ± 14.0	58.7 ± 19.6	46.3 ± 13.4	74.2 ± 22.4	61.8 ± 8.2	63.3 ± 19.0
Differential BAL cell count, %						
Macrophages	92 ± 7	71 ± 15	66 ± 16	57 ± 17	93 ± 5	69 ± 17
Lymphocytes	7 ± 7	13 ± 9	15 ± 17	35 ± 14	2 ± 1	18 ± 10
Neutrophils	1 ± 1	10 ± 8	13 ± 7	3 ± 4	3 ± 3	9 ± 10
Eosinophils	0 ± 0	6 ± 7	4 ± 4	2 ± 5	1 ± 3	3 ± 2

* Except where indicated otherwise, values are the mean ± SD. BAL = bronchoalveolar lavage; IPF = idiopathic pulmonary fibrosis; NSIP = nonspecific interstitial pneumonia; COP = cryptogenic organizing pneumonia; DIP = desquamative interstitial pneumonia; SSc = systemic sclerosis; TLC = total lung capacity; DLCO = diffusion capacity for carbon monoxide.

Pulmonary function tests were routinely performed using standard methods, in accordance with the ATS recommendations (15). The diffusion capacity for carbon monoxide (DLCO) was measured by a single-breath method using a gas mixture of 0.2% CO and 8% helium, with correction for hemoglobin.

In addition, serum samples and pulmonary function data were collected from a separate cohort of 40 patients with pulmonary fibrosis whose condition was attributable to various interstitial lung diseases (8 patients with COP, 5 patients with NSIP, 17 patients with IPF, 5 patients with DIP, and 5 patients with SSc). These patients were followed up for at least 6 months, during which at least 4 serum samples and 4 sets of pulmonary function data were obtained from each patient. Serum samples were tested in duplicate. During this observation period, patients received various drugs, including steroids, acetylcysteine, azathioprine, and cyclophosphamide. The patients were separated into 3 groups according to the ATS/ERS criteria for IIPs (1), categorized by whether patients had progressive, stable, or improving disease. Any improvement during the observation period was defined as an increase in total lung capacity of more than 10%, while deterioration was defined as a decrease in total lung capacity of more than 10%. For comparisons, changes in total lung capacity and changes in CCL18 concentrations were calculated by subtracting the data at the beginning of the observation period from the data at the end of the observation period.

Preparation of BAL cells and cell culture. Bronchoscopy, BAL, and culture of BAL cells were performed using standard techniques as previously described (16,17). In detail, BAL cells (1×10^6 cells/ml) were placed in 24-well culture plates in RPMI (Gibco, Karlsruhe, Germany) with 2% heat-inactivated human AB serum and antibiotics (50 units/ml penicillin and 50 μg/ml streptomycin; Biochrom, Berlin, Germany) in a humidified atmosphere containing 5% CO_2 at 37°C for 24 hours. At the end of the culture period, supernatants were harvested and stored at −70°C until assayed for CCL18 concentrations. The viability of the cells was determined by Trypan blue exclusion, and always exceeded 95%. The mean frequency of each subset of BAL cells in the study groups is shown in Table 1; data were not corrected for differing cell cytologic features.

Differential BAL cell count. BAL cells were stained with May-Grünwald stain. The differential cell count for each subset of BAL cells was then determined by counting at least 200 cells on the cell smear.

Flow cytometry. Freshly isolated BAL cells from healthy volunteers and from patients with pulmonary fibrosis were fixed in 4% paraformaldehyde and stained using biotinylated goat anti-human CCL18 antibody and streptavidin/phycoerythrin (DakoCytomation, Carpinteria, CA). Not all patients were included in this experiment. Cells were analyzed by flow cytometry (FACSCalibur; BD Biosciences, San Jose, CA). The percentage of CCL18-positive cells was determined using isotype-matched biotinylated antibodies as a control. Results are expressed as the relative fluorescence intensity, calculated by subtracting the intensity of CCL18 staining from the intensity of isotype-matched control antibody staining.

Enzyme-linked immunosorbent assay (ELISA). CCL18 was quantified using a DuoSet ELISA Development System kit (R&D Systems Europe, Abingdon, UK). The detection limit for the CCL18 ELISA was 7 pg/ml. For duplicate samples, an intraassay coefficient of variation (CV) of <10% and an interassay CV of <20% were accepted. Results were not adjusted for the differential BAL cell count.

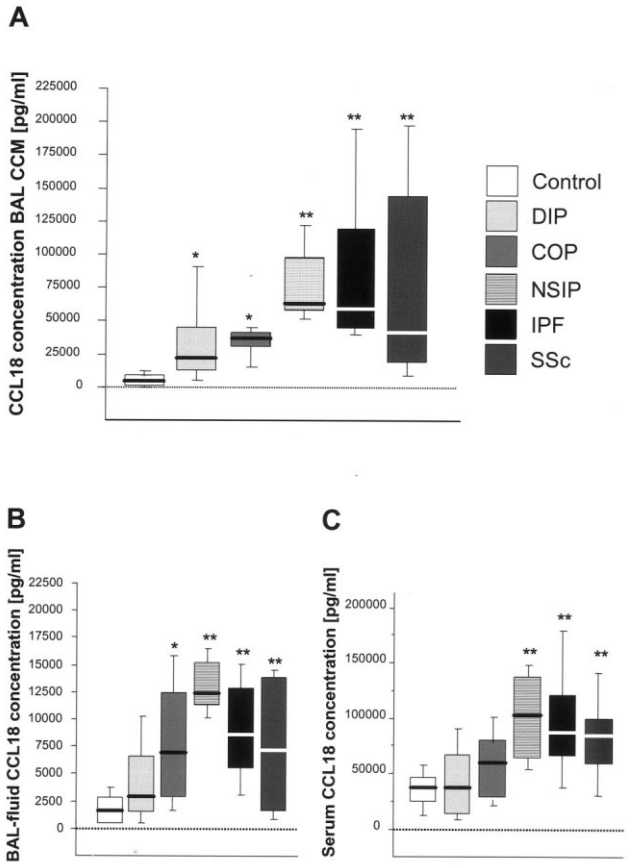

Figure 1. Spontaneous production of CCL18 by bronchoalveolar lavage (BAL) cells (**A**) and concentrations of CCL18 in BAL fluid (**B**) and serum (**C**) from patients with idiopathic interstitial pneumonias (desquamative interstitial pneumonia [DIP; n = 10], cryptogenic organizing pneumonia [COP; n = 10], nonspecific interstitial pneumonia [NSIP; n = 7], and idiopathic pulmonary fibrosis [IPF; usual pattern; n = 16]), patients with systemic sclerosis (SSc) with lung involvement (n = 12), and healthy volunteers as controls (n = 23). Values are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median, and the lines outside the boxes represent the 10th and 90th percentiles. $* = P < 0.05$; $** = P < 0.001$, versus controls. CCM = conditioned culture medium.

Statistical analysis. Data on CCL18 are expressed as the median (interquartile range). Statistical comparisons between experimental and control data in the in vitro studies were made by analysis of variance with post hoc Fisher's protected least significant difference test. Correlations were determined using a simple regression model. Prior to these analyses, binominal distribution data were logarithmically transformed. *P* values less than 0.05 were considered significant.

RESULTS

Marked increase in CCL18 production by BAL cells derived from patients with pulmonary fibrosis. BAL cells derived from patients or healthy volunteers were cultured for 24 hours in culture medium. Spontaneous CCL18 production in the cell culture supernatants was measured by ELISA. BAL cells from patients with IPF, patients with NSIP, and patients with SSc produced the highest concentrations of CCL18 (Figure 1A), with 100-fold higher concentrations in these cells than in those from healthy volunteers ($P < 0.001$). BAL cells from patients with COP and patients with DIP also showed an increased production of CCL18 ($P < 0.05$) (Figure 1A), but the median concentrations of CCL18 in these patients were statistically significantly lower than in the cohorts of patients with IPF or NSIP ($P < 0.05$) (Figure 1A).

Increased CCL18 concentrations in BAL fluid and serum from patients with IIPs and patients with SSc. The concentrations of CCL18 in BAL fluid and serum (Figure 1B) resembled that described for BAL cells. CCL18 concentrations in BAL fluid were highest in patients with IPF, those with NSIP, and those with SSc (Figure 1B). The CCL18 concentrations in patients with IPF and patients with NSIP were also up to 100-fold higher than in healthy controls. Four cohorts of patients with fibrotic lung tissue remodeling (IPF, NSIP, COP, and SSc) showed a statistically significant increase in CCL18 concentrations in the BAL fluid. In addition, there was a close correlation between the CCL18 concentrations measured in the BAL fluid and those in the conditioned medium of BAL cells cultured for 24 hours ($r = 0.64$, $P < 0.0001$).

With regard to serum concentrations, CCL18 was significantly increased in 3 cohorts of patients with fibrotic lung tissue remodeling (NSIP, IPF, and SSc) (Figure 1C). There was a close correlation between the CCL18 concentrations in the serum and those in the BAL fluid ($r = 0.64$, $P < 0.0001$) and between the serum concentrations and those in the conditioned BAL cell culture medium ($r = 0.56$, $P < 0.0001$).

Increase in the percentage of CCL18-positive alveolar macrophages and CCL18 fluorescence intensity per cell in patients with fibrotic lung diseases. Not all patients were included in the flow cytometry experiments. Flow cytometric analyses of BAL cells from patients and healthy controls revealed that only large BAL cells stained positive for CCL18. These cells could be identified as alveolar macrophages on the basis of cell granularity and size. BAL lymphocytes showed no staining for CCL18 (Figure 2A). We observed an increase in the percentage of CCL18-positive alveolar macrophages and in the CCL18 fluorescence intensity per cell in patients with fibrotic lung diseases in comparison with healthy controls (Figures 2G and H).

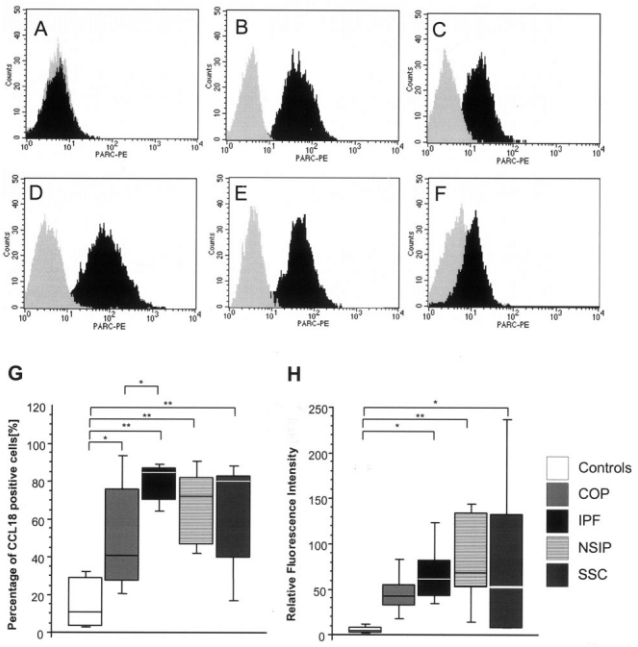

Figure 2. Flow cytometric analyses of spontaneous CCL18 expression. Immunostaining for CCL18 was performed on bronchoalveolar lavage (BAL) lymphocytes from a patient with idiopathic pulmonary fibrosis (IPF) **(A)** and on BAL macrophages from a patient with IPF **(B),** a patient with cryptogenic organizing pneumonia (COP) **(C),** a patient with nonspecific interstitial pneumonia (NSIP) **(D)**, a patient with systemic sclerosis (SSc) **(E)**, and a healthy volunteer **(F)**. Findings in **A–F** are representative of the flow cytometry results in all subjects. In addition, the percentage of CCL18-positive alveolar macrophages **(G)** and the relative fluorescence intensity for CCL18 on alveolar macrophages **(H)** were determined in 10 healthy control subjects, 5 patients with COP, 7 patients with IPF, 5 patients with NSIP, and 5 patients with SSc with lung involvement. Values in **G** and **H** are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median, and the lines outside the boxes represent the 10th and 90th percentiles. $* = P < 0.05$; $** = P < 0.005$. PARC-PE = pulmonary and activation-regulated chemokine (CCL18) with phycoerythrin staining.

Close correlation of spontaneous CCL18 production by BAL cells and serum CCL18 concentrations with BAL neutrophil and BAL eosinophil cell counts. BAL neutrophil and BAL eosinophil cell counts are established indicators of the extent of fibrotic remodeling in the lavaged lung area. Furthermore, both are known to have prognostic value in patients with pulmonary fibrosis. We observed a close correlation between spontane-

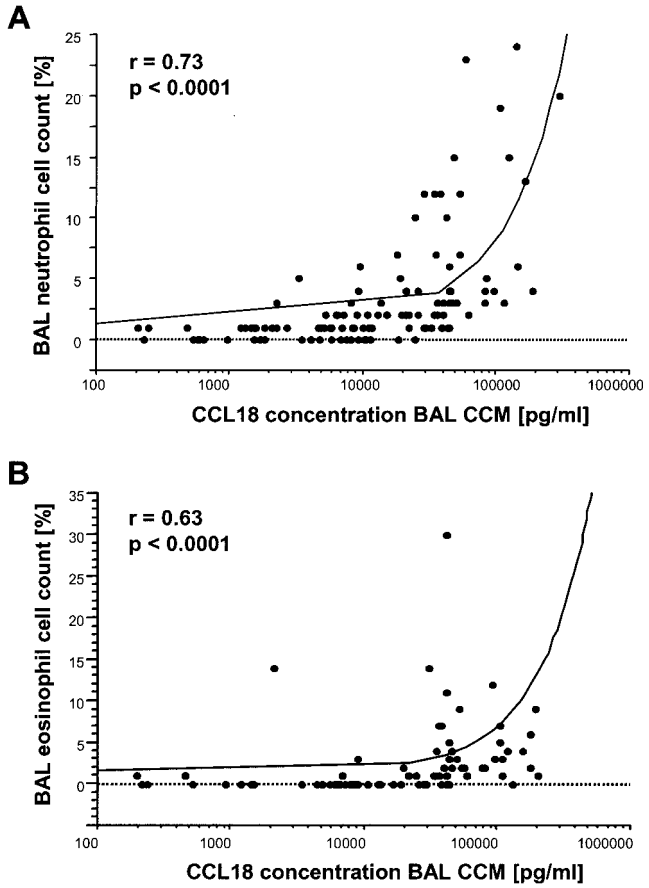

Figure 3. Correlation of spontaneous production of CCL18 by bronchoalveolar lavage (BAL) cells with BAL neutrophil (**A**) and BAL eosinophil (**B**) cell counts in the total study cohort. Circles indicate individual values. CCM = conditioned culture medium.

ous CCL18 production by BAL cells and both the BAL neutrophil cell count ($r = 0.73$, $P < 0.0001$) and the BAL eosinophil cell count ($r = 0.63$, $P < 0.0001$) (Figures 3A and B). There was a weak correlation between the percentage of alveolar macrophages and spontaneous CCL18 production by BAL cells ($r = 0.38$, $P < 0.0001$), and there was no correlation between the percentage of BAL lymphocytes and spontaneous CCL18 production by BAL cells. Similar to the observations in BAL cell culture medium, there was a correlation between CCL18 concentrations in the serum and both the BAL neutrophil cell count ($r = 0.58$, $P < 0.0001$) and BAL eosinophil cell count ($r = 0.62$, $P < 0.0001$) (results not shown).

Correlation of serum CCL18 concentrations with pulmonary function data. A close negative correlation was observed between the production of CCL18 by BAL cells and the total lung capacity ($r = -0.66$, $P < 0.0001$) (Figure 4A). In addition, there was a close negative correlation between the DLCO and the production of CCL18 by BAL cells ($r = -0.66$, $P < 0.0001$) (Figure 4B). Furthermore, there was a negative correlation between CCL18 concentrations in the serum and both the total lung capacity ($r = -0.52$, $P < 0.0001$) and the DLCO ($r = -0.36$, $P = 0.0044$) (results not shown).

Serum CCL18 concentration as an indicator of fibrotic activity. We collected serum samples and pulmonary function data, in parallel, from an additional cohort of 40 patients with pulmonary fibrosis. In these patients, there was a close negative correlation between

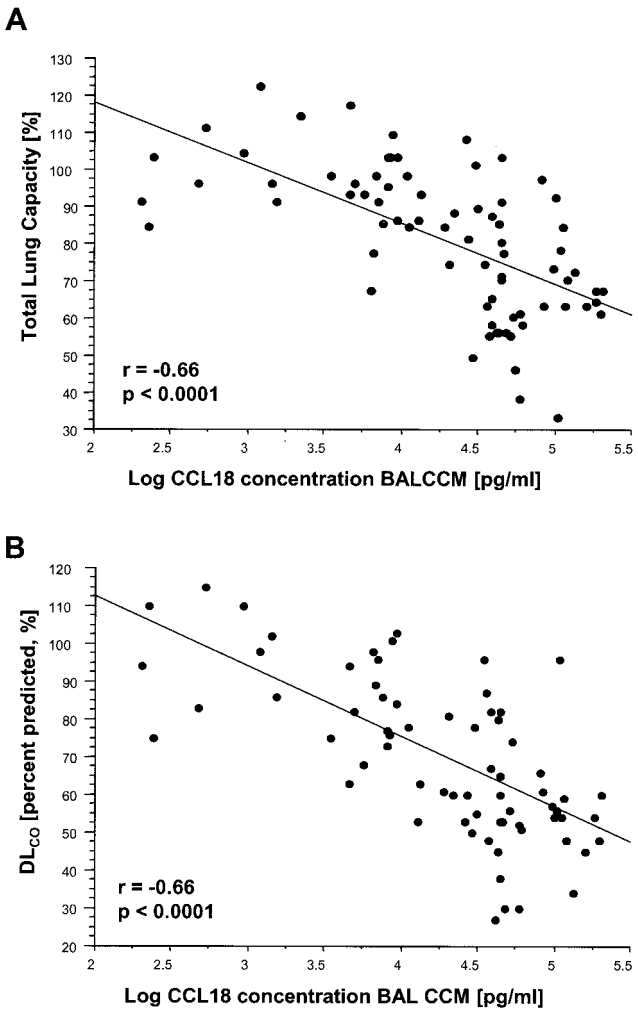

Figure 4. Negative correlation of CCL18 concentrations in bronchoalveolar lavage (BAL) cell supernatants with the total lung capacity (**A**) and the diffusion capacity for carbon monoxide (DLCO) (**B**) in the total study cohort. For normalization, CCL18 concentrations are depicted on a logarithmic scale. CCM = conditioned culture medium.

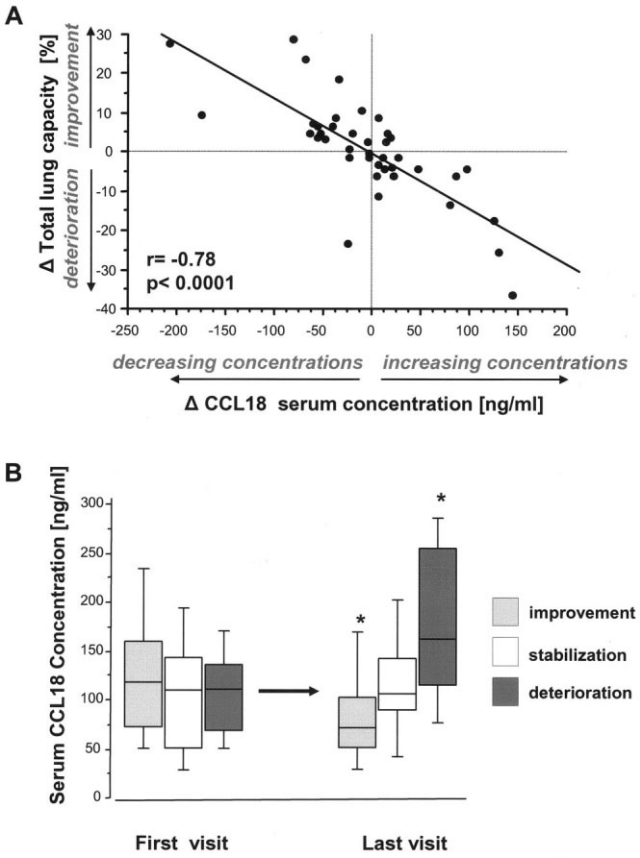

Figure 5. Close correlation between the course of serum concentrations of CCL18 and course of total lung capacity in 40 patients with various interstitial lung diseases associated with pulmonary fibrosis. **A,** Correlation between changes in total lung capacity and changes in serum CCL18 concentrations during the period of observation. **B**, Correlations with serum CCL18 concentrations among patients grouped according to the course of pulmonary function during the observation time, classified by improvement, stabilization, or deterioration of disease using the American Thoracic Society/European Respiratory Society criteria (1). Values in **B** are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median, and the lines outside the boxes represent the 10th and 90th percentiles. $* = P < 0.05$ versus patients with stable disease.

the change in total lung capacity and the change in serum production of CCL18 ($r = -0.78$, $P < 0.0001$) (Figure 5A). Patients whose total lung capacity improved over the followup period (at least 6 months) had decreasing serum CCL18 concentrations. In these patients, the change in CCL18 production was expressed as a negative value, because serum CCL18 concentrations at the beginning were higher than serum CCL18 concentrations at the end of the observation period.

We also separated these 40 patients into 3 different groups on the basis of the ATS/ERS criteria, with categories according to whether the patients had progressive, stable, or improving disease (1). The serum CCL18 concentrations in patients with progressive disease increased during the observation period and were significantly different from those in patients with stable or improving disease (Figure 5B).

DISCUSSION

Pulmonary fibrosis represents a final pathway of various pulmonary diseases, including lung involvement in rheumatic diseases. In a recent report we showed that different interstitial lung diseases, such as radiologic types III and IV sarcoidosis, hypersensitivity pneumonitis, and IPF, share the up-regulation of CCL18 production as a common mechanism, and that a vicious circle involving macrophages and fibroblasts and their products, CCL18 and collagen, perpetuates pulmonary fibrosis (3). In this study we have shown that CCL18 is abundantly produced by BAL cells from patients with IIPs and patients with SSc. Production of CCL18 by BAL cells was correlated with pulmonary function results and BAL neutrophil cell counts, which are both known markers of disease progression. In addition, in another group of 40 patients with fibrotic lung disease, we observed an extraordinarily close correlation between the course of total lung capacity and changes in serum CCL18 levels.

Our data clearly demonstrate that CCL18 production by BAL cells is markedly enhanced in patients with fibrotic lung disease in the IIPs or SSc. BAL cells from patients with IPF (UIP pattern), patients with NSIP, or patients with SSc produced up to 100-fold more CCL18 than did the BAL cells of healthy control subjects. In addition, BAL cells from patients with other diseases associated with pulmonary fibrosis, such as COP or DIP, produced a higher level of CCL18 than was found in controls. We have recently shown that there is a strong link between CCL18 production and the evolution of pulmonary fibrosis in such different diseases as sarcoidosis, IPF, and hypersensitivity pneumonitis. We herein show that there is also an increase in CCL18 protein production in IIPs such as DIP, NSIP, COP, and IPF (UIP pattern), as well as in SSc. An increase in CCL18 mRNA expression in patients with SSc (4) and patients with IPF (18) has been described previously. However, we are the first to comprehensively describe CCL18 production by BAL cells in a broad spectrum of fibrotic lung diseases. In a recent study, we found that CCL18 produced by alveolar macrophages enhances

collagen production by lung fibroblasts (3), and conversely, fibroblast production of collagen up-regulates CCL18 production by alveolar macrophages. The present study demonstrates that this vicious circle is a common mechanism in all tested fibrotic lung diseases. Thus, our data suggest that an increase in CCL18 production by BAL cells indicates the evolution of pulmonary fibrotic remodeling.

CCL18 concentrations were also highly increased in the BAL fluid from all tested patient cohorts with fibrotic lung disease. In patients with IPF, SSc, or NSIP, CCL18 concentrations in BAL fluid were up to 100-fold higher than is considered normal, similar to the results obtained on CCL18 production by BAL cells. Consistent with our previous findings and those by Pardo et al (3,18), we were able to show, by in situ hybridization and immunohistochemistry and by assessment of isolated cells, that CCL18 is mainly produced by alveolar macrophages. The close correlation between the levels of CCL18 in BAL fluid and those in BAL cell cultures suggests that the main CCL18-producing cells can be recovered by BAL. According to previously published results from our group (3), flow cytometric analysis of BAL cells revealed that only alveolar macrophages, and not BAL lymphocytes, produce CCL18. The difference in CCL18 production between patients and controls is caused by an increase in the percentage of CCL18-producing cells as well as an increase in the CCL18 production per cell in patients with fibrotic lung diseases.

Several factors could have an influence on the observed differences in CCL18 production by BAL cells between different patient cohorts. We have previously shown that collagen itself up-regulates CCL18 production by alveolar macrophages, in a concentration-dependent manner. Therefore, differences in fibrotic activity, which is tantamount to increased collagen production and fibroblast proliferation, might be responsible for the observed differences in CCL18 production between patient cohorts. Since there was heterogeneity in disease severity, particularly in the cohort of patients with SSc, this might also explain the broad range of CCL18 production within the patient cohorts.

In this context we wondered whether CCL18 levels in BAL cell supernatants and in serum were indicators of disease severity. To test this, we analyzed the relationship between parameters of pulmonary function and differential BAL cell counts. We observed a negative correlation between CCL18 concentrations in BAL cell supernatants or serum and the total lung capacity, and a negative correlation between CCL18 concentrations and the DLCO. Both of these pulmonary function markers are known to reflect disease severity and are associated with the risk of death in patients with pulmonary fibrosis (19–22). In addition, there was a correlation between CCL18 concentrations in BAL cell supernatants or serum and BAL neutrophil and eosinophil cell counts, which have been described as markers of disease progression and fibrotic activity in patients with pulmonary fibrosis and SSc (23–27). The possibility that CCL18 has a direct influence on peripheral blood neutrophils via its chemotactic activity was recently excluded (28). CCL18 is described as a chemokine capable of attracting naive T cells, activated T cells, monocytes, eosinophils, and dendritic cells (9,28,29). Nevertheless, despite the correlations with BAL eosinophil cell counts, there was no correlation between BAL cell lymphocyte counts and concentrations of CCL18 in BAL cell supernatants, and there was only a weak correlation with BAL macrophage cell counts.

We showed a close correlation between the concentrations of CCL18 in BAL fluid and those in serum. Interestingly, CCL18 levels in the serum were similarly high, and detectable within the 100-ng range. This correlation between CCL18 levels in BAL fluid and those in the serum from all examined disease groups suggests that the level of CCL18 production within the lung is reflected in the serum production of CCL18. However, we cannot exclude the possibility that other factors might influence CCL18 levels in the serum. In our experiments, alveolar macrophages produced ~100-fold more CCL18 compared with freshly isolated monocytes. None of our patients had a disease, such as atopic dermatitis (30) or Gaucher disease, that is known to be associated with increased CCL18 serum levels (31). The increase in serum CCL18 concentrations in patients with fibrotic lung diseases might be associated with multiple mechanisms. First, there might be spillover from the lung. Second, results from several studies have suggested that there is also an increase in serum collagen concentrations in patients with fibrotic lung disease (32,33). We have shown that macrophages produce more CCL18 following stimulation with collagen (3). Therefore, it might be possible that the increase in serum collagen concentrations will cause an increase in CCL18 production by extrapulmonary macrophages that are exposed to serum collagen. Furthermore, it is tempting to speculate that there is systemic aberrant CCL18 production by macrophages in patients with fibrotic lung diseases. This issue requires further study.

In other experiments, we analyzed the relationship between serum CCL18 concentrations and disease

progression. We observed a close negative correlation between the changes in serum CCL18 concentrations and the changes in total lung capacity. This effect was observed regardless of the given therapy. More precisely, in patients in whom pulmonary function improved with the use of steroids (with or without further immunosuppressive therapy), CCL18 levels decreased. In contrast, in patients with IPF or SSc whose pulmonary function declined despite the use of therapy with immunosuppressants such as steroids, CCL18 serum concentrations increased. This finding suggests that changes in serum CCL18 concentrations reflect disease activity rather than the severity of the disease. In patients in whom disease activity was obviously ameliorated by steroids, such as in patients with COP, the CCL18 concentrations dramatically decreased. Therefore, serum CCL18 levels could serve as an indicator of disease activity in patients with pulmonary fibrosis who are being treated with a distinct drug regimen.

The observed correlation between the course of predicted total lung capacity and changes in serum CCL18 concentrations was extraordinarily close, with a correlation coefficient of −0.78. To our knowledge, the correlation between other known serum markers, such as KL-6, interleukin-8, or surfactant D, and pulmonary function data is far lower. Recently, Kodera et al (5) showed that serum CCL18 concentrations in patients with SSc were indicative of the evolution of pulmonary fibrosis, and that serum CCL18 levels better reflected the pulmonary outcome in patients with SSc than did, for instance, the levels of KL-6. In 21 patients with SSc who showed an improvement in their fibrosis activity score on HRCT following immunosuppressive therapy, Kodera et al (5) found decreased serum CCL18 concentrations in parallel with a change in pulmonary fibrotic activity in all but 1 of the patients. Combining these data with our findings, we propose that increasing serum CCL18 concentrations can be used as an indicator of the evolution of fibrotic lung remodeling, and that serum CCL18 concentrations reflect the fibrotic lung activity in patients with pulmonary fibrosis independent of the cause of the disease.

In conclusion, our studies show that the production of CCL18 within the lung is dramatically upregulated in patients with IIPs and in those with SSc, both of which are diseases known to be associated with fibrotic lung tissue remodeling. We demonstrate that there is a close correlation between CCL18 production and other known factors of disease progression in patients with pulmonary fibrosis. Serum CCL18 concentrations are strongly correlated with CCL18 production by BAL cells. In contrast to an approach utilizing BAL neutrophil cell counts, serum CCL18 concentrations are easily determined and repeatable. Furthermore, in contrast to pulmonary function tests, no cooperation of the patient is needed for determinations of CCL18. We believe that repeated measurements of serum CCL18 concentrations can provide additional and valid information. Monitoring serum CCL18 concentrations in patients with rheumatic diseases might reveal the evolution of pulmonary fibrotic remodeling. Furthermore, monitoring serum CCL18 concentrations during the course of the disease and at the time when pulmonary fibrosis is already established might be an extraordinarily useful tool in clinical practice and in studies evaluating new antifibrotic approaches to treatment.

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AUTHOR CONTRIBUTIONS

Dr. Prasse had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Prasse, Pechkovsky, Toews, Müller-Quernheim.

Acquisition of data. Prasse, Pechkovsky, Eggeling, Kollert, Germann, Schäfer, Ludwig, Zissel.

Analysis and interpretation of data. Prasse, Pechkovsky, Toews, Eggeling, Kollert, Zissel, Müller-Quernheim.

Manuscript preparation. Prasse, Toews, Eggeling, Müller-Quernheim.

Statistical analysis. Prasse, Zissel.

Recruitment of study cohort. Prasse, Müller-Quernheim.

REFERENCES

1. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. Am J Respir Crit Care Med 2002;165:277–304.
2. Vassallo R, Thomas CF. Advances in the treatment of rheumatic interstitial lung disease. Curr Opin Rheumatol 2004;3:186–91.
3. Prasse A, Pechkovsky DV, Toews GB, Jungraithmayer W, Kollert F, Goldmann T, et al. A vicious circle of alveolar macrophages and fibroblasts perpetuates pulmonary fibrosis via CCL18. Am J Respir Crit Care Med 2006;173:781–92.
4. Luzina IG, Atamas SP, Wise R, Wigley FM, Xiao HQ, White B. Gene expression in bronchoalveolar lavage cells from scleroderma patients. Am J Respir Cell Mol Biol 2002;26:549–557.
5. Kodera M, Hasegawa M, Komura K, Yanaba K, Takehara K, Sato S. Serum pulmonary and activation-regulated chemokine/CCL18 levels in patients with systemic sclerosis: a sensitive indicator of active pulmonary fibrosis. Arthritis Rheum 2005;52:2889–96.
6. Atamas SP, Luzina IG, Choi J, Tsymbalyuk N, Carbonetti NH, Singh IS, et al. Pulmonary and activation-regulated chemokine stimulates collagen production in lung fibroblasts. Am J Respir Cell Mol Biol 2003;29:743–7.
7. Kodelja V, Muller C, Politz O, Hakij N, Orfanos CE, Goerdt S.

Alternative macrophage activation-associated CC-chemokine-1, a novel structural homologue of macrophage inflammatory protein-1 α with a Th2-associated expression pattern. J Immunol 1998;160:1411–8.
8. Schutyser E, Richmond A, Van Damme J. Involvement of CC chemokine ligand 18 (CCL18) in normal and pathological processes. J Leukoc Biol 2005;78:14–26.
9. Hieshima K, Imai T, Baba M, Shoudai K, Ishizuka K, Nakagawa T, et al. A novel human CC chemokine PARC that is most homologous to macrophage-inflammatory protein-1 α/LD78 α and chemotactic for T lymphocytes, but not for monocytes. J Immunol 1997;159:1140–9.
10. Raghu G, Brown KK, Bradford WZ, Starko K, Noble PW, Schwartz DA, et al, for the Idiopathic Pulmonary Fibrosis Study Group. A placebo-controlled trial of interferon gamma-1b in patients with idiopathic pulmonary fibrosis. N Engl J Med 2004; 350:125–33.
11. White B, Moore WC, Wigley FM, Xiao HQ, Wise RA. Cyclophosphamide is associated with pulmonary function and survival benefit in patients with scleroderma and alveolitis. Ann Intern Med 2000;132:947–54.
12. King TE Jr, Safrin S, Starko KM, Brown KK, Noble PW, Raghu G, et al. Analyses of efficacy end points in a controlled trial of interferon-gamma1b for idiopathic pulmonary fibrosis. Chest 2005;127:171–7.
13. Johnson WC, Raghu G. Clinical trials in idiopathic pulmonary fibrosis: a word of caution concerning choice of outcome measures. Eur Respir J 2005;26:755–8.
14. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581–90.
15. American Thoracic Society. Standardization of spirometry—1987 update: statement of the American Thoracic Society. Am Rev Respir Dis 1987;136:1285–98.
16. Ziegenhagen MW, Zabel P, Zissel G, Schlaak M, Muller-Quernheim J. Serum level of interleukin 8 is elevated in idiopathic pulmonary fibrosis and indicates disease activity. Am J Respir Crit Care Med 1998;157:762–8.
17. Prasse A, Georges CG, Biller H, Hamm H, Matthys H, Luttmann W, et al. Th1 cytokine pattern in sarcoidosis is expressed by bronchoalveolar CD4+ and CD8+ T cells. Clin Exp Immunol 2000;122:241–8.
18. Pardo A, Smith KM, Abrams J, Coffman R, Bustos M, McClanahan TK, et al. CCL18/DC-CK-1/PARC up-regulation in hypersensitivity pneumonitis. J Leukoc Biol 2001;70:610–6.
19. King TE Jr, Tooze JA, Schwarz MI, Brown KR, Cherniack RM. Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. Am J Respir Crit Care Med 2001;164:1171–81.
20. Gay SE, Kazerooni EA, Toews GB, Lynch JP III, Gross BH, Cascade PN, et al. Idiopathic pulmonary fibrosis: predicting response to therapy and survival. Am J Respir Crit Care Med 1998;157:1063–72.
21. Martinez FJ, Safrin S, Weycker D, Starko KM, Bradford WZ, King TE Jr, et al, for the IPF Study Group. The clinical course of patients with idiopathic pulmonary fibrosis. Ann Intern Med 2005;142:963–7.
22. Gross TJ, Hunninghake GW. Idiopathic pulmonary fibrosis. N Engl J Med 2001;345:517–25.
23. Ziegenhagen MW, Schrum S, Zissel G, Zipfel PF, Schlaak M, Muller-Quernheim J. Increased expression of proinflammatory chemokines in bronchoalveolar lavage cells of patients with progressing idiopathic pulmonary fibrosis and sarcoidosis. J Investig Med 1998;46:223–31.
24. Hunninghake GW, Gadek JE, Lawley TJ, Crystal RG. Mechanisms of neutrophil accumulation in the lungs of patients with idiopathic pulmonary fibrosis. J Clin Invest 1981;68:259–69.
25. Carre PC, Mortenson RL, King TE Jr, Noble PW, Sable CL, Riches DW. Increased expression of the interleukin-8 gene by alveolar macrophages in idiopathic pulmonary fibrosis: a potential mechanism for the recruitment and activation of neutrophils in lung fibrosis. J Clin Invest 1991;88:1802–10.
26. Wells AU, Hansell DM, Haslam PL, Rubens MB, Cailes J, Black CM, et al. Bronchoalveolar lavage cellularity: lone cryptogenic fibrosing alveolitis compared with the fibrosing alveolitis of systemic sclerosis. Am J Respir Crit Care Med 1998;157:1474–82.
27. Fulmer JD, Roberts WC, von Gal ER, Crystal RG. Morphologic-physiologic correlates of the severity of fibrosis and degree of cellularity in idiopathic pulmonary fibrosis. J Clin Invest 1979;63: 665–76.
28. Schraufstatter I, Takamori H, Sikora L, Sriramarao P, DiScipio RG. Eosinophils and monocytes produce pulmonary and activation-regulated chemokine, which activates cultured monocytes/macrophages. Am J Physiol Lung Cell Mol Physiol 2004;286: L494–501.
29. Adema GJ, Hartgers F, Verstraten R, de Vries E, Marland G, Menon S, et al. A dendritic-cell-derived C-C chemokine that preferentially attracts naive T cells. Nature 1997;387:713–7.
30. Gunther C, Bello-Fernandez C, Kopp T, Kund J, Carballido-Perrig N, Hinteregger S, et al. CCL18 is expressed in atopic dermatitis and mediates skin homing of human memory T cells. J Immunol 2005;174:1723–8.
31. Boot RG, Verhoek M, de Fost M, Hollak CE, Maas M, Bleijlevens B, et al. Marked elevation of the chemokine CCL18/PARC in Gaucher disease: a novel surrogate marker for assessing therapeutic intervention. Blood 2004;103:33–9.
32. Kirk JM, Bateman ED, Haslam PL, Laurent GJ, Turner-Warwick M. Serum type III procollagen peptide concentration in cryptogenic fibrosing alveolitis and its clinical relevance. Thorax 1984; 39:726–32.
33. Allanore Y, Borderie D, Lemarechal H, Cherruau B, Ekindjian OG, Kahan A. Correlation of serum collagen I carboxyterminal telopeptide concentrations with cutaneous and pulmonary involvement in systemic sclerosis. J Rheumatol 2003;30:68–73.