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Clinical significance of anti-GM-CSF antibodies in idiopathic pulmonary alveolar proteinosis

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Abstract

Background: To serve as a diagnostic marker, the role of anti-GM-CSF (granulocyte-macrophage colony-stimulating factor) antibodies involved in idiopathic pulmonary alveolar proteinosis (iPAP) remains unclear.

Methods: Anti-GM-CSF antibodies were detected in blood and bronchoalveolar lavage fluid (BALF) in 13 iPAP patients. To serve as controls, 3 patients with secondary PAP, 35 patients with other pulmonary disorders and 10 subjects without lung lesions were studied. Blood samples only were obtained from 30 healthy medical personnel. Anti-GM-CSF antibodies were detected using immunoblotting and measured semiquantitatively by serial dilution or concentration methods. The relation between antibodies and reported severity indicators for iPAP was analyzed.

Results: Anti-GM-CSF antibodies could be detected in both blood and BALF samples in 12 of 13 iPAP patients. Anti-GM-CSF antibodies were undetectable in blood and/or BALF from other subjects studied. BALF levels of anti-GM-CSF antibodies highly correlated with the data of severity indicators for iPAP, including serum lactate dehydrogenase, arterial oxygen tension, alveolar-arterial oxygen tension difference ($AaPO_2$), diffusing capacity of the lung, and some lesion scores on chest radiograms and computed tomograms. In contrast, blood anti-GM-CSF antibodies did not significantly correlate with the severity indicators evaluated. Additionally, iPAP patients who required subsequent therapeutic lung lavage had significantly higher values of serum LDH, $AaPO_2$, and BALF anti-GM-CSF antibodies, and significantly lower values of PaO_2.

Conclusions: Like serum LDH, PaO_2 and $AaPO_2$, BALF levels of anti-GM-CSF antibodies might reflect the disease severity and predict the need of subsequent therapeutic lung lavage. The findings may expand the role of anti-GM-CSF antibodies in iPAP.

Key words: Anti-granulocyte-macrophage colony-stimulating factor antibodies; Granulocyte-macrophage colony-stimulating factor; Lactate dehydrogenase; Pulmonary alveolar proteinosis; Pulmonary function testing

INTRODUCTION

Pulmonary alveolar proteinosis (PAP) is a rare disease characterized by accumulation of surfactant within the alveoli and terminal airways. Two types of PAP can be recognized: congenital and acquired, while acquired PAP can be divided further into idiopathic and secondary forms. Clinically, more than 90% of PAP patients occurred with unknown etiology, namely idiopathic PAP (iPAP).[1][2]

Macrophage dysfunction has been thought to be responsible for the surfactant accumulation in iPAP, however, the exact etiology and pathogenesis remain obscure.[1][2][3] In 1994, the investigations on experimental hematology unexpectedly established a link between granulocyte-macrophage colony-stimulating factor (GM-CSF) and surfactant homeostasis.[4][5] Without changes in basal hematopoiesis, mice deficient in either GM-CSF or its receptor developed pulmonary lesions histologically similar to PAP.[4][5][6] Further studies demonstrated that local availability of GM-CSF was sufficient to correct surfactant accumulation in GM-CSF knockout mice, either by aerosolized administration[7] or selective expression of GM-CSF in respiratory epithelial cells.[8] Subsequently, exogenous GM-CSF treatment was applied to iPAP patients with a response rate of 40-50%.[1][2] [9][10][11]

Unlike animal model, the defects in gene expression of GM-CSF or its receptor have not yet been found in human iPAP.[1] [12][13] There were no intrinsic cellular defects in synthesizing and secreting GM-CSF,[14] however, the bioavailability of GM-CSF appeared to be blocked by neutralizing antibodies.[14][15] High levels of neutralizing antibodies against GM-CSF could be detected in blood and bronchoalveolar lavage fluid (BALF) of iPAP patients but not in those of patients with congenital or secondary PAP, patients with other pulmonary diseases, or in healthy subjects, suggesting that iPAP may be an autoimmune disease.[1][2][3] [15] By blocking the binding of GM-CSF to its receptor, and thereby inhibiting the differentiation and function of macrophages, anti-GM-CSF antibodies are considered to be a causal factor of iPAP.[16] Nevertheless, the clinical significance of anti-GM-CSF antibodies in iPAP remains obscure as evidenced by mixed results of anti-GM-CSF antibodies in predicting treatment response to GM-CSF and lack of correlation between blood anti-GM-CSF antibodies and reported severity markers for iPAP.[11] [17] An increase of arterial oxygen tension (PaO_2) accompanied by a decrease of anti-GM-CSF antibodies in blood and/or bronchoalveolar lavage fluid (BALF) was reported recently in 3 iPAP patients after inhaled GM-CSF treatment.[18] Based on these findings, the clinical relevance of anti-GM-CSF antibodies in iPAP deserves further studies to elucidate. In this study, we measured anti-GM-CSF antibodies in paired specimens of blood and BALF in iPAP patients and investigated their relation to severity markers for iPAP in usual practice, including serum lactate dehydrogenase (LDH), pulmonary function parameters, arterial blood gases, and radiological findings.[1][2][3] [11] [19]

METHODS

Patients with iPAP

The Institutional Review Board of Taipei Veterans General Hospital approved the study, and informed consent was obtained from the patients for the study of blood and BALF samples. Thirteen consecutive iPAP patients diagnosed by cytological examination of BALF[20] and pathologic examination of the lung tissues at Taipei Veterans General Hospital were enrolled in this study. The etiologies reported to be associated with secondary PAP were not found in these patients. All patients studied are alive at the time of manuscript preparation. One of 13 iPAP patients had diabetes mellitus and hypertension treated with hypoglycemic and anti-hypertensive agents.

Others denied major medical illnesses.

All iPAP patients were followed up for more than one year except for the one diagnosed in March 2005. The disease improved spontaneously in three, and remained stationary in five patients. Therapeutic lung lavage was required in the remaining five patients. The criteria for therapeutic lung lavage in this study were dyspnea with restriction of daily activity and/or occupation or PaO_2 < 60 mm Hg at room air. Nearly complete resolution was observed in one patient after 3 cycles of therapeutic whole lung lavage. Repeated therapeutic lung lavage was necessary for another one patient. Because of poor response and intolerance to therapeutic whole lung lavage, therapeutic bronchoscopic lobar/segmental lavage was adopted subsequently in this patient.[21] The remaining three received one cycle of bronchoscopic lobar/segmental lavage with remarkable improvement as evidenced by follow-up imaging studies and pulmonary function testing. None of our patients received GM-CSF therapy.

Serum LDH, pulmonary function testing, chest radiography, and high-resolution computed tomography (HRCT) of chest were performed prior to diagnostic bronchoalveolar lavage (BAL) at an interval of less than 3 days. Only BALF obtained from diagnostic BAL at the time of initial diagnosis were included for analysis.

Pulmonary function testing

Pulmonary function testing including spirometry, plethysmography, diffusing capacity of the lung for carbon monoxide (DLco), and analyses of arterial blood gases were performed in all patients. Blood samples for pH, PaO_2 and $PaCO_2$ (arterial CO_2 tension) values were analyzed at room air, using ABL III (Radiometer, Copenhagen, Denmark). PAO_2 (alveolar oxygen tension) is calculated by the following equation. PAO_2 = (barometric pressure - 47) X FiO_2 - $PaCO_2$/R. R, an exchange ratio, is assumed as 0.8 in this study. The alveolar-arterial PO_2 difference ($AaPO_2$) is calculated by subtracting PaO_2 from PAO_2.

Image scoring

Frontal chest radiographs and HRCT of chest were graded according to the visual scoring system proposed by Lee et al.[19] Scores were determined for degree, extent and severity of lung opacity. The opacity score was evaluated by means of a three-point scale as follows: mild ground-glass opacity = 1; moderate ground-glass opacity = 2; consolidation opacity = 3. The “ground-glass opacity” refers to the presence of increased lung opacity associated with partial obscuration of normal vascular structures. The extent of lung opacity was estimated by means of four-point scale: opacity involving < 25% of hemithorax =1; $\geq$ 25 to < 50% = 2; $\geq$ 50 to < 75% = 3; $\geq$ 75% = 4. The severity score was calculated by multiplying the opacity and extent scores. The final values for these scores were the sum of each hemithorax. The chest radiograms were read and interpreted independently by two chest physicians (S-C C and F-C L) who were blind to the clinical data and results of pulmonary function testing. The mean values obtained from the two readers were used for analysis.

The images of HRCT were photographed at window levels appropriately for demonstrating the pulmonary parenchyma, and were read and interpreted by two chest physicians (S-C C and F-C L) and one chest radiologist (M-S C) in a blind fashion. Every individual scan was evaluated for the degree, extent and severity of lung opacity, and the extent of reticulation. The degree and extent of opacity were scored using the same scales as described above. The severity score was calculated by multiplying the opacity and extent scores. The extent of reticulation was measured as the extent of lung opacity. The score for each lung was the average of all HRCT slices, and the sum of each

hemithorax became the final. The mean values obtained from the three readers were used for analysis.

BALF and blood

The fiberoptic bronchoscope (model BF 20 or P20; Olympus, Tokyo, Japan) was wedged into the orifice of lobar or segmental bronchus of the right middle or lingular division, or other appropriate location. Diagnostic BAL was performed prior to transbronchial lung biopsy, using 3 aliquots of a 50-mL sterile isotonic sodium chloride solution. The aspirates were pooled into a siliconized container and kept on ice during transport. Ten-ml venous blood was collected immediately after BAL. The supernatants of blood and BALF were frozen and stored at -70º C until analyzed.

Measurement of GM-CSF

The levels of GM-CSF in blood and BALF were measured by commercially available enzyme-linked immunosorbent assay (ELISA) kit (R&D system, Minneapolis, MN, USA), with the lower limit 3 pg/mL.

Measurement of anti-GM-CSF antibodies

The levels of anti-GM-CSF antibodies were detected and measured by immunoblotting. In brief, human recombinant GM-CSF (*E.coli* derived; molecular mass of 14 kD; Calbiochem, Affiliate of Merck KGaA, Darmstadt, Germany) was subjected to 8% polyacrylamide gel electrophoresis under reducing condition, with 0.1μg rhGM-CSF loaded in each lane. Then the proteins were transferred to polyvinylidene fluoride (PVDF) membrane by diffusion blotting.[22] With appropriate blocking, incubation and washes, horseradish peroxidase-conjugated anti-human IgG antibodies (Jackson Immuno Research Lab Inc. Pa, USA) was added and the immunoreactive bands were developed by 3.3-diaminobenzidine, H_2O_2 and $NiCl_2$ enhancement method.

For the blood and BALF samples with positive results, the concentrations of anti-GM-CSF antibodies were determined semiquantitatively by serial dilution. In case of negative results, the BALF samples were concentrated for further measurement.

Control groups

To serve as diseased controls, paired specimens of blood and BALF were obtained from 3 patients with secondary PAP and 35 patients with other pulmonary diseases, including collagen vascular diseases in 12, cytomegalovirus pneumonitis in 12, idiopathic pulmonary fibrosis in 6, and sarcoidosis in 5 patients. The underlying diseases of secondary PAP were acute myeloid leukemia in 2 and renal transplantation in one case. Blood and BALF were obtained from 10 patients without pulmonary lesions to serve as lung controls. Peripheral blood only was obtained from 30 healthy hospital personnel to serve as normal controls.

Statistical analysis

Data were expressed as median and range. Paired data comparisons were performed using a Wilcoxon signed-rank test. Specific comparisons of data between two groups were made using the Mann-Whitney U test. The correlations between variables were determined by Spearman rank correlation coefficients. Significance was defined as $p < 0.05$. Statistical analysis was done using SPSS version 10 (SPSS, Chicago, IL, USA).

RESULTS

From 1995 January to 2005 March, 13 consecutive patients of iPAP with varying

degrees of severity were enrolled in this study. The demographical characteristics and clinical data are summarized in Table 1.

Correlations between severity markers for iPAP

The relations between the severity indicators for iPAP, including serum LDH, PaO_2 and $AaPO_2$, results of pulmonary function testing, and image scores are given in Table 2. By and large, iPAP patients had varying degrees of restrictive ventilatory defect. Gas exchange was impaired, as evidenced by a reduction of DLco or PaO_2, or a widening of $AaPO_2$. The levels of serum LDH were abnormally elevated in 11 of 13 cases. The values of PaO_2 and $AaPO_2$ correlated significantly with those of serum LDH ($r = -0.69$, $p = 0.010$, $r = 0.60$, $p = 0.029$, respectively). The data of ventilatory function did not significantly correlated with those of serum LDH, PaO_2 and $AaPO_2$, however, a negative correlation was found between the values of DLco and $AaPO_2$.

The common findings of iPAP on chest radiographs were bilateral air-space ground-glass opacity and/or consolidation, with relatively symmetrical distribution. The severity score on chest radiographs was highly correlated with serum LDH, PaO_2, and $AaPO_2$ (Table 2). The extent score on chest radiographs was highly correlated with serum LDH and $AaPO_2$. A negative correlation was found between total lung capacity (TLC) and all lesion scores (opacity score, $r = -0.65$, $p = 0.017$; extent score, $r = -0.64$, $p = 0.020$; severity score, $r = -0.69$, $p = 0.009$) on chest radiographs. The severity score on chest radiographs correlated negatively ($r = -0.55$, $p = 0.049$) with forced vital capacity (FVC). However, the data of DLco did not highly correlated with lesion scores on chest radiographs.

Characteristic findings of iPAP on thoracic HRCT revealed patchy ground-glass opacity with varying degrees of intra- and inter-lobular septal thickening. The extent, severity and reticulation scores of HRCT were highly correlated with those of serum LDH, PaO_2 and $AaPO_2$ (Table 2). All lesion scores on HRCT were highly correlated with TLC (opacity score, $r = -0.82$, $p = 0.001$; extent score, $r = -0.69$, $p = 0.009$; severity score, $r = -0.77$, $p = 0.002$; reticulation score, $r = -0.84$, $p < 0.001$). The severity and reticulation scores on HRCT were negatively correlated with FVC ($r = -0.62$, $p = 0.025$ and $r = -0.57$, $p = 0.042$, respectively). Similarly, the lesion scores on HRCT did not highly correlate with the data of DLco.

GM-CSF in iPAP patients and control groups

In iPAP patients, there was no significant difference in blood and BALF levels of GM-CSF. Compared with those of secondary PAP, iPAP patients had significantly lower blood and BALF levels of GM-CSF ($p = 0.025$, respectively)(fig 1). The blood levels of GM-CSF were significantly higher in iPAP patients than in normal controls, lung controls and in patients with CMV pneumonitis. In contrast, there was no significant difference in BALF levels of GM-CSF among the patients with iPAP, those with other pulmonary disease and lung controls.

Anti-GM-CSF antibodies in iPAP patients and control groups

Anti-GM-CSF antibodies could be detected in both blood and BALF samples in 12 of 13 iPAP patients. By contrast, anti-GM-CSF antibodies could not be detected in blood or BALF samples obtained from disease controls, lung controls and normal controls. Accordingly, the sensitivity and specificity of anti-GM-CSF antibodies determined by immunoblotting in aiding a diagnosing iPAP were 92% and 100%, respectively.

For samples with positive results, the levels of anti-GM-CSF antibodies were determined semi-quantitatively by serial dilution. In case of negative results, BALF was

concentrated for further evaluation. For the subjects with undetectable antibodies, the levels of blood and BALF antibodies were defined arbitrarily as 1:1 (1) and 50:1 (0.02), respectively. The distribution of anti-GM-CSF antibodies in iPAP patients and various control groups were given in fig 2.

Correlations between anti-GM-CSF antibodies and severity markers for iPAP

The relationship between anti-GM-CSF antibodies and severity markers for iPAP was analyzed and shown in Table 3. The levels of blood anti-GM-CSF antibodies did not correlate with those of clinical parameters evaluated. By contrast, the values of BALF anti-GM-CSF antibodies were significantly correlated with those of serum LDH, PaO_2, $AaPO_2$, DLco, opacity score on chest radiographs, and extent and reticulation scores on HRCT. In terms of evaluating the disease severity of iPAP, the local or BALF anti-GM-CSF antibodies were superior to the circulating ones.

The levels of anti-GM-CSF antibodies and the need of therapeutic lung lavage

To further explore the clinical significance of anti-GM-CSF antibodies in iPAP, 13 patients were divided into subgroups based on the need of subsequent therapeutic lung lavage. The patients who required therapeutic lung lavage tended to have higher values of serum LDH, $AaPO_2$, and HRCT scores, and lower values of PaO_2, forced expiratory volume in one second (FEV1), FVC, TLC, and DLco (Table 4). The differences in serum LDH, PaO_2, $AaPO_2$, and extent score and reticulation score of HRCT were statistically significant. Furthermore, these patients had significantly higher titers of anti-GM-CSF antibodies in BALF at the time of diagnosis of iPAP.

Serial changes of anti-GM-CSF antibodies in two iPAP patients with different clinical course

Serial changes of anti-GM-CSF antibodies and severity markers for iPAP in two patients are shown in Figure 3. In the patient with spontaneous improvement, the improvement of serum LDH, $AaPO_2$ and PaO_2 were found in association with a decrease of anti-GM-CSF antibodies in blood and BALF. In the patient who required repeated therapeutic lung lavage, the changes of BALF and blood anti-GM-CSF antibodies measured before each cycle of therapeutic lung lavage were relatively parallel to those of severity markers.

Discussion

This was the first study to demonstrate a relation between BALF levels of anti-GM-CSF antibodies and severity indicators for iPAP, including serum LDH, DLco, PaO_2, $AaPO_2$, and some lesion scores on chest radiographs and HRCT. In terms of evaluating the severity of iPAP, BALF anti-GM-CSF antibodies were superior to the circulating ones. BALF levels of anti-GM-CSF antibodies might predict the need of therapeutic lung lavage in iPAP patients. In addition, the present study supported that the presence of anti-GM-CSF antibodies in blood and/or BALF could serve as a useful diagnostic marker for iPAP. These findings may expand the roles of anti-GM-CSF antibodies in iPAP.

Several studies indicated that circulating anti-GM-CSF antibodies could serve as a serological marker for a diagnosis of iPAP, and iPAP is regarded as an autoimmune disease accordingly.[1][2][3] [15] [23][24] The present study indicated that, using immunoblotting, anti-GM-CSF antibodies could be detected in both blood and BALF in 12 of 13 patients with iPAP. Furthermore, anti-GM-CSF antibodies could not be detected in blood and/or BALF in patients with secondary PAP and other pulmonary disease and in subjects without lung diseases (lung controls) and normal controls. These findings

were consistent with previous reports[23][24] that anti-GM-CSF antibodies were highly specific for iPAP. However, one of our patients who had undetectable anti-GM-CSF antibodies detected by immunoblotting in both blood and BALF could not fit into this pathophysiological group. Similarly, no significant increase of anti-GM-CSF antibodies was reported in two of seven patients with iPAP in a recent study.[25] The reasons why anti-GM-CSF antibodies could not be detected or not significantly increase in blood and/or BALF in some patients with iPAP remain unknown. There are several possibilities. First, the methods used to detect anti-GM-CSF antibodies are not sensitive enough. Second, iPAP may be a syndrome rather than a disease. Finally, as in collagen vascular diseases, autoantibody specific for a given collagen vascular disease is not exclusively present in the patients. Further studies are required to clarify these issues.

To further verify the accuracy of immunoblotting, in-house ELISA kit was performed as described by Schoch et al[26] with some modification. By and large, a good correlation was observed between the levels of anti-GM-CSF antibodies measured by immunoblotting and ELISA methods in both blood ($r = 0.77$, $p = 0.002$) and BALF ($r = 0.70$, $p = 0.008$). Compared with those of the control groups, significantly higher levels of anti-GM-CSF antibodies were detected in blood and BALF samples in 12 of 13 patents with iPAP. The lowest values were found in the one patient who had undetectable anti-GM-CSF antibodies in both blood and BALF using immunoblotting. Further details are provided in an online supplement.

Except for serving as a diagnostic marker for iPAP, the roles of anti-GM-CSF antibodies involved in etiology and pathogenesis, monitoring disease activity, reflecting the disease severity, predicting and/or monitoring the treatment response, and in predicting outcome of the patients remain unknown. Previous study reported that the circulating anti-GM-CSF antibodies did not significantly correlate with the severity indicators for iPAP.[11] In agreement with the previous report,[11] the present study indicated that blood levels of anti-GM-CSF antibodies did not highly correlate with the data of $AaPO_2$, PaO_2, serum LDH and lesion scores on chest radiographs and HRCT (Table 3). By contrast, the BALF values of anti-GM-CSF antibodies were significantly correlated with those of serum LDH, PaO_2, $AaPO_2$, DLco, and some lesion scores on chest radiographs and HRCT. Accordingly, BALF levels of anti-GM-CSF antibodies were better than the circulating ones in reflecting the disease severity of iPAP.

To further explore the clinical values of anti-GM-CSF antibodies in iPAP, the patients were divided into subgroups based on the need of therapeutic lung lavage (Table 4). Significantly higher levels of serum LDH, $AaPO_2$ and BALF anti-GM-CSF antibodies, lower value of PaO_2, and more severe lung lesions on chest CT were found in iPAP patients who required therapeutic lung lavage. As a result, the BALF levels of anti-GM-CSF antibodies measured at the time of diagnosis may be of value in predicting the need of subsequent therapeutic lung lavage in iPAP patients.

There are some limitations in this study. The cases studied were limited because iPAP is a rare disease. The power of the test will increase up to ≥ 0.8 to detect a significant difference if we double the case number. However, the results can be of clinical relevance since all studied patients came from the same institute, and all samples were collected and processed with the same protocol. These may decrease or avoid the inherent bias caused by different methods used in collecting and/or processing the samples as seen in multi-center studies. Further studies with larger population are needed to verify these issues.

In summary, the current study supported that the presence of anti-GM-CSF antibodies in blood or BALF can serve as a diagnostic marker for iPAP. Furthermore, BALF anti-GM-CSF antibodies might reflect the disease severity and predict the need of

subsequent therapeutic lung lavage in patients with iPAP. These findings may expand the role of anti-GM-CSF antibodies in iPAP.

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Table 1. Clinical Characteristics of Patients with Idiopathic Pulmonary Alveolar Proteinosis

Case	Age at Dx. (yrs)	Sex (M/F)	Serum LDH (IU/L)*	PaO_2 (mm Hg)†	$AaPO_2$ (mm Hg)†	Length of F-U (yrs)	Clinical course	Status at last F-U
1	53	M	239	81.0	25.6	4	Stationary	Symptom free
2	40	F	389	47.9	58.6	8	TLL at Dx.	O_2 Tx, repeated TLL
3	40	M	269	84.4	11.5	5	Stationary	Symptom free
4	31	M	292	72.7	24.9	4	TLL at Dx.	Symptom free
5	16	M	280	65.7	38.9	3	SI	Symptom free
6	18	F	289	80.7	25.7	6	SI	Symptom free
7	54	F	242	80.2	21.2	6	Stationary	Mild DOE
8	36	F	175	82.1	23.0	3	Stationary	Symptom free
9	59	F	265	60.7	44.9	4	TLL 2 yrs after Dx.	Symptom free
10	33	M	191	75.6	26.4	6	SI	Symptom free
11	34	M	243	70.3	31.1	2	Stationary	Mild DOE
12	45	M	389	49.0	60.2	1	TLL at Dx.	Symptom free
13	42	M	392	39.0	71.3	0.5	TLL at Dx.	Symptom free

*LDH = lactate dehydrogenase, reference value: 95-213 IU/L.

†PaO_2 and $AaPO_2$ were measured in sitting position at room air.

$AaPO_2 = PAO_2 – PaO_2$; PAO_2 = (barometric pressure – 47) X $FiO_2 – PaCO_2/0.8$.

Dx. = diagnosis; F-U = follow-up; TLL = therapeutic lung lavage; O_2 Tx. = oxygen treatment; SI = spontaneous improvement; DOE = dyspnea on exertion

Table 2. Relation between Clinical, Radiological and Physiological Parameters in Idiopathic Pulmonary Alveolar Proteinosis (iPAP) Patients

Variables	LDH		PaO_2*		$AaPO_2$*	
	Correlation Coefficient	P value	Correlation Coefficient	P value	Correlation Coefficient	P value
FEV1, % pred.	-0.10	0.775	0.24	0.426	-0.38	0.197
FVC, % pred.	-0.17	0.571	0.35	0.247	-0.47	0.108
TLC, % of pred.	-0.39	0.190	0.51	0.078	-0.51	0.076
DLco, % of pred.	-0.51	0.076	0.36	0.231	-0.61	0.027
Radiograms						
Opacity score	0.41	0.169	-0.59	0.033	0.53	0.062
Extent score	0.56	0.045	-0.48	0.096	0.59	0.035
Severity score	0.56	0.045	-0.57	0.040	0.60	0.033
Chest HRCT						
Opacity score	0.54	0.056	-0.51	0.072	0.36	0.232
Extent score	0.77	0.002	-0.57	0.042	0.64	0.019
Severity score	0.56	0.045	-0.60	0.032	0.60	0.029
Reticulation score	0.75	0.003	-0.57	0.042	0.56	0.047

*PaO_2 and $AaPO_2$ were measured in the sitting position at room air.
$AaPO_2 = PAO_2 - PaO_2$, $PAO_2 = (\text{barometric pressure} - 47) \times FiO_2 - PaCO_2/0.8$.
LDH = lactate dehydrogenase; FEV1 = forced expiratory volume in one second; FVC = forced vital capacity; TLC = total lung capacity; DLco = diffusing capacity of lungs for carbon monoxide; HRCT = high resolution computed tomography

Table 3. Relation between Anti-GM-CSF Antibodies and Severity Indicators for iPAP

Variables	Anti-GM-CSF Antibodies in Blood		Anti-GM-CSF Antibodies in BALF	
	Correlation Coefficient	P value	Correlation Coefficient	P value
Serum LDH, IU/L	0.04	0.906	0.58	0.039
PaO_2, mm Hg*	-0.27	0.376	-0.69	0.009
$AaPO_2$, mm Hg*	0.38	0.201	0.77	0.002
FEV1, % pred.	-0.15	0.629	- 0.11	0.738
FVC, % pred.	-0.21	0.498	-0.22	0.471
TLC, % of pred.	- 0.02	0.949	-0.44	0.133
DLco, % of pred.	-0.06	0.856	-0.62	0.024
Chest radiograms				
Opacity score	0.32	0.283	0.65	0.016
Extent score	0.17	0.578	0.42	0.158
Severity score	0.12	0.678	0.50	0.080
Chest HRCT				
Opacity score	0.08	0.805	0.35	0.242
Extent score	0.01	0.978	0.74	0.004
Severity score	0.07	0.812	0.48	0.100
Reticulation score	-0.04	0.898	0.58	0.038

*PaO_2 and $AaPO_2$ were measured in the sitting position at room air.
$AaPO_2 = PAO_2 – PaO_2$, $PAO_2 = (\text{barometric pressure} – 47) \times FiO_2 – PaCO_2/0.8$.
Anti-GM-CSF antibodies = anti-granulocyte-macrophage colony-stimulating factor antibodies; BALF = bronchoalveolar lavage fluid; LDH = lactate dehydrogenase; FEV_1 = forced expiratory volume in one second; FVC = forced vital capacity; TLC = total lung capacity; DLco = diffusing capacity of the lungs for carbon monoxide; HRCT = high resolution computed tomography

Table 4. Comparisons Between iPAP Patients Divided into Groups Based on Therapeutic Lung Lavage

Variables*	Therapeutic Lung Lavage		P value
	Yes (N = 5)	No (N = 8)	
LDH, IU/L	389 (265 - 392)	243 (175 - 289)	0.011
PaO_2, mm Hg†	49.0 (39.0 - 72.7)	80.7 (65.7 - 84.4)	0.006
A-aPO_2, mm Hg†	58.6 (24.9 - 71.3)	25.7 (11.5 - 38.9)	0.030
FEV1, % of pred.	67 (61 - 77)	83 (32 - 89)	0.222
FVC, % of pred.	67 (55 - 75)	82 (47 - 96)	0.171
TLC, % of pred.	74 (60 - 75)	89 (68 - 95)	0.093
DLco, % of pred.	29 (23 - 62)	51 (33 - 88)	0.093
Anti-GM-CSF Ab in blood			
Immunoblotting (dilution)	1000 (200 - 2000)	1600 (1 - 4000)	0.622
Anti-GM-CSF Ab in BALF			
Immunoblotting (dilution)	32 (2 - 64)	4 (0.02 - 8)	0.045
Chest radiograms			
Opacity score	4 (2 - 6)	4 (2 - 4)	0.171
Extent score	6 (2 - 8)	4 (2 - 6)	0.284
Severity score	11 (5 - 18)	8 (4 - 10)	0.127
Chest HRCT			
Opacity score	4 (2 - 5)	3 (2 - 6)	0.284
Extent score	7 (5 - 8)	5 (2 - 6)	0.030
Severity score	15 (6 - 22)	8 (2 - 12)	0.093
Reticulation score	6 (4 - 7)	4 (3 - 6)	0.045

* Values of median and range (in parenthesis) are given.

†PaO_2 and $AaPO_2$ were measured in the sitting position at room air.

$AaPO_2 = PAO_2 - PaO_2$; PAO_2 = (barometric pressure – 47) X $FiO_2 - PaCO_2/0.8$.

iPAP = idiopathic pulmonary alveolar proteinosis; LDH = lactate dehydrogenase; FEV_1 = forced expiratory volume in one second; FVC = forced vital capacity; TLC = total lung capacity; DLco = diffusing capacity of lungs; GM-CSF = granulocyte-macrophage colony-stimulating factor; BALF = bronchoalveolar lavage fluid; anti-GM-CSF Ab = anti-granulocyte-macrophage colony-stimulating factor antibodies; HRCT = high resolution computed tomography

Figure legends:

Figure 1:

Box and whisker plots for the levels of GM-CSF in blood (A) and BALF (B) are given. The box represents the interquartile range that contains 50% of values. The whiskers are lines that extend from the box to the highest and lowest values.
GM-CSF = granulocyte-macrophage colony-stimulating factor; BALF = bronchoalveolar lavage fluid; iPAP = idiopathic pulmonary alveolar proteinosis; IPF = idiopathic pulmonary fibrosis; CVD = collagen vascular disease; CMV = cytomegalovirus pneumonitis; NC = normal controls

Figure 2:

Anti-GM-CSF antibodies measured by immunoblotting in blood (A) and BALF (B) are shown. The values of median and range are given in iPAP patients.
Anti-GM-CSF antibodies = anti-granulocyte-macrophage colony-stimulating factor antibodies; BALF = bronchoalveolar lavage fluid; iPAP = idiopathic pulmonary alveolar proteinosis; LC = lung controls; IPF = idiopathic pulmonary fibrosis; CVD = collagen vascular disease; CMV = cytomegalovirus pneumonitis; NC = normal controls

Figure 3:

Serial data of anti-GM-CSF in blood and BALF and severity indicators are given in 2 iPAP patients with different clinical course, including (A) a patient improved spontaneously and (B) a patient required repeated therapeutic lung lavage. In figure B, all data were obtained before each cycle of therapeutic lung lavage.
*Treated by therapeutic whole lung lavage.
** Treated by therapeutic bronchoscopic lobar/segmental lavage
Anti-GM-CSF antibodies = anti-granulocyte-macrophage colony-stimulating factor antibodies; BALF = bronchoalveolar lavage fluid; iPAP = idiopathic pulmonary alveolar proteinosis