# Autoantibodies to IL-1α in sera from rapidly progressive idiopathic pulmonary fibrosis

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**Abstract: To clarify the clinical significance of autoantibodies to interleukin-1α (IL-1α autoantibodies) in rapidly progressive idiopathic pulmonary fibrosis ( IPF ), we measured the level of IL-1α autoantibodies in serum of 11 patients on the first hospital day, when patients were admitted due to severe symptoms, and on the 21st hospital day. IL-1α autoantibodies in serum were measured using radioimmunoassay, and the limitation of this assay for IL-1α autoantibodies was 5 ng/ml. These antibodies were detected in 5 of 11 patients on the first hospital day. On the 21st hospital day, these antibodies were detected in all patients, and its level was increased compared with that on the first hospital day. IL-1α autoantibodies that appeared in patients corresponded to that of IgG. The half life of exogeneous autoantibodies was investigated following administration of autoantibody rich plasma obtained from healthy blood donors to 6 control patients (CP) and 6 progressive IPF patients. These autoantibody levels in their serum were less than 5 ng/ml before administration. Serum was obtained at the indicated time after administration of IL-1α autoantibodies and the level of these autoantibodies in serum was measured, then the half life was calculated. Half life of exogeneous IL-1α autoantibodies in progressive IPF patients was significantly shorter than that in CP (71.3±31.8 hr vs 352.0±98.3 hr, $p<0.01$). These findings suggested that IL-1α autoantibodies were generated in response to the inflammatory process of rapidly progressive IPF and may act as a regulatory factor for IL-1α. J. Med. Invest. 48 : 181-189, 2001**

***Keywords*** : *autoantibodies, IL-1 α, idiopathic pulmonary fibrosis, half life, radioimmunoassay*

## INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is an interstitial lung disease characterized by the accumulation of inflammatory cells in the air spaces, and fibrosis of alveolar walls and the interstitium (1, 2). Despite a worldwide effort, the pathogenesis of IPF is still unclear. The prognosis in IPF is poor, and there is currently no fundamental treatment for the disease (1, 3). The clinical course of IPF is not uniform among patients with the disease (3-5). Although in most patients, the diseases are of slow progression on a yearly basis, it is, in occasional patients, in a phase of subacute progression on a weekly basis (3-5). We have also observed that some patients whose clinical course is chronically progressive suddenly

Abbreviations : IL-1 ; interleukin-1, IL-1ra ; IL-1receptor antagonist, BSA ; bovine serum albumin, PBS ; phosphate buffered saline, EDTA ; ethylenediaminotetraacetic acid, FFP ; fresh frozen plasma

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show a rapid clinical deterioration (6). Such exacerbation often proves fatal.

Interleukin-1 (IL-1) exists in two genomic forms, IL-1 α and IL-1 β, which share many biologic activities (7-11). Although the precursor for IL-1 β requires cleavage to reveal its IL-1α activity, the proIL-1α is fully active as a precursor and remains intracellular (7-9). IL-1 is recognized as the essential mediators of inflammatory reactions (7-9), and is also suspected of being implicated in fibrogenic processes, since it can increase both the growth of fibroblasts and the collagen secretion rate (12, 13). Therefore, IL-1 is thought to play an important role in the pathogenesis of interstitial pneumonia and fibrosis (14-16). However, the regulated mechanism neutralizing IL-1 α activity *in vivo* is known to exist in interstitial lung diseases, including IPF. Indeed, it was reported that IL-1ra, which neutralizes IL-1α activity, was increased in interstitial lung diseases, and the IL-1/IL-1ra ratio was thought to be important when investigating these diseases (14, 15, 17). However, it is also thought important to investigate the mechanism of IL-1α regulation in IPF patients, because alveolar macrophages, which are key cells in the pathogenesis of IPF, contained much IL-1 α which has an active precursor form (8, 18).

Autoantibodies to several cytokines, including IL-2 (19), TNF-α (20), IFN-γ (21), IL-6 (22), MCP-1 (23), IL-8 (24) and IL-10 (25) have recently been identified in normal human plasma. Autoantibodies to IL-1α (IL-1α autoantibodies) were also found in sera of patients with inflammatory process, such as rheumatoid arthritis and juvenile chronic arthritis, in addition to asymptomatic subjects (26-34). It is known that IL-1 α autoantibodies neutralize IL-1 α activity specifically *in vitro* (29-31, 35), while IL-1ra blocks the activity of both IL-1α and IL-1β (7-8). IL-1 α autoantibodies in healthy subjects are known to be mostly of the IgG ( IgG1 and IgG4) and these antibodies have remarkably high binding affinities to IL-1α ($Kd=10^{-9}$-$10^{-11}$M) (28, 31, 36, 37). Therefore, it is conceivable that these antibodies play a regulatory role *in vivo*, controlling immunoinflammatory reactions (38). Thus, it has been suggested that these autoantibodies may be involved in modulating or in the pathogenesis of certain diseases.

In this study, to clarify the role of IL-1α autoantibodies in patients with rapidly progressive IPF, we investigated the IL-1α autoantibodies in serum from these patients, and also investigated the changes in titers of the IL-1α autoantibodies in serum from progressive IPF patients. Moreover, fresh frozen plasma (FFP) obtained from normal healthy donors, which contained rich IL-1 α autoantibodies, was administered intravenously to progressive IPF patients, intravenously and its half life was calculated and compared with that in control patients.

## MATERIALS AND METHODS

### *Study Population*

The subjects studied consisted of 17 patients with rapidly progressive IPF. The patients were selected on the basis of two criteria : (1) Rapidly progressive IPF. Rapid progression was defined as an increased dyspnea (an increase of more than 1 grade in the Hugh-Jones classification system) or a decrease in $PaO_2$ (>10 mmHg in the same condition) during the previous 1 month, and the presence of ground glass attenuation in high resolution computed tomography (HRCT). Since clinical deterioration may result from infection, heart failure, cancer, our thromboembolism, rather than disease progression (3), we excluded such patients after clinical evaluation, including CT, sputum examination and echo cardiography. (2) The likelihood of treatment with weekly high-dose corticosteroids of at least 3 cycles. A cycle of high dose corticosteroid pulse therapy consisted of intravenous methylprednisolone (1000 mg/day) for 3 consecutive days, followed by oral prednisolone 60 mg/day for 4 days. After 3 cycles of pulse therapy and/or immunosuppressive drugs were administrated to most of the non-responders, and a gradual tapering of the oral corticosteroid was started for the responders.

Of the 17 patients, 12 were men and 5 were women. The age range was 46 to 73 yr (mean±SD : 67.6±7.3) (Table 1). Diagnosis of IPF was based on the histological features in specimens obtained from postmorten examination, open lung biopsy, video-associated thoracoscopic biopsy, or clinical and radiological features including honeycombing in HRCT with histology-proven interstitial pneumonia, which did not contradict the diagnosis of IPF, in specimens obtained from transbronchial lung biopsy. Patients who fulfilled the diagnostic criteria of collagen vascular diseases were excluded. Fine crackles in both lungs were noted in all patients. None of the patients had been treated with oral corticosteroids or cytotoxic agents in the past or at the time of deterioration. The patients were classified as current smokers (S) if they had smoked within 1 yr ; ex-smokers (EX) if they had not smoked for 1 yr but had smoked previously ; and

Table 1. Background and outcome of patients

| Patient No. | Age/Sex | Smoking# | Dyspnea (Hugh-Jones) | %VC | AaDo2 | LDH (IU/L) | CRP (mg/dl) | Outcome |
|---|---|---|---|---|---|---|---|---|
| 1 K. O. | 65/M | S | IV | 60 | 73.6 | 520 | 3.20 | Deceased |
| 2 T. S. | 71/M | S | III | 64 | 74.6 | 480 | 0.26 | Surviving |
| 3 M. N. | 70/M | EX | III | 56 | 101.0 | 685 | 4.25 | Surviving |
| 4 S. H. | 61/M | EX | IV | 54 | 126.2 | 618 | 5.28 | Deceased |
| 5 I. H. | 73/F | NS | IV | NA* | 164.6 | 564 | 1.56 | Deceased |
| 6 O. N. | 70/M | EX | III | 58 | 52.0 | 522 | 0.92 | Surviving |
| 7 Y. M. | 70/M | S | IV | 68 | 48.5 | 600 | 4.15 | Surviving |
| 8 Y. K. | 46/M | EX | III | 67 | 75.3 | 540 | 1.82 | Deceased |
| 9 O. M. | 70/M | EX | IV | NA | 612.7 | 1856 | 2.73 | Deceased |
| 10 K. H. | 57/F | NS | IV | 44 | 82.6 | 872 | 15.76 | Deceased |
| 11 T. K. | 70/M | EX | IV | NA | 91.6 | 549 | 2.60 | Surviving |
| 12 Y. M. | 70/M | S | IV | 58 | 226.5 | 600 | 4.15 | Surviving |
| 13 Y. H. | 70/M | S | IV | NA | 450.6 | 520 | 3.20 | Deceased |
| 14 M. H. | 77/M | S | IV | 60 | 134.0 | 650 | 3.55 | Deceased |
| 15 O. T. | 73/F | NS | IV | NA | 320.2 | 823 | 1.30 | Deceased |
| 16 Y. T. | 72/F | NS | IV | NA | 109.3 | 492 | 3.50 | Deceased |
| 17 U. S. | 65/F | NS | III | 40 | 91.6 | 630 | 4.30 | Surviving |

* NA ; not available
\# smoking status as defined in Materials and Methods

never-smokers (NS). The degree of dyspnea was described according to the Hugh-Jones classification. From arterial blood gas analysis at less than 24 h before treatment, the alveolar-arterial difference of oxygen (A-aDo2) was calculated. It should be noted that the percent vital capacity shown in Table 1 was obtained up to 3 weeks before treatment, not specifically just prior to treatment.

The patients outcome were shown in Table 1. Seven patients responded to the treatment as evidenced by an increased $PaO_2$ with the same condition (>10 mmHg) and decreased dyspnea (a decrease of more than 1 grade in the Hugh-Jones classification). They survived for at least 3 months after treatment. Ten patients failed to respond to the treatment and died from progressive respiratory failure within the 2 months.

In eleven cases (case 1 to 11), venous blood was obtained on the first hospital day (before treatment), when the patients were admitted to our hospital due to severe symptoms, and on the 21st hospital day. In six cases (case 12 to 17), venous blood was obtained before treatment. Serum was obtained and stored at -80°C. Serum LDH levels were determined according to the method of Wroblewski La Due (normal range : 220-450 IU/L).

The control group consisted of 6 patients (5 patients with chronic obstructive pulmonary disease and 1 patient with lung cancer). The age range was 54 to 75 yr (68.0±8.5), and 5 were male and one was female. The patients of the control group had no inflammatory findings.

*Measurement of IL-1α autoantibodies*

Human recombinant IL-1α (rIL-1α) was prepared as described previously (39). IL-1α autoantibodies in serum were measured using radioimmunoassay (RIA) as described previously (31). Briefly, duplicate samples of 50 ml of serum or standard dilutions of monoclonal antibodies to IL-1 α, ranging from 5 to 640 ng/ml, were mixed with $^{125}$I-rIL-1 α. Each sample was mixed with 0.1% bovine serum albumin/phosphate buffer saline (BSA/PBS) containing 5 mM ethylenediaminotetraacetic acid (EDTA)/0.05% $NaN_3$, and each standard dilution of rhIL-1α was mixed with 0.1% BSA/PBS containing 5 mM EDTA/0.05% $NaN_3$ and carrier buffer (2% bovine gamma globulin). When antigen-antibody binding had reached equilibrium after overnight incubation at room temperature, each sample was mixed with 25% polyethylene glycol (MW 6000) and incubated for 1h. Then, the tubes were centrifuged at 3000 rpm for 15 min, and the radioactivities of the precipitates were counted in an automated gamma-spectrometer. The sensitivity of this assay for IL-1 α autoantibodies was 5 ng/ml.

*Characterization of IL-1α autoantibodies in serum from patients with rapidly progressive IPF*

To characterized IL-1α autoantibodies that appeared in serum of patients on the 21st hospital day, we used

serum containing high IL-1α autoantibody levels obtained from IPF, and investigated the immunoglobulin class of the IL-1α autoantibodies. The serum was applied to a protein A agarose column. After washing with binding buffer (50 mM sodium borate with 3M NaCl, pH9.0), the bound material was eluted with elution buffer (100 mM glycine, pH 2.5) and collected in 2 ml fractions. The IL-1α autoantibodies level in each fraction was measured using RIA. The serum IL-1 α autoantibodies were also fractionated using gel filtration with a Superose 12 HR 10/30 column (Pharmacia, Uppsala Sweden). Serum was applied to a column preequilibrated with 50 mM Tris-HCl containing 0.5 M NaCl and 5 mM EDTA (pH 8.0) and eluted with the same buffer. The elute was collected in 0.5 ml fractions and the IL-1α autoantibody concentration of each fraction were measured using RIA.

*Preparation of IL-1α autoantibodies rich plasma*

Fresh frozen plasma (FFP) was obtained from healthy blood donors at the Tokushima Blood Transfusion Center (Tokushima, Japan). This plasma is permitted to be used for blood transfusion. The level of IL-1α autoantibodies in plasma was measured, and the plasma with high titers of IL-1α autoantibodies, i.e., a level over 640 ng/ml, was selected and stored at -80°C until the transfusion.

*Half life of exogeneous IL-1α autoantibodies in rapidly progressive IPF*

We investigated serum IL-1 α autoantibody levels after intravenous administration of FFP with high levels of IL-1 α autoantibodies at the indicated time intervals, and measured the half life (T1/2). We studied 12 patients including 6 IPF patients (case 12-17) and 6 control patients. In the IPF patients, 5 units of FFP were administered after the first pulse therapy. IL-1 α autoantibody levels of serum from these patients were less than 5 ng/ml before IL-1 α autoantibody rich plasma administration. Serum was obtained on days 1, 3, 7 and 14 after intravenous administration of FFP. This investigation was approved by the Tokushima University ethics committee. Informed consent was obtained from each patient or the patient's family.

*Statistical Analysis*

Statistical analysis was performed using the Mann-Whitney U test, and p values of 0.05 or less were regarded as statistically significant.

## RESULTS

*IL-1 α autoantibodies in serum from patients with rapidly progressive IPF*

We measured IL-1α autoantibodies in serum from patients with progressive IPF on the first hospital day and the 21st hospital day. On the first hospital day, IL-1α autoantibodies in serum were detected in 5 of 11 patients with progressive IPF, with a level between 10 and 168 ng/ml, while they were not detected in the remaining 6 patients. On the 21st hospital day, IL-1α autoantibodies were detected in all IPF patients, its level was between 17 and 1273 ng/ml and was increased compared with that on the first hospital day (Figure 1). The level of IL-1α autoantibodies on the 21st hospital days was not significantly different among the outcome of patients.

*Characterization of IL-1α autoantibodies appeared in serum of patients with progressive IPF*

To characterize IL-1α autoantibodies that appeared in serum of progressive IPF patients, we used serum with high levels of IL-1α autoantibodies obtained from 5 patients. The sample was applied to a protein A agarose column. After washing with the binding buffer, we obtained the IgG fraction using an elution buffer. RIA showed that the high titer of IL-1α autoantibodies was presented only in the IgG fraction (Figure 2A). Furthermore, the serum was fractionated using gel filtration, and the molecular size of IL-1 α autoantibodies corresponded to that of IgG (Figure 2B). All samples from the 5 patients which were examined showed similar findings.

*Half life of exogeneous IL-1α autoantibodies in patients with rapidly progressive IPF and control patients*

IL-1α autoantibodies in rich FFP was administrated to 6 progressive IPF patients and 6 control patients. All IPF patients were treated with high dose steroid therapy and all control patients were stable. Although IL-1α autoantibodies which might be produced in the process of exacerbation, could not be distinguished from IL-1 α autoantibodies in FFP, the serum IL-1α autoantibody levels were measured after administration of IL-1 α autoantibodies in rich plasma at the indicated interval, and the half life (T1/2) of the exogenous IL-1 α autoantibodies was calculated. As shown in Figure 3, T1/2 in progressive IPF patients was significantly shorter than that in control patients (mean±SD;71.1±31.8hr v.s. 352±98.3 hr, $p<0.001$). Clinical change in these pa-

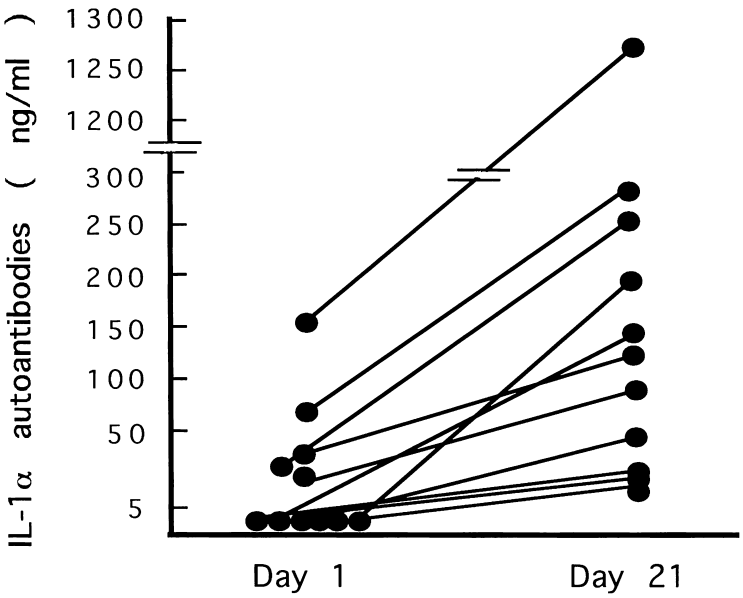
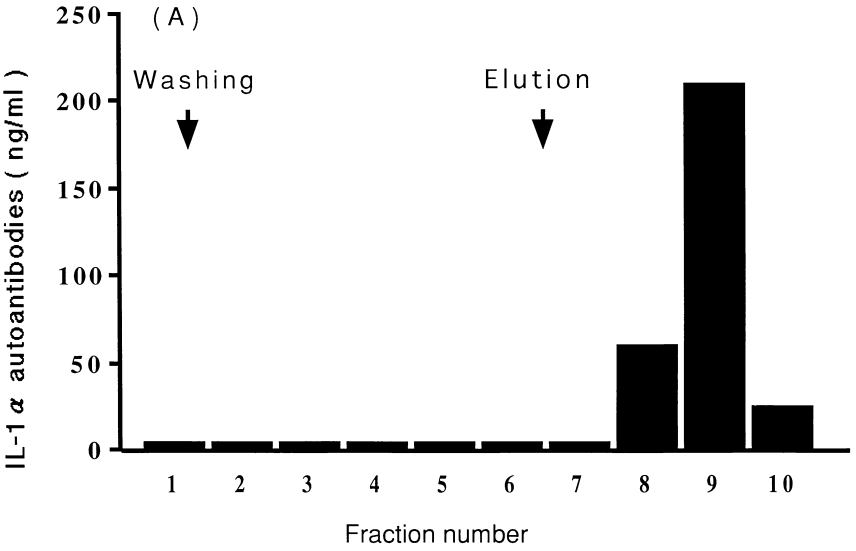



Fig. 1. The change of IL-1 α autoantibody levels in serum from rapidly progressive IPF patients. Serum from IPF patients was obtained on the first hospital day (A) and 21st hospital day (B), and the level of IL-1α autoantiboides were measured using RIA with a detection limit of 5 ng/ml as described in Materials and Methods.



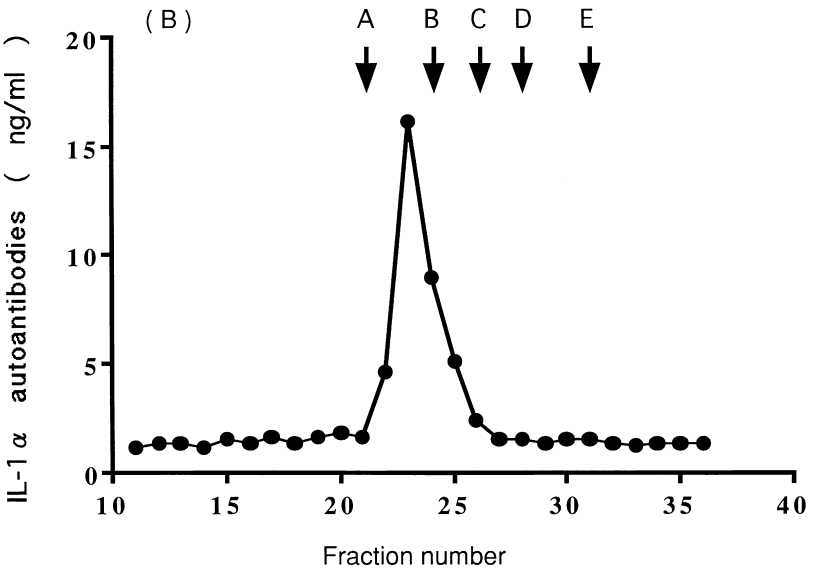




Fig. 2. Characterization of IL-1α autoantibodies appeared in serum of patients with rapidly progressive IPF. (A) Fractionation of serum IL-1α autoantibodies using a protein A agarose column. Serum of patients were applied to the protein A column. After washing with binding buffer, protein was eluted in 2 ml fractions using an elution buffer. (B) Analysis of IL-1 α autoantibodies via gel filtration. Serum of patients was fractionated using gel filtration with a Superose 12 HR 10/30 column. Molecular marker : A ; glutamate dehydrogenase (290kD), B ; lactate dehydrogenase (142 kD), C ; enolase (67 kD), D ; adenylate kinase (32 kD), E ; cytochrome C (12.4 kD). The titer of the IL-1 α autoantibodies in each fraction was measured using RIA as described in Materials and Methods. The same findings were observed in 5 different cases.

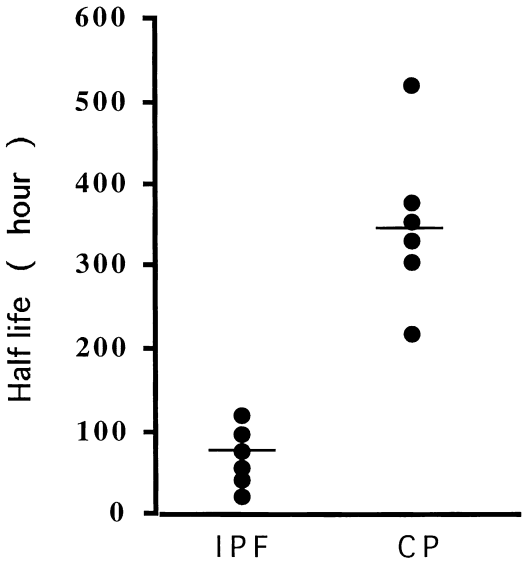



Fig. 3 Half life of exogenous IL-1 α autoantibodies in patients with progressive IPF and control patients. IL-1 α autoantibodies rich FFP was administrated to 6 patients and 6 controls. The concentration of IL-1 α autoantibodies in serum was measured at the indicated time using RIA, as described in Materials and Methods, and the half life was calculated. Significant difference between the IPF group and the control group, $p<0.01$.

tients could not be estimated after IL-1α autoantibody administration.

## DISCUSSION

The present study demonstrated that the level of IL-1α autoantibodies in serum obtained from patients with rapidly progressive IPF was increased during their clinical course, despite administration of high dose steroids. Furthermore, IL-1α autoantibodies which appeared in serum, were eluted at a molecular size which corresponded to IgG. IL-1α autoantibodies in rich FFP was administrated intravenously to patients with rapidly progressive IPF, and the T1/2 was calculated. The T1/2 obtained from IPF patients was shorter than that from control patients.

IL-1α autoantibodies were shown to be capable of specifically inhibiting the activity of IL-1α (30, 31, 33, 40). These antibodies bind to natural proIL-1α as well as 17 kD recombinant IL-1α (41). In this regard, IL-1α autoantibodies might be present to modulate immune inflammatory reaction by inhibition of IL-1α activity, and this is also thought to play an important role in the pathogenesis of progressive IPF. The level of IL-1 α autoantibodies was increased in serum from patients with progressive IPF on the 21st hospital day compared with that on the first hospital day, despite treatment. The IL-1α autoantibodies might be presented in response to lung tissue injury and their presence might reflect the degree of tissue injury. The IL-1 α autoantibodies that appeared in serum obtained from progressive IPF patients on the 21st hospital day were eluted with an apparent molecular weight of 100,000-200,000, which corresponds to a subclass of the IgG fraction. Moreover, a substantial amount of this material was bound to protein A, which binds with high specificity to the Fc regions of IgG1, IgG2 and IgG4. These observations suggest that IL-1 α autoantibodies were of IgG class and our findings were similar to that of normal subjects (28, 31).

Next, we administrated intravenously IL-1α autoantibodies in rich FFP to rapidly progressive IPF patients, and estimated the half life of IL-1α autoantibodies. Svenson *et al.* reported that pharmaceutical preparations of human IgG contain specific and neutralizing, high affinity antibodies against IL-1α and IL-6, and the presence of these autoantibodies may help explain why high dose IgG therapy is beneficial in a number of pathogenetically obscure immunoinflammatory disorders (42). Moreover, Ross *et al*. demonstrated that *in vivo* antibodies against IFN-α, IL-1α and IL-6 were increased after high dose IgG therapy (43). Interestingly, the half life of exogeneous IL-1 α autoantibodies in progressive IPF patients was shorter than that in control patients. However, the half life of exogenous IL-1α autoantibodies might not be correct, because we could not distinguish between exogeneous and endogeneous IL-1α autoantibodies, which appeared during disease progression. This is an important limitation of the present study. Actually, the IL-1α autoantibody levels increased faster after administration of IL-1 α autoantibodies in rich FFP was decreased than that of control patients. Exogenous IL-1α autoantibodies are thought to be consumed to neutralize IL-1α. In this regard, the level of IL-1 α autoantibodies that appeared in serum from patients might be decreased in the disease condition. These findings suggest that excess extracellular IL-1α ctivity including mature and proIL-1 α were present in progressive IPF patients and it is likely that IL-1 α autoantibodies might be present to regulate IL-1α activity. Although the cells which produce IL-1α in progressive IPF were unclear, monocytes/macrophages, endothelial cells, dendritic cells and fibroblasts were shown to express IL-1α (7). Isolated peripheral blood monocytes expressed more IL-1β than IL-1α (44-45). However, alveolar macrophages released more cell associated IL-1α than did blood monocytes (45). Recently, Janson *et al.* reported that cell lysate in alveolar macrophages

stimulated with LPS contained high levels of IL-1α (46). For this, alveolar macrophages were thought to be a crucial source of IL-1α production in rapidly progressive IPF. Since exogenous IL-1α autoantibodies inhibit IL-1 α activity, the effect of these antibodies on disease activity in progressive IPF patients might be expected. However, we could not estimate the clinical effects of these antibodies in the administrated cases, because all of these patients were treated with high dose steroid therapy, and IL-1α autoantibodies in rich FFP might have contained autoantibodies to other cytokines, such as IL-2, IL-6, IL-8, IL-10 and IFN-γ, or other factors.

Although the physiological or pathophysiological roles of autoantibodies to IL-1 α are still unknown, IL-1α autoantibodies can be a natural inhibitor and may play a highly significant role in regulation of IL-1 α action *in vivo*. Additionally, it may play a significant role in the kinetics of IL-1 α, although it is still unknown whether the immune complexes are scavenged more quickly than free IL-1 α or whether autoantibodies act as an IL-1α reservoir to prolong its half life (37). The role of IL-1α autoantibodies *in vivo* remains to be determined.

In conclusion, IL-1 α autoantibodies presented in serum from progressive IPF might be generated in response to the inflammatory processes of this disease and may act as a regulatory factor for IL-1α. In this regard, recent isolation of a high affinity human monoclonal antibody to IL-1 α (47) might provide a new means for treatment of progressive IPF.

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