Clin Exp Immunol 1995; **100**:325–329

Evaluation of soluble IL-6 receptor concentration in serum and epithelial lining fluid from patients with interstitial lung diseases

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(Accepted for publication 10 January 1995)

SUMMARY

We measured soluble IL-6 receptor (sIL-6R) levels in serum and bronchoalveolar lavage fluids (BALF) from patients with interstitial pneumonia of unknown etiology (IP) ($n = 17$), sarcoidosis ($n = 8$) and normal control subjects ($n = 10$), to investigate its role in pulmonary diseases. Soluble IL-6R was determined by an ELISA. The volume of epithelial lining fluid (ELF) in BALF was estimated using an urea method. We found that levels of sIL-6R in serum, BALF, and ELF from patients with IP or sarcoidosis were significantly higher than those from normal subjects. Furthermore, levels of sIL-6R in BALF or ELF were significantly correlated with those of albumin, indicating that sIL-6R, together with albumin, may enter ELF as a result of the increased permeability caused by pulmonary inflammation. Thus most of the sIL-6R in ELF would be from serum, and relatively small amounts of it might be produced locally. However, sIL-6R levels in ELF, but neither serum nor BALF, were significantly correlated with levels of C-reactive protein in patients with IP. These results suggest that both systemic and local production of sIL-6R are increased, and raised sIL-6R is involved in the modulation of systemic and local inflammatory responses in patients with IP and sarcoidosis.

Keywords IL-6 soluble IL-6 receptor bronchoalveolar lavage epithelial lining fluid interstitial lung disease

INTRODUCTION

IL-6 is a multifunctional cytokine, and is especially known as a key factor in acute-phase reactions [1,2]. It has been shown to act on a variety of target cells through surface IL-6 receptors identified as two different membrane glycoproteins, i.e. an 80-kD protein as ligand-binding receptor, and a 130-kD protein as non-ligand-binding but signal transducing receptor [3,4].

Generation of soluble forms of cell surface receptors is a general phenomenon, such as that of CD21 [5], CD23 [6] and tumour necrosis factor (TNF) receptor [7]. The soluble form of human IL-6R (sIL-6R) has been characterized as an IL-6 binding 53-kD molecule in normal human urine [8]. The means of sIL-6R generation are considered to involve shedding [9]. Although IL-6 is implicated in various human diseases, there have been few reports concerning the clinical significance of sIL-6R. Recently, elevated serum sIL-6R levels were reported in patients with HIV infection [10] and multiple myeloma [11]. In contrast, the soluble IL-2 receptor (sIL-2R) has been much studied [12,13]. Elevated serum levels of sIL-2R have been found in patients with a variety of pulmonary diseases including sarcoidosis, lung transplantation, tuberculosis, and HTLV-1-associated bronchopneumopathy [14].

In the present study, we measured sIL-6R levels in both serum and epithelial lining fluids (ELF) to evaluate its role in patients with interstitial lung diseases (ILD).

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PATIENTS AND METHODS

Subjects

Bronchoalveolar lavage fluids (BALF) and sera from 35 subjects were investigated. None of these subjects was initially taking inhaled or systemic steroids, and none had clinical evidence of infection: (i) control group: seven men and three women referred with non-specific respiratory symptoms. Bronchoscopic examinations, with normal results, were indicated by the past history of haemosputum in seven, persistent cough in three. Five were current smokers; (ii) ILD group: 10 men and seven women with biopsy-proven interstitial pneumonia of unknown etiology (IP), and three men and five women with sarcoidosis. Ten of these patients were currently smoking and one was an ex-smoker.

Table 1. Bronchoalveolar lavage findings of the subject groups

Subject group	M/F	Age (years)	BALF (ml)	ELF (ml)	Cell density ($\times 10^{-5}$/ml)	Cell differentials (%)			
						Macrophages	Lymphocytes	Neutrophils	Eosinophils
Normal ($n = 10$)	7/3	54·7 ± 10·7	83·5 ± 9·7	1·5 ± 0·6	1·4 ± 1·0	83·7 ± 6·1	14·2 ± 5·2	2·0 ± 1·8	0·1 ± 0·2
ILD									
Sar ($n = 8$)	3/5	48·1 ± 18·2	88·0 ± 19·2	1·6 ± 0·8	2·5 ± 1·0*	63·9 ± 19·2**	31·6 ± 15·4**	3·9 ± 9·1	0·1 ± 0·2
IP ($n = 17$)	10/7	54·5 ± 13·2	76·3 ± 17·9	2·1 ± 1·3	3·3 ± 1·3*	69·1 ± 21·8	23·2 ± 16·8	5·4 ± 7·0	2·8 ± 4·8

*$P < 0.05$; **$P < 0.01$ compared with normal controls.
BALF, Bronchoalveolar lavage fluid; ELF, epithelial lining fluids; IP, interstitial pneumonia.

There were no significant differences in age, sex, and smoking history among these groups. BALF findings of the above patients are shown in Table 1.

The levels of C-reactive protein (CRP) and albumin were determined by turbidimetric immunoassay and radioimmunoassay, respectively. In the IP group, the values of CRP, erythrocyte sedimentation rate (ESR), and platelet counts were available at the same time as bronchoscopy in 15 patients. Thus the correlation between these values and sIL-6R levels were determined in these 15 patients.

Bronchoalveolar lavage

BAL was performed using 50 ml × 3 (total 150 ml) instillation of warmed saline. The right middle lobe was the lavage site chosen for all subjects. The lavage samples were filtered through a single layer of sterile gauze to remove gross contamination. The cellular component was removed by centrifugation at 1000*g* for 5 min and supernatants were stored at −80°C until use. Total cell counts and cell differentials were obtained by haemocytometer and Wright–Giemsa staining, respectively.

Measurement of sIL-6R

The sIL-6R was measured by a specific ELISA according to the method described by Nakajima *et al.* [15]. Briefly, an anti-IL-6R MoAb (100 µl of 2 µg/ml), MT-18 [16], was coated onto ELISA plates at 4°C overnight. After blocking with 1% bovine serum albumin (BSA; Sigma, St Louis, MO), samples of recombinant sIL-6R (Tosoh Corporation, Ayase, Kanagawa, Japan) were incubated with buffer (0·1% BSA–TBS) for 2 h at room temperature. Then they were further incubated with biotinylated polyclonal guinea pig anti-sIL-6R IgG fraction at a concentration of 1 µg/ml for an additional 2 h at room temperature. The plates were then incubated with horseradish peroxidase-coupled streptavidin (Zymed Incorporated, South San Francisco, CA) for 30 min before colour development with *o*-phenylendiamine substrate. The absorbance was measured at 492/630 nm. The detection limit of this assay was 0·1 ng/ml. The assay is specific for human IL-6R because of non-reactivity with murine IL-6R, human IL-1β, IL-2, IL-3, IL-4, interferon-gamma (IFN-γ), TNF-α, or sIL-2R. Per cent coefficient of variation (CV) of intra- and interassay precision were 2·2 and 4·7, respectively.

Estimation of ELF volume and concentration of sIL-6R and albumin in ELF

Urea concentration was determined using a commercial kit (a-gent BUN; Abbott Laboratory, North Chicago, IL). Using each concentration of urea in BALF and serum, the volume of ELF in BALF was calculated by the method of Rennard *et al.* [17] as follows:

$$\text{Volume ELF} = \frac{\text{total amount of urea in BALF (mg)}}{\text{serum urea concentration (mg/ml)}}$$

Then the concentration of sIL-6R or albumin in ELF was calculated as follows:

$$\text{ELF sIL-6R concentration (ng/ml)} = \frac{\text{total amount of sIL-6R in BALF (ng)}}{\text{volume of ELF (ml)}}$$

$$\text{ELF albumin concentration (mg/ml)} = \frac{\text{total amount of albumin in BALF (mg)}}{\text{volume of ELF (ml)}}$$

Statistical analysis

Data are shown as mean ± s.d. Intergroup comparisons were made by using the Kruskal–Wallis test or the one-way ANOVA, and in addition the Mann–Whitney *U*-test or Student's *t*-test, respectively. Spearman's rank sum test was used for correlation analysis. Statistical significance was defined as $P < 0.05$.

RESULTS

The BAL findings of the study subjects are shown in Table 1. There were significant differences in cell density and percentage of both alveolar macrophages and lymphocytes (both $P < 0.05$; one-way ANOVA) but not neutrophils or eosinophils among groups. The cell density of patients with sarcoidosis or IP was significantly greater than that of normal subjects (both $P < 0.05$; Student's *t*-test). The percentage of alveolar macrophages was significantly lower in patients with sarcoidosis than in normal controls ($P < 0.01$; Student's *t*-test), reflecting that the percentage of lymphocytes was significantly increased ($P < 0.01$; Student's *t*-test). There were no differences in age and recovered volume of BALF or ELF among the three (by one-way ANOVA).

The levels of sIL-6R could be detected in the non-concentrated BALF of all cases except one sample from a control subject. Table 2 shows the concentration of sIL-6R

Table 2. Levels of sIL-6R in serum, bronchoalveolar lavage fluid (BALF) and epithelial lining fluid (ELF) of the subject groups

Subject group	Serum sIL-6R (ng)		BALF sIL-6R (ng)		ELF sIL-6R (ng)	
	/ml fluid	/mg albumin	/ml fluid	/mg albumin	/ml fluid	/mg albumin
Normal	110·5 ± 21·2	2·5 ± 0·7	0·35 ± 0·19	8·5 ± 2·9	21·2 ± 9·5	8·8 ± 3·0
ILD						
Sar	155·4 ± 33·1*	3·6 ± 1·0*	0·54 ± 0·21*	12·7 ± 4·6	36·4 ± 15·2*	12·4 ± 4·5
IP	138·7 ± 39·7*	3·5 ± 1·4	0·85 ± 0·62**	13·6 ± 10·5	35·2 ± 19·0*	13·1 ± 7·1

*$P < 0·05$; **$P < 0·01$ compared with normal controls.
IP, Interstitial pneumonia.

in serum, BALF, and ELF from all subjects. The levels of sIL-6R in serum, BALF and ELF in patients with sarcoidosis or IP were all significantly higher than those in the control group ($P < 0·01$ and $P < 0·05$ in serum samples, respectively, and both $P < 0·05$ in both BALF and ELF; Mann–Whitney *U*-test). These significant increases over normal levels were negated in BALF and ELF when sIL-6R concentrations were standardized in terms of sIL-6R/mg of albumin, although ILD groups still tended to be increased. Only serum levels in both IP and sarcoidosis were significantly raised compared with the control group (both $P < 0·05$).

There were no significant correlations between sIL-6R concentrations and concentrations of the particular types of cells (alveolar macrophages, lymphocytes, neutrophils or eosinophils) in BALF. The levels of sIL-6R in BALF and ELF were significantly correlated with those of albumin (Fig. 1; $r_s = 0·55$ and $r_s = 0·63$, respectively; both $P < 0·01$; Spearman's rank sum test).

In patients with IP ($n = 15$), the levels of sIL-6R in ELF, but neither serum nor BALF, were significantly correlated with serum CRP concentration (Table 3; $r_s = 0·56$, $P < 0·05$; Spearman's rank sum test). None of these levels was correlated with ESR or platelet counts, although both of them are considered to be connected with IL-6 function.

DISCUSSION

The present study showed significantly elevated sIL-6R levels in serum, BALF and ELF from patients with interstitial lung diseases. This finding is consistent with the implication that the ligand of the receptor, IL-6, plays a role in the pathogenesis of interstitial lung diseases [18]. Since sIL-6R is only generated in culture supernatants of constitutively IL-6R-bearing cells [15], and T lymphocytes and macrophages are able to express IL-6R, sIL-6R would be produced by such inflammatory cells in patients with sarcoidosis and IP. This is why we expected that the levels of sIL-6R would be much higher in ELF than in serum in patients with inflammatory lung diseases, as is demonstrated in sIL-2R, a well known soluble cytokine receptor [14]. However, our results demonstrated that sIL-6R levels in ELF were invariably lower than those in serum, indicating that sIL-6R, unexpectedly, did not seem to be actively produced at the site of local inflammation. This is also in contrast to the findings that ELF levels of IL-6, and KL-6, a mucin-like glycoprotein, which is demonstrated to be produced in the lower respiratory tracts, are several hundred times higher than those levels in serum in patients with ILD [18,19].

This discrepancy may be explained by the fact that activation of human T and B cells does not markedly increase their surface IL-6 receptors [20], and the main site of IL-6R mRNA expression is hepatocytes, at least in mice. Although IL-6R mRNA was detected in the whole lung after *in vivo* stimulation with human IL-6, the degree of expression was much less than that in the liver [21]. Thus, the increased serum sIL-6R could be due to exaggerated production by hepatocytes.

Table 3. Correlation between sIL-6R levels and clinical parameters in patients with interstitial pneumonia (IP)

sIL-6R levels in	CRP (mg/dl)		ESR (mm/h)		Platelet count (10^4/mm^3)	
	r_s	P	r_s	P	r_s	P
Serum	0·18	NS	0·07	NS	0·13	NS
BALF	0·33	NS	0·12	NS	0·04	NS
ELF	0·56	<0·05	0·05	NS	0·03	NS

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; BALF, bronchoalveolar lavage fluid; ELF, epithelial lining fluid.

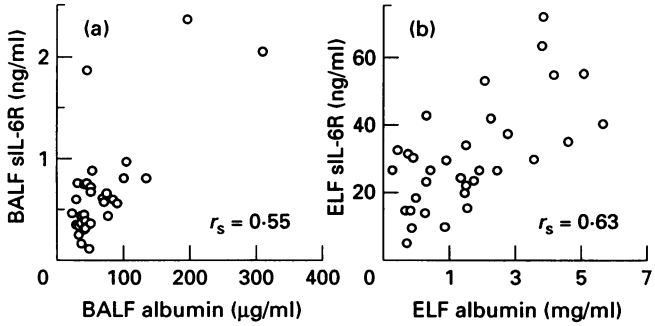

Fig. 1. Correlation between albumin and sIL-6R concentrations in bronchoalveolar lavage fluid (BALF) (a) and epithelial lining fluid (ELF) (b). There are significant correlations between them (both $P < 0·01$).

Furthermore, we demonstrated that sIL-6R levels in BALF and ELF were significantly correlated with the albumin concentration (Fig. 1). Since albumin concentration in BALF is considered to reflect permeability through the air–blood barrier in the lower respiratory tracts [22], increased permeability by lung inflammation presumably contributes to the entrance of sIL-6R into ELF. Thus, most sIL-6R in ELF seems to originate from serum, where sIL-6R is abundant as a result of presently unknown mechanism(s). This notion could be supported by our observation that the levels of sIL-6R in synovial fluids and pleural effusions are also invariably lower than those in serum in patients with rheumatoid arthritis and tuberculous pleurisy, respectively (unpublished). Increased levels of albumin may explain the negation of significant differences of sIL-6R levels in BALF and ELF between the ILD and control groups, when further standardized with the amount of albumin. Since small but significant amounts of sIL-6R would be produced in patients with ILD, as described above, such a local production might explain the fact that the levels of sIL-6R/mg albumin in ELF are several times higher than those in serum.

The role of increased sIL-6R should be considered from three aspects. First, unlike other soluble cytokine receptors, sIL-6R can act as an agonist; i.e. sIL-6R may serve in potentiating IL-6 function by complex formation with IL-6 and binding with cell surface gp130 [3,4]. Second, high levels of IL-6 lead to down-regulation of the gp80-IL-6 receptor, resulting in unresponsiveness of the cells to IL-6. This refractory state could be overcome by the presence of sIL-6R [23]. On the other hand, a high amount of sIL-6R inhibits dose-dependently the concanavalin A (Con A)-induced proliferative response of peripheral blood mononuclear cells by presently unknown mechanism(s) [10]. These observations indicate that the increased sIL-6R in ELF would be involved in local immune regulation.

We tried to determine the correlation between sIL-6R and CRP, ESR, and platelet counts in peripheral blood, all of them considered to be related to IL-6 function [1,2], in patients with ILD. We found that only ELF levels of sIL-6R are significantly correlated with CRP levels. This observation may indicate that sIL-6R has a role in acute-phase reaction, and local production of sIL-6R is critical for induction of CRP in patients with IP. It should be noted, however, that the concentration in ELF is determined by both local production and the intensity of local inflammation (abundant sIL-6R in blood would enter ELF through the blood–air barrier, together with blood albumin), as mentioned above. Thus the correlation between sIL-6R in ELF and CRP indicates that the intensity of local inflammation, but not increased local sIL-6R production, may contribute to the production of CRP.

In conclusion, both local and systemic levels of sIL-6R are increased, and raised sIL-6R may cause an immunomodulatory effect on both the local and systemic inflammatory reaction in patients with IP and sarcoidosis.

ACKNOWLEDGMENTS

This study was supported in part by Grant-in-Aid for Scientific Research no. 05807053 from the Ministry of Education, Science and Culture, Japan.

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