Acta Med. Okayama, 2001
Vol. 55, No. 4, pp. 205-211

Acta Medica Okayama
http://www.lib.okayama-u.ac.jp/www/acta/

Original Article

Intercellular Adhesion Molecule-1 in Patients with Idiopathic Interstitial Pneumonia

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This study focuses on a possible role of intercellular adhesion molecule-1 (ICAM-1) in interstitial pulmonary diseases. We determined a soluble form of ICAM-1 in serum and bronchoalveolar lavage fluid (BALF) using ELISA in patients with usual interstitial pneumonia (UIP), bronchiolitis obliterance organizing pneumonia (BOOP), or nonspecific interstitial pneumonia (NSIP). In addition, we investigated the expression of ICAM-1 in the lung tissues of these patients by means of immunohistochemical staining. Serum levels of soluble ICAM-1 were significantly higher in patients with UIP or NSIP than in healthy subjects, and were also high in patients with BOOP. The soluble ICAM-1 in BALF tended to be higher in patients with UIP, BOOP, or NSIP than in normal subjects. A significant correlation was seen between soluble levels of ICAM-1 in serum and BALF. In the immunostaining of ICAM-1 of the lung tissues, ICAM-1 expression was more pronounced in patients with UIP than in those with BOOP or NSIP. The increased expression of ICAM-1 was seen in type II alveolar epithelium and vascular endothelium in patients with interstitial pneumonia. A positive correlation was observed between the degree of ICAM-1 expression in the lung tissues and the BALF levels of soluble ICAM-1. The expression of ICAM-1 in type II alveolar epithelium suggests that ICAM-1 plays a specific role in the fibrotic process of the lung, and that the measurement of soluble ICAM-1 in sera and BALF could be a useful marker for evaluating the progression of fibrosis.

Key words: usual interstitial pneumonia, bronchiolitis obliterance organizing pneumonia, nonspecific interstitial pneumonia

Intercellular adhesion molecule-1 (ICAM-1) is a member of the immunoglobulin supergene family and its ligand is lymphocyte function-associated antigen-1 (LFA-1). ICAM-1 is expressed in various tissues including vascular endothelial cells and fibroblasts, and its expression at the site of inflammation is regulated by proinflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α) and interferon-γ (INF-γ) at the site of inflammation [1], [2]. ICAM-1 has been reported to play an important role in the migration of leukocytes from blood vessels to the inflammatory site [3]. However, the exact role of adhesion molecules including ICAM-1 in lesions of interstitial pulmonary diseases has not been fully elucidated.

In the present study, we investigated a soluble form of ICAM-1 in serum and bronchoalveolar lavage fluid (BALF), in patients with idiopathic interstitial pneumonia (IIP). Using an ELISA method, along with the immuno-

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histochemical staining of ICAM-1 expressed in the lung tissues, we investigated the roles of ICAM-1 in the development of interstitial pulmonary diseases.

Materials and Methods

Subjects. Eleven patients with IIP, which had been diagnosed clinically and histopathologically by thoracoscopic lung biopsy, were enrolled in the study (Table 1). In terms of histological classification, 4 patients were diagnosed as having usual interstitial pneumonia (UIP), 4 as having nonspecific interstitial pneumonia (NSIP), and the remaining 4 as having bronchiolitis obliterans organizing pneumonia (BOOP). The control group consisting of 18 healthy subjects (20 to 32 years old) with no history of respiratory diseases also underwent BAL. Noncancerous portions of lungs resected from 5 lung cancer patients were used as a control.

Serum Specimen. Blood samples were collected from 12 patients with IIP and 18 healthy volunteers as controls, Serum was separated and stored at $-20\,^{\circ}C$ until measurement of soluble ICAM-1.

Bronchoalveolar Lavage. Bronchoalveolar lavage was performed in 10 patients with IIP and 10 healthy controls. Following local anesthesia with 4% lidocaine, a bronchoscope was wedged into B5 of the right middle lobe, and 50 ml of normal saline was infused and then collected immediately. The procedure was repeated 4 times, using a total volume of 200 ml of saline. Recovered BALF was used for measurement of soluble ICAM-1. The BALF was then centrifuged ($4\,^{\circ}C$, 250 xg, 10 min), and the supernatant was collected and stored at $-20\,^{\circ}C$ until use. A differential cell count of BALF cells was performed on Giemsa-stained smears prepared by a cytocentrifuge.

Assay of Soluble ICAM-1. Levels of soluble ICAM-1 in serum and BALF were measured by sandwich enzyme linked immunoassay using an ELISA kit (T cell Diagnosis Inc., Cambridge, MA, USA) in which a 96-well microtiter plate coated with anti-ICAM-1 antibody was used. Serum and BALF samples were stored at $-20\,^{\circ}C$ until use. After thawing the samples, serum was diluted 100 times and BALF was diluted 10 times with antibody diluent. All measurements of the samples and the standard curve were performed in duplicate. Two wells were used for the blank and 10 wells for drawing the standard curve. Twenty-five microliters of the ICAM-1 standard solution (0, 0.56, 2.86, 5.89, and 8.94 ng/ml) and diluted samples were added to wells coated with anti-ICAM-1 antibody. Horse-radish peroxidase (HRP)-labeled anti-ICAM-1 antibody (75 μl) was added to each well except for the blank wells. The microplate was incubated at room temperature on a rotator set at 150 rpm for 2 h. After 3 washings, 100 μl of chromogen solution

Table I Characteristics of patients with IIP

Type of histology	Sex	Age	Serum s ICAM-1	BALF s ICAM-1	lisease duration	Steroid therapy	Disease activity	Pulmonary function		Aterial blood gas		Chest X-ray honeycomb pattern
								%VC	%DLCO	PaO_2 (Torr)	$AaDO_2$ (Torr)	
UIP	M	51	493.3	61.6	48 months	(−)	Not active	88.0	49.0	76.0	25.8	(+)
UIP	M	24	505.5	75.0	18 months	(−)	Active	53.7	57.6	89.6	10.4	(+)
UIP	M	60	364.3	31.3	48 months	(+)	Not active	49.0	38.9	73.4	22.2	(+)
UIP	M	70	424.4	43.8	26 months	(−)	Not active	37.5	23.0	77.5	28.3	(+)
NSIP	M	44	564.8	91.2	14 months	(+)	Active	74.0	94.0	79.6	26.3	(−)
NSIP	M	62	397.3	30.5	29 months	(−)	Active	81.1	90.1	71.9	29.3	(−)
NSIP	F	75	407.0		2 months	(−)	Active	83.3	68.0	74.9	27.8	(−)
NSIP	F	26	614.3	127.3	16 months	(+)	Active	40.7	37.5	72.0	27.2	(−)
BOOP	F	53	380.6	10.6	2 months	(−)	Active	65.9	ND	73.0	29.9	(−)
BOOP	F	68	386.5	41.9	3 months	(−)	Active	84.6	95.8	63.4	39.5	(−)
BOOP	F	67	302.6	23.7	2 months	(−)	Active	122.0	ND	58.5	42.2	(−)
BOOP	F	65	418.6		4 months	(−)	Active	50.2	26.1	77.2	24.3	(−)

$AaDO_2$, alveolar-arterial oxygen pressure gradient; BOOP, bronchiolitis oblitarance organizing pneumonia; DLco, diffuse capacity of carbon monooxide; ND, not determined; NSIP, nonspecific interstitial pneumonia; PaO_2, aterial oxygen pressure; UIP, usual interstitial pneumonia; VC, vital capacity.

was added to all wells prior to a 30-min incubation. After adding 50 μl of stop solution (2N H_2SO_4), soluble ICAM-1 was measured at 490 nm absorbance.

Immunohistochemical Analysis. For staining of paraffin sections, specimens were fixed in 10% formalin for 4 h, preserved in phosphate-buffered saline (PBS) overnight, embedded in paraffin, sectioned, and placed on slides. After deparaffinization in xylene and ethanol, the specimens were boiled in target retrieval solution (Target Retrieval Solution, DAKO Japan, Kyoto, Japan) for 40 min, and stained using the Catalyzed Signal Amplification method (CSA System, DAKO Japan, Kyoto, Japan). Endogenous peroxidase was eliminated by reacting with 3% hydrogen peroxide for 30 min, and then nonspecific staining with the primary antibody was blocked by 10% rabbit serum. The slides were incubated for 60 min at 4 °C with primary anti-ICAM-1 mouse monoclonal antibody (Mouse Anti-Human ICAM-1, DAKO Japan, Kyoto, Japan) followed by biotinylated secondary antibody (anti-mouse IgG) for 30 min and streptavidin-biotin-peroxidase complex for 15 min, biotinylated tyramide (amplification reagent) for 15 min, and finally streptavidin-peroxidase for 15 min. Several changes of Tris buffered saline (TBS) were carefully performed between all steps of the procedure. After washing with TBS, peroxide-labeled streptavidin was reacted with biotin. The antigen-antibody complex was made visualized using 0.05% solution of diaminobenzidine tetrahydrochloride (DAB) in PBS for 5 min. Subsequently, the slides were counterstained with Mayer hematoxylin, and dehydrated and cleared in xylene. Controls were performed on identically prepared tissues and slides using the appropriately matched isotype (mouse IgG1) as the test primary antibody.

Immunoreactivity was evaluated visually and expressed as follows: Grade 0, negative; Grade I, weakly positive; Grade II, positive; and Grade III, strongly positive.

Statistical analysis. The results were expressed as the mean ± standard deviation (SD). Statistical analysis was performed using Student's t-test and correlation coefficients were determined using Pearson's method.

Evaluation of disease activity. The disease activity of IIP was evaluated by assessing changes in symptoms (Hugh-Jones classification), interstitial shadows of chest X-ray, and respiratory function (FVC) within the previous 3 months on admission. Exacerbation of more than 2 of these parameters was regarded as the active stage while no change or exacerbation of one parameter was regarded as non-active [4].

Results

Soluble ICAM-1 Levels in Serum. The serum level of soluble ICAM-1 in patients with IIP was 438.3 ± 89.3 ng/ml, which was significantly higher than the control value of 306.5 ± 66.4 ng/ml in healthy subjects. According to histologic type, serum levels of soluble ICAM-1 were as follows: 446.9 ± 65.6 ng/ml in patients with UIP, 495.9 ± 110.1 ng/ml in patients with NSIP and 372.1 ± 49.2 ng/ml in patients with BOOP. Serum levels of soluble ICAM-1 in both UIP and NSIP patients were significantly higher than those in healthy subjects (Fig. 1).

Cellular Component in BALF. The total cell density was 203.4 ± 158.0 × 10^3/ml in UIP patients, 349.2 ± 257.4 × 10^3/ml in NSIP patients and 348.7 ± 333.1 × 10^3/ml in BOOP patients. Although these values fluctuated, there was no significant difference among these different groups of patients.

Soluble ICAM-1 in BALF. The soluble ICAM-1 level in BALF from patients with IIP was 53.4 ± 35.5 ng/ml, which was higher than that from

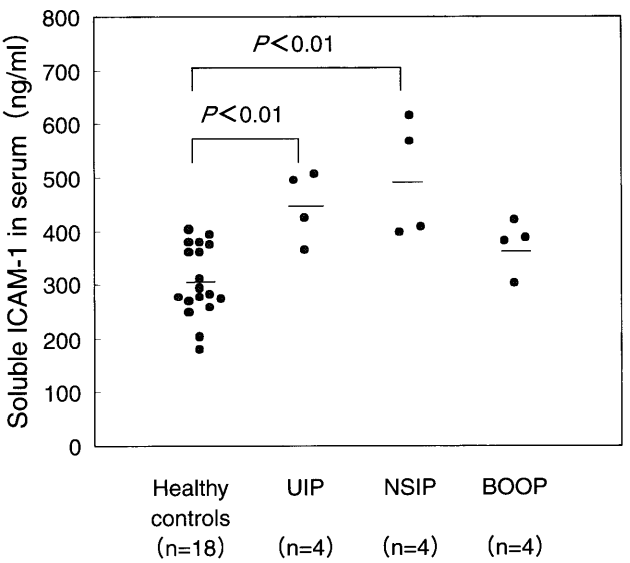

Fig. 1 Soluble ICAM-1 levels in serum of patients with IIP. Serum soluble ICAM-1 was significantly higher in patients with UIP or BOOP than in healthy subjects, and also high in patients with NSIP.

healthy controls (36.1 ± 13.7 ng/ml). Soluble ICAM-1 levels in BALF from the different IIP groups were as follows: 53.1 ± 19.1 ng/ml in UIP patients, 83.0 ± 48.9 ng/ml in NSIP patients and 25.4 ± 15.7 ng/ml in BOOP patients. BALF levels of soluble ICAM-1 in UIP and NSIP patients tended to be higher than those in healthy subjects (Fig. 2).

Correlation Between Soluble ICAM-1 in Sera and in BALF. A significant positive correlation ($r = 0.94$, $P < 0.01$) was also found between the soluble ICAM-1 levels in BALF and sera obtained simultaneously (Fig. 3).

Soluble ICAM-1 and Clinical Features. No significant correlation was seen between soluble ICAM-1 levels in sera or BALF and certain parameters of pulmonary function such as percent vital capacity (% VC) and arterial oxygen pressure (PaO_2). The serum soluble ICAM-1 in patients with long-term (≧ 12 months) illness was significantly higher than that in patients with short-term (< 12 months) illness (Fig. 4). Furthermore, the soluble ICAM-1 level in BALF from patients with long-term illness was significantly higher than that from patients with short-term illness (Fig. 5). No correlation was found between the serum levels of soluble ICAM-1 in IIP patients with active and non-active forms of the disease.

Immunohistochemical Staining of Lung Tissue. Localization of ICAM-1 in the lung tissues was investigated using immunohistochemical staining (Table 2). In control lung tissues, type I alveolar epithelia

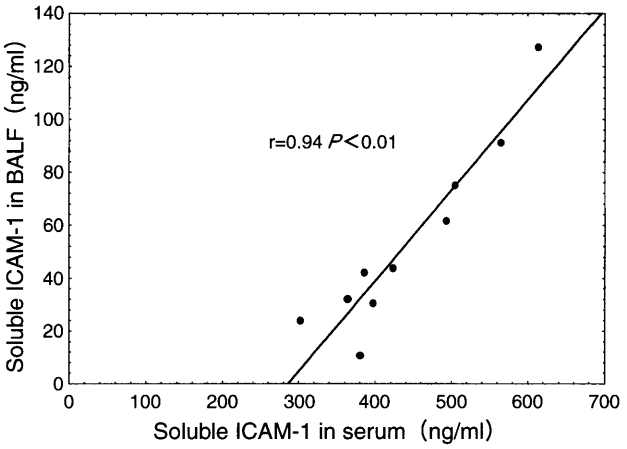

Fig. 3 Correlation between soluble ICAM-1 levels in BALF and serum of patients with IIP. A significant positive correlation is seen between the soluble ICAM-1 level in BALF and serum in patients with IIP.

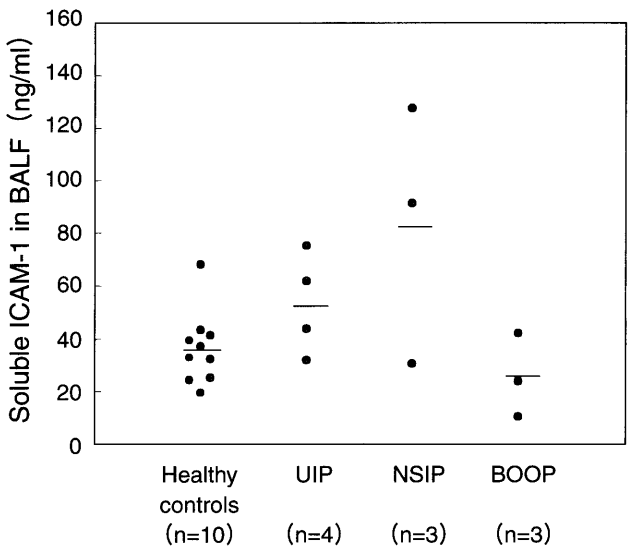

Fig. 2 Soluble ICAM-1 levels in BALF of patients with IIP. BALF soluble ICAM-1 tended to be higher in patients with UIP or NSIP than in normal controls.

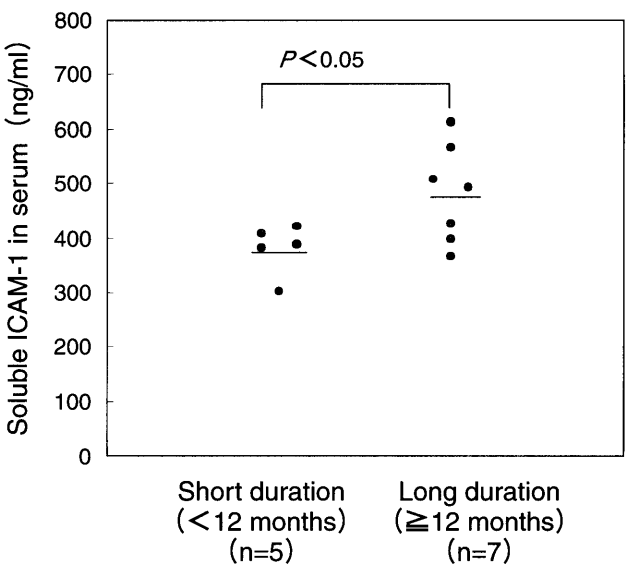

Fig. 4 Soluble ICAM-1 levels in serum of patients with long-term and short-term illness. Serum soluble ICAM-1 levels in patients with long-term illness was significantly higher than those in patients with short-term illness.

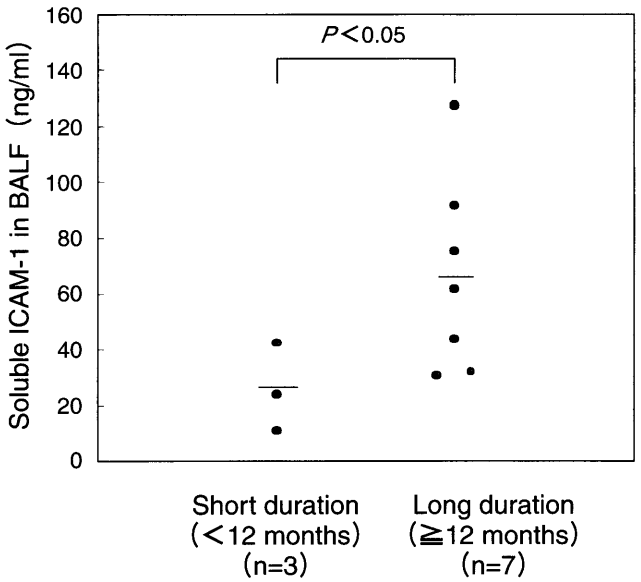

Fig. 5 Soluble ICAM-1 levels in BALF of patients with long-term and short-term illness. The soluble ICAM-1 levels in BALF from patients with long-term illness was significantly higher than those from patients with short-term illness.

were stained, while type II alveolar epithelia and vascular endothelial cells were not stained (Fig. 6-a). In the lung tissues of IIP patients, type I alveolar epithelia were not stained in patients with UIP, NSIP and BOOP, while type II alveolar epithelia were intensely stained (Fig. 6-b). Vascular endothelial cells in patients with UIP, NSIP, and BOOP were weakly stained. The regions of respiratory bronchioles and surrounding connective tissues were not stained. Type II alveolar epithelia were most strongly stained in UIP patients, when compared to NSIP or BOOP patients, while no difference was seen in the staining of vascular endothelial cells among them. Staining intensity tended to be stronger in IIP patients with higher levels of soluble ICAM-1 in BALF.

Discussion

Idiopathic interstitial pneumonia comprises diseases with different clinical courses and different histopathologic characteristics [5]. Most cases of IIP are classified pathologically into UIP [6], BOOP [7], or NSIP [8]. With any of these histologies, the etiology is unknown and the mechanism of the inflammatory process or fibrotic

Table 2 Distribution and localization of ICMA-1 in lung tissue of IIP as determined by immunohistochemitsry

Case	Type of histology	Pulmonary epithelium		Vascular endothelium	Bronchiolar epithelium	Interstitium
		Type II	Type II			
1	Control	I	0	0	0	0
2	Control	I	0	0	0	0
3	Control	I	0	0	0	0
4	Control	I	0	0	0	0
5	Control	I	0	0	0	0
6	UIP	0	II	II	0	0
7	UIP	0	III	II	0	0
8	UIP	0	III	II	0	0
9	UIP	0	III	II	0	0
10	NSIP	0	III	II	0	0
11	NSIP	0	II	II	0	0
12	NSIP	0	II	II	0	0
13	NSIP	0	II	II	0	0
14	BOOP	0	II	II	0	0
15	BOOP	0	II	II	0	0
16	BOOP	0	II	II	0	0
17	BOOP	0	II	II	0	0

Grade 0, negative; Grade I, weekly positive; Grade II, positive; Grade III, storongly positive.

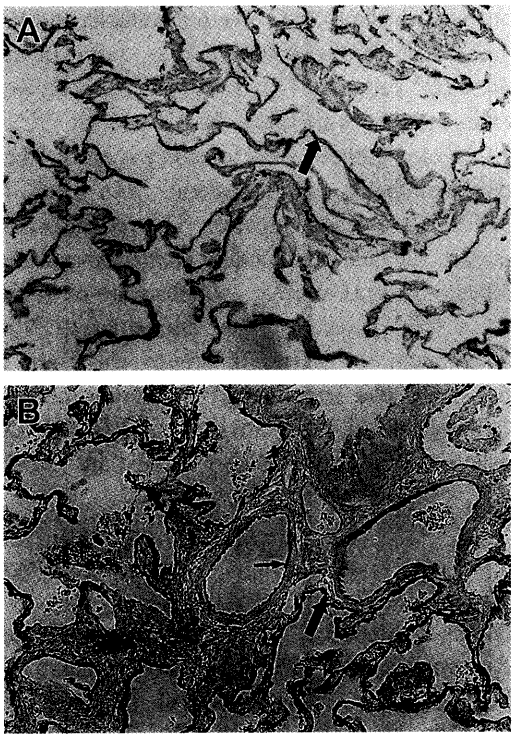

Fig. 6 A, photomicrograph of lung tissue obtained from control lung tissue and stained with the monoclonal antibody against ICAM-1. ICAM-1 was found to be weakly expressed on the surface of type I pulmonary epithelia (large arrow); B, photomicrograph of lung tissue obtained from UIP lung tissue and stained with the monoclonal antibody against ICAM-1. ICAM-1 was found to be strongly expressed on the surface of type II pulmonary epithelia (large arrow) and also expressed on vascular endothelial cells (small arrow).

changes has not been elucidated.

In 12 patients with IIP, soluble ICAM-1 was measured in serum and BALF and the expression of ICAM-1 in the lung tissues was investigated. The serum level of soluble ICAM-1 in all IIP patients was significantly higher than that in healthy controls. Shijubo [9] and Ashitani [10] also reported that the serum level of soluble ICAM-1 was higher in patients with interstitial pulmonary fibrosis (IPF) than in normal controls. Soluble ICAM-1 in BALF from all IIP patients also tended to be higher than that from normal controls. However, Ishii *et al.* [11] reported that soluble ICAM-1 in BALF from patients with clinically-diagnosed IPF was not different from that from healthy controls even after correction for the BAL recovery rate. These differences are probably due to heterogeneity in the stage and histologic type of IPF in the above studies.

In regard to the role of ICAM-1 in the inflammatory process of the lung, an increased level of soluble ICAM-1 in BALF was reported in bleomycin-induced pneumonia in rats [12]. In a mouse model, the lung tissues injured by high concentrations of oxygen also showed increased expression of ICAM-1 protein and mRNA [13]. In the immunohistochemical staining of the lung tissue in our study, a high expression of ICAM-1 was observed especially in alveolar type II epithelium of IIP patients. The higher expression of ICAM-1 in the lung tissue was related to the higher level of soluble ICAM-1 in BALF. The significant correlation between BALF and serum levels of soluble ICAM-1 in IIP patients suggests that ICAM-1 expression in the lung tissues may be a common source of soluble ICAM-1 in BALF and serum.

In the relation of soluble ICAM-1 in serum and BALF from IIP patients to their clinical courses, parameters of pulmonary functions and chest x-ray findings revealed that soluble ICAM-1 in serum and BALF was increased along with duration of illness, but not with disease activity. Vanhee [14] reported that ICAM-1 expression in vascular endothelial cells was transient after acute stimulation by a high concentration of TNF-α, while continuous expression of ICAM-1 was observed after chronic stimulation by a low concentration of TNF-α. Therefore, these observations suggest that increased expression of ICAM-1 and elevated levels of soluble ICAM-1 may be closely related to chronic and progressive processes of fibrosis rather than to acute inflammatory process. Immunohistochemical staining of ICAM-1 in the lung tissues revealed that only type I cells of the alveolar epithelium in the control were weakly stained. In contrast, type II cells were strongly stained with anti ICAM-1 antibody in IIP patients, while type I cells remained unstained. The vascular endothelial cells in UIP patients also showed weak staining of ICAM-1. Kasper *et al.* [12] reported that ICAM-1 was expressed in type I alveolar epithelia in control mice, while its expression in type II alveolar epithelia was increased in mice with bleomycin-induced pneumonia with fibrosis. In our data, the expression of ICAM-1 on type II alveolar epithelial cells was much stronger in UIP than in BOOP or NSIP patients. This observation is consistent with findings reported by Vanhee [14] and Kasper *et al.* [12], because the increase of type II epithelial cells is chronic in lung injury and the disease progression of UIP, whereas it is acute or subacute in the disease progression of BOOP or NSIP.

In conclusion, the increased ICAM-1 expression on type II alveolar epithelia in the lung tissue of patients with interstitial pneumonia could be a source of the increased soluble ICAM-1 in sera and BALF. ICAM-1 may play an important role in the persistent fibrotic changes of the lung and can be a useful marker for evaluating the progression of fibrosis.

References

1. Springer TA: Adhesion receptor of the immune system. Nature (1990) **346**, 425-434.
2. Rothlein R, Czajkowski M, O'Neill MM, Marlin SD, Mainolfi E and Merluzzi VJ: Induction of Intercellular adhesion molecule I on primary and continuous cell lines by pro-inflammatory cytokines. Regulation by pharmacologic agents and neutralizing antibodies. J Immunol (1988) **141**, 1665-1669.
3. Butcher EC: Leukocyte-endothelial cell recognition: Three (or more) steps to specificity and diversity. Cell (1991) **67**, 1033-1036.
4. Matsuo K, Tada S, Shibayama T, Ueno Y, Miyake T, Takehara H, Kataoka M, Harada M and Kimura I: Factors affecting prognosis of idiopathic interstitial pneumonia. Acta Med Okayama (1996) **50**, 37-46.
5. Nagai S, Kitaichi M and Izumi T: Idiopathic interstitial pneumonia: Profiles of the subsets, and prognosis. Nihon Kyobu Shikkan Gakkai Zasshi (1996) **34**, S200-S203.
6. Carrington CB, Gaensler EA, Coutu RE, FitzGerald MX and Gupta RG: Natural history and treated course of usual and desquamative interstitial pneumonia. N Engl J Med (1978) **298**, 801-809.
7. Epler GR, Colby TV, McLoud TC, Carrington CB and Gaensler EA: Bronchiolitis obliterans organizing pneumonia. N Engl J Med (1985) **312**, 152-158.
8. Katzenstein AL and Fiorelli RF: Nonspecific interstitial pneumonia/fibrosis. Histologic features and clinical significance. Am J Surg Pathol (1994) **18**, 136-147.
9. Shijubo N, Imai K, Aoki S, Hirasawa M, Sugawara H, Koba H, Tsujisaki M, Sugiyama T, Hinoda Y, Yachi A, Asakawa M and Suzuki A: Circulating intercellular adhesion molecule-1 (ICAM-1) antigen in sera of patients with idiopathic pulmonary fibrosis. Clin Exp Immunol (1992) **89**, 58-62.
10. Ashitani J, Mukae H, Ihiboshi H, Hiratsuka T, Taniguchi H, Mashimoto H and Matsukura S: Serum-soluble adhesion molecules in patients with idiopathic pulmonary fibrosis and acute respiratory distress syndrome. Nihon Kyobu Shikkan Gakkai Zasshi (1997) **35**, 942-947.
11. Ishii Y and Kitamura S: Elevated levels of soluble ICAM-1 in serum and BAL fluid in patients with active sarcoidosis. Chest (1995) **107**, 1636-1640.
12. Kasper M, Koslowski R, Luther T, Schuh D, Muller M and Wenzel KW: Immunohistochemical evidence for loss of ICAM-1 by alveolar epithelial cells in pulmonary fibrosis. Histochem Cell Biol (1995) **104**, 397-405.
13. Welty SE, Rivera JL, Elliston JF, Smith CV, Zeb T, Ballantyne CM, Montgomery CA and Hansen TN: Increases in lung tissue expression of intercellular adhesion molecule-1 are associated with hyperoxic lung injury and inflammation in mice. Am J Respir Cell Mol Biol (1993) **9**, 393-400.
14. Vanhee D, Delneste Y, Lassalle P, Gosset P, Joseph M and Tonnel AB. Modulation of endothelial cell adhesion molecule expression in a situation of chronic inflammatory stimulation. Cell Immunol (1994) **155**, 446-456.