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Serum Levels of Soluble L-Selectin and Interstitial Changes in Patients with IgA Nephropathy

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The purpose of the present study was to assess the correlations among expression of L-selectin in renal tissues, serum levels of soluble L-selectin, and renal injuries in patients with IgA nephropathy (IgAN). The serum levels of soluble L-selectin from 37 IgAN patients and 30 healthy controls were measured by the human soluble L-selectin immunoassay. To examine the localization of L-selectin, we immunohistochemically stained three serial sections from all biopsy specimens of 37 IgAN patients. The expression of L-selectin in renal tissues was detected by L-strepto-avidin biotin method. We observed marked expression of L-selectin in the cells infiltrated into interstitium, but not in those without interstitial cell infiltration. The serum levels of soluble L-selectin in IgAN patients (1.2 ± 0.2 μg/ml) was significantly higher than those in healthy controls (0.8 ± 0.1 μg/ml). There was a significant positive correlations between grade of interstitial cell infiltration and serum levels of L-selectin ($r = 0.44$, $p < 0.01$). However, there was no significant correlation between serum levels of soluble L-selectin and clinical parameters such as extent of proteinuria, creatinine clearance and serum IgA levels. These findings suggest that L-selectin may play some roles in the interstitial changes and measurement of serum soluble L-selectin may be useful to evaluate the extent of interstitial cell infiltration in IgAN patients.

Key words: IgA nephropathy, L-selectin, interstitial injury, cell infiltration, ELISA

Introduction

IgA nephropathy (IgAN) is generally recognized worldwide as one of the most common primary glomerulonephritides and characterized by glomerular mesangial deposition of IgA and C3 in renal specimens[1]. Surveys of patients with primary glomerulonephritis that were conducted in 1985 and 1993 by the Research Group on Progressive Renal Diseases in Japan revealed a high prevalence of IgAN and a relatively poor prognosis for patients[2]. Accumulation of leukocytes within the glomerulus and interstitium of the kidney is now accepted as one of the key pathogenetic mechanisms in both experimental and human glomerulonephritis[3]. The three main groups of adhesion molecules involved in leukocyte-endothelial cell interactions are the selectins, the integrins and cetain members of the immuno-

globulin supergene family. At least, three selectins have been characterized. E-selectin is expressed predominantly by cytokine activated endothelium, L-selectin by circulating leukocytes and P-selectin by activated endothelial cells and platelets[4]. Chaudhury et al[5] reported a marked increase in E-selectin and P-selectin expression on the extraglomerular vascular endothelium, with prominent interstitial infiltrates, in some biopsy samples from IgAN patients. They also showed a large number of L-selectin-positive cells were present within glomerular and interstitial infiltrates in affected kidneys. However, the role of the selectin family on the progression of IgAN has not been fully understood.

The purpose of the present study was to evaluate the correlation among the expression of L-selectin in renal tissues, serum levels of soluble L-selectin, and renal injuries in IgAN patients.

Materials and Methods

Patient

Thirty-seven IgAN patients (18 males and 19 females) were examined in this study. The mean age at renal biopsy was 38.4 ± 2.2 years. Routine microscopic, electron microscopic, and immunofluorescence analyses were performed for the diagnosis of IgAN. Patients whose biopsy specimens stained predominently for IgA in the glomerular mesangial areas were included in this study after the exclusion of patients with systemic lupus erythematosus, Henoch-Schönlein purpura, cirrhosis, or other systemic diseases. The clinical and laboratory data of the 37 patients at renal biopsy were evaluated in regard to disease duration (months), blood pressure (mmHg), urinary red cell sediment (count/high power field), 24-hour urinary protein excretion (g/day), 2 hour-creatinine clearance (ml/min), and serum IgA levels (mg/dl). Informed consent was obtained prior to the study. All serum samples obtained from patients without an infectious process were stored at −20 ℃ until assay.

Thirty age-matched healthy controls were enrolled in this study for measurement of serum levels of soluble L-selectin.

Histological examination

The histological findings of IgAN were assessed by two renal pathologists without any knowledge of the clinical data. Each specimens were examined light microscopically for glomerular, interstitial, and vascular changes as previously described[6]. The percentage of glomeruli exhibiting glomerular obsolescence, crescent formation, and glomerular tuft adhesion to Bowman's capsule were examined. Interstitial changes were evaluated on the basis of inflammatory cell infiltration was graded into four degrees of severity: grade 0, no abnormality; grade 1, mild; grade 2, moderate; grade 3, severe. Vascular changes concerning arterio- and arteriolosclerosis were also evaluated using a similar grading scale: grade 0, no abnormality; grade 1, mild; grade 2, moderate; grade 3, severe.

Serial frozen sections of 2-μm thickness were prepared from 37 cases of IgAN. As the normal control, we used renal tissues obtained from 5 patients with minor glomerular abnormality. As previously described[7], for immunohistochemistry to detect L-selectin, 2-mm sections were incubated with a monoclonal antibody to human L-selectin (CD62L, DAKO Japan, Kyoto, Japan) at a dilution of 1: 40 for 60 min at room temperature. After washing, the sections were stained using LSAB method and observed using a light microscopy. Negative controls comprised both normal and diseased kidneys in which the primary antibody was omitted or in which an irrelevant antibody was used instead of antibody to L-selectin.

Measurement of serum soluble L-selectin concentration

To quantify L-selectin levels in the serum, enzyme-linked immunoadsorbent assay (ELISA)

Table 1 Clinical findings of patients with IgA nephropathy

Age (years)	38.4 ± 2.2	(15 ~ 68)
Sex (males/females)	18/19	
Duration at renal biopsy (months)	94.5 ± 15.8	(0 ~ 408)
Blood pressure (mmHg)		
Systoric	125.5 ± 2.8	(94 ~ 176)
Diastoric	78.5 ± 1.5	(63 ~ 100)
Urinary protein (g/day)	1.3 ± 0.2	(0.1 ~ 3.7)
Urinary red blood cells (/HPF)	206.5 ± 64.0	(1 ~ 1,000)
Creatinine clearance (ml/min)	84.0 ± 4.5	(40.1 ~ 149.0)
Serum IgA (mg/dl)	324.8 ± 29.4	(112 ~ 1,250)

was applied according to the manufacturer's protocols (R & D Systems, Minneapolis, MN, USA). Briefly, the samples of serum were diluted to 1: 100 with sample buffer. A murine monoclonal anti-human antibody specific for L-selectin was precoated onto a microtiter plates. Standards and samples were pipetted into the wells and any L-selectin present were bound by the immobilized antibody. After being washed away any unbound compounds, an enzyme linked polyclonal antibody specific for human L-selectin was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of L-selectin bound in the initial step. The color reaction was stopped and the intensity of the color was measured. Results were expressed as μg/ml. The lower detection limit of this assay was less than 0.3 ng/ml.

Statistical analysis

Data are presented mean ± SEM. Statistical analyses were performed using commercially available personal computer software, Stat-View 4.5. Mann-Whitney U test was employed for analyzing differences between the two groups. Pearson's correlation analysis was used to observe the relationship between serum L-selectin concentration and other clinical or pathological parameters. P less than 0.05 is considered statistically significant.

Table 2 Pathological findings of patients with IgA nephropathy

Glomerus		
Global sclerosis (%)	24.3 ± 3.8	(0 ~ 76)
Crescent (%)	9.9 ± 2.1	(0 ~ 50)
Adhesion (%)	23.3 ± 3.4	(0 ~ 75)
Interstitium		
Fibrosis (0 ~ 3)	1.5 ± 0.2	-
Cell infiltration (0 ~ 3)	1.4 ± 0.3	-
Vascular change (0 ~ 3)	0.8 ± 0.2	-

Results

Clinical and pathological findings of IgAN patients

Table 1 shows the clinical and laboratory data of 37 patients. Disease duration at renal biopsy ranged from 0 to 408 months (mean: 94.5 ± 15.8 months). Urinary protein loss ranged from 0.1 g/day to 3.7 g/day, with the mean being 1.3 ± 0.2 g/day. Creatinine clearance was from 40.1 to 149.0 ml/min, with the mean being 84.0 ± 4.5 ml/min, so renal function varied from normal to renal failure.

Table 2 demonstrates the results of histological findings in IgAN patients enrolled in this study. The percentages of glomerular changes were global sclerosis: 24.3 ± 3.8%, crescent: 9.9 ± 2.1%, adhesion: 23.3 ± 3.4%, and the grades of interstitial changes were fibrosis: 1.5 ± 0.2, cell infiltration: 1.4 ± 0.3, vascular change: 0.8 ± 0.2.

Immunohistochemical localization of L-selectin in IgAN patients

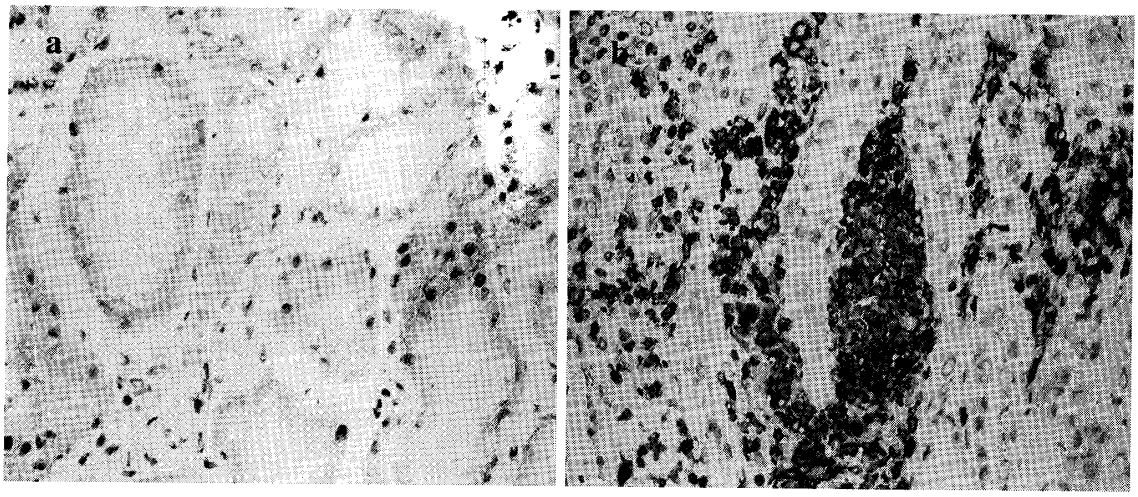

Fig. 1 Immunohistochemical localization of L-selectin in a control kidney (a) and in a kidney from a IgAN patient (b), ×200.

To examine the localization of L-selectin, we immunohistochemically stained three serial sections from all biopsy specimens of 37 IgAN patients. A few numbers of L-selectin-positive cells were present in the interstitium in a control kidney (Fig. 1a). In an IgAN patient, the expression of L-selectin was remarkably increased in periglomerular region, interstitium, and in focal cellular infiltrates (Fig. 1b).

Serum concentration of soluble L-selectin in IgAN patients

As shown in Fig. 2, a significant increase in serum concentrations of soluble L-selectin was found in IgAN patients (1.2 ± 0.2 μg/ml) as compared with healthy controls (0.8 ± 0.1 μg/ml) ($p < 0.05$).

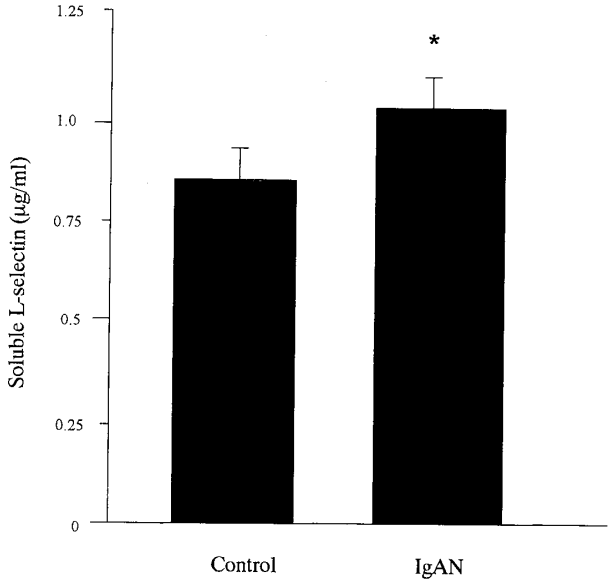

Fig. 2 Serum concentrations of soluble L-selectin in IgAN patients (n = 37) and in age-matched healthy controls (n = 30)
Results represent the mean ± SEM, *$p < 0.05$ vs. controls.

Correlation of serum soluble L-selectin concentrations with hematological parameters in IgAN patients

To evaluate the significance of serum L-selectin concentration on renal function in IgAN patients, we performed a simple regression analysis between serum concentration of soluble L-selectin and other clinical parameters. There was no correlation between serum concentrations of soluble L-selectin and clinical parameters such as grade of urinary red cell sediment ($r = -0.127$, $p = 0.4619$), and serum IgA levels ($r = -0.135$, $p = 0.432$). As shown in Fig. 3 and Fig. 4, no significant correlation was detected between serum concentrations of soluble L-selectin and renal functions estimated by Ccr ($r = -0.158$, $p = 0.3604$) and proteinuria ($r = 0.087$, $p = 0.6143$).

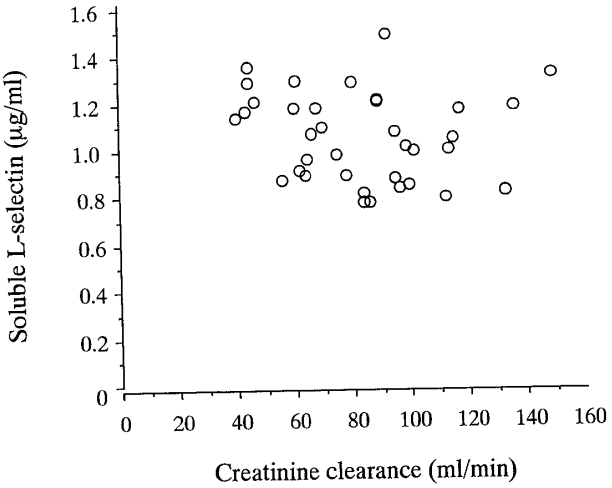

Fig. 3 Correlation between serum concentration of soluble L-selectin and creatinine clearance (Ccr) in 37 IgAN patients.

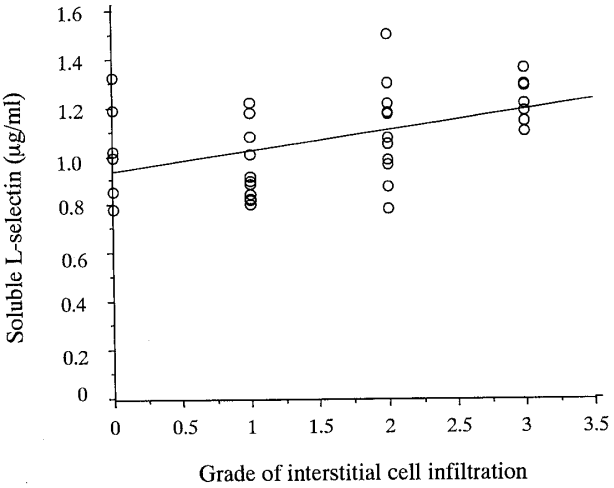

Fig. 5 Relationship between serum concentration of soluble L-selectin and grade of interstitial cell infiltration in 37 IgAN patients.

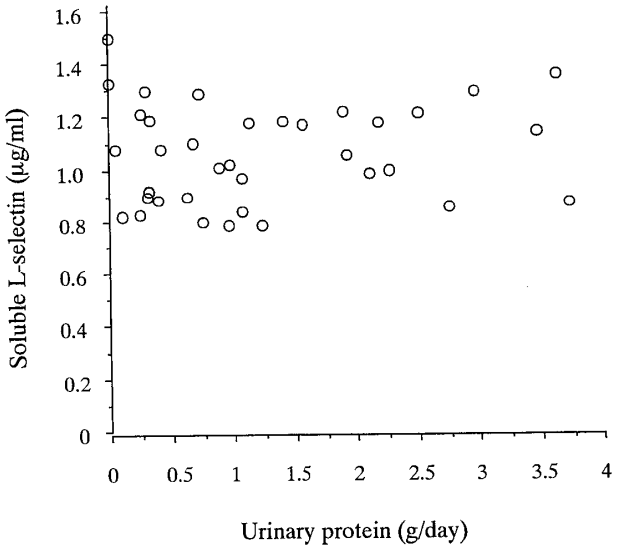

Fig. 4 Correlation between serum concentration of soluble L-selectin and urinary protein excretion (Up) in 37 IgAN patients.

Relationship between serum soluble L-selectin concentration and histological parameters in IgAN patients

To confirm the role of L-selectin on histological injury in IgAN patients, we examined the correlation between serum concentration of soluble L-selectin and histological parameters in 37 IgAN patients. As shown in Fig. 5, there was a positive correlation between serum concentration of soluble L-selectin and grade of interstitial cell infiltration ($r = 0.44$, $p < 0.01$). There was no significant correlation between serum concentration of soluble L-selectin and extent of other histological parameters such as crescent formation ($r = -0.099$, $p = 0.569$) and adhesion ($r = -0.039$, $p = 0.8236$) in glomeruli and interstitial fibrosis ($r = 0.215$, $p = 0.078$).

Discussion

In the present study we found that the expression of L-selectin was remarkably increased in periglomerular region, interstitium, and in focal cellular infiltrates in IgAN patients. Moreover, a significant increase in serum concentration of soluble L-selctin in IgAN patients as compared with healthy controls. There was a positive correlation between serum concentration of soluble L-selectin and grade of interstitial cell infiltration. These findings suggest that L-selectin may play some roles in the interstitial changes in IgAN patients.

Infiltration of leukocytes into glomerular and interstitial regions of the kidney is a key event in the pathogenesis of human glomerulonephritis.

This process is mediated by specific adhesion molecules, some of which are expressed in a coordinated fashion following endothelial cell activation. L-selectin plays a critical role in the attachment of leukocytes to activated endothelium[8]. This adhesion molecule is constitutively expressed on leukocytes, including granulocytes, monocytes and most lymphocytes[9], and is lost at the end of cell activation[10]. As previously reported by Chaudhury et al[5], biopsies with advanced IgAN with significant interstitial cell infiltration and strong expression of L-selectin in the interstitial infiltrates. On the other hand, biopsies from patients with early IgAN without interstitial infiltrates had negligible expression of the L-selectin. Regardless of the nature and severity of the initial insult, there may be a common pathway that is responsible for progressive renal dysfunction followed by interstitial changes in IgAN. This could involve altered adhesion molecule expression.

A recent study[11] reported higher proportions of T and B lymphocytes expressing higher amounts of L-selectin in IgAN patients. Conversely, serum levels of soluble L-selectin were not different from controls, but significantly higher than serum levels in patients suffering from other renal diseases. They suggested that this over-expression of L-selectin might be involved in an enhanced efficiency of lymphoid cell homing to lymphoid tissues in IgAN. The present study has shown a significant increase in serum concentration of soluble L-selectin in IgAN patients as compared with healthy controls. Even though the causes of the discrepancy are not obvious, patient populations of the two studies may be different, especially concerning the grade of interstitial changes.

Previous reports showed that the L-selectin ligands detected by a rat L-selectin and human IgG chimeric molecule (rLEC-IgG) were expressed in the distal tubules of kidneys, whereas no leukocyte traffic was seen under physiological conditions in animal models. The role of L-selectin ligands in leukocyte infiltration into the kidney interstitium was investigated using an ureteric obstruction model[12)~14)]. Interstitial infiltration of mononuclear cells was observed not only in obstructive nephropathy, but also in glomerulonephritis including IgAN and rejection of transplanted kidney[15]. Infiltrating inflammatory cells were likely to contribute to the progression of renal tissue injury[16)17)]. Thus, a therapeutic strategy based on blocking L-selectin-ligand interactions might be an effective new treatment for renal inflammatory diseases. Indeed, mononuclear cell infiltration was significantly inhibited by intravenous injection of a neutralizing monoclonal antibody against L-selectin, indicating the possible involvement of a L-selectin-mediated pathway[12].

In this study, we have clearly shown that elevated serum concentration of soluble L-selectin is significantly correlated with grade of interstitial cell infiltration. Taken together, L-selectin may play some roles in the interstitial changes and measurement of serum soluble L-selectin may be useful to evaluate the extent of interstitial cell infiltration in IgAN patients.

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References

1) **D' Amico G**: The commonest glomerulonephritis in the world: IgA nephropathy. Q J Med **245**: 707–727, 1987
2) **Koyama A, Igarashi M, Kobayashi M**: Natural history and risk factors for immunoglobulin A nephropathy in Japan. Am J Kidney Dis **29**: 526–532, 1997
3) **Brady HR**: Leukocyte adhesion molecules. Kidney Int **45**: 1285–1300, 1994

4) **Adler S, Brady HR**: Cell adhesion molecules and the glomerulopathies. Am J Med **107**: 371–386, 1999
5) **Roy-Chaudhury P, Wu B, King G et al**: Adhesion molecule interactions in human glomerulonephritis: Importance of the tubulointerstitium. Kidney Int **49**: 127–134, 1996
6) **Taneda S, Honda K, Nitta K et al**: Clinicopathological study of IgA nephropathy in adults: The influence of onset age on clinical and renal histological findings and on the effect of steroid therapy. Clin Exp Nephrol **3**: 96–103, 1999
7) **Nitta K, Horita S, Okano K et al**: Tubular osteopontin expression in patients with ANCA-associated glomerulonephritis. Clin Nephrol **56**: 459–466, 2001
8) **Osborn L**: Leukocyte adhesion to endothelium in inflammation. Cell **62**: 3–6, 1990
9) **Gallatin WM, Weissman IL, Butcher EC**: A cell surface molecule involved in organ-specific homing of lymphocytes. Nature **304**: 30–34, 1983
10) **Kanof ME, James SP**: Leu 8 antigen is diminished during cell activation but does not correlate with effector function of activated T lymphocytes. J Immunol **140**: 3701–3706, 1988
11) **Kennek-de March MN, Bene MC, Renoult E et al**: Enhanced expression of L-selectin on peripheral blood lymphocytes in patients with IgA nephropathy. Clin Exp Immunol **115**: 542–546, 1999
12) **Watanabe T, Song Y, Hirayama Y et al**: Sequence and expression of a rat cDNA for LECAM-1. Biochem Biophys Acta **1131**: 321–324, 1992
13) **Tamatani T, Kuida K, Watanabe T et al**: Molecular mechanisms underlying lymphocyte recirculation. III. Characterization of the LECAM-1 (L-selectin)-dependent adhesion pathway in rats. J Immunol **150**: 1735–1745, 1993
14) **Shikata K, Suzuki Y, Wada J et al**: L-selectin and its ligands mediate inflitration of mononuclear cells into kidney interstitium after ureteric obstruction. J Pathol **188**: 93–99, 1999
15) **Poeter KA**: Renal transplantation. *In* Pathology of the Kidney (Heptinstall RH ed), pp1799–1934, Little Brown, Boston (1992)
16) **Saito T, Atkins RC**: Contribution of mononuclear cells to the progression of experimental focal glomerular sclerosis. Kidney Int **37**: 1076–1083, 1990
17) **McClusky RT**: Immunologic aspects of renal disease. *In* Pathology of the Kidney (Heptinstall RH ed), pp169–260, Little Brown, Boston (1992)

IgA 腎症における可溶性 L-セレクチンの血清濃度と腎間質病変

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本研究の目的は，IgA 腎症患者における腎組織における L-セレクチン発現および可溶性 L-セレクチンの血清濃度と腎間質病変との関連性を検討することである．対象は，腎生検の結果と臨床所見から IgA 腎症と診断された 37 例（男性 18 例，女性 19 例）で，平均年齢は 38.4±2.2 歳である．可溶性 L-セレクチンの血清濃度は，市販の ELISA キットを用いて測定した．腎生検組織における L-セレクチンの発現は，凍結切片を用いて LSAB 法で免疫染色して確認した．IgA 腎症患者における可溶性 L-セレクチンの血清濃度（1.2±0.2 μg/ml）は，年齢および性別を一致させた健常者のそれ（0.8±0.1 μg/ml）に比し有意に高値を示した．腎生検組織における L-セレクチンは，間質における浸潤細胞に多量に発現していた．血清 L-セレクチン濃度は，間質における細胞浸潤の程度と正の相関を示したが，腎機能や蛋白尿量との間には有意な相関性は認められなかった．これらの結果から，IgA 腎症患者において，L-セレクチンは間質への細胞浸潤に関与していると推測された．また，可溶性 L-セレクチンの血清濃度の測定は，腎間質病変の存在を予想するための指標になると考えられた．