

Elevated serum L-selectin levels and abnormal regulation of L-selectin expression on leukocytes in atopic dermatitis: Soluble L-selectin levels indicate disease severity

Yuka Shimada, MD,^a Shinichi Sato, MD, PhD,^a Minoru Hasegawa, MD, PhD,^a
Thomas F. Tedder, PhD,^b and Kazuhiko Takehara, MD, PhD^a Kanazawa, Japan, and
Durham, NC

Background: L-selectin mediates leukocyte rolling on endothelium at sites of inflammation, suggesting that L-selectin may be involved in the development of cutaneous lesions of atopic dermatitis (AD). After leukocyte activation, L-selectin is rapidly shed from the cell surface.

Objective: The purpose of this study was to assess leukocyte L-selectin expression and quantitate levels of serum soluble L-selectin (sL-selectin) in patients with AD.

Methods: Serum sL-selectin levels in patients with AD (n = 70), contact dermatitis (n = 18), and psoriasis (n = 23), as well as normal control subjects (n = 30), were examined by using an ELISA. The L-selectin expression on leukocytes in heparinized blood samples from patients with AD (n = 18) and normal control subjects (n = 10) was also examined by flow cytometry.

Results: Serum levels of sL-selectin in patients with AD were significantly higher than those found in normal control subjects. Furthermore, sL-selectin levels correlated positively with disease severity and total serum IgE levels in AD. The expression of L-selectin on B cells, monocytes, and neutrophils was significantly decreased in patients with AD compared with normal control subjects, although those on CD4⁺ or CD8⁺ T cells from patients with AD were similar to those from normal control subjects.

Conclusion: Elevated sL-selectin levels and the abnormal expression of L-selectin on some leukocyte subsets in patients with AD may correlate with inflammation associated with AD. Furthermore, the level of sL-selectin may be a useful immunologic indicator for disease activity in AD. (*J Allergy Clin Immunol* 1999;104:163-8.)

Key words: L-selectin, atopic dermatitis, soluble L-selectin, leukocyte rolling, leukocyte activation

The development of cutaneous lesions in atopic dermatitis (AD) is closely related to the accumulation of specific leukocytes migrating from blood into the affected skin through the endothelium. The skin lesions in AD are characterized histologically by infiltration of activated T cells, predominantly CD4⁺ T cells, and monocyte/macrophages.^{1,2} However, abnormalities in the regulation of the migration of leukocytes into the skin lesions of AD are not fully understood.

Leukocyte trafficking between blood and skin is dependent on the expression of numerous cell surface adhesion molecules, including members of the selectin families.³ L-selectin (CD62L), a member of the selectin family, is expressed on most circulating leukocytes, including neutrophils, monocytes, eosinophils, basophils, and most lymphocytes.³ L-selectin has been suggested to interact with inducible ligand(s) on the nonlymphoid vascular endothelium, thereby mediating the initial step (rolling) of leukocyte migration.^{3,4} Recent studies that used L-selectin-deficient mice have demonstrated that L-selectin plays a functionally significant role in cutaneous inflammation because L-selectin-deficient mice have impaired delayed hypersensitivity responses⁵ and delayed allogeneic skin graft rejection.⁶

After leukocyte activation, cell surface L-selectin is rapidly modulated, which results in an increased affinity for its ligand, followed by the endoproteolytic release of L-selectin.⁷⁻⁹ Soluble L-selectin (sL-selectin) is functionally active and is present at high levels in human plasma.^{9,10} Levels of sL-selectin are elevated in patients with sepsis, HIV, Kawasaki disease, hemodialysis, and leukemia, although levels of sL-selectin are decreased in the serum of patients in whom adult respiratory distress syndrome develops.¹⁰⁻¹⁴ Although the biologic significance of shedding of L-selectin from the cell surface and circulating sL-selectin remains unclear, it is likely that circulating sL-selectin serves as a biologic buffer system

From ^athe Department of Dermatology, Kanazawa University School of Medicine, Kanazawa; and ^bthe Department of Immunology, Duke University Medical Center, Durham.

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Reprint requests: Shinichi Sato, MD, PhD, Department of Dermatology, Kanazawa University School of Medicine, 13-1 Takaramachi, Kanazawa, Ishikawa 920-8641, Japan.

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Abbreviations used

AD:	Atopic dermatitis
LDH:	Lactate dehydrogenase
NK:	Natural killer
sL-selectin:	Soluble L-selectin
TBS:	Tris-buffered saline solution

to prevent leukocyte rolling at sites of subacute inflammation.^{3,9} Thus the combination of regulated L-selectin expression with regulated levels of circulating sL-selectin is likely to play a major role in directing leukocyte migration.

In this study we examined serum levels of sL-selectin and L-selectin expression on peripheral blood leukocytes in patients with AD. Our data suggest that the elevated sL-selectin levels and abnormal L-selectin expression on some leukocyte subsets in patients with AD correlate with inflammation associated with AD and that the serum level of sL-selectin is a serologic indicator of disease severity in AD.

METHODS**Patients and control subjects**

Serum levels of sL-selectin were examined in 70 patients with AD (28 female, 42 male; age, 5 to 42 years; mean age, 22.8 ± 6.5 years). All patients fulfilled the criteria for AD proposed by Hanifin and Rajka.¹⁵ The clinical severity of AD was evaluated with the scoring system proposed by Rajka and Langeland¹⁶: 15 patients had mild-type AD; 27 patients had moderate-type AD; and 28 patients had severe-type AD. The clinical and laboratory data and serum samples were obtained at the same time. The laboratory data used in this study were serum total IgE levels, blood eosinophil numbers, and lactate dehydrogenase (LDH). All patients were treated with topical steroids in combination with oral antiallergic drugs. However, none of the patients were treated with systemic steroids. In addition, patients with contact dermatitis ($n = 18$) and psoriasis vulgaris ($n = 23$) were examined. The control subjects were 30 healthy age- and sex-matched individuals. Serum specimens were aliquoted and kept frozen at -40°C before use.

L-selectin expression levels by peripheral blood leukocytes were examined in 18 patients with AD (10 female, 8 male; age, 14 to 33 years; mean age, 25.0 ± 5.1 years). The patients consisted of 8 moderate-type cases and 10 severe-type cases. Ten healthy age- and sex-matched individuals were used as normal control subjects.

The protocol was approved by the Committee at Kanazawa University School of Medicine and Kanazawa University Hospital, and informed consent was obtained from all patients.

ELISA for sL-selectin

The serum levels of sL-selectin were measured with an ELISA, as described previously.¹⁰ Briefly, 96-well microtiter plates (Costar, Cambridge, Mass) were coated with an anti-L-selectin mAb (LAM1-5) in 0.1 mol/L borate buffer, pH 8.4, at 4°C for 18 hours. After 2 washes with Tris-buffered saline solution (TBS), the wells were blocked with 2% BSA and 1% gelatin in TBS for 1 hour at 37°C . After washes with TBS containing 0.05% Tween 20, serum samples diluted 1:40 were incubated in triplicate wells for 90 minutes at 20°C . Each assay included a previously quantified serum sample for generating a standard curve. After being washed with TBS containing 0.05% Tween 20, the plates were incubated with

biotinylated anti-LAM1-3 mAb ($1 \mu\text{g/mL}$) for 60 minutes at 20°C and then with avidin-horseradish peroxidase (Sigma Chemical Co, St Louis, Mo) for 30 minutes at 4°C . The plates were developed with *o*-phenylenediamine (Sigma Chemical Co) as a substrate in 0.1 mol/L citrate buffer, pH 4.5, in the presence of H_2O_2 . The plates were read at 450 nm in a Titertek plate reader (Flow Laboratories, McLean, Va). The relative concentration of sL-selectin in individual samples was calculated by a comparison of the mean optical density to a semilog standard curve of the titrated standard serum sample by linear regression analysis.

Flow cytometric analysis

Heparinized blood samples were immediately placed on ice at 4°C to avoid the spontaneous shedding of L-selectin from leukocytes. Phycoerythrin-conjugated mAb to L-selectin was purchased from Coulter Corp (Miami, Fla). FITC-conjugated anti-CD4, CD8, CD20, CD14, and CD16 mAbs and isotype-matched control mAbs were also purchased from Coulter Corp. The blood samples were stained at 4°C with predetermined optimal concentrations of the test mAb for 20 minutes, as previously described.¹⁷ Blood erythrocytes were lysed after staining with the Coulter Whole Blood Immunolysis kit as detailed by the manufacturer (Coulter Corp). Cells were washed and analyzed with a FACScan flow cytometer (Becton Dickinson, San Jose, Calif). Cells with the forward and side light scatter properties of lymphocytes, monocytes, and granulocytes were analyzed for each sample. Five thousand cells were analyzed for each sample, with fluorescence intensity shown on a 4-log scale. Fluorescence histograms are shown as 50% log density plots. Positive and negative populations of cells were determined with unreactive isotype-matched mAbs (Coulter Corp) as control cells for background staining. Background levels of staining were delineated with gates positioned to include 98% of the control cells.

Statistical analysis

Mann-Whitney *U* tests were used to compare variables between 2 groups because most of the data were not normally distributed. Spearman's rank correlation coefficient was used to examine the relationship between 2 continuous variables. Probability (*P*) values of less than .05 were considered statistically significant. All data are shown as means $\pm$ SD.

RESULTS**Serum sL-selectin levels in patients with AD**

Patients with contact dermatitis and psoriasis vulgaris showed mean serum sL-selectin levels similar to those of control subjects (Fig 1). By contrast, sL-selectin levels in patients with AD were significantly higher than those found in normal control subjects ($1.54 \pm 1.25 \mu\text{g/mL}$ vs $0.99 \pm 0.45 \mu\text{g/mL}$; $P < .05$). Patients with severe AD showed significantly increased mean levels of sL-selectin (Fig 2) when compared with patients with moderate AD ($1.93 \pm 1.34 \mu\text{g/mL}$ vs $1.32 \pm 0.65 \mu\text{g/mL}$; $P < .05$) and mild AD ($1.93 \pm 1.34 \mu\text{g/mL}$ vs $0.98 \pm 0.26 \mu\text{g/mL}$; $P < .01$). Similarly, patients with moderate AD had significantly elevated mean sL-selectin levels compared with patients with mild AD ($1.32 \pm 0.65 \mu\text{g/mL}$ vs $0.98 \pm 0.26 \mu\text{g/mL}$; $P < .01$). sL-selectin levels in patients with mild AD and control subjects were similar. Thus elevated mean serum levels of sL-selectin correlated with the severity of AD. A significant correlation was found between sL-selectin levels and total serum IgE levels in patients with AD ($r = 0.495$; $P < .0005$; Fig 3). However,

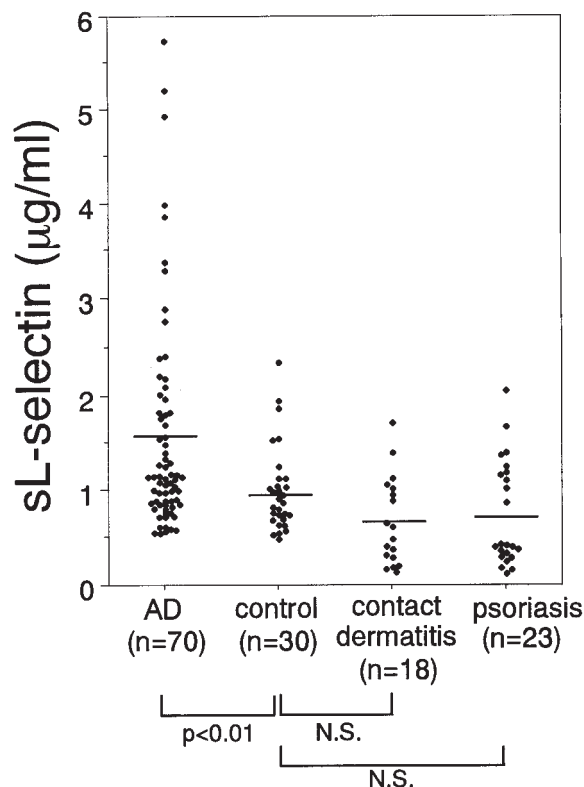


FIG 1. Serum levels of sL-selectin in patients with AD, contact dermatitis, and psoriasis. sL-selectin levels were determined by ELISA. N.S., Not significantly different.

sL-selectin levels did not correlate with blood eosinophil numbers or LDH.

Frequency of L-selectin⁺ blood leukocytes in patients with AD

The frequency of L-selectin-expressing leukocytes from patients with AD was assessed. Although a subpopulation of CD4⁺ or CD8⁺ T cells was L-selectin⁻ in both patients with AD and normal control subjects, the frequency of L-selectin⁺ cells among CD4⁺ or CD8⁺ T cells from patients with AD was similar to that found in normal control subjects (Fig 4). In contrast, the frequency of L-selectin⁺ cells among CD16⁺ natural killer (NK) cells was significantly elevated in patients with AD when compared with that found in normal control subjects ($45.2\% \pm 15.6\%$ vs $37.3\% \pm 6.9\%$; $P < .05$; Fig 4). In both patients with AD and normal control subjects, almost all CD20⁺ B cells, CD14⁺ monocytes, and CD16⁺ neutrophils expressed L-selectin (data not shown). Similarly, almost all eosinophils from patients with AD with peripheral blood eosinophilia expressed L-selectin (data not shown). However, sufficient numbers of eosinophils were not obtained from normal control subjects for analysis. Therefore the frequency of L-selectin⁺ cells among the leukocyte subpopulations in patients with AD appeared to be similar to that found in normal control subjects, except for L-selectin⁺ NK cells.

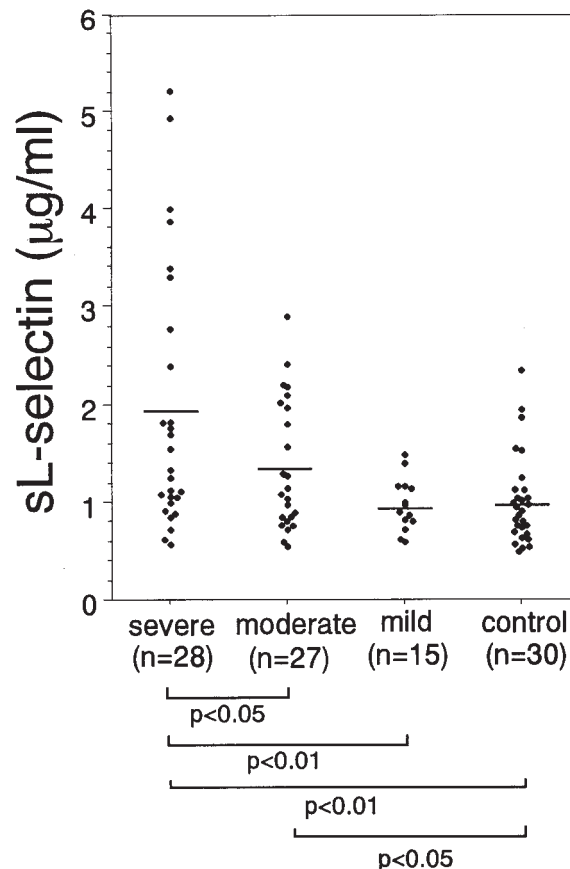


FIG 2. Serum levels of sL-selectin ranked according to the severity of AD.

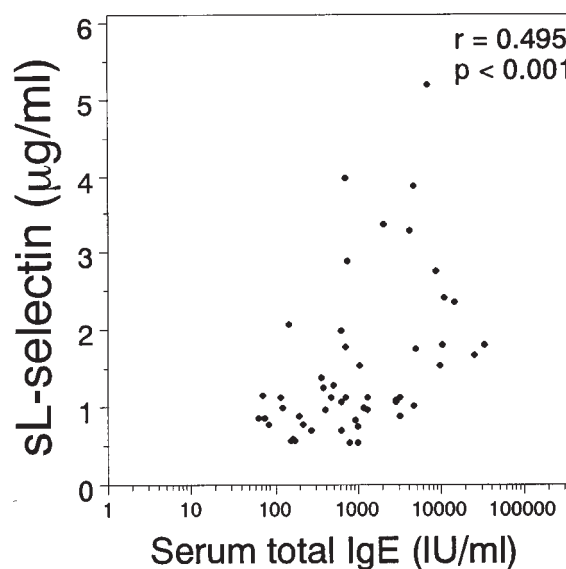


FIG 3. sL-selectin levels versus total serum IgE levels in patients with AD.

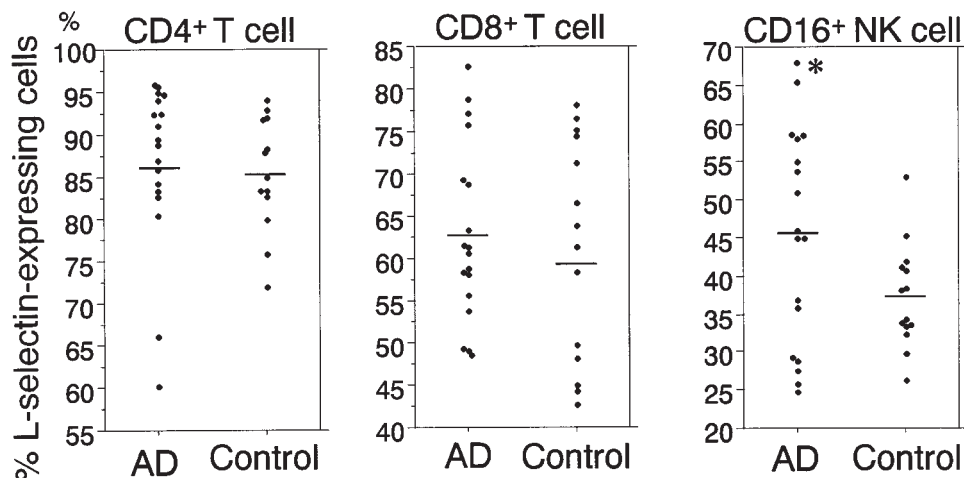


FIG 4. The frequencies of L-selectin⁺ leukocyte subpopulations from patients with AD. The frequencies of L-selectin⁺ lymphocytes were determined by 2-color immunofluorescence staining with flow cytometric analysis. All samples were stained and analyzed sequentially by flow cytometry in parallel with the use of identical instrument settings.

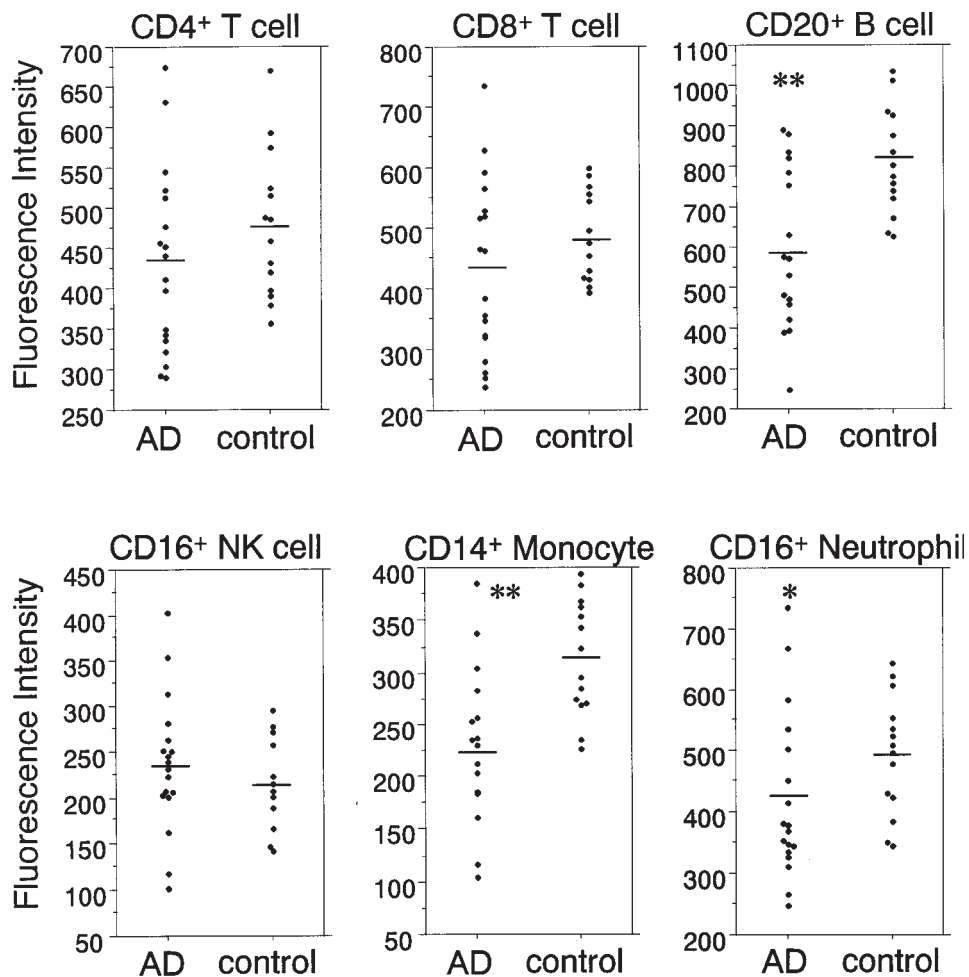


FIG 5. Expression of cell-surface L-selectin on leukocyte subpopulations from patients with AD. L-selectin expression levels were assessed by 2-color immunofluorescence staining with flow cytometric analysis. All samples were stained and analyzed sequentially by flow cytometry in parallel with the use of identical instrument settings.

L-selectin expression on blood leukocytes from patients with AD

L-selectin expression on L-selectin⁺ CD4⁺ or CD8⁺ T cells and L-selectin⁺ NK cells was similar for patients with AD and normal control subjects (Fig 5). By contrast, L-selectin expression on L-selectin⁺ B cells and L-selectin⁺ monocytes was significantly lower (27% to 28%; $P < .01$) in patients with AD. Similarly, L-selectin expression was significantly lower (15%; $P < .05$) on L-selectin⁺ neutrophils from patients with AD than from normal control subjects. L-selectin expression on monocytes positively correlated with total serum IgE levels in patients with AD ($r = 0.667$; $P < .01$; data not shown). However, L-selectin expression levels did not correlate with blood eosinophil numbers or LDH.

DISCUSSION

This study showed that serum levels of sL-selectin in patients with AD were significantly elevated compared with normal control subjects (Fig 1). It has been reported that serum levels of sE-selectin, which is another member of the selectin family and is shed from activated endothelial cells, are elevated and correlate with disease activity in patients with AD.^{18,19} However, serum levels of soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1 do not increase in patients with AD.¹⁹ Levels of sL-selectin correlated with both the severity of AD and total serum IgE levels (Figs 2 and 3), suggesting that the serum level of sL-selectin is a useful immunologic indicator for monitoring disease activity in subjects with AD. Although the functional significance of sL-selectin is still unknown, the increased levels of sL-selectin in AD might function as a biologic adhesion inhibitor for downregulating excessive cutaneous inflammation. However, further studies will be needed to clarify the role of sL-selectin in IgE production and disease severity in patients with AD.

Consistent with the increased serum levels of sL-selectin, the surface expression levels of L-selectin on B cells, monocytes, and neutrophils were significantly decreased in patients with AD (Fig 5). It has been suggested that the decreased L-selectin expression reflects the augmented activation of leukocytes because cellular activation rapidly induces the proteolytic shedding of the extracellular domain of L-selectin from leukocytes.^{20,21} In this respect downregulated L-selectin expression on B cells, monocytes, and neutrophils in AD suggests that these leukocytes are activated in vivo. Recently, various immunologic abnormalities have been identified in patients with AD. B cells from patients with AD have enhanced expression of B7.2 (CD86), which may be involved in IgE synthesis.²² In addition, the enhanced production of GM-CSF by keratinocytes from patients with AD is suggested to activate monocytes, leading to enhanced antigen-presenting capacity.²³ Therefore the decreased L-selectin expression of some leukocyte subsets from patients with AD may be related to these

immunologic abnormalities observed in patients with AD. Alternatively, it is possible that the downregulated expression of L-selectin is the result of extensive rolling of leukocytes on the endothelium. However, it has not been shown whether the extensive rolling of leukocytes results in the decreased expression of L-selectin. In both possibilities, decreased L-selectin expression would diminish the migration capacity of leukocytes because a recent study²⁴ that used L-selectin-deficient mice has shown that migration capacity of lymphocytes is regulated in part by subset-specific differences in L-selectin expression.

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