Serum-Soluble Selectin Levels in Patients with Rheumatoid Arthritis and Systemic Sclerosis

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Abstract

Soluble forms of selectins may play a regulatory role in inflammatory responses that are key to the pathophysiology of rheumatic diseases such as rheumatoid arthritis (RA) and systemic sclerosis (SSc). The aim of this study was to examine whether the elevated serum-soluble (s) selectin levels are associated with RA or SSc. Serum sE-, sL- and sP-selectin levels were measured by sandwich enzyme-linked immunosorbent assay in 34 RA patients, 30 SSc patients and 16 healthy subjects. The levels of sE-selectin were significantly higher in RA and SSc patients than those in healthy subjects. The sL-selectin level was significantly lower in RA patients compared to healthy subjects. Serum sP-selectin levels were not significantly different among the study groups. The active RA patients had significantly higher serum sE- and sL-selectin levels compared to inactive RA patients. Also, some correlations were observed between the serum selectin levels and measures of disease activity such as erythrocyte sedimentation rate and C-reactive protein in RA patients. The higher levels of sE-selectin were found in SSc patients with pulmonary fibrosis, and there was also a negative correlation between diffusion capacity for carbon monoxide and serum sE-selectin. Serum levels of selectins may provide a useful additional marker for disease activity in RA patients and for disease severity in SSc patients.

Introduction

Adhesion molecules have been shown to play important roles in cellular interactions involved in the acute and chronic inflammatory responses that are key to the pathophysiology of rheumatic diseases such as rheumatoid arthritis (RA), systemic sclerosis (SSc) and systemic lupus erythematosus [1]. A number of cell adhesion molecules, including selectins, integrins and immunoglobulin superfamilies, have been detected as soluble, circulating forms in human serum and other body fluids. Leucocyte recruitment promoted by adhesion molecules is a regulated process and occurs through a series of events that include leucocyte rolling along the endothelial cell surface, firm adhesion and activation, and extravasation into tissue during an inflammatory response [2–5]. The endothelial cell has a potential key role in inflammatory disease because of its unique location and demonstrated role in regulating vascular tone, permeability, coagulation, inflammatory reactions and the immune response. In early inflammatory reactions, endothelial cells express leucocyte adhesion proteins, which coordinate the migration of leucocytes to extravascular sites involved in inflammation [6].

Leucocyte rolling is principally mediated by the selectins [5]. Three selectins have been described. E-selectin, also known as endothelial leucocyte adhesion molecule-1, is a member of the selectin family of adhesion molecules and appears on the vascular luminal cell surface of endothelial cells. It is considered as a marker of endothelial cell activation. E-selectin appears to be important in the adhesion of granulocytes, monocytes and the memory CD4 subpopulation of T cells to activated endothelium [7]. L-selectin, which is constitutively expressed on the majority of all leucocytes, is rapidly shed via proteolytic cleavage following leucocyte activation [4]. P-selectin is a 140 kDa membrane protein stored both in the α-granules of platelets and in the Weibel–Palade bodies of endothelial cells [8, 9]. Upon the activation of these cells by strong agonists, e.g. thrombin, histamine and inflammatory cytokines such as interleukin-1 and tumour necrosis factor-α, P-selectin is expressed on endothelial cells and plays an important role

in mediating rolling of leucocytes on the activated endothelial cells of the blood vessel wall in the early phases of inflammatory reactions [10]. Inhibition or loss of one or more selectins significantly attenuates rolling and emigration in several inflammatory models [4].

Increased levels of serum-soluble (s) E-, L- and P-selectins have been reported in some connective tissue diseases [11–20]. It was suggested that the measurement of levels of soluble, circulating forms of selectins is useful as an indicator of connective tissue diseases [11, 14–17, 21, 22]. It may provide valuable insights into the pathophysiology of rheumatic diseases.

In the present study, we examined whether high levels of serum sE-, sL- and sP-selectins were found in patients with RA and SSc. Moreover, we evaluated the associations between the levels of these molecules and the indices of disease activity and that between pulmonary involvement in RA patients and disease severity in SSc patients.

Materials and methods

Thirty-four patients with RA and 30 patients with SSc, recruited from a hospital-based sample, were included in the study. The patients fulfilled the respective classification criteria of the American College of Rheumatology [23, 24]. Patients with acute or chronic infection, malignancy, environmental lung disease and renal disease, as well as active smokers, were excluded from the study. Patients receiving any drugs that may cause pulmonary fibrosis, such as methotrexate, were also excluded. Demographic and clinical features of patient groups are summarized in Table 1. The number of patients with RA having pulmonary involvement is higher than the expected frequency in this population. One of the aims of this study was to determine whether serum-soluble selectins levels are associated with pulmonary involvement in patients with RA or SSc. Therefore, the RA patients having pulmonary involvement were particularly selected for this study. Sixteen healthy subjects, 12 females and four males, were evaluated as the control group. Mean age was 50.2 ± 10.5 years in healthy subjects.

Table 1 The demographic, clinical and laboratory features of patients with rheumatoid arthritis (RA) and systemic sclerosis (SSc)

	RA patients	SSc patients
Age (years)	52.6 ± 13.6	47.8 ± 11.8
Sex (female/male)	24/10	27/3
Median follow-up (months)	40 (3–240)	44 (2–210)
Pulmonary involvement [*n* (%)]	17 (50.0)	15 (50.0)
Larsen's score		
LS 1, 2, 3 [*n* (%)]	19 (55.9)	
LS 4, 5 [*n* (%)]	15 (44.1)	
Serum C-reactive protein (mg/l)	57 ± 52	
Erythrocyte sedimentation rate (mm/h)	65 ± 30	
Haematocrit (%)	35.9 ± 3.7	
White blood cell count (mm^3)	7765 ± 1765	
Platelet count ($\times 10^3/mm^3$)	302 ± 99	
Diffusion capacity for carbon monoxide (%)		79 ± 28

Serum levels of sE-, sL- and sP-selectins in the study groups were measured by sandwich enzyme-linked immunosorbent assay using commercial kits (Bender MedSystems, Vienna, Austria). Serum C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), haematocrit, white blood cell (WBC) count and platelet count were measured in RA patients. Serum CRP level was measured by a nephelometric immunoassay.

The patients with RA were classified according to disease activity, anatomical joint damage and the presence of pulmonary involvement. Disease activity was assessed according to preliminary criteria for clinical remission in RA from Pinals *et al.* [25]. Anatomical joint damage was classified using Larsen's score (LS) [26].

The patients with SSc were classified according to the presence of pulmonary fibrosis. Pulmonary fibrosis was evaluated using high-resolution computed tomography. Diffusion capacity for carbon monoxide (DLCO) was also determined in patients with SSc. The SSc patients having isolated pulmonary hypertension were not included in the study.

Results are expressed as mean $\pm$ SD. Comparison of means has been carried out with Mann–Whitney *U*-test. Correlations were analysed using Spearman's correlation coefficient. A Bonferroni correction was applied for multiple comparisons to all novel associations, with a correction factor derived from the number of selectins tested. P_c indicates where the Bonferroni correction was applied. The difference was considered significant when the *P*-value was <0.05.

Results

The clinical and laboratory findings of patients with RA or SSc are summarized in Table 1. Twenty-seven patients (79.4%) with RA had active disease. Seventeen RA patients (50%) had pulmonary involvement. The LSs of RA patients are as follows: LS 1, six patients (17.6%); LS 2, six patients (17.6%); LS 3, seven patients (20.6%); LS 4, eight patients (23.5%); and LS 5, seven patients (20.6%). The patients were classified according to LS as mild-to-moderate (LS 1, LS 2 and LS 3) and severe anatomical joint damage (LS 4 and LS 5). Fifteen patients with SSc (50%) had pulmonary fibrosis.

The mean serum sE-, sL- and sP-selectin levels in the study groups are summarized in Table 2. The levels of sE-selectin were significantly higher in RA and SSc patients than those in healthy subjects ($P < 0.01$ and $P < 0.001$, respectively). The sL-selectin level was significantly lower in RA patients compared to SSc patients ($P = 0.001$) and healthy subjects ($P < 0.001$), but it did

Table 2 Mean serum-soluble selectins levels (ng/ml) in study groups

	RA	SSc	Healthy controls
sE-selectin	43.7 ± 30.9*	44.9 ± 22.3†	24.9 ± 12.9
sL-selectin	378 ± 168†‡	552 ± 224	672 ± 140
sP-selectin	383 ± 198§	246 ± 163	292 ± 199

RA, rheumatoid arthritis; SSc, systemic sclerosis.
*$P < 0.01$ versus healthy controls.
†$P < 0.001$ versus healthy controls.
‡$P = 0.001$ versus SSc.
§$P < 0.01$ versus SSc.

not significantly differ between the patients with SSc and healthy subjects ($P > 0.05$). The patients with RA had significantly higher serum sP-selectin level compared to the patients with SSc ($P < 0.01$). However, serum sP-selectin levels in patients with RA or SSc were not significantly different compared to healthy subjects ($P > 0.05$).

Comparisons of serum-soluble selectin levels between active and inactive RA patients are shown in Figs 1 and 2. The levels of serum sE- and sL-selectin were significantly higher in patients with active RA compared to inactive patients (47.9 ± 32.9 versus 27.4 ± 14.0 ng/ml, $P < 0.05$ and 406 ± 162 versus 266 ± 152 ng/ml, $P < 0.05$, respectively). Although sP-selectin level was higher in active RA patients than those in inactive patients, the difference did not reach statistically significant level (414 ± 183 versus 260 ± 220 ng/ml, $P = 0.08$).

The correlations between serum sE-, sL- and sP-selectin levels and laboratory parameters in RA patients are summarized in Table 3. Serum sP-selectin level was significantly correlated with CRP ($r = 0.463$, $P_c < 0.05$) and ESR ($r = 0.475$, $P_c < 0.05$). Although serum sE- and sL-selectin levels were significantly correlated with CRP ($r = 0.361$, $P < 0.05$ and $r = 0.369$, $P < 0.05$, respectively), significance was lost after Bonferroni correction ($P_c > 0.05$). There was a significant correlation between serum sL-selectin level and WBC count ($r = 0.461$, $P_c < 0.05$). Serum sE-, sL- and sP-selectin levels were not significantly associated with haematocrit level. Serum selectin levels were not found to be significantly associated with anatomical joint damage and the presence of pulmonary involvement in patients with RA (Table 4).

Serum sE-selectin level was significantly higher in SSc patients with pulmonary fibrosis compared to other SSc patients (56.4 ± 24.8 versus 38.0 ± 12.5 ng/ml, $P < 0.05$). There was a significant negative correlation between sE-selectin level and DLCO ($r = -0.536$, $P_c < 0.01$). Serum sL- and sP-selectin levels were not associated with pulmonary fibrosis.

Discussion

Cell-surface adhesion molecules mediate important cellular interactions in acute and chronic inflammatory responses. The function of soluble forms of adhesion molecules is unknown, but they may have regulatory function in inflammatory responses by binding counter receptors to interacting cell types, thereby mediating the influx of leucocytes into tissue [1]. The selectin family is known to participate in the first stage, called rolling phenomenon, of cell-to-cell adhesion [5].

We have found increased levels of sE-selectin in patients with RA and SSc. Previously, increased sE-selectin level was found in patients with RA [13, 18, 20] and in patients with SSc [11, 13, 14, 16, 19] relative to controls. The association of serum sE-selectin level with the indicators of

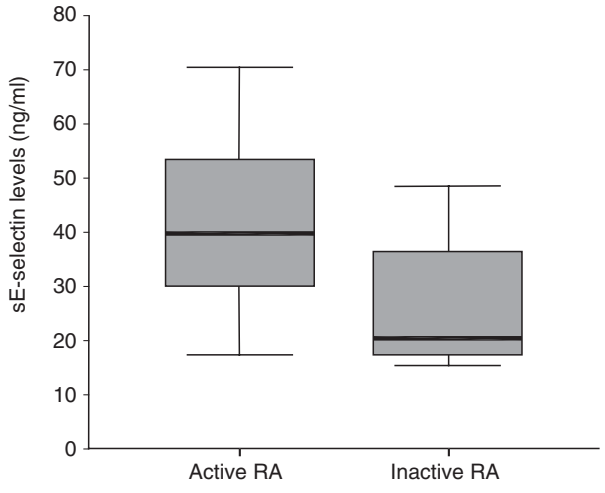

Figure 1 Serum sE-selectin levels in patients with active and inactive rheumatoid arthritis (RA).

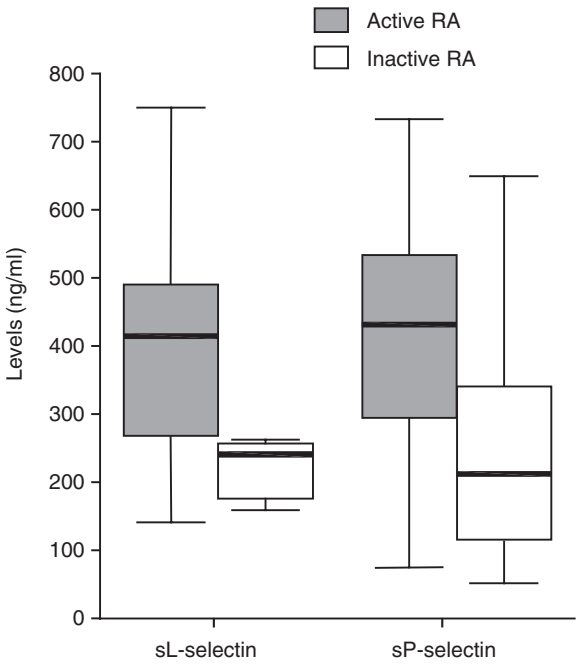

Figure 2 Serum sL- and sP-selectin levels in patients with active and inactive rheumatoid arthritis (RA).

Table 3 Correlations between serum-soluble selectins levels and laboratory parameters in patients with rheumatoid arthritis

	sE-selectin		sL-selectin		sP-selectin	
	r	P/P_c	*r*	P/P_c	*r*	P/P_c
C-reactive protein	0.361	0.04/0.12	0.369	0.032/0.096	0.463	0.009/0.027
ESR	0.296	Not significant	0.262	Not significant	0.475	0.007/0.021
White blood cell count	0.275	Not significant	0.461	0.009/0.027	0.215	Not significant
Platelet count	0.117	Not significant	0.395	0.028/0.084	0.289	Not significant
Haematocrit	−0.212	Not significant	−0.199	Not significant	−0.248	Not significant

ESR, erythrocyte sedimentation rate; P_c, corrected *P*-value.

disease activity in RA patients is controversial. In our study, serum sE-selectin level was significantly higher in active RA patients than that in inactive RA patients. Although it was correlated with serum CRP, the significance was lost after Bonferroni correction. However, serum sE-selectin level was not significantly associated with severity of anatomical joint damage and the presence of pulmonary involvement. Kuuliala *et al.* [22] examined serum sE-selectin levels in a cohort of 85 patients with early RA followed-up for 5 years, and they found that sE-selectin level was significantly correlated with CRP, active joint count, progression of joint destruction and Health Assessment Questionnaire score, but not with extra-articular involvement. However, other authors [13, 15, 18, 20] did not show a significant association between serum sE-selectin level and disease activity indicators.

In our study, the higher levels of sE-selectin were found in SSc patients with pulmonary fibrosis, and there was also a significant negative correlation between DLCO and serum sE-selectin. Gruschwitz *et al.* [14] found the highest levels of sE-selectin in those SSc patients with the most severe disease. Denton *et al.* [21] reported that in SSc patients, change in circulating E-selectin levels was associated with disease severity, falling with improvement in renal function or skin score and rising with deterioration in pulmonary function tests. They suggested that serial measurements of sE-selectin levels might have potential value as surrogate markers for clinical progression or remission in this disease [21]. Ihn *et al.* [16] found that the serum levels of sE-selectin were significantly elevated in the patients with SSc compared to the control subjects. In this study, the frequency of pulmonary fibrosis was also significantly higher in the patients with elevated levels of sE-selectin than in those with normal levels (62 versus 26%). Moreover, the frequency of each of decreased per cent vital capacity and decreased per cent DLCO was also higher in the patients with elevated levels of sE-selectin [16]. Andersen *et al.* [19] found that sE-selectin level was significantly increased in SSc patients compared with controls. They showed that E-selectin was expressed on the surface of the luminal endothelial cells from patients with SSc. Importantly, E-selectin shedding is restricted to activated endothelial cells. Therefore, the increased levels of sE-selectin could be an indicator of activated endothelial cells. The expression of E-selectin on the vascular endothelium in skin biopsy specimens from their SSc patients is consistent with the finding of elevated sE-selectin and strongly supports the notion of an activated endothelium in SSc. The presence of leucocyte infiltration near the blood vessels indicates that the E-selectin participates in directing the extravasation of leucocytes to the perivascular space [19].

In the present study, the serum sL-selectin level was significantly lower in RA patients compared to healthy subjects, but it did not significantly differ between the patients with SSc and healthy subjects. The results of previous studies regarding serum sL-selectin level in patients with RA and SSc are controversial. Serum sL-selectin level was found to be significantly lower in patients with RA and SSc compared to healthy controls in the study of Blann *et al.* [27], but Sfikakis *et al.* [28] did not find a significant difference in serum sL-selectin level between the patients with RA and SSc and controls. Littler *et al.* [15] have reported significantly higher level of sL-selectin in RA patients compared to healthy subjects. Reduced level of sL-selectin in serum from patients with RA is a surprising finding of unclear reason. As an adhesion molecule involved in leucocyte transmigration across

Table 4 Mean serum-soluble selectin levels (ng/ml) according to anatomical joint damage and the presence of pulmonary involvement in patients with rheumatoid arthritis

	Larsen's score (LS)		Pulmonary involvement	
	LS 1–3	LS 4, 5	Present	Absent
sE-selectin	43.5 ± 25.9	44.6 ± 24.4	47.9 ± 36.9	39.4 ± 20.5
sL-selectin	354 ± 147	394 ± 176	416 ± 187	339 ± 142
sP-selectin	310 ± 206	389 ± 172	405 ± 214	361 ± 183

the endothelium, cell membrane-bound L-selectin may be expected to be upregulated in activated leucocytes which are participating in the process of rolling and adhesion [29]. Levels in the controls may reflect the shedding of the molecule at the end of its lifespan, as it is likely that leucocytes shed their adhesion ligands by metalloproteinase digestion as they pass through the endothelium [30]. Consequently, low levels in patients with RA may be due to reduced shedding (i.e. retention) or increased internalization of cell-bound L-selectin. Soluble L-selectin may also be binding with stronger than usual affinity to its ligand(s) on the endothelial cell. Humbria *et al.* [31] reported that the expression of L-selectin on neutrophils isolated from synovial fluid of patients with inflammatory joint diseases, including RA, has been found to be reduced compared to peripheral blood neutrophils. However, sL-selectin was detected at similar levels in synovial fluid and peripheral blood of RA patients and was not elevated relative to normal controls [31]. Additionally, Bond and Hay [32] showed that lower levels of expression of L-selectin on lymphocytes, monocytes and granulocytes were found in RA patients compared to controls. The expression of L-selectin on T and B cells was found to correlate with disease activity in RA [32]. They suggested that the apparent downregulation of L-selectin expression on circulating lymphocytes in RA may be seen as an end-stage mechanism to halt any further migration. Another possibility for lowered expression may be immunosuppressive agents undertaking for RA. It has been shown that methotrexate and corticosteroids decreases the expression of L-selectin and decreases E-selectin function [32, 33].

Although we found lower levels of sL-selectin in patients with RA, the levels of sL-selectin were significantly higher in patients with active RA compared to inactive patients in our study, similar to the results of Bond and Hay [32]. There was a significant correlation between serum sL-selectin level and WBC count. Bloom *et al.* [34] also reported a positive correlation between sL-selectin level and WBC count in patients with juvenile RA.

It was suggested that there are higher levels of sP-selectin in the serum of patients with connective tissue diseases, especially RA and mixed connective tissue disease [12, 15, 17, 28]. Littler *et al.* [15] observed a significant correlation between serum sP-selectin levels and disease activity markers, such as CRP and ESR, in patients with RA. Ertenli *et al.* [17] found that serum sP-selectin level was significantly correlated with Ritchie articular index, morning stiffness and platelet count in patients with RA and thrombocytosis. A significant correlation between serum sP-selectin and platelet count was also reported by Takeda *et al.* [12]; however, in this study, serum sP-selectin level was not found to be significantly correlated with WBC, CRP and ESR. In our study, serum sP-selectin levels were not significantly different among the study groups. Serum sP-selectin level was higher in active RA patients than that in inactive patients, but the difference did not reach a statistically significant level. Serum sP-selectin level was significantly correlated with CRP and ESR in our RA patients.

In conclusion, we found higher levels of sE-, sL- and sP-selectins in RA patients during active disease than those in RA patients during inactive disease. Also, some correlations were observed between the serum selectin levels and measures of disease activity such as ESR and CRP in RA patients. In our study, the higher levels of sE-selectin were found in SSc patients with pulmonary fibrosis, and there also was a significant negative correlation between DLCO and serum sE-selectin. These findings suggest that serum levels of these molecules may provide a useful additional marker for disease activity in RA patients and for disease severity in SSc patients. Activation/injury to the endothelium through the selectins may be important in connective tissue diseases. Especially, measurement of sE-selectin may be an important marker for clinical progression or remission in SSc patients. Measurement of these selectins may increase our understanding of the relationship between *in vivo* endothelial activation and inflammation involved in rheumatic diseases.

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