INCREASED SERUM LEVELS OF SOLUBLE VASCULAR CELL ADHESION MOLECULE-1 AND E-SELECTIN IN PATIENTS WITH SYSTEMIC SCLEROSIS

H. IHN, S. SATO,* M. FUJIMOTO, K. TAKEHARA* and K. TAMAKI
Department of Dermatology, Faculty of Medicine, University of Tokyo, Tokyo and *Department of Dermatology, Kanazawa University of Medicine, Kanazawa, Japan

SUMMARY

Objective. To determine the serum levels of soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble E-selectin (sE-selectin) in patients with systemic sclerosis (SSc).

Methods. Serum samples from 80 patients with SSc and 20 healthy control subjects were examined by a sensitive enzyme-linked immunosorbent assay.

Results. The serum levels of sVCAM-1 and sE-selectin were significantly higher in the patients with SSc than in the healthy controls. The serum levels of sVCAM-1 were correlated with the presence of pulmonary fibrosis, joint involvement and elevated erythrocyte sedimentation rate levels. The serum levels of sE-selectin were correlated with the presence of pulmonary fibrosis.

Conclusion. These results suggest that endothelial activation is involved in the development of this disease.

KEY WORDS: Systemic sclerosis, Adhesion molecules, Pulmonary fibrosis, Endothelial cell activation.

SCLEDODERMA, OR systemic sclerosis (SSc), is a generalized connective tissue disease which is characterized by microvascular obliteration and increased deposition of collagen, resulting in fibrotic lesions [1, 2]. It had been suggested that a vascular alteration might be the triggering factor in its pathogenesis [3]. Many investigators have suggested that the adhesive interactions of leucocytes with the endothelial cells and with the extracellular matrix have a central role in the function of the immune system [4]. These interactions are crucial in all diseases with immune dysregulation, including SSc [5].

In recent years, a number of the molecules which mediate leucocyte–endothelial adhesion have been identified; these include vascular cell adhesion molecule-1 (VCAM-1) [6] and E-selectin [7].

VCAM-1 is a 105–110 kDa single-chain glycoprotein belonging to the immunoglobulin supergene family [6]. VCAM-1 acts as a ligand for the \( \alpha_4 \beta_1 \) (VLA-4) [8] and \( \alpha_4 \beta_7 \) [9], and thereby mediates the cell adhesion of lymphocytes, monocytes, eosinophils and basophils [10, 11]. VCAM-1 is expressed by activated endothelial cells, germinal centre dendritic cells, Kupffer cells, synovial lining cells and renal proximal tubule cells [12]. The expression by cultured endothelial cells of VCAM-1 is induced by stimulation with interleukin-1 (IL-1), tumour necrosis factor alpha (TNF-\( \alpha \)), lipopolysaccharide (LPS) or IL-4 [13, 14].

E-Selectin is a member of the selectin family with a lectin-like N-terminal domain capable of recognizing the tetrasaccharide sialyl-Lewis\( ^x \) or sialyl-Lewis\( ^a \) of monocytes and granulocytes, which appears to be important in the adhesion of granulocytes, monocytes and the memory CD4 subpopulation of T cells to activated endothelium [7, 15, 16]. Unlike that of other endothelial adhesion molecules such as VCAM-1, E-selectin expression appears to be restricted to endothelial cells. The inflammatory cytokines TNF-\( \alpha \), IL-1, interferon gamma (IFN-\( \gamma \)) and LPS are known to act as inducers and enhancers of E-selectin [17].

Recently, soluble forms of these molecules have been identified [17, 18]. High levels of soluble VCAM-1 (sVCAM-1) and soluble E-selectin (sE-selectin) have been detected in a variety of inflammatory and neoplastic conditions [18–22].

In the present study, we measured the serum levels of sVCAM-1 and sE-selectin in patients with SSc, and investigated whether these levels were correlated with the clinical or serological features of this disease.

PATIENTS AND METHODS

Patients

Serum samples were obtained from 80 patients with SSc and 20 healthy control subjects matched with SSc patients according to sex and age. The patients with SSc were classified into two subgroups according to the classification system proposed by LeRoy et al. [23]: 34 patients had limited cutaneous SSc (lcSSc) and 46 patients had diffuse cutaneous SSc (dcSSc), as previously described [24]. All patients met the American College of Rheumatology (formerly, American Rheumatism Association) criteria for the diagnosis of SSc (scleroderma) [25].

Clinical assessment

The clinical and laboratory data reported in this study are those obtained at the time the blood samples were drawn. The patients were evaluated for the presence of gastrointestinal, pulmonary, cardiac, renal or muscle involvement as described previously [24, 26]. Joint involvement was evaluated by Thompson’s articular index.

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Correspondence to: H. Ihn, Department of Dermatology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.

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Antinuclear antibodies (ANA) were detected by immunofluorescence using HEp-2 cells and double immunodiffusion [27, 28].

Enzyme-linked immunosorbent assays of soluble adhesion molecules

Aliquots of serum were frozen at −80°C until assayed. Soluble VCAM-1 and E-selectin levels were measured with specific ELISA kits (British Biotechnology Products, Oxford) according to the manufacturer’s protocol. The intra-assay and interassay coefficients of variation were <6% and <8%, respectively. Levels of circulating intercellular adhesion molecule-1 (ICAM-1) were also measured with a specific ELISA kit (T cell Diagnostics, Cambridge, MA, USA), as described previously [29]. Serum levels of these soluble adhesion molecules >2 s.d. greater than the mean level in the normal control subjects were regarded as elevated.

Statistical analysis

Statistical analysis was carried out with Student’s t-test for the comparison of means and Fisher’s exact probability test for the analysis of frequency. Correlations with clinical data were assessed by using Spearman’s rank correlation coefficient. Two-tailed P values of <0.05 were considered significant.

RESULTS

Serum levels of sVCAM-1

Serum levels of sVCAM-1 in samples obtained from the patients with SSc and healthy control subjects are demonstrated in Fig. 1a. Compared with the levels in the control subjects (mean ± s.d.: 506.8 ± 127.3 ng/ml), those in the patients with SSc were significantly elevated (786.6 ± 297.6 ng/ml; P < 0.001). Of the patients with SSc, those with dcSSc had significantly higher serum sVCAM-1 levels (845.4 ± 314.0 ng/ml) than those with lcSSc (706.9 ± 257.3 ng/ml; P < 0.02). Each of these subgroups had significantly higher serum sVCAM-1 levels than control subjects (P < 0.001 and P < 0.01, respectively). The cut-off value (2 s.d. above the mean in the control subjects) was set at 761.4 ng/ml. Elevated serum levels of sVCAM-1 were found in 24 (52%) of the 46 patients with dcSSc, and in 15 (44%) of the 34 patients with lcSSc.

Serum levels of sE-selectin

The serum levels of sE-selectin were significantly elevated in the patients with SSc (83.7 ± 30.7 ng/ml) compared to the control subjects (53.5 ± 14.6 ng/ml; P < 0.001). The significant elevation was also found in the patients with dcSSc (86.8 ± 30.6 ng/ml; P < 0.001) and in those with lcSSc (79.5 ± 30.9 ng/ml; P < 0.01). There was no significant difference between

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Fig. 1.—(a) Serum levels of sVCAM-1. The dashed line represents the cut-off value (mean ± 2 s.d.), which was set at 761.4 ng/ml, based on the data for the 20 healthy control subjects. The subgroups of the patients with diffuse cutaneous systemic sclerosis (dcSSc) and with limited cutaneous systemic sclerosis (lcSSc) had significantly higher serum sVCAM-1 levels compared with the control subjects (P < 0.001 and P < 0.01, respectively). Elevated levels of sVCAM-1 were found in 24 (52%) of the 46 patients with dcSSc, and in 15 (44%) of the 34 patients with lcSSc. (b) Serum levels of sE-selectin. The dashed line represents the cut-off value, which was set at 81.7 ng/ml. Compared with the levels in the control subjects, the sE-selectin levels were significantly elevated in patients with dcSSc (86.8 ± 30.6 ng/ml; P < 0.001) and in those with lcSSc (79.5 ± 30.9 ng/ml; P < 0.01). There were no significant differences between the sE-selectin levels in the patients with dcSSc and those with lcSSc. Elevated sVCAM-1 levels were found in 23 (50%) of the 46 patients with dcSSc, and in 18 (53%) of the 34 patients with lcSSc.
the levels in these two subgroups of patients. Elevation of the sE-selectin level above the cut-off value (81.7 ng/ml) was found in 23 (50%) of the 46 patients with dcSSc, and in 18 (53%) of the 34 patients with lcSSc (Fig. 1b).

**Relationships among serum levels of soluble adhesion molecules**

The serum cICAM-1 levels were significantly correlated with the sVCAM-1 levels in patients with SSc ($r = 0.521, P < 0.001$). However, the sE-selectin levels showed no correlation with either the cICAM-1 levels ($r = 0.254$) or the sVCAM-1 levels ($r = 0.224$).

**Relationships of serum levels of soluble adhesion molecules with clinical and serological features of patients with SSc**

The clinical and serological features in patients with elevated and normal levels of soluble adhesion molecules are summarized in Table I. There were no significant differences between these groups in sex, age or duration of disease. Pulmonary fibrosis was significantly more frequent in the patients with high levels of sVCAM-1 than in those with normal levels (61% vs 32%, $P < 0.02$). The frequency of reduced per cent vital capacity (%VC) or decreased diffusion capacity for carbon monoxide (%DLco) was also greater in the patients with high levels of sVCAM-1 (45% vs 19%, $P < 0.05$; 73% vs 47%, $P < 0.05$, respectively).

Additionally, the incidence of joint involvement in the patients with elevated sVCAM-1 levels (49%) was significantly higher than in the patients with normal levels (26%, $P < 0.05$). An elevated erythrocyte sedimentation rate (ESR) was significantly correlated with elevated sVCAM-1 level ($P < 0.01$). Moreover, patients with elevated levels of sVCAM-1 had significantly higher ESR than those with normal levels (33.9 ± 21.7 mm/h vs 21.7 ± 17.5 mm/h, $P < 0.01$). An elevated C-reactive protein (CRP) level was also significantly correlated with elevated sVCAM-1 level ($P < 0.01$).

The frequency of pulmonary fibrosis was significantly higher in the patients with elevated levels of sE-selectin than in those with normal levels (62% vs 26%, $P < 0.01$). Moreover, the frequency of each of decreased %VC and decreased %DLco was also higher in the patients with elevated levels of sE-selectin (42% vs 18%, $P < 0.05$; 69% vs 43%, $P < 0.05$, respectively).

**DISCUSSION**

In this study, we measured the levels of soluble adhesion molecules in serum samples from a large number of patients with SSc, in order to examine the relationship of these levels to the clinical and sero-

| TABLE I |
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| Clinical and serological features of patients with SSc with elevated and normal serum levels of soluble adhesion molecules |
| Elevated sVCAM-1 (n = 39) | Normal sVCAM-1 (n = 41) | Elevated sE-selectin (n = 42) | Normal sE-selectin (n = 38) |
|------------------|
| **Sex (male:female)** | 5:34 | 3:38 | 5:37 | 3:35 |
| **Age (yr)** | 52.3 | 47.8 | 51.5 | 48.4 |
| **Duration of disease (yr)** | 7.8 | 6.7 | 7.8 | 6.5 |
| **Clinical features (%)** | | | | |
| Pitting scars/ulcers | 68 | 51 | 52 | 66 |
| Short sublingual frenulum | 55 | 59 | 52 | 66 |
| Contracture of phalanges | 53 | 44 | 43 | 53 |
| Pigmentation | 58 | 51 | 55 | 53 |
| Calcinoic | 18 | 28 | 27 | 16 |
| Telangiectasia | 73 | 63 | 72 | 62 |
| Raynaud’s phenomenon | 90 | 93 | 91 | 92 |
| **Organ involvement (%)** | | | | |
| Lung | 61** | 32** | 62*** | 26*** |
| Decreased %DLco | 73* | 47** | 69* | 43* |
| Decreased %VC | 45* | 19* | 42* | 18* |
| Oesophagus | 70 | 67 | 72 | 60 |
| Heart | 13 | 0 | 8 | 3 |
| Kidney | 9 | 3 | 3 | 9 |
| Muscle | 21 | 8 | 20 | 7 |
| Joint | 49* | 26* | 38 | 35 |
| **ANA specificity (%)** | | | | |
| Anti-topo I | 33 | 42 | 40 | 34 |
| ACA | 31 | 29 | 29 | 32 |
| Anti-U1RNP | 21 | 12 | 17 | 16 |
| **Laboratory findings (%)** | | | | |
| Elevated CRP | 27*** | 7*** | 24 | 9 |
| Elevated ESR | 83*** | 49*** | 63 | 67 |
| ESR (mm/h) | 33.9*** | 21.7*** | 29.0 | 24.2 |
| Values are percentages, except where indicated otherwise. DLco, diffusion capacity for carbon monoxide; VC, vital capacity; ANA, antinuclear antibody; anti-topo I, anti-topoisomerase I antibody; ACA, anticientromere antibody; anti-U1RNP, anti-U1RNP antibody; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate. |

* P < 0.05, ** P < 0.02, *** P < 0.01.
logical features in patients with this disease. We found that the patients showed significantly higher serum levels of sVCAM-1 and sE-selectin compared to the healthy control subjects. This suggests upregulation of VCAM-1 and E-selectin in patients with SSc. Indeed, recent studies have also demonstrated that the levels of soluble circulating forms of these molecules are elevated in patients with SSc [30–33]. Moreover, increased expression of cell-bound VCAM-1 and E-selectin on endothelial cells in the skin has been documented immunohistochemically in patients with SSc [31]. These studies, including our own, provide further evidence for endothelial cell activation in SSc, and suggest the clinical importance of these soluble adhesion molecules in patients with SSc. Furthermore, several studies have revealed that upregulation of these adhesion molecules is induced by activation signals and cytokines such as TNF-α, IL-1 and IFN-γ [13, 14, 17]. Thus, the elevated serum levels of these adhesion molecules may be a consequence of chronic exposure to these cytokines in patients with SSc.

Another study revealed that serial levels of sVCAM-1 and sE-selectin reflect the disease severity in SSc [33]. However, there are no reports clearly discussing the possible association of clinical features with these adhesion molecules based on data for a large number of patients with SSc. In our patients, an elevated serum sVCAM-1 level was closely correlated with elevated frequency of joint involvement, elevated ESR level and elevated CRP level. It could be postulated that the serum sVCAM-1 level may reflect the level of inflammation in patients with SSc. Interestingly, the serum sE-selectin level was closely correlated with the presence and extent of pulmonary fibrosis specifically, but not with other clinical or serological features of the patients with SSc.

Measurement of the levels of sVCAM-1 and sE-selectin may offer an important approach to the evaluation of endothelial activation in vivo, and may increase our understanding of the endothelial activation involved in autoimmune disorders.

References

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