Serum Levels of Soluble TNFα Receptor Type I and the Severity of Systemic Sclerosis

SLAWOMIR MAJEWSKI1,2, ANNA WOJAS-PELC3, MAGDALENA MALEJCZYK 1,2, ELZBIETA SZYMANSKA4 and STEFANIA JABLONSKA1

1Department of Dermatology, Warsaw School of Medicine, 2Institute of Venereology, Warsaw, 3Department of Dermatology, Jagiellonian University, Krakow and 4Medical Research Centre of the Polish Academy of Sciences, Warsaw, Poland

Several abnormalities of cytokines have been shown to occur in systemic scleroderma; however their correlation with clinical parameters is controversial. Since serum concentrations of cytokine receptors have been shown to correlate with inflammatory processes, including systemic sclerosis, the aim of our study was to compare serum concentrations of TNFα receptor type 1 with the concentrations of soluble intercellular adhesion molecule-1 (sICAM-1), soluble interleukin-2 receptor (sIL-2R) and aminoterminal propeptide of procollagen type III (PIII NP). The findings were correlated with the clinical parameters and antibody patterns, and with the disease severity. Serum samples were studied with the use of enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) for sTNFα RI, sICAM-1, sIL-2R and PIII NP. The series comprised 36 patients with systemic scleroderma: 13 with diffuse variety and 23 with limited variety, and 7 with Raynaud’s disease. Healthy volunteers (n = 25) were chosen from doctors and/or other laboratory staff. Increased levels of sTNFα-receptor type 1 were found in 77% of patients with diffuse variety and in only 30% of patients with limited form. Increased serum concentrations in patients with diffuse scleroderma and limited variety were found for sICAM-1 54% and 65%, for sIL-2R 46% and 15%, and for PIIINP 77% and 50%, respectively. There were significant correlations between serum levels of sTNFα-receptor type 1 and PIIINP (r = 0.653, p < 0.0001), and sTNFα-receptor type 1 and sIL-2R (r = 0.625, p < 0.0001), but not between sTNFα-receptor type 1 and sICAM-1 (r = 0.127, p < 0.526). Clinical analysis revealed that serum concentrations of sTNFα-R1 seem to correlate best with the severity of the disease and, as the only parameter, correlated with lung involvement. The study showed that, in addition to recognized parameters of scleroderma severity (IL-2R, PIIINP), a new important marker appears to be sTNFα-receptor type 1. Key words: cytokine receptors; disease severity; scleroderma.

(Accepted November 26, 1998.)

S. Majewski MD, Department of Dermatology and Venereology, Warsaw School of Medicine, Koszykowa PL-82A02-008, Warsaw, Poland.

Patients with systemic sclerosis (SSc) show, in addition to tissue fibrosis, a variety of distinct immunological and vascular abnormalities (1, 2). The process of fibrosis in SSc seems to be a secondary phenomenon and may result from migration of peripheral blood mononuclear cells (PBMC) into the perivascular spaces, leading to cell activation, production of various proinflammatory cytokines (3–5) and upregulation of some adhesion molecules involved in the enhancement of immune reactions (6–8).

Evaluation of serum levels of interleukins (IL-1, IL-2, IL-4, IL-6), interferon gamma (IFNγ) and TNFα revealed some abnormalities, but no evident clinical correlations were found between cytokine levels and the extent or severity of organ involvement (3–5). Recently, however, it was reported that increased levels of TNFα may reflect the inflammatory stage and also the extent of internal involvement (9).

One of the most important mechanisms controlling serum levels of cytokines and their biological activity is the generation of soluble forms of cytokine receptors, whose concentrations in body fluids could also serve as parameters of inflammation and immune system involvement. In SSc, serum levels of sIL-2R were found to be a more sensitive parameter than IL-2 concentrations, and serum levels of sIL-2R correlated with disease severity, mortality and recent disease onset (10, 11). Increased levels of TNFα-R were reported in a single study on a small cohort of SSc patients, however the prognostic value was not evaluated and no correlations with type of SSc and visceral involvement were shown (12). In addition to cytokine receptors, soluble forms of various adhesion molecules involved in inflammatory processes could be detected in the sera of SSc patients, and this may reflect vascular and immune involvement (6, 13, 14).

The aim of this study was to examine serum concentrations of TNFα-receptor type 1 (sTNFα-R1), as related to soluble ICAM-1 (sICAM-1), sIL-2R, and amino-terminal propeptide of procollagen (PIIINP) in 36 patients with SSc, and correlate this with the clinical parameters of the disease severity. In addition, 7 patients with Raynaud’s disease and 25 healthy individuals were studied as controls.

MATERIAL AND METHODS

Patients
All patients with SSc in this study fulfilled the criteria of the American Rheumatism Association (15). Diffuse scleroderma (dSSc) was diagnosed if indurations of the skin involved the face, extremities and central trunk. Limited scleroderma (lSSc) was diagnosed according to criteria described by Le Roy et al. (16) in patients with skin changes confined to the face and forearms. The dSSc group comprised 13 individuals (mean age 51.7 years, range 35–66 years, 6 males and 7 females). The lSSc group comprised 23 patients (mean age 53.2 years, range 21–71 years, 2 males and 21 females). The group of patients with Raynaud’s disease comprised 7 individuals (mean age 38.9 years, range: 17–52 years, 2 males and 5 females). As control, sera from 25 adult healthy volunteers were studied. Informed consent was obtained for all patients and controls.

In all patients the internal organ involvement was evaluated. Patients were considered to have oesophageal involvement if they had symptoms of oesophageus dysmotility on radiography or scintigraphy. Lung involvement was assessed by X-ray examination and lung func-
tion tests. Patients were considered to have heart involvement if they had any of the following findings on electrocardiography and/or echocardiography: cardiac arrhythmia, conduction disturbances, right heart failure secondary to pulmonary hypertension or pericarditis.

Kidney involvement was considered if patients had abnormal creatinine clearance and/or changes in urinalysis. Patients were considered to have muscle involvement if they had myalgia and electromyographic abnormalities and/or increased levels of the muscle enzymes, creatine-phosphokinase and aldolase. None of the patients received systemic therapy for 2 weeks prior to or during the study.

All sera were studied by immunofluorescence assays and immunodiffusion and some of them additionally by immunoblot for the presence of various autoantibodies, including Scl70, anticentromere, anti-U1-RNP, anti-ribonucleoprotein, anti-KU, anti-Ro/La and others, as described previously (17, 18). Scl70 antibodies were found in 9/13 (69\%) patients with dSSc, and 14/23 (61\%) in lSSc, and a similar high prevalence of these antibodies in both SSc varieties was reported consistently in our cohort (18, 19). Anticentromere antibodies were disclosed in 3/23 (13\%) patients with lSSc, i.e. in a lower prevalence than found by us in a large series of patients with this variety (29.7\%) (20). The difference might be due to a much smaller number of cases studied for cytokines. Other antibodies were detected in single cases.

Evaluation of serum levels of sTNFα-RI, sIL-2R, sICAM-1 and PIIINP

Serum concentrations of sTNFα-RI, sIL-2R, sICAM-1 and PIIINP were assessed in the patients with lSSc and Raynaud's disease (RD) with the use of ELISA kits (R & D Systems, Minneapolis, MN, USA), according to the manufacturer’s protocols. Sera were diluted 1:10 and with the use of reagents from Orion Diagnostica, Oulunsalo, Finland.

**RESULTS**

None of the 25 control sera from healthy donors tested showed significantly increased or decreased values for sTNFα-RI, sIL-2R, sICAM-1 and PIIINP. The mean values for sTNFα-RI, sIL-2R, sICAM-1 and PIIINP in healthy individuals were: 820±220 pg/ml; 720±112 U/ml; 207±63 ng/ml; and 3.01±0.75 mcg/l, respectively. Serum levels of sTNFα-RI were found to be significantly higher in patients with dSSc than in the controls (Table I). The mean value of sTNFα-RI concentration in serum of patients with dSSc was significantly higher than in patients with lSSc (p<0.01). Individual levels of sTNFα-RI were significantly increased in 10/13 (77%) patients with dSSc, in 6/20 (30%) with lSSc and in 0/7 (0%) patients with Raynaud’s disease.

Serum concentrations of sIL-2R were found to be higher in patients with dSSc than in the controls. The mean value of sIL-2R was significantly higher in serum of patients with dSSc than in serum of patients with lSSc (p<0.03 (Table I). The levels of sIL-2R were significantly increased in 6/13 (46\%) patients with dSSc, much less in lSSc, 3/20 (15\%), and in only 1 patient with Raynaud’s disease.

Serum levels of sICAM-1 were found to be higher in patients with dSSc than in the control. Mean values of sICAM-1 concentration in sera of patients with dSSc did not differ from those of patients with lSSc (Table I). Individual levels of sICAM-1 were significantly increased in 7/13 (54%) patients with dSSc and in 15/23 (65%) with lSSc, but not in patients with Raynaud’s disease.

Mean values of PIIINP concentrations in serum of patients with dSSc were significantly higher than in the patients with lSSc (p<0.008) (Table I). Individual concentrations of PIIINP were significantly increased in 10/13 (77\%) patients with dSSc and in a high proportion of patients with lSSc (9/18, 50\%). One patient with Raynaud’s disease also had an increased level of serum PIIINP.

Linear regression analysis of the data showed significant correlations between serum concentrations of sTNFα-RI and sIL-2R (p=0.001) as well as PIIINP (p=0.001) and PIIINP (p=0.001) and sIL-2R (p=0.002), whereas there was no correlation between levels of sICAM-1 and other parameters.

Serum levels of TNFαRI showed the best clinical correlations with skin, muscle and lung involvement (Table II). Increased TNFαRI serum concentration was the only parameter which significantly correlated with the lung disease in SSc. Increased values of all parameters studied, except for ICAM-1, i.e. sTNFα-RI, sIL-2R and PIIINP, also correlated with muscle involvement and central skin sclerosis. There was no correlation between these parameters and arthralgia, duration of Raynaud’s phenomenon and duration of skin sclerosis. Concentrations of sTNFα-RI, sIL-2R, sICAM-1 and PIIINP did not correlate with titres and types of ANA (data not shown).
Table II. Correlations of clinical and laboratory findings in patients with SSc

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Laboratory parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sTNFα-RI</td>
</tr>
<tr>
<td>R (duration of Raynaud’s phenomenon)</td>
<td>ns</td>
</tr>
<tr>
<td>S (duration of skin sclerosis)</td>
<td>ns</td>
</tr>
<tr>
<td>Less than 2 year interval between R and S</td>
<td>p = 0.01</td>
</tr>
<tr>
<td>Central skin involvement</td>
<td>p = 0.01</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>ns</td>
</tr>
<tr>
<td>Internal organ involvement:</td>
<td></td>
</tr>
<tr>
<td>Oesophagus</td>
<td>ns</td>
</tr>
<tr>
<td>Lungs</td>
<td>p = 0.006</td>
</tr>
<tr>
<td>Heart</td>
<td>ns</td>
</tr>
<tr>
<td>Muscles</td>
<td>p = 0.001</td>
</tr>
</tbody>
</table>

The statistical significance was determined by chi² test. ns = non-significant.

DISCUSSION

This study on selected laboratory parameters reflecting immune, fibrotic and inflammatory vascular processes in patients with SSc confirmed previous findings on increased levels of sIL-2R (6, 11), sICAM-1 (9, 13, 14) and PIIINP (21–23) in these patients. The highest mean values of all parameters (except for sICAM-1) were found in patients with dSSc, significantly different from the mean values in patients with lSSc. Most importantly, we found a significant increase of sTNFα-R1 in a high proportion of sera from patients with SSc, especially in dSSc. Increased serum levels of TNFα type I and II receptors were reported in SSc patients in a single study, and these authors found a significant correlation between the concentrations of the two types of receptors (12). This was the reason for studying only one type of TNFα receptor (R1), but, in addition, we correlated its levels with other parameters of inflammation and clinical data.

Analysis of individual patients’ data revealed that serum levels of both sTNFα-R1 and PIIINP were most frequently increased in dSSc (in 77% of cases), compared with the concentrations of sIL-2R and sICAM-1. In our series, increased levels of sIL-2R appeared to be a less sensitive parameter of disease severity than did the concentrations of sTNFα-R1 and PIIINP. In lSSc, increased concentrations of sTNFα-R1 and sIL-2R were about three-fold less frequently detected than in dSSc, whereas increased serum levels of sICAM-1 were found with equal frequency in dSSc and lSSc. There was also no correlation between serum levels of sICAM-1 and extent of skin involvement. Similar data were reported by Denton et al. (24) who found that sICAM-1 level did not reflect clinical changes in SSc patients. This would suggest that sICAM-1 is not only related to fibrosis, in spite of its pronounced expression on SSC fibroblasts (6). The soluble ICAM-1 might also be released from activated endothelial cells and mononuclear cells expressing this molecules (6, 14).

In contrast, serum levels of sTNFα-R1, sIL-2R and PIIINP correlated significantly with each other. No correlation of TNFα-R and IL-2R levels was found by Heilig et al. (12), who suggested that these could be independent parameters. However, in our study on a large cohort of SSc patients, all these parameters correlated significantly between themselves but, most importantly, with the extent of cutaneous sclerosis (central skin involvement). It should be stressed that levels of sTNFα-R1 showed better correlations with lung involvement than did PIIINP. Expression of TNFα-R1 is not restricted to lymphocytes, but is also detected on epithelial cells and macrophages (25). This could explain the positive correlation of increased serum level of TNFα-R1 with lung involvement in which the activation of macrophages and possibly also of epithelial alveolar cells is of great importance for development of the interstitial fibrosis.

Disparate results were reported for the levels of TNFα in the sera of SSc patients (4, 9). However, these authors did not evaluate serum concentrations of sTNFα-R1, which, as an inhibitor of TNFα, could substantially affect the levels of TNFα.

Although the pathogenic significance of soluble cytokine receptors is not known, it is conceivable that high levels of soluble TNFα-R, by formation of complexes with TNFα, may inhibit the biological activity of endogenous TNFα, which is known to decrease collagen synthesis and stimulate collagenase (26, 27). In this way, TNFα-R could contribute to the enhancement of the fibrotic process. Another possibility is that the increased level of TNFα-R is only reflecting cytokine activation in the immunologically induced fibrosis. Thus, in addition to recognized parameters of SSc severity (IL-2R, PIIINP), a new important marker appears to be sTNFα-R1. All three parameters of inflammation and fibrosis might reflect the clinical condition and could be helpful in prognostication and monitoring the therapies. In this study TNFα-R1 proved to be the most sensitive indicator of severity of SSc.

ACKNOWLEDGEMENT

The study was supported by a grant 4 PO5 B04410 from the Polish Committee for Scientific Research.

REFERENCES

4. Needleman BW, Wigley FM, Stair RW. Interleukin-1, interleukin-2, interleukin-4, interleukin-6, tumor necrosis factor-α, and inter-


