

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

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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 TNF α 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 (PIIINP). 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 sTNF α RI, sICAM-1, sIL-2R and PIIINP. 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 sTNF α -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 sTNF α -receptor type 1 and PIIINP ($r=0.653$, $p<0.0001$), and sTNF α -receptor type 1 and sIL-2R ($r=0.625$, $p<0.0001$), but not between sTNF α -receptor type 1 and sICAM-1 ($r=0.127$, $p<0.526$). Clinical analysis revealed that serum concentrations of sTNF α -RI 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 sTNF α -receptor type 1. **Key words:** cytokine receptors; disease severity; scleroderma.

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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 (IFN γ) and TNF α 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 TNF α 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 TNF α 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 TNF α -receptor type 1 (sTNF α -RI), 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 oesophagus dysmotility on radiography or scintigraphy. Lung involvement was assessed by X-ray examination and lung func-

Table I. Serum concentrations of sTNF α -RI, sIL2-R, sICAM-1 and PIINP in patients with SSc and Raynaud's disease (RD)

Groups	Parameters			
	sTNF α -RI mean $\pm$ D (pg/ml)	sIL2-R mean $\pm$ SD (U/ml)	sICAM-1 mean $\pm$ SD (ng/ml)	PIINP mean $\pm$ D (mcg/l)
dSSc	1771 $\pm$ 769 ^{ab} 77% (10/13) ^c	1592 $\pm$ 1167 ^{ab} 46% (6/13)	389 $\pm$ 119 ^a 54% (7/13)	6.0 $\pm$ 1.5 ^{ab} 77% (10/13)
ISSc	1283 $\pm$ 250 ^a 30% (6/20)	935 $\pm$ 504 ^a 15% (3/20)	367 $\pm$ 125 ^a 65% (15/23)	4.5 $\pm$ 1.4 ^a 50% (9/18)
RD	994 $\pm$ 229 0%	798 $\pm$ 796 14% (1/7)	228 $\pm$ 56 0%	+0.5 14% (1/7)
Controls means	820 $\pm$ 220	720 $\pm$ 112	207 $\pm$ 63	3.01 $\pm$ 0.75
normal ranges	380 – 1260	496 – 944	121 – 333	1.5 – 4.5

^aSignificantly different from the control.

^bSignificantly different from ISSc, as assessed by Student's *t*-test.

The normal ranges, i.e. cut off values were based on the data of 25 healthy control sera (mean $\pm$ 2 SD).

^cPercentage and numbers of patients with increased serum levels (relates to all patients groups).

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 creatine 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 Scl 70, anticentromere, anti-U1-RNP, anti-fibrillarin, 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 ISSc, 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 ISSc, 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 sTNF α -RI, sIL2-R, sICAM-1 and PIINP

Serum concentrations of sTNF α -RI, sIL-2R, sICAM-1 were assessed with the use of ELISA kits (R & D Systems, Minneapolis, MN, USA), according to the manufacturer's protocols. Sera were diluted 1 : 10 and studied in three replicates and mean values were used for the statistical analysis. PIINP serum levels were determined by radioimmunoassay 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 sTNF α -RI, sIL-2R, sICAM-1 and PIINP. The mean values for sTNF α -RI, sIL-2R, sICAM-1 and PIINP in healthy individuals were: 820 $\pm$ 220 pg/ml; 720 $\pm$ 112 U/ml; 207 $\pm$ 63 ng/ml; and 3.01 $\pm$ 0.75 mcg/l, respectively.

Serum levels of sTNF α -RI were found to be significantly higher in patients with dSSc than in the controls (Table I). The mean value of sTNF α -RI concentration in serum of patients with dSSc was significantly higher than in patients with ISSc ($p < 0.01$). Individual levels of sTNF α -RI were signif-

icantly increased in 10/13 (77%) patients with dSSc, in 6/20 (30%) with ISSc 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 concentration in serum of patients with dSSc was significantly higher than in serum of patients with ISSc ($p < 0.03$) (Table I). The levels of sIL-2R were significantly increased in 6/13 (46%) patients with dSSc, much less in ISSc, 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 ISSc (Table I). Individual levels of sICAM-1 were significantly increased in 7/13 (54%) patients with dSSc and in 15/23 (65%) with ISSc, but not in patients with Raynaud's disease.

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

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

Serum levels of TNF α -RI showed the best clinical correlations with skin, muscle and lung involvement (Table II). Increased TNF α -RI 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. sTNF α -RI, sIL-2R and PIINP, 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 sTNF α -RI, sIL-2R, sICAM-1 and PIINP did not correlate with titres and types of ANA (data not shown).

Table II. Correlations of clinical and laboratory findings in patients with SSc

Clinical parameters	Laboratory parameters			
	sTNF α -RI	sIL2-R	sICAM-1	PIIINP
R (duration of Raynaud's phenomenon)	ns	ns	ns	ns
S (duration of skin sclerosis)	ns	ns	ns	ns
Less than 2 year interval between R and S	ns	$p=0.01$	ns	ns
Central skin involvement	$p=0.01$	$p=0.03$	ns	$p=0.007$
Arthralgia	ns	ns	ns	ns
Internal organ involvement:				
Oesophagus	ns	ns	ns	ns
Lungs	$p=0.006$	ns	ns	ns
Heart	ns	ns	ns	ns
Muscles	$p=0.001$	$p=0.01$	ns	$p=0.001$

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 sTNF α -RI in a high proportion of sera from patients with SSc, especially in dSSc. Increased serum levels of TNF α 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 TNF α receptor (RI), 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 sTNF α -RI 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 sTNF α -RI and PIIINP. In lSSc, increased concentrations of sTNF α -RI 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 sTNF α -RI, sIL-2R and PIIINP correlated significantly with each other. No correlation of TNF α -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

sTNF α -RI showed better correlations with lung involvement than did PIIINP. Expression of TNF α -RI 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 sTNF α -RI 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 TNF α in the sera of SSc patients (4, 9). However, these authors did not evaluate serum concentrations of sTNF α R, which, as an inhibitor of TNF α , could substantially affect the levels of TNF α .

Although the pathogenic significance of soluble cytokine receptors is not known, it is conceivable that high levels of soluble TNF α R, by formation of complexes with TNF α , may inhibit the biological activity of endogenous TNF α , which is known to decrease collagen synthesis and stimulate collagenase (26, 27). In this way, TNF α R could contribute to the enhancement of the fibrotic process. Another possibility is that the increased level of TNF α 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 sTNF α -RI. 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 TNF α -RI proved to be the most sensitive indicator of severity of SSc.

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