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Serum Cytokine and Anti-FcγR Autoantibody Measurements in Patients with Systemic Sclerosis

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The serum IL-1β, IL-2, IL-4, IL-6, IL-8, TNF-α and soluble IL-2 receptor levels were measured, and the presence of anti-Fcγ receptor (FcγR) antibodies was investigated in the sera of 18 patients with systemic sclerosis (SSc). An increase of TNF-α was detected in 8 of the 18 cases. Il-1β was elevated in all the 18 patients. Both IL-2 and IL-4 were elevated in 7 cases. The IL-6 level was elevated in 17 patients, while IL-8 was increased in all cases. The soluble IL-2 receptor level was elevated in 11 patients. FcγR-specific antibodies were detected in the sera of 6 patients, and there was a significant association between anti-FcγR antibody positivity and IL-4 elevation. The presence of anti-FcγR antibodies may influence several cell functions and may contribute to the remarkable variability of cytokine levels in SSc. ***Key words: interleukin; anti-Fcγ receptor antibody; scleroderma.***

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Systemic sclerosis (SSc) is an autoimmune disease, characterized by fibrosis and vasculopathy of the skin and certain internal organs. In the early stage of SSc, a mononuclear cell infiltrate appears in the skin, demonstrating the importance of the altered cellular immunity in this disorder.

Several serum cytokines have been tested in SSc. The results show a remarkable variability, indicating that the cytokine levels are influenced by several different factors. Serum interleukin 1β levels were described as elevated in several cases (1, 2), but normal values were also described (3, 4). Similarly, TNF-α, IL-2, IL-4, IL-6 were also found to be normal or elevated in several patients' sera (1, 3–10). The serum IL-8 levels have also been described as increased in several cases with SSc (9, 11). An activation of the cellular immunity can also be demonstrated by an increase in the serum soluble IL-2 receptor levels in this disorder (1, 4, 5, 7–9, 12, 13).

The cause of the high variability in the serum cytokine patterns remains unclear. Beside the geographic differences and the different selection of patients, serum inhibitory factors, autoantibodies directed against the different cytokines can substantially influence the results. Some recent publications have described the presence of autoantibodies against Fcγ receptors (FcγR) in SSc (14). The binding of these antibodies to a series of cells including monocytes/macrophages, B lymphocytes, neutrophil granulocytes and natural killer cells can also modify the serum cytokine levels in this disorder. The interaction of FcγR (CD16) ligands of natural killer cells and monocytes induces a transcription and/or secretion of several cytokines (15, 16).

In this study, we detected anti-FcγR autoantibodies and measured the IL-1, IL-2, IL-4, IL-6, IL-8, TNF-α and soluble IL-2 receptor levels in the sera of 18 patients with SSc.

PATIENTS AND METHODS

Patients

Eighteen patients with SSc encountered at the 3rd Department of Internal Medicine of University Medical School of Debrecen (Hungary) were investigated. Clinical and laboratory data of the patients were evaluated by a standard protocol, as previously described (17). All patients fulfilled the diagnostic criteria for SSc (18). For the classification of patients, the two-subset model was used (19). The mean age ± SD of the patients was 53.9 ± 6.2 years and the mean disease duration 12.3 ± 8.4 years. The mean follow-up of the cases was 6.8 ± 3.8 years. Four patients were classified as having diffuse scleroderma. Seventeen patients had lung involvement. Diffuse lung fibrosis was detected in 4 cases; bibasilar pulmonary fibrosis was found in 11 patients. Signs of restrictive ventilatory failure were found in 11 cases. Oesophageal dysmotility was detected in 10 cases. Sicca syndrome was observed in 6 cases and myositis in one case. Cardiac involvement was found in 7 patients and subcutaneous calcinosis in 2 patients. None of the patients had renal involvement. An obvious sign of disease activity (progression of the skin involvement) was detected in 2 cases with diffuse scleroderma. Antinuclear antibodies were detected by indirect immunofluorescence on HEp-2 cells in 17 patients. Anticentromere antibodies were found in 2 cases. Eleven patients were positive for anti-topoisomerase antibody.

With regard to the therapy, nifedipine (20–40 mg daily) was administered in 16 cases. Pentoxyfylline (800–1200 mg daily) was used in 15 patients. Four patients received D-penicillamine (150–600 mg/day) therapy. Vitamin E therapy (400 mg/week) was administered in 10 cases. H_2 receptor blocking agents (cimetidine, ranitidine, famotidine) were used in 10 patients.

The sera of 25 healthy volunteers were also investigated. There was no significant difference between the patients and the controls in terms of racial distribution or female/male ratio.

Detection of FcγR-specific autoantibodies

The presence of FcγR-specific autoantibodies was determined, as previously described (14, 20). Recombinant mouse FcγR (21) was bound to polystyrene plates (2.5 μg/ml, carbonate buffer, pH 9.7). This receptor can detect antibodies against all the three types of human FcγR (14). To prevent non-specific binding of IgG through the Fc region, the mouse FcγR was reduced and alkylated. After an incubation with 1:100 and 1:400 dilution of the samples (1 h, room temperature), the specific antibodies bound to the plates were detected with peroxidase-labelled anti-human IgG and IgM secondary antibodies produced in sheep (5 μg/ml, Sigma Chemical Co.). The murine TSK-23 IgM monoclonal antibody directed against mouse FcγR was used as positive control. Serum samples showing a reactivity above this positive control were considered as positive if the reactivity decreased by a serial dilution of the particular sample(s) indicating the specificity of the binding. No positive sample was found among the 25 healthy controls tested.

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Measurement of cytokine levels

The cytokine levels were determined by the ELISA method. Sera of patients with SSc and 25 controls were also evaluated. For the detection of serum IL-1β, TNF-α, IL-6 and IL-8 levels, Quantikine immunoassay (R&D Systems Inc, Minneapolis, MN, USA) was used. For the detection of IL-2 and IL-4, Intertest-2 and Intertest-4 were used (Genzyme, Cambridge, MA, USA). The soluble IL-2 receptor level was determined by RIL2s ELISA kit (Immunotech S.A., Marseille, France). Duplicates were evaluated by a standard curve consisting of 7 points. The mean ± 2 SD values of the controls were taken as an elevated serum cytokine level.

RESULTS

An increased serum TNF-α level was detected in 8 of the 18 cases. IL-1β was increased in all the 18 patients. IL-2 and IL-4 were evaluated in 7 cases. IL-6 was elevated in 17 patients, while IL-8 was increased in all cases. The soluble IL-2 receptor level was elevated in 11 cases (Table I). The elevation of TNF-α and IL-4 levels, compared to the elevation of the other cytokines, was modest.

Six of the 18 cases showed anti-FcγR IgG antibody positivity. No IgM antibody was detected, and none of the control sera contained FcγR-specific antibody. Table II shows the number of sera with elevated cytokine levels in the anti-FcγR antibody positive and negative group. Five of the anti-FcγR antibody positive sera exhibited simultaneously an elevated IL-4 level as well (Table II). The association between anti-FcγR antibody positivity and increased IL-4 level was statistically significant ($p < 0.03$ by chi-square test). We failed to detect any correlation of the elevated levels of any investigated cytokine with sex, age, type of scleroderma, gastrointestinal, pulmonary, cardiac, renal or muscle involvement, the presence of antinuclear, anti-Scl-70, or anticentromere antibodies. Furthermore, no correlation was detected between disease duration, activity, therapy and cytokine levels (data not shown).

Table I. *Serum cytokine levels in 18 patients with systemic sclerosis*

	Controls[1] (25) (mean ± SD)	SSc (18) (mean)	SSc (18) (No. of positive cases[2])
TNFα (pg/ml)	5.8 ± 2.3	11.0 ± 6.6	8
IL-1β (pg/ml)	4.2 ± 1.2	36.3 ± 18.5	18
IL-6 (pg/ml)	14.3 ± 7.9	159.6 ± 120.5	17
IL-8 (pg/ml)	3.5 ± 0.9	321.0 ± 204.1	18
IL-2 (pg/ml)	100.0 ± 20	248.8 ± 20.2	7
IL-4 (ng/ml)	0.75 ± 0.1	1.2 ± 1.25	7
sIL-2R (pg/ml)	4704 ± 1400	6026 ± 4300	11

[1]Cases showing no activity in the sera were taken with a value of 0.
[2]The number of positive cases was defined as values above the mean ELISA extinction values of the controls + 2 SD.

Table II. *The distribution of FcγR positivity and increased cytokine levels in the sera of 18 patients with SSc*
D: diffuse cutaneous systemic sclerosis, L: limited cutaneous systemic sclerosis (21).

Patients	TNF[1]	IL-1	IL-2	IL-4	IL-6	IL-8	sIL-2R	FcγR	SSc
1.	−	+	+	+	+	+	+	+	D
2.	+	+	+	+	+	+	+	+	L
3.	+	+	−	+	+	+	+	+	L
4.	−	+	+	+	+	+	+	+	L
5.	−	+	−	−	+	+	−	+	L
6.	−	+	+	+	+	+	+	+	L
7, 13.	+	+	−	−	+	+	−	−	L
8.	−	+	−	−	+	+	+	−	L
9.	+	+	+	−	+	+	+	−	L
10.	+	+	−	−	+	+	−	−	L
11.	−	+	+	−	+	+	−	−	L
12.	−	+	−	−	+	+	+	−	L
14.	−	+	−	−	+	+	+	−	L
15.	−	+	−	−	−	+	+	−	D
16.	+	+	−	+	+	+	+	−	D
17.	+	+	−	+	+	+	−	−	D
18.	−	+	+	−	+	+	−	−	L

[1]Positive samples were defined as ELISA extinction values above the mean of the controls + 2 SD.

DISCUSSION

In the present study, the serum IL-1β, IL-2, IL-4, IL-6, IL-8, TNF-α and soluble IL-2 receptor levels were investigated in 18 patients with SSc. Our results are basically similar to those of previous investigators, with the exception of the very high proportion of elevated IL-1β, IL-6 and IL-8 levels among our cases. We measured an increased IL-1β level in the sera of all patients, while Needleman et al. (3) and Kantor et al. (4) did not detect IL-1β elevation in their cases. Reitamo et al. (11) showed an elevated IL-8 level in 24 of 134 patients, while we found an IL-8 increase in all patients. IL-1β and IL-6 are among those cytokines that have been reported to alter various fibroblast activities, such as growth, production of extracellular matrix components, production of collagenase or prostaglandins and expression of major histocompatibility molecules (3). IL-8 is produced by a variety of cell types and is chemotactic for neutrophil granulocytes and possibly also for T lymphocytes. The second function may be important in SSc, as in this disorder the inflammatory cell infiltration consists primarily of mononuclear cells including T lymphocytes.

In our patients TNF-α, soluble IL-2R and IL-2 levels were evaluated in 28–56%. Similarly to Needleman et al. (3) and Reitamo et al. (11) we also failed to detect a positive correlation between IL-1, IL-4, IL-6, IL-8 and anti-IL-8 antibody levels and the disease activity in patients with SSc.

Previous findings in the literature show a remarkable variability in the cytokine levels. Geographical differences, different subset, distribution, and/or disease activity of the cases investigated and the presence of autoantibodies against the different cytokines (11, 22, 23) may contribute to this variability. As another secondary phenomenon, anti-FcγR autoantibodies can also contribute to the variability of cytokine levels detected in SSc (15, 16). Anti-FcγR antibodies were detected against all the three types of human FcγR in the sera of patients with SSc, SLE and Sjögren's syndrome (14, 20). Since these anti-

bodies can bind to all the three types of human FcγR, they can possibly influence the functions of monocytes/macrophages, B lymphocytes, neutrophil granulocytes and natural killer cells. These antibodies can contribute to the maintenance of the high level of circulating immune complexes by blocking immune complex phagocytosis via the FcγR and can trigger the release of lysosomal enzymes and reactive oxygen intermediates from granulocytes (24, 25). Besides, these antibodies possibly enhance the release of certain cytokines from these cells and may have an impact on the inflammatory mechanisms occurring in different organs.

In this study, the sera of 6 patients contained specific antibody against FcγR. We compared the cytokine level in the anti-FcγR antibody positive and negative group, and we found a significant association between anti-FcγR antibody positivity and IL-4 elevation. The IL-4 level was elevated in almost all cases (5/6) with anti-FcγR antibody, while in the negative group only 2 of 12 patients produced an increased IL-4 level. The reason for the association of FcγR autoantibodies and increased IL-4 level needs further clarification. This cytokine is produced by the T helper type two (TH2) lymphocytes, which can express FcγR after activation. The other cytokine which is also a TH2 product is IL-6, but it can also be produced by several non-immune cells including fibroblasts; therefore an elevated serum IL-6 level does not necessarily originate from the TH2 lymphocytes. To our knowledge, this is the first study in which the levels of different cytokines and anti-FcγR antibody production in SSc are compared, and we think that the presence of these antibodies may influence several cell functions and may contribute to the remarkable variability of cytokine levels in this disorder.

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