Circulating levels of Th1/Th2 cytokines in patients with primary Sjögren's syndrome: Correlation with clinical and immunological features

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Abstract

Objective

To analyse the circulating levels of Th1 and Th2 cytokines in patients with primary Sjögren's syndrome (SS), as well as to investigate their association with clinical and immunological manifestations.

Methods

We included 62 consecutive patients (58 women and 4 men) seen in our Unit. All patients fulfilled 4 or more of the European diagnostic criteria for SS. Serum levels of IL-6 (pg/mL), IL-2 (pg/mL), srIL-2 (pM), TNF (pg/mL) and IL-10 (pg/mL) were determined using a solid phase enzyme immunoassay performed on microtiter plate.

Results

When compared with the control group, high levels of Th1 (Il-2, srIL-2) and Th2 (IL-6, IL-10) cytokines were detected in SS patients, although only IL-6 levels reached statistical significance. On the other hand, analysis of the mean serum concentrations of cytokines showed distinct patterns of elevated cytokines according to the organ involved, and elevated levels of IL-6 (126.5 v 20.6 pg/mL, $p < 0.05$) and IL-10 (10.6 v 2.2 pg/mL, $p < 0.005$) were observed in those patients with liver involvement. Analysis of the cytokine levels according to the presence of immunological features showed: higher levels of srIL-2 (95.6 v 54.0 pM, $p < 0.05$) in patients with anti-Ro/SS-A antibodies; increased levels of srIL-2 (111.4 v 59.4 pM, $p < 0.05$) in patients with antiLa/SS-B antibodies; higher levels of srIL-2 (90.4 vs 50.8 pM, $p < 0.05$) and TNF (37.9 v 22.6 pg/mL, $p = 0.001$) in patients with RF and higher levels of IL-6 (88.0 v 23.1 pg/mL, $p < 0.05$) in patients with cryoglobulins and in those with hypocomplementemia (130.3 vs 21.0 pg/mL, $p < 0.05$).

Conclusion

We found a significant elevation of several circulating cytokines in some clinical and immunological subsets of patients with primary SS. These cytokine patterns may be markers for specific extraglandular involvement in SS and could be of interest in assessing the response to treatment protocols or in monitoring the disease evolution.

Key words

Interleukin 2, soluble receptor of interleukin 2, tumor necrosis factor, interleukin 6, interleukin 10, primary Sjögren's syndrome.

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Introduction

Sjögren's syndrome (SS) is a systemic autoimmune disease that mainly affects the exocrine glands and usually presents as a persistent dryness of the mouth and eyes. This is caused by diminished salivary and lachrymal secretory function resulting from a loss of glandular parenchyma due to chronic mononuclear cell infiltrate (1). The histological hallmark is a focal lymphocytic infiltration of the exocrine glands. The disease spectrum extends from organ-specific autoimmune disease (autoimmune exocrinopathy) (2) to systemic processes (musculoskeletal, pulmonary, gastric, hematological, vascular, dermatological, renal and neurological involvement). In the absence of any associated systemic autoimmune disease, patients with this condition are classified as having "primary" SS. Because of this heterogeneity, attempts have been made to identify subsets of patients that would permit a more reliable prediction of the course of primary SS in affected individuals (3, 4).

Recent studies have suggested that cytokine-mediated mechanisms may play an important role in the pathogenesis of primary SS (5). Firstly, the mutual stimulation of Th1 cells and the target organ, via the production of various cytokines, may play a key role in the induction and/or maintenance of the disease, giving rise to the eventual destruction of the target organ (6). Additionally, Th2 cytokines stimulate B cells to differentiate, proliferate and produce immunoglobulins, thus playing a role in the lymphoaggressiveness that characterises SS.

Several studies have analysed the pattern of cytokine production in the labial salivary gland of SS patients (6-14), as well as in peripheral blood lymphocytes or saliva (7, 11, 12, 15, 16), but the incidence and potential correlation of circulating cytokine levels with the clinical and serologic manifestations of SS have been little studied (17-20). The objective of this study was to analyse the circulating levels of Th1 and Th2 cytokines in patients with primary Sjögren's syndrome (SS), as well as to investigate their association with clinical and immunological manifestations.

Patients and methods

Patients

We investigated 62 consecutive patients (58 women and 4 men; mean age 61 years; range 40 to 80 years) in our Unit. All patients fulfilled four or more of the preliminary diagnostic criteria for SS proposed by the European Community Study Group in 1993 (21). In addition, a positive minor salivary gland biopsy result or a positive immunological test (antinuclear antibodies, rheumatoid factor, anti-Ro/SS-A and/or anti-La/SS-B) was required for the diagnosis of SS. None of the patients presented clinical or immunological evidence of other systemic autoimmune disease.

All patients underwent a complete history and physical examination, as well as diagnostic tests for SS applied according to the recommendations of the European Community Study Group (21). Twenty (33%) patients had disease limited to the exocrine glands, and the remaining 42 (67%) had 1 or more of the following extraglandular manifestations: arthralgia and/or arthritis in 25 (40%) patients, autoimmune thyroiditis (positive autoantibodies and altered thyroid function) in 15 (24%), cutaneous vasculitis (demonstrated by cutaneous purpura and skin biopsy) in 10 (16%), Raynaud's phenomenon in 9 (15%), pulmonary involvement (demonstrated by the clinical picture, altered chest radiography and/or spirometry) in 8 (13%), peripheral neuropathy (demonstrated by the clinical picture and nerve conduction tests) in 6 (10%), and liver involvement (altered liver function tests and/or liver biopsy, unrelated to hepatitis B or hepatitis C virus infection) in 5 (8%). Those patients with peripheral neuropathy were treated with oral corticosteroids (0.5-1 mg/kg/day), as were 5 patients with cutaneous vasculitis (< 0.5 mg/kg/day).

Controls

Twenty-eight outpatients of similar age and gender (25 women and 3 men, mean age 56 years, range 21-78 years) were also included in the study as control subjects. None of them showed clinical evidence of autoimmune or infectious diseases.

Laboratory studies

Serum levels of IL-6 (pg/mL), IL-2 (pg/mL), srIL-2 (pM), TNF (pg/mL) and IL-10 (pg/mL) were determined using a solid phase enzyme immunoassay performed on microtiter plate (Medgenix, BioSource Europe, Fleurus, Belgium, for IL-2, Il-6 and TNF ; Immunotech, Marseille, France, for srIL-2; and Perseptive, Framingham, MA, USA, for IL-10). These techniques are sandwich assays performed on microtiter plates in which the wells are coated with the first monoclonal antibody. After the addition of a second monoclonal antibody labelled with an enzyme, the microtiter plate is washed to remove unbound enzyme labelled antibodies. The revealing solution is added and incubated. The intensity of the developed coloring is proportional to the concentration of cytokine.

Other immunological tests included antinuclear antibodies (ANA) (indirect immunofluorescence using mouse liver as substrate), antibodies to dsDNA (determined by Farr's ammonium sulphate precipitation technique), precipitating antibodies to the extractable nuclear antigens Ro/SS-A and La/SS-B (counterimmunoelectrophoresis) and rheumatoid factor (RF) (ELISA). Complement components (C3 and C4) were estimated by the radial immunodiffusion method and CH50 by Lachmann's haemolytic technique. Serum cryoglobulinemia was measured after centrifugation; serum supernatant was removed, incubated at 4° for 8 days and examined for cryoprecipitation. The type of cryoglobulinemia was identified by agarose gel electrophoresis, combined with immunofixation.

Statistical analysis

We used conventional chi-square and Fisher's exact tests to analyse qualitative differences, Student's test for the comparison of means in large samples of similar variance, and the non-parametric Mann-Whitney U test for small samples. Values of quantitative variables are expressed as mean ± standard error of the mean (SEM). A value of $p < 0.05$ was taken to indicate statistical significance. In order to correct the p values for the number of variables tested, we applied Bonferroni's correction. This statistical analysis was performed by means of the SPSS program using the information stored in the data-base program.

Results

Comparison of soluble cytokine levels between SS patients and control group

Patients with primary SS showed the following mean values of soluble cytokine levels: 155.5 pg/ml ± 43.2 pg/ml for IL-2, 66.6 pM ± 4.9 pM for srIL-2, 28.6 pg/ml ± 2.4 pg/ml for TNF , 29.4 pg/ml ± 8.3 pg/ml for IL-6 and 2.8 pg/ml ± 0.5 pg/ml for IL-10. When compared with the control group, high levels of Th1 (Il-2, srIL-2) and Th2 (IL-6, IL-10) cytokines were detected in SS patients, although only IL-6 levels reached statistical significance (29.4 pg/ml vs 4.5 pg/ml, $p = 0.04$) (Table I).

Soluble cytokine levels and extraglandular manifestations of SS

We analysed the mean serum concentrations of cytokines in SS patients according to the presence or absence of extraglandular manifestations (Table II). Distinct patterns of elevated cytokines were observed according to the organ involved. In patients with liver involvement, elevated levels of IL-6 (126.5 pg/ml vs 20.6 pg/ml, $p < 0.05$) and IL-10 (10.6 pg/ml vs 2.2 pg/ml, $p < 0.05$) were observed. Patients with pulmonary involvement showed higher levels of IL-6 (73.8 pg/ml vs 20.6 pg/ml) and TNF (43.2 pg/ml vs 26.4 pg/ml), and those with cutaneous vasculitis higher levels of IL-6 (63.0 pg/ml vs 20.1 pg/ml) and srIL-2 (89.6 pM vs 62.2 pM), although p values were under 0.05 significance after Bonferroni's correction.

Soluble cytokine levels and immunological features of SS

We also analysed the mean serum concentrations of cytokines in SS patients according to the presence of immunological features (Table III). Patients with anti-Ro/SS-A antibodies showed higher levels of srIL-2 (95.6 pM vs 54.0 pM, $p < 0.05$),and those with anti-La/SS-B antibodies showed increased levels of srIL-2 (111.4 pM vs 59.4 pM, $p < 0.05$). Patients with positive RF

Table I. Mean serum ± SEM concentrations of cytokines levels in SS patients, compared with control group.

	Patients (n=62)	Controls (n=28)	P
IL-2 (pg/ml)	155.5 ± 43.2	80.8 ± 15.6	-
srIL-2 (pM)	66.6 ± 4.9	53.0 ± 5.7	-
TNF (pg/ml)	28.6 ± 2.4	29.8 ± 1.7	-
IL-6 (pg/ml)	29.4 ± 8.3	4.5 ± 1.3	0.04
IL-10 (pg/ml)	2.8 ± 0.5	2.4 ± 0.5	-

Table II. Mean serum concentrations of cytokines in SS patients grouped according to the presence of extraglandular manifestations.

Extraglandular manifestation	Patients (n)	IL-2 (pg/ml)	srIL-2 (pM)	TNF (pg/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)
Articular involvement	25	148.6	65.8	23.9	15.8	3.2
Autoimmune thyroiditis	15	115.4	67.4	29.6	22.5	2.2
Cutaneous vasculitis	10	20.6	89.6	31.8	63.0	4.9
Raynaud's phenomenon	9	156.9	87.4	33.0	21.9	2.9
Pulmonary involvement	8	291.8	84.8	43.2	73.8	2.7
Peripheral neuropathy	6	11.7	75.5	29.3	10.8	2.2
Liver involvement	5	10.2	84.2	37.0	126.5*	10.6*

* $p < 0.05$ (Bonferroni's correction).

Table III. Mean serum concentrations in SS patients grouped according to the presence of immunological features.

Immunological feature	Patients (n)	IL-2 (pg/ml)	srIL-2 (pM)	TNF (pg/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)
ANA	42	145.6	72.4	31.0	33.7	3.2
RF	25	115.4	90.4*	37.9*	50.2	4.2
Anti-Ro/SS-A	19	83.2	95.6*	35.5	30.1	4.2
Anti-La/SS-B	9	28.0	111.4*	40.8	50.8	3.6
Cryoglobulins	6	76.7	93.5	46.0	88.0*	3.0
Hypocomplementemia	4	17.0	115.8*	39.2	130.3*	4.2

*$p < 0.05$ (Bonferroni's correction).

showed higher levels of rIL-2 (90.4 pM vs 50.8 pM, $p < 0.05$) and TNF (37.9 pg/ml vs 22.6 pg/ml, $p < 0.05$). In patients with cryoglobulins, higher levels of IL-6 (88.0 pg/ml vs 23.1 pg/ml, $p < 0.05$) were observed. Finally, patients with hypocomplementemia showed higher levels of IL-6 (130.3 pg/ml vs 21.3 pg/ml, $p < 0.05$).

Discussion

In this study, higher levels of Th1 (IL-2, srIL-2) and Th2 (IL-6 and IL-10) were found in SS patients when compared with the control group, although the difference was statistically significant only in the case of IL-6 levels. In addition, we found that IL-6 levels were higher in patients with RF, cryoglobulins or hypocomplementemia, while patients with positive anti-Ro/SS-A and anti-La/SS-B antibodies showed mainly higher levels of srIL2. Some authors have previously studied circulating levels of cytokines in SS patients. Grisius *et al*. (17) determined the salivary and serum IL-6 in 31 patients with primary SS and found that mean IL-6 concentrations were elevated in the SS patients compared with healthy control subjects. Two other groups have also reported elevated levels of IL-6 and IFN- in patients with SS (16, 22), and other authors have found higher levels of sIL-2r in patients with SS (18, 20).

We found increased levels of IL-6 and IL-10 in SS patients with liver involvement. A possible relationship between IL-6 and liver disease has been postulated (23). Kakumu *et al*. (24) found that locally produced IL-6 contributes to the inflammatory process and immunological response in acute and chronic liver disease and other authors (25) have found an increased IL-6 concentration in response to hepatic resection. Moreover, Muller *et al*. (26) described higher IL-6 production by peripheral blood monocytes in patients with primary biliary cirrhosis and Sun *et al*. (27) found elevated IL-6 levels in patients with acute hepatitis. All the studies indicate that IL-6 seems to be a specific marker of liver inflammation and may play a role in hepatocyte injury. Additionally, the role of IL-10 in hepatic disease has been considered in one study (28).

The wide variety of immunoglobulins produced in SS patients includes several autoantibodies such as RF, antibodies directed to specific nuclear/cytoplasmic antigens of the RNA-protein complex and antibodies to organ-specific antigens present in different tissues. This overproduction of immunoglobulins and autoantibodies is due to a B lymphocyte activation, which is considered to be the most characteristic immunoregulatory abnormality in patients with SS (29). We found several significant correlations of cytokine levels with the main immunological features of SS patients. Patients with anti-Ro/SS-A and anti-La/SS-B antibodies showed higher levels of circulating srIL-2. This correlation was studied by Tomas *et al*. (18) which found a correlation between the srIL-2 level and the presence of SS-A and SS-B antibodies in patients with SS. However, our SS population shows a low prevalence of anti-ENA autoantibodies. This may be related to the technique used by our Immunology Laboratory (counterimmunoelectrophoresis), which has a high specificity but a low sensitivity (80%), using purified 60 kDa Ro and La antigens. In addition, McCauliffe *et al*. (30) described the existence of anti 52 kDa autoantibodies in 5 (28%) of 18 SS patients with undetectable Ro and La autoantibodies by conventional techniques, and Beer *et al*. (31) described the existence of nonprecipitating anti-La/SS-B autoantibodies in 19% of their SS patients.

In this study, the strong association of cryoglobulinemia and hypocomplementemia with elevated serum levels of IL-6 suggests a possible role for this cytokine in the immune complex mediated pathology implicated in the pathogenesis of several extraglandular manifestations in SS patients. Some studies (32) have suggested that IL-6 might promote the production of autoantibodies such as RF, the main component of mixed cryoglobulins (33).

In conclusion, we found different patterns of elevated circulating cytokines in clinical and immunological subsets of patients with primary SS. It is possible that these circulating cytokines are secreted by infiltrating inflammatory cells located in extraglandular sites, and that a distinct pattern of circulating cytokine elevation might be correlated with the immunological damage of the affected organs. A more complete understanding of the complex consequences of SS might also lead to the possible therapeutic application of cytokine agonists and antagonists in order to disrupt the cytokine network, as a further means of inhibiting the initiation and/or progression of the disease. Further studies are needed to evaluate the role of cytokine levels in order to monitor disease evolution and assess clinical response to treatment.

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