Elevated interleukin-6 plasma levels are regulated by the promoter region polymorphism of the IL6 gene in primary Sjögren’s syndrome and correlate with the clinical manifestations of the disease

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Abstract

Objectives. To determine whether plasma interleukin-6 (IL-6) and G/C base exchange polymorphism at position −174 of the IL6 gene have an effect on the clinical manifestations of primary Sjögren’s syndrome (pSS).

Methods. Levels of circulating IL-6 protein and polymorphism of the IL6 gene were analysed in 66 patients with pSS and in 400 healthy subjects. These data were studied in relation to clinical data on the pSS patients.

Results. Plasma IL-6 was elevated in pSS patients compared with healthy controls. pSS patients with coeliac disease, pulmonary fibrosis or alveolitis or peripheral nervous system symptoms had significantly higher IL-6 levels than patients without these manifestations. IL-6 levels increased in parallel with the histological grade of minor salivary gland biopsy and the number of pSS criteria fulfilled. IL6 allele frequencies were similar in patients and normal subjects. Plasma IL-6 levels were regulated by the IL6 genotype in pSS patients.

Conclusions. The G/C polymorphism of the IL6 gene does not predispose patients to pSS, but the circulating IL-6 concentration is related to specific manifestations of the disease and the levels of IL-6 are regulated by the IL6 promoter polymorphism in pSS.

Key words: Autoimmunity, IL-6, Sjögren’s syndrome, Cytokines, Genetics, Polymorphism.

Primary Sjögren’s syndrome (pSS) is a systemic autoimmune disorder that primarily affects the salivary and lacrimal glands. The most important manifestations of the disease are keratoconjunctivitis sicca and oral dryness caused by diminished lacrimal and salivary gland secretory activity. Classically, this glandular dysfunction is associated with progressive lymphocytic infiltration, which leads to the destruction of acinar and ductal epithelial cells and the loss of glandular parenchyma [1]. Extraglandular manifestations also occur, affecting e.g. the skin, joints, muscles, peripheral and central nervous system, kidneys and lungs [2–4]. Sjögren’s syndrome patients often have other autoimmune diseases, such as autoimmune thyroiditis, primary biliary cirrhosis and coeliac disease [5–8]. Antibodies against ribonucleoproteins (SS-A/Ro and SS-B/La) are frequently present in the sera of SS patients [9]. Several cytokines, such as interleukin (IL)-1, IL-1 receptor antagonist, IL-6, IL-10 and interferon γ (IFN-γ), have been proposed to play a role in the pathogenesis of the disease [10–13].

IL-6 is known to be a B-cell growth and differentiation factor and it is generally found to be highly expressed in many autoimmune diseases (for a review, see [14]). IL-6 also has direct and indirect haematopoietic activity, mainly promoting megakaryocyte maturation, B-cell development and immunoglobulin synthesis [15, 16]. Elevated IL-6 concentrations have been found in the serum, saliva and tear fluid of SS patients [17–19].

Recent findings suggest that the transcriptional activity of the gene for IL-6 (IL6) and the plasma levels of IL-6 protein are associated with a single G/C base
exchange polymorphism sited at the 5’ flanking region of the IL6 gene [20]. Homozygotes for allele G and G/C heterozygotes have been shown to have higher plasma IL-6 levels, higher IL6 gene transcriptional activity and higher inducible IL-6 responses than subjects homozygous for allele C. An imbalance of this base exchange polymorphism is seen in patients with early-onset juvenile arthritis [20].

In this study we analysed plasma levels of IL-6 and promoter region polymorphism of the IL6 gene in 66 pSS patients. The analysed polymorphisms and the measured IL-6 concentrations were compared with normal values obtained from 400 healthy controls. The plasma IL-6 levels and IL6 polymorphisms were compared with several clinical and immunological parameters in order to evaluate the association of IL-6 with clinical manifestations of pSS.

Subjects and methods

Patients
All patients fulfilling three or more modified [21] Californian criteria for pSS [22] were selected from the records of patients with sicca symptoms examined in the Section of Rheumatology of the Department of Internal Medicine at Tampere University Hospital, Finland, during the years 1977–1992 (n = 111). Histological findings were graded on the Chisholm–Mason scale; grades 3 and 4 were regarded as diagnostic [23]. All patients also fulfilled the European criteria for pSS [24]. Those who were alive were invited by letter for cytokine and cytokine gene polymorphism determinations and specimens were obtained after informed consent from 66 pSS patients. Clinical data and patient characteristics at study entry are summarized in Table 1.

Normal controls
Blood samples (n = 400) from healthy adults were obtained from the Finnish Red Cross Blood Transfusion Centre, Tampere (the mean age of the blood donors was 40.5 yr, age range 18–60 yr, male:female ratio 1). The ethnic origin was the same in the controls and the pSS patients (i.e. Finnish Caucasian).

Clinical methods
The patients had recently had a careful clinical examination that included an in-depth interview covering previous and concurrent diseases, the duration of sicca symptoms, the existence of recurrent parotid or submandibular gland swellings, and present sicca symptoms of the eyes and mouth [21]. Special emphasis was laid on possible extraglandular symptoms of SS (dermatological, endocrine, gastrointestinal, lymphoproliferative, musculoskeletal, neurological, renal, respiratory and vascular symptoms). Purpura was defined as a history of typical episodic palpable purpura lesions in the lower limbs, or as revealed by skin biopsy histology. The diagnosis of coeliac disease was based on reticulin antibody determinations and small bowel biopsy histology. Lymphadenopathy was defined as lymph node enlargement so persistent as to have indicated a nodal biopsy. Arthritis was defined as articular swelling observed by a clinician. Peripheral and central neurological symptoms were recorded from the history given by the patients and from data on possible neurological investigations from the case histories. The diagnosis of pulmonary fibrosis was based on findings in chest radiographs; the diagnosis of alveolitis had been established by thorough investigations in a pulmonary unit.

Laboratory tests
Rheumatoid factor was determined with an immuno- turbimetric assay and antinuclear antibodies with an indirect immunofluorescence test using Hep-2 cells. Antibodies against the ribonuclear antigens SS-A (Ro) and SS-B (La) were detected by enzyme-linked immunosorbent assay (ELISA).

IL-6 determination
Plasma IL-6 concentrations were determined from plasma samples obtained between 08.00 and 12.00 h by the use of a commercially available ELISA (Pelikine human IL-6 ELISA kit; CLB, Amsterdam, The Netherlands), following the manufacturer’s instructions. The optical density of individual wells was determined with a Multiscan Bichromatic 348 (Labsystems, Helsinki, Finland) spectrophotometer. The detection limit of the assay was 0.6 pg/ml.

Table 1. Demographic and medical characteristics of 66 pSS patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
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<tbody>
<tr>
<td><strong>Demographic characteristics; mean ± s.d.</strong></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>60 ± 12</td>
</tr>
<tr>
<td>pSS duration after diagnosis (yr)</td>
<td>9.1 ± 4.2</td>
</tr>
<tr>
<td>Age at onset of pSS (yr)</td>
<td>51 ± 11</td>
</tr>
<tr>
<td>Females/males</td>
<td>64/2</td>
</tr>
<tr>
<td><strong>Other characteristics; number of patients (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Positive for rheumatoid factor</td>
<td>47 (73)</td>
</tr>
<tr>
<td>Positive for antinuclear antibodies</td>
<td>56 (85)</td>
</tr>
<tr>
<td>Positive for SS-A antibody</td>
<td>45 (70)</td>
</tr>
<tr>
<td>Positive for SS-B antibody</td>
<td>34 (53)</td>
</tr>
<tr>
<td>Coeliac disease</td>
<td>8 (12)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>10 (15)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>18 (27)</td>
</tr>
<tr>
<td>Lung fibrosis or alveolitis</td>
<td>8 (12)</td>
</tr>
<tr>
<td>Pleurisy</td>
<td>7 (11)</td>
</tr>
<tr>
<td>Joint swelling</td>
<td>14 (21)</td>
</tr>
<tr>
<td>Joint pain</td>
<td>43 (65)</td>
</tr>
<tr>
<td>PNS symptoms</td>
<td>13 (20)</td>
</tr>
<tr>
<td>CNS symptoms</td>
<td>6 (9)</td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>36 (55)</td>
</tr>
<tr>
<td>Purpura</td>
<td>12 (18)</td>
</tr>
<tr>
<td>Parotid gland swelling</td>
<td>29 (45)</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>8 (12)</td>
</tr>
</tbody>
</table>

CNS, central nervous system; PNS, peripheral nervous system.
DNA isolation
DNA was isolated from blood samples using the salting-out method [25]. Intact DNA samples were obtained from 61 patients.

Genotype analysis
The NlaIII polymorphic site at the promoter region position –174 of the IL6 gene was amplified by the polymerase chain reaction (PCR) using oligonucleotides 5' TGACCTCAGCTTTACTCTTG 3' and 5' CTGAT-TGGGAAACCTTATAAG 3' [20]. After the restriction enzyme digestions, the PCR products were identified by electrophoresis (9% polyacrylamide gel) and ethidium bromide staining. The cycling conditions were similar to those published previously [20].

Statistical analysis
Because of the small number of cases (<10) in some subgroups of pSS patients, the plasma IL-6 values were compared using the Mann–Whitney U-test. Allele frequencies were compared using the χ² test and Fisher’s exact test. Correlations were calculated as the Spearman rank order correlation. Findings were considered statistically significant at P < 0.05.

Results

Plasma levels of IL-6
The plasma levels of IL-6 were elevated in the pSS patients compared with the healthy controls (Table 2). Plasma IL-6 levels were significantly higher among the pSS patients with coeliac disease, in patients with peripheral nervous system (PNS) symptoms and in patients with pulmonary fibrosis or alveolitis than in pSS patients without these findings. None of the patients had all three manifestations, and only five patients had two simultaneous manifestations. Thus, the observed high IL-6 levels in these discrete disease groups were not attributable to a subgroup of patients with all of these manifestations. Plasma IL-6 levels increased in parallel with increasing lesion grade in minor salivary gland biopsies, and were significantly higher in patients with grades 3–4 (4.47 ± 2.50 pg/ml, n = 49) than in patients with grades 0–2 (2.78 ± 2.61 pg/ml, n = 17, P < 0.005). Furthermore, the plasma IL-6 levels were higher in patients with definite pSS (four Californian criteria) than in those with possible pSS (three criteria). Among pSS groups, the plasma IL-6 levels were lower in patients with purpura than in patients without purpura. SS-A antibody-positive patients had lower IL-6 concentrations than SS-A antibody-negative patients. No correlations between IL-6 plasma levels and age, disease duration, SS-A or SS-B antibody titres were found.

IL6 (∆174) G/C base exchange polymorphisms
The allele frequencies were similar in pSS patients and in control subjects (Table 3). The IL-6 plasma levels were higher in pSS patients with G/G (5.35 ± 3.01 pg/ml) than in those with G/C (3.96 ± 2.71 pg/ml) or C/C (3.52 ± 2.40 pg/ml; G/G vs C/C, P < 0.05). No significant differences were observed in the control group when the subjects were categorized on the basis of IL6 genotype. When subjects were categorized according to allele G carrier state, the allele G-positive control subjects had a slightly higher plasma IL-6 (1.86 ± 2.86 pg/ml) than allele G-negative subjects (1.67 ± 1.50 pg/ml, P = 0.276). Although some of the pSS subgroups were too small for reliable frequency analysis, the frequency of allele G seemed to be increased in subgroups with coeliac disease (0.429), PNS manifestation (0.500) and pulmonary manifestation (0.642) compared with groups without these manifestations (0.417, 0.400 and 0.389 respectively). A decreasing trend in the frequency of allele G was seen in subgroups with purpura (0.364) or SS-A antibodies (0.400) compared with non-purpura and SS-A antibody-negative groups (0.430 and 0.474 respectively). Thrombocyte counts (given in 10⁹/l) were higher in patients with allele G (273 ± 42.1, n = 41) than in allele G-negative patients (235 ± 44.8, n = 20, P < 0.05).

Discussion
In this study we showed that plasma levels of IL-6 were elevated in primary Sjögren’s syndrome patients, which
IL-6 and IL6 polymorphism in primary Sjögren’s syndrome

Table 3. Allelic frequencies of IL6 (−174) polymorphism in healthy controls and in pSS patients

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>G/G</th>
<th>G/C</th>
<th>C/C</th>
<th>Allele frequency</th>
<th>P between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>400</td>
<td>81 (20)</td>
<td>201 (50)</td>
<td>118 (30)</td>
<td>0.454</td>
<td>0.546</td>
</tr>
<tr>
<td>pSS patients</td>
<td>61</td>
<td>10 (16)</td>
<td>31 (51)</td>
<td>20 (33)</td>
<td>0.418</td>
<td>0.582</td>
</tr>
</tbody>
</table>

*Numbers in parentheses are percentages of cases. *b*2 test.

is in agreement with results by Pettersson et al. [17]. For the first time, we have shown an association of higher IL-6 levels with specific extraglandular manifestations of pSS. Moreover, we analysed the promoter region polymorphism of the IL6 gene and found that plasma IL-6 concentrations were dependent on IL6 allelic state in the pSS patients but not in healthy controls. The allelic distribution of the IL6 (−174) polymorphism was similar in patients and controls.

Little is known about the mechanisms by which IL-6 contributes to the development of the various disease manifestations of pSS. In the development of coeliac disease, the pathological features of gluten sensitivity are associated with local and systemic increases in IL-6 and other proinflammatory cytokines [26, 27]. There is some evidence that the gluten-specific T-cell clones secrete predominantly Th0 profile cytokines (IFN-γ, IL-4, IL-5, IL-6, IL-10), tumour necrosis factor α, transforming growth factor β) [28]. Similar Th0 cytokine responses to gliadin have been observed in peripheral blood mononuclear cell cultures of normal and coeliac subjects [29]. On the basis of these data, it is possible that the high IL-6 concentrations observed in pSS patients with coeliac disease are caused by increased Th0 cytokine production. It is also possible that IL-6 is primarily elevated and promotes the autoimmune process that leads to coeliac disease in a subgroup of pSS patients who are prone to the development of this state.

The role of IL-6 in the development of PNS symptoms in pSS and other autoimmune diseases [e.g. systemic lupus erythematosus (SLE)] in humans is also mostly unknown. Recent studies in animal models suggest that IL-6 has a local effect on nicotinic and noradrenergic neurotransmission and is also capable of inducing the production of a subset of neuropeptides and transmitters in sympathetic neurones [30, 31]. In addition, neurodegenerative mechanisms may also be driven by IL-6, as locally administered IL-6 has been shown to have demyelinating activity in rats [32]. Within the limitations of this study, we can only speculate about whether the increased IL-6 contributes to these mechanisms and to the appearance of PNS symptoms in pSS. Thus, more extensive studies are needed to elucidate the exact role of IL-6 in neuropathies associated with pSS.

It is probable that the mechanisms causing pulmonary fibrosis and alveolitis in pSS resemble those that are responsible for the development of reactive fibrosis in other autoimmune diseases. An association of IL-6 with fibrosis similar to that described here has also been found in systemic sclerosis, in which the elevated serum IL-6 is related to the occurrence of pulmonary fibrosis and a decline in vital capacity [33]. In addition, IL-6 has been suggested to play a role in alveolar fibroblast proliferation and fibrogenesis in patients with diffuse interstitial fibrosis [34]. The consequences of lung exposure to excess IL-6 have also been studied in a rat model, in which the locally introduced over-expression of IL-6 and IL-6 receptor genes leads to histologically distinctive interstitial pneumonia and lymphocytic alveolitis [35].

The presence of extraglandular manifestations of pSS has been linked to more severe forms of the disease, as has the high lymphocyte infiltration grade of the minor salivary gland biopsy [36]. We observed an almost linear correlation between the lymphocyte infiltration grade and plasma IL-6 level, and a correlation between the number of pSS criteria and plasma IL-6 concentration. These complementary histological and clinical findings suggest that IL-6 has a biological role in the development of the more severe forms of pSS and some forms of extraglandular disease.

Although the high plasma IL-6 concentration seems to be related to several unfavourable phenomena in pSS, it may nevertheless possess some protective effects. As mentioned in the introduction, IL-6 plays a role in megakaryocyte maturation in vivo [16, 37]. The mechanism of purpura in pSS is generally believed to be non-thrombocytopenic, but we speculate that the low level of IL-6 production observed in pSS patients with purpura could, to some extent, predispose some patients to this complication. This megakaryopoietic effect of IL-6 was also seen at the genetic level, as thrombocyte counts were lower among allele G-negative pSS patients.

In addition to purpura, low plasma IL-6 was observed in patients with SS-A antibodies. It has been shown that SS-A antibody positivity is more common in pSS patients with more severe disease [38, 39]. In the patients in our study, no such association was observed, and no correlation between SS-A or SS-B antibody titre and plasma IL-6 concentration was found. We do not know the reason for this discrepancy, but it is obvious that the methods of detecting SS-A antibodies vary widely. It has been shown that only the presence of SS-A antibody with specificity against the 60-kDa component of the Ro antigen correlates with the quantity of extraglandular manifestations [40]. Moreover, the patients in our study were selected according to the Californian criteria [22], which are more strict than the European criteria [24]. The use of the Californian criteria probably excludes some patients with the mildest forms of the disease.
(and increases the proportion of patients with extra-
glandular disease).

As there was no difference in the distribution of the IL6 (~174) polymorphism between normal controls and pSS patients, it would appear that the IL6 polymorphism is not a predisposing factor for pSS. Similar results concerning the IL6 (~174) polymorphism in SLE patients have been described recently [41]. The allele G seems partly to determine the plasma level of IL-6, suggesting that the presence of this allele may be a risk factor for some extraglandular manifestations associated with a high IL-6 concentration in pSS. In contrast to pSS, the IL6 genotype had no effect on the IL-6 plasma level in healthy controls, suggesting that IL6 polymorphism regulates primarily the inducible IL-6 responses, or that more sensitive methods are needed to detect the genotype effect on basal IL-6 production. Factors other than allelic imbalance of the IL6 gene account for the elevated plasma IL-6 baseline value in pSS. The balance of IL-6-inducible and -inhibitory cytokines remains to be ascertained.

Acknowledgements

This study was supported by grants from the Medical Research Fund of Tampere University Hospital. We thank Sinikka Repo-Koskinen for technical assistance.

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