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High circulating levels of soluble scavenger receptors (sCD5 and sCD6) in patients with primary Sjögren's syndrome

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Abstract

Objective. To determine the existence of circulating levels of soluble scavenger receptors (sCD5 and sCD6) in patients with primary Sjögren's syndrome (SS), and to analyse the correlation with clinical and immunological features of SS.

Methods. Ninety consecutive patients with primary SS were studied. All patients fulfilled four or more of the European diagnostic criteria for SS. sCD5 and sCD6 levels were determined using a specific enzyme-linked immunosorbent assay (ELISA) developed in our laboratory.

Results. Detectable levels of sCD5 were found in 39 (43%) SS patients. The mean ± standard error values of sCD5 were 3.5 ± 0.5 ng/ml for patients with SS and 1.9 ± 0.1 ng/ml for healthy blood donors ($P < 0.001$). We found higher levels of sCD5 in patients with hypocomplementaemia (6.5 *vs* 3.5 ng/ml, $P = 0.03$) and cryoglobulinaemia (6.9 *vs* 2.6 ng/ml, $P = 0.001$). On the other hand, detectable levels of sCD6 were found in 60 (67%) SS patients. The mean ± standard error values of sCD6 were 25.5 ± 7.8 ng/ml in SS patients and 5.27 ± 1.40 ng/ml in healthy blood donors ($P = 0.01$). When the sCD6 levels were compared according to the presence or absence of immunological features, patients with cryoglobulinaemia showed higher levels of circulating sCD6 (77.3 *vs* 17 ng/ml, $P = 0.01$) than those without cryoglobulinaemia.

Conclusion. Patients with primary SS showed higher levels of circulating sCD5 and sCD6 when compared with controls. Moreover, the existence of some immunological features (hypocomplementaemia and cryoglobulinaemia) was associated with high levels of both soluble scavenger receptors. These facts may reflect an enhanced lymphocytic activation in patients with primary SS.

KEY WORDS: Soluble CD5, Soluble CD6, Primary Sjögren's syndrome.

Sjögren's syndrome (SS) is an autoimmune disease that mainly affects exocrine glands and usually presents as a persistent dryness of the mouth and the eyes caused by functional impairment of the salivary and lachrymal glands [1]. The disease spectrum extends from an organ-specific autoimmune disease (autoimmune exocrinopathy) to a systemic process [2]. Due to this heterogeneity, attempts have been made to identify subsets of patients who would permit more reliable prediction of the course of primary SS in affected individuals [3–5].

The membrane-associated CD5 and CD6 glycoproteins belong to the ancient family of receptors with extracellular scavenger receptor cysteine-rich (SRCR) domains [6]. CD5 and CD6 are type 1 membrane proteins with three extracellular SRCR domains, they share a similar pattern of cellular expression and are genetically linked in human chromosome 11 [7]. CD5 is a 67-kDa co-receptor found on thymocytes, mature T lymphocytes, B1a normal B-cell subset and B-cell chronic lymphocyte leukaemias [8], and CD6 is a

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105/130-kDa monomeric surface glycoprotein also expressed predominantly by the monocytes and mature T lymphocytes. A minority of B cells express the CD5 molecule, which is found on virtually all T cells. This B-cell subset produces large amounts of autoantibodies and is responsible for the production of most serum IgM antibodies. The CD5+ B-cell subset is expanded in some systemic autoimmune diseases [9].

At present, there are no reports on circulating soluble forms of the transmembrane SRCR family members in primary SS. This study was undertaken to determine the existence of soluble CD5 (sCD5) and/or CD6 (sCD6) in patients with primary SS and to evaluate a possible relationship with clinical or immunological features of SS.

Patients and methods

Patients

We investigated 90 consecutive patients with primary SS 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 [10], and none of the patients presented clinical or immunological evidence of other systemic autoimmune disease. We evaluated the following extraglandular features: articular involvement (arthralgias and/or non-erosive arthritis), Raynaud's phenomenon, cutaneous vasculitis (demonstrated by cutaneous purpura and skin biopsy), autoimmune thyroiditis (positive autoantibodies and/or thyroid dysfunction), peripheral neuropathy (demonstrated by the clinical picture and nerve conduction tests) and interstitial pneumonitis (demonstrated by the clinical picture, X-ray and computed tomography scan).

The serum levels of sCD5 and sCD6 were also analysed in 50 healthy blood donors from the blood bank of our hospital.

CD5/CD6 enzyme-linked immunosorbent assay (ELISA)

The presence of sCD5/sCD6 was determined using a specific sandwich ELISA developed in our laboratory. F96 Maxisorp plates (Nunc, Kamstrup, Denmark) were coated overnight at 4°C with 500 ng of purified anti-CD5/CD6 monoclonal antibody. After three washes with Tris-buffered saline (TBS)–Tween 0.05%, the wells were blocked for 30 min at 37°C with phosphate-buffered saline (PBS)/5% non-fat milk. Test samples (100 μl) were incubated for 2 h at room temperature in blocking solution. After another washing step, the wells were incubated for 1 h at room temperature with biotinylated anti-CD5/CD6 monoclonal antibody. After another wash, the assay was developed with the ELISA Amplification System (Life Technologies, Carlsbad, CA, USA) following the manufacturer's instructions. Absorbance at 495 nm was measured using a Titertek ELISA reader (Flow Laboratories, Rockville, MD, USA) and mean optical density readings calculated. As the standard we used serial dilutions of purified recombinant sCD5/sCD6-Rg in blocking solution [11, 12]. The lower detection limit was 0.4 ng/ml for CD5 and 4 ng/ml for CD6.

Laboratory studies

Immunological tests included antinuclear antibodies (ANA) (indirect immunofluorescence using mouse liver as the substrate), precipitating antibodies to the extractable nuclear antigens Ro/SS-A and La/SS-B (counter-immunoelectrophoresis) and rheumatoid factor (RF; latex fixation and Waaler-Rose tests). Complement factors (C3 and C4) were estimated by nephelometry (Behring BNA nephelometer). Serum cryoglobulinaemia was measured after centrifugation.

Statistical analysis

We used the χ^2 test and Fisher's exact test 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. A value of $P < 0.05$ was taken to indicate statistical significance. This statistical analysis was performed by means of the SPSS program using the information stored in the database program.

Results

General characteristics

We studied 90 patients with primary SS (83 women and seven men). The SS onset occurred between 20 and 79 yr (mean 53 yr) and, at the time of study, the mean age of the patients was 60 yr (range 23–80 yr) and the mean disease duration was 80 months (range 6–280 months). All patients were positive for ocular diagnostic tests. Parotid scintigraphy was positive in 57/80 patients (71%) and labial minor salivary gland biopsy showed lymphocytic infiltrates grade 3 or 4 (according to Chisholm-Mason classification) in 53/78 (68%) patients. The most common extraglandular manifestations were articular involvement in 37 (41%), cutaneous vasculitis in 13 (14%), autoimmune thyroiditis in 13 (14%), Raynaud's phenomenon in 10 (11%), interstitial pneumonitis in 10 (11%) and peripheral neuropathy in six (7%) patients. The most frequent immunological features were ANA in 63 (70%), RF in 36 (40%), anti-Ro/SS-A in 30 (33%), anti-La/SS-B in 17 (20%), type II cryoglobulinaemia in 10/75 (13%) and hypocomplementaemia in 9/77 (12%) patients.

Circulating sCD5 levels

Detectable levels of sCD5 were found in 39 (43%) SS patients, and positive sera signals ranged from 0.4 to 13.6 ng/ml. The mean $\pm$ standard error values of sCD5 were 3.5 ± 0.5 ng/ml in SS patients and 1.9 ± 0.1 ng/ml in controls ($P < 0.001$) (Fig. 1). In SS patients, we observed no significant differences in the sCD5 levels according to the presence or absence of the main clinical SS features. We found higher sCD5 levels in patients with hypocomplementaemia (6.5 *vs* 3.5 ng/ml, $P = 0.03$) and mixed cryoglobulinaemia (6.9 *vs*

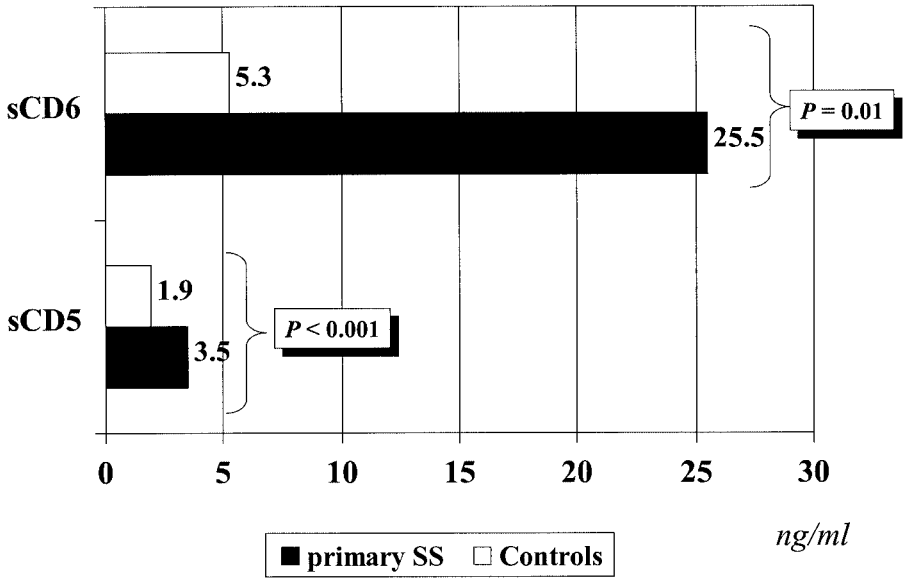

FIG. 1. Serum levels of sCD5 and sCD6 in patients with SS and controls.

2.6 ng/ml, $P = 0.001$) when compared with patients without these immunological features.

Circulating sCD6 levels

Detectable levels of sCD6 were found in 60 (67%) SS patients, and positive sera signals ranged from 4 to 234 ng/ml. The mean ± standard error values of sCD6 were 25.5 ± 7.8 ng/ml in SS patients and 5.27 ± 1.40 ng/ml in healthy blood donors ($P = 0.01$) (Fig. 1). In SS patients, we observed no significant differences in the sCD6 levels according to the presence or absence of the main clinical SS features. When the sCD6 levels were compared according to the presence or absence of immunological features, patients with mixed cryoglobulinaemia showed higher levels of circulating sCD6 (77.3 *vs* 17 ng/ml, $P = 0.01$) than those without cryoglobulinaemia.

Discussion

During the past several years, a great deal of attention has been focused on the possible role of CD5+ (ly-1) B cells in the pathogenesis of autoimmunity (a small B-cell subset involved in the production of polyreactive autoantibodies and RF) [13–15]. The importance of CD5/CD6 expression in the pathogenesis of SS has been scarcely studied. On one hand, several studies [16, 17] have demonstrated that circulating CD5+ B cells are increased in SS patients, and Dauphinée *et al.* [17] demonstrated that almost half of SS patients had both decreased CD5 expression on T cells and increased CD5 expression on B cells. On the other hand, a previous study [18] determined the presence of a 'cell-free' CD5 in the sera of patients with autoimmune diseases. Jamin *et al.* [18] found that the mean level of 'cell-free' CD5 was significantly higher in SS patients than in the normal controls, but several differences exist between our study and Jamin's work. First, Jamin *et al.* [18] studied a very small population of SS patients (21 SS patients and 14 controls *vs* 90 SS patients and 50 controls in our study). Thus, Jamin *et al.* used a different ELISA system (polyclonal *vs* monoclonal antiserum as the primary coating antibody and membrane solubilized CD5 *vs* rsCD5 as the internal standard) and, finally, no molecular characterization of the 'cell-free' CD5 form was performed in Jamin's work. Despite these differences, both studies demonstrate the existence of high levels of a soluble/cell-free form of CD5 in SS.

In this study, we found high circulating levels of soluble scavenger receptors (CD5 and CD6) in the serum of SS patients. Interestingly, sCD6 seems to circulate in higher proportion than sCD5 (sCD6 levels were 7-fold higher than sCD5 levels) and its presence in serum is more easily detectable (67 *vs* 43% for sCD5). The origin of these soluble forms is not known. It is possible that the sCD5 and sCD6 molecules are shed in the serum by activated lymphocytes that subsequently infiltrate glandular or extraglandular structures. We may hypothesize that these raised levels might represent an unsuccessful mechanism of counter-regulation of an enhanced lymphocytic activation, considering that both soluble molecules are released when these lymphocytes are activated.

In our SS patients, a close relationship between sCD5 and sCD6 levels and the presence of mixed cryoglobulinaemia, which contains monoclonal IgM-RF, was found. This fact suggests enhanced activity of CD5/CD6 + B cells related to the production of cryoglobulinaemic IgM in patients with primary SS. However, a possible interference of RF in our ELISA might have occurred, although we observed no significant differences in the CD5/CD6 levels in SS patients

with and without RF. High levels of CD5 and CD6 have also been observed in patients without RF and, conversely, some patients with high RF levels have negative determination of CD5/CD6. Additionally, we analysed the possible existence of a correlation between RF levels and CD5 or CD6 levels, and found no significant correlation. Nevertheless, we cannot totally exclude the possibility that RF of any isotype may interfere in some patients with high levels of CD5/CD6.

Moreover, we also found a relationship between hypocomplementaemia and high levels of sCD5. Few data about the association of CD5 and complement factors are available to date. In CD5+ B-cell leukaemias, CD5 might play a role with CD21 (the CD3 d complement receptor) in adhesion to complement-coated substrates [19]. Thus, CD5 may be involved in adhesion and tissue localization of B cells in patients with primary SS.

Finally, new therapeutic strategies could emerge from the investigation of CD5 and CD6 molecules in primary SS. In other autoimmune diseases, such as rheumatoid arthritis, previous open-label studies with a murine anti-T cell, ricin-linked immunoconjugate directed against the CD5 antigen have been interpreted as showing therapeutic benefit in patients with rheumatoid arthritis and other autoimmune disorders [20, 21].

In conclusion, patients with primary SS studied cross-sectionally showed higher levels of circulating sCD5 and sCD6 when compared with controls, and circulating levels of sCD6 were 7-fold higher than sCD5 levels. Moreover, the existence of some immunological features (hypocomplementaemia and cryoglobulinaemia) is associated with high levels of these soluble scavenger receptors in the sera of SS patients. These facts may reflect an enhanced activity of peripheral lymphocytes, mainly of the CD5+ B-cell subset, which is frequently expanded in SS and is responsible for the production of polyreactive RF-like autoantibodies.

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