Lymphocytotoxic Antibody in Sjögren's Syndrome

Yukinobu ICHIKAWA, Masatoshi TAKAYA and Shigeru ARIMORI

The Fourth Department of Internal Medicine, School of Medicine, Tokai University
(Received March 14, 1980)

The autoimmune mechanism in Sjögren's syndrome (SjS) and the close association of this syndrome with systemic lupus erythematosus (SLE) have been suggested in several reports.

Although the pathological significances of lymphocytotoxic antibody is obscure, possible roles of this antibody in the pathogenesis of autoimmunity have been speculated.

The purposes of the present study were to demonstrate lymphocytotoxic antibody in sera of patients with SjS, and then to analyse various correlations of this antibody with clinical parameters of SjS.

We detected cold-reactive lymphocytotoxic antibody not only in most (86.4%) of the patients with SLE, but also in many patients with SjS (51.6%; excluding SjS patients with SLE), rheumatoid arthritis (RA; 31.6%) and myasthenia gravis (MG; 36.4%), which are considered to be autoimmune diseases. On the other hand, lymphocytotoxic antibody was detected in only one (5.6%) of eighteen healthy persons. We also confirmed that the cold-reactive lymphocytotoxic antibody detected in SLE is a 2-mercaptoethanol sensitive and T-lymphocytotoxic antibody. Frequent demonstration (50%) of this antibody even in SjS patients without any associated diseases supports the immunological basis of SjS as well as virological participation in SjS.

Although this antibody did not significantly correlate with most of the clinical parameters in SjS patients, this antibody may become a hallmark as one of immunological abnormalities in SjS, even though it was produced by non-specific lymphocyte activation.

(Key Words: Lymphocytotoxic Antibody, Sjögren's Syndrome, Systemic Lupus Erythematosus, Rheumatoid Arthritis, Myasthenia Gravis)

INTRODUCTION

Sjögren's syndrome (SjS) is a rheumatic disease which has been found widely in association with various autoimmune diseases (1). Cardinal manifestations of this syndrome consisted of xerostomia, xerophthalmia and enlargements of the salivary glands. Although the pathogenesis of the syndrome is obscure, an autoimmune mechanism has been suggested by several investigations concerned with humoral or cellular immunity (2, 10). In addition, close association of this syndrome with systemic lupus erythematosus (SLE) has been supported by evidence such as SLE-like serological abnormalities detected in SjS and frequent overlapping of these two diseases (1, 5, 7.)

Detection of lymphocytotoxic antibody (LCA) in the sera of most SLE patients and many patients with other autoimmune diseases suggested possible associations of this antibody with the pathogenesis of autoimmunity (16).

Investigations of LCA may also contribute to a further etiological or immunological analysis of SjS. The purposes of the present study,
therefore, were to demonstrate LCA in sera of patients with SjS, and then to analyse various correlations of LCA with clinical parameters of SjS.

MATERIALS AND METHODS

(1) Patients: Forty-one patients with SjS diagnosed from the presence of either keratoconjunctivitis sicca, typical sialographical changes (punctate, or more severe sialectasis) in the parotid gland, or lymphoid cell infiltration consistent with SjS in the sublingual gland, were studied. All of the patients with SjS were females, and their associated diseases were SLE (SjS + SLE; ten patients), rheumatoid arthritis (SjS + RA; eight patients), chronic thyroiditis (SjS + CT; four patients) and progressive systemic sclerosis (SjS + PSS; three patients); the remaining sixteen patients had no associated diseases (SjS alone).

Controls consisted of nineteen patients with classical or definite RA, three patients with SLE, eleven patients with myasthenia gravis (MG) and eighteen healthy persons.

All sera were stored at −80°C, and heat-inactivated at 56°C for 30 min. before use.

(2) Preparations of peripheral blood lymphocytes as target cells: The lymphocytes were isolated from heparinized peripheral blood obtained from healthy persons on Ficoll-Conray gradients (specific gravity: 1.077, at 400 g, for 30 min.). After washing three times in Dulbecco's phosphate buffered saline (PBS), the lymphocytes were resuspended in RPMI1640 supplemented with penicillin (105 U/ml), streptomycin (100 mg/l) and HEPES (5.97 g/l), and adjusted to 3 × 106 cells/ml.

(3) Rabbit sera as a complement source: Fresh sera (Nos. 1–4) obtained from four Japanese White rabbits (females, body weight: 2 kg) were stored at −80°C until use.

(4) Lymphocytotoxicity assays: Assays for lymphocytotoxicity were performed as described by Koike et al (8). Briefly, 20 μl of target cell suspensions was incubated with 50 μl of patient's serum in plastic tubes (10 × 70 mm) at 15°C for 30 min. After addition of 50 μl of rabbit complement, the target cells were further incubated at 15°C for 180 min. All incubations were performed in a water bath with continuous and gentle shaking. Percentages of dead cells were determined by trypan blue dye exclusion. Serum showed 20% cytotoxicity or more against all target cells obtained from three different healthy donors was considered as positive for lymphocytotoxic antibody (LCA). The average cytotoxicity against three different target cells was calculated as the percent-cytotoxicity of the patient's serum.

(5) E-rosette forming activity: Assays for E-rosette forming activity of serum-treated lymphocytes were also performed as described by Koike, T. et al (9). Briefly, sheep red blood cells (SRBC; preserved in Alsever's solution at 4°C) were washed three times in PBS, and adjusted to 1% suspension in fetal bovine serum (GIBCO; heat-inactivated at 56°C for 30 min.).

After treatment with patient's serum followed by the addition of rabbit serum as described above, the treated lymphocytes were washed once in PBS, resuspended in 100 μl of RPMI1640, and then mixed with
100μl of 1% SRBC suspension. This mixture was centrifuged for 5 min. at 200g, and then incubated for 120 min. at room temperature. The mixture was resuspended gently and percentages of E-rosette forming cells (E-RFC) were determined microscopically.

(6) Treatment of patient's serum with 2-mercaptoethanol (2-ME): One milliliter of patient's serum was mixed with an equal volume of 0.2 M 2-ME in PBS, incubated for 120 min. at 37°C, and then dialysed against PBS at 4°C for 24 hours (500 ml × 4 times). The volume untreated serum was adjusted to be the same as the final volume of 2-ME treated serum with PBS, and these equally diluted sera were used for assays.

RESULTS

(1) Effects of rabbit sera on target cells: First, the effects of incubation with rabbit sera at 15°C for 180 min on target cells were determined as shown in Fig. 1. Four to seven percent of the target cells were killed by rabbit sera (Nos. 1, 2 and 3), but 47% of the target cells were killed by No. 4 rabbit serum. Therefore, No. 4 serum was discarded for further lymphocytotoxicity assays.

![Fig. 1 Effect of rabbit sera on target cells](image)

(2) Complement activities of rabbit sera: Nos. 2 and 3 rabbit sera were used preliminarily as a complement source for the detection of LCA in sera of patients with SLE. Definite cytotoxicity against the three different target cells was detected in two of the eight patient's sera using No. 2 rabbit serum. However, definite cytotoxicity was detected in all SLE patient's sera when No. 3 rabbit serum was also used as a complement source. Complement activities of three rabbit sera for lymphocytotoxicity assays in one SJS patient with SLE were determined as shown in Fig. 2. Either No. 1 or No. 3 rabbit serum was considered as an adequate complement source, and No. 3 rabbit serum was used in further lymphocytotoxicity assays.
(3) Nature of LCA in serum of SLE patients: Both dead cells and E-RFC of the three different target cells were examined respectively in duplicated samples after incubation with SLE serum and No. 3 rabbit serum. Percentages of dead cells were inversely correlated with those of E-RFC (Fig. 3). Following treatment of SLE serum with 2-ME, its lymphocytotoxic activity was eliminated completely, and percentages of E-RFC were recovered (Fig. 4).
(4) Incidences and cytotoxic activities of LCA in patients: Fig. 5 shows the incidences and cytotoxic activities detected in undiluted sera of patients and healthy persons. LCA was detected in 16 (51.6%) out of 31 patients with SjS (excluding ten SjS patients with SLE), eleven (84.6%) out of 13 patients with SLE (including ten patients previously excluded), six (31.6%) out of 19 patients with RA, four (36.4%) out of 11 patients with MG, and one (5.6%) out of 18 healthy persons. All of these incidences of LCA in patients were significantly higher than that in healthy persons (p<0.05). In addition, incidences in patients with SLE (including ten SjS patients with SLE) were significantly higher than those in patients with SjS (p<0.05) although no significant difference was demonstrated between the incidence in patients with SjS and those in patients with RA (0.1< p<0.2).

(5) Correlations of LCA with various clinical parameters in patients with SjS: With respect to associated diseases, LCA was detected in eight (50%) of the 16 SjS patients without associated diseases, eight (80%) of the 10 SjS patients with SLE, four (50%) of the eight SjS patients with RA, three (75%) of the four SjS patients with CT, and one (33.3%) of the SjS patients with PSS (Fig. 6). The incidences in SjS patients without associated diseases were not significantly different from those in SjS patients with associated diseases.
Lymphocytotoxicity (%)

<table>
<thead>
<tr>
<th></th>
<th>%positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>SjS * (31)</td>
<td>51.6</td>
</tr>
<tr>
<td>SLE ** (13)</td>
<td>84.6</td>
</tr>
<tr>
<td>RA (19)</td>
<td>31.6</td>
</tr>
<tr>
<td>MG (11)</td>
<td>36.4</td>
</tr>
<tr>
<td>Controls (18)</td>
<td>5.6</td>
</tr>
</tbody>
</table>

* SjS patients without SLE
** Including ten patients with SjS

**Fig. 5** Lymphocytotoxic antibody in patients; ●; LCA positive, ×; LCA negative

Lymphocytotoxicity (%)

<table>
<thead>
<tr>
<th></th>
<th>%positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>SjS alone (16)</td>
<td>50</td>
</tr>
<tr>
<td>SjS + SLE (10)</td>
<td>80</td>
</tr>
<tr>
<td>SjS + RA (8)</td>
<td>50</td>
</tr>
<tr>
<td>SjS + CT (4)</td>
<td>75</td>
</tr>
<tr>
<td>SjS + PSS (3)</td>
<td>33.3</td>
</tr>
</tbody>
</table>

**Fig. 6** Lymphocytotoxic antibody in Sjögren's syndrome and associated diseases

Among patients with SjS, activities of LCA were not significantly correlated with lymphocyte counts in the peripheral blood \((r = 0.2, p > 0.05)\), serum complement levels \((CH50; r = 0.26, p > 0.05)\), serum IgM concentrations \((r = 0.22, p > 0.05)\), volumes of mixed saliva secreted by stimulation per 10 min. \((r = 0.14, p > 0.05)\), or ages of the patients \((r = 0.2, p > 0.05)\). Although incidences of LCA among SjS patients were correlated significantly with those of antinuclear antibodies by the indirect immunofluorescence technique \((p < 0.02)\), no significant correlation was demonstrated with those of rheumatoid factors (RA test) or with those of anti-RNP antibodies.
in sera of SjS patients. In addition, incidences of LCA were not correlated with severities of lymphocytic infiltrations in sublingual glands biopsied from SjS patients. When compared with clinical symptoms of SjS patients, incidences of LCA were correlated significantly with Raynaud's phenomenon \( (p<0.05) \) although no significant correlation with facial erythema or with polyarthritis was demonstrated.

(6) Effect of LCA on autologous lymphocytes: the cytotoxic effect of LCA on autologous lymphocytes was evaluated in three LCA-positive patients to determine whether it is an autoantibody or not. All sera from patients showed definite cytotoxicities in autologous target cells although their activities were lower than those in heterologous lymphocytes obtained from healthy persons (Table 1).

**Table 1** Cytotoxic effect of LCA on autologous lymphocytes in three LCA-positive patients

<table>
<thead>
<tr>
<th>Lymphocytotoxicity (%)</th>
<th>SLE</th>
<th>SjS + RA</th>
<th>SjS + SLE</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient's serum + patient's lymphocytes + C'</td>
<td>92</td>
<td>34</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>Patient's serum + donor's lymphocytes + C'</td>
<td>94</td>
<td>78</td>
<td>88</td>
<td>1</td>
</tr>
<tr>
<td>Patient's lymphocytes + C'</td>
<td>10</td>
<td>14</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>Patient's lymphocytes in RPMI 1640</td>
<td>15</td>
<td>17</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Sjögren's syndrome (SjS) is characterized by chronic lymphocytic inflammation in either the salivary or lachrymal glands (12). This syndrome would be recognized more widely than before if its subclinical states are included (1, 6). Autoimmunity in SjS has been also supported by various autoantibodies frequently detected in sera of patients with this syndrome and by frequent overlapping of this syndrome with other autoimmune diseases such as SLE, RA, PSS and CT (1, 2, 7). In addition, abnormal cellular immunity in SjS has been also suggested by several investigations concerned with lymphocyte responsibility (10) and lymphocyte subpopulations (15).

Lymphocytotoxic antibody (LCA) was detected in most patients with SLE and many patients with RA by Terasaki, P.I., et al (16) This antibody has been confirmed as a cold-reactive and complement-dependent antibody of the IgM type (3). Associations of this antibody with several clinical parameters such as lymphocytopenia, hypocomplementemia and CNS symptoms, have been reported in SLE (3, 4, 18). Relative T-lymphocyte specificity of this antibody was shown in SLE (11). Therefore, the similarity of this antibody (in human SLE) to natural thymocytotoxic autoantibody (NTA) in NZB mice, a relative suppressor T-lymphocyte specificity, has been suggested (14). Suppressive factors for some lympho-
cytotoxic functions, however, were demonstrated not only in IgG of human SLE sera, but were also shown to be active at 37°C, independent of complement (15, 17). In addition to this heterogeneity of antilymphocyte antibody, the pathological significance of LCA was obscure as indicated above, but some possible roles of LCA in autoimmune diseases have been speculated in association with the viral etiology of autoimmunity and elimination of specific lymphocyte functions leading to the production of various autoantibodies. Therefore, investigations of LCA may contribute to further etiological or immunological analysis of SjS.

In the present study, we also confirmed that cold-reactive LCA is a widely detectable antibody in SLE and it is 2-ME sensitive and T-lymphocytotoxic as previously reported. We detected LCA not only in most patients with SLE, but also in many patients with SjS, RA and MG, which are considered to be autoimmune diseases. In addition, frequent demonstration of LCA even in SjS patients without any associated diseases supports the autoimmune basis as well as the virological etiology in SjS. In SjS patients, however, this antibody was not significantly correlated with various clinical parameters as follows: lymphocyte counts in the peripheral blood, serum complement levels, serum IgM concentrations, severities of salivary gland involvement, and ages of the patients. Antinuclear antibody and Raynaud's phenomenon were significantly correlated with LCA in a total of 41 patients with SjS, although all patients with positive antinuclear antibody were associated with SLE, and correlations of LCA with Raynaud's phenomenon in SjS patients without associated diseases was not significant (0.05 < p < 0.1).

Definite cytotoxicity of LCA in autologous lymphocytes was demonstrated in three patients with SLE and SjS, and the LCA detected in these patients was confirmed as an autoantibody. Lower cytotoxic activity of LCA against autologous cells was observed in all patients when compared with that against heterologous lymphocytes. These results indicate that these lower cytotoxic activities of LCA in autologous cells were associated with a previous elimination of LCA-sensitive lymphocytes in vivo, or differences in the affinity of LCA between autologous and heterologous lymphocytes.

Although the definitive roles of LCA could not be clarified in SjS from the above results, this antibody can be expected to be a hallmark in SjS, as one of the various autoantibodies produced by non-specific lymphocyte activation.

REFERENCES
Lymphocytotoxic Antibody in Sjögren's Syndrome

Arth Rheum 15: 251, 1972


