Cellular Immunity to Secretory IgA (As a Common Duct Antigen of Exocrine Glands) in Sjögren's Syndrome

Yukinobu ICHIKAWA, Masatoshi TAKAYA and Shigeru ARIMORI

The Fourth Department of Internal Medicine, School of Medicine, Tokai University

(Received November 1, 1979)

Autoimmunity to salivary duct cells has been suggested in Sjögren's syndrome (SjS). We were interested in the secretory component (SC) of secretory IgA (s-IgA) as a common duct cell antigen of systemic exocrine glands because of the possible analogy between SC in SjS and thyroglobulin in Hashimoto's thyroiditis. Therefore, we isolated s-IgA as a source of SC from human milk and investigated lymphocyte responses to s-IgA in patients with SjS.

Higher mitogenic indices against s-IgA were demonstrated in SjS than in rheumatoid arthritis, other autoimmune diseases and normal persons. In addition, mitogenic indices in patients with SjS alone were significantly higher than those in SjS patients with associated diseases.

These results suggest both the role of SC as a common antigen of exocrine glands in the pathology of SjS, and the presence of an etiologically different subgroup in patients with SjS.

(Key Words: Cellular Immunity, Secretory IgA, Exocrine Glands, Sjögren's Syndrome)

INTRODUCTION

An autoimmune basis of salivary gland destruction in Sjögren's syndrome (SjS) has been suggested by the glandular infiltration of lymphoid cells around the excretory ducts (11). In addition, anti-salivary duct antibodies in sera (1, 3, 6, 9) and cellular immune responses to salivary gland homogenate (2, 13) or to the protein fraction of saliva (9, 14) have been reported in patients with this disorder.

On the other hand, it is well-known that SjS is characterized by systemic involvement of exocrine glands in addition to the salivary glands (4, 5). Therefore, it has been suggested that autoimmunity to the duct antigen of exocrine glands is responsible for the tissue damage in this disorder. For this reason, we became interested in the secretory component (SC) of secretory IgA (s-IgA) as a common duct antigen of systemic exocrine glands.

The purpose of the present study was to demonstrate the status of cellular immune responses against s-IgA in patients with SjS.

MATERIAL AND METHODS

(1) Patients:

Twenty patients with SjS diagnosed from the presence of either keratoconjunctivitis sicca, typical sialographical changes (punctate or globular sialectasis) in the parotid gland, or lymphoid cell infiltrations consistent with SjS in the sublingual gland were studied. Controls consisted of ten patients with classical or definite rheumatoid arthritis (RA), ten patients...
with other autoimmune diseases such as chronic thyroiditis (six patients),
systemic lupus erythematosus (two patients), polyarteritis nodosa (one
patient) and ulcerative colitis (one patient); and ten normal persons.

(2) Isolation of s-IgA:
Normal human milk was collected for 2-7 days after delivery and stored at
−20°C. Then, s-IgA was isolated by the method of Kobayashi (8). Briefly,
the collected milk was centrifuged at 7,000rpm for 120min. After removal
of the fat layer, the precipitating fraction at 50% saturation with ammonium
sulfate was collected by centrifugation and dissolved in distilled water.
Following acidification at pH4.6 with 1N acetic acid, the precipitating
casein was removed by centrifugation. Then, the concentrated material was
dialysed against 0.01M sodium phosphate buffer (pH7.6) and chromato-
graphed on a DEAE-cellulose column by stepwise elution with 0.01M
(pH7.6) and 0.1M (pH6.4) sodium phosphate buffer. The fraction eluted
by 0.1M (pH6.4) buffer was chromatographed on a CM-cellulose column
by linear gradient elution between 0.005M and 0.5M sodium acetate buffer
(pH5.0). Isolated s-IgA was further purified by gel-filtration on a Sephadex-
G 200 column with Tris-HCl buffer (μ = 0.05, pH8.0) supplemented with
1 M NaCl, and was dialysed against distilled water. All procedures described
above were performed at 2−4°C. s-IgA was identified by Ouchterlony
immunodiffusion and immunoelectrophoresis with rabbit anti-human IgA
serum, rabbit anti-human SC serum and anti-human serum from horses
(Hoechst).

(3) Lymphocyte transformation:
The lymphocytes were isolated from heparinized peripheral blood on Ficoll-
Conray gradients (specific gravity of 1.077, at 400 g for 30 min.). After wash-
ing three times with Dulbecco’s phosphate buffered saline, the lymphocytes
were resuspended in RPMI 1640 supplemented with penicillin (105U/l),
streptomycin (100 mg/l), HEPES (5.97 g/l) and 15% inactivated normal
human AB serum, and were adjusted to 1 × 10⁶ cells/ml.

s-IgA isolated from human milk was diluted to 3 mg protein/ml with
RPMI 1640, and sterilized through a millipore-filter.

Then, 200μl of the lymphocyte suspension was cultured with 20μl of
s-IgA for 5 days at 37°C in a 5% CO2-incubator. On day 5, 1μCi of (Methyl-
³H) Thymidine (Amersham; specific activity of 8.3 mCi/mg)/well was added
to the cultures, and they were further incubated for 7 hours. The lymphocytes
were harvested with a multiple automated sample harvester (7), and counted
by a liquid scintillation spectrophotometer. Antigen-free cultures were pre-
bred by the addition of RPMI 1640 instead of s-IgA, and a lymphocyte-free
background was also prepared. All cultures were studied in triplicate and the
mitogenic index (M.I.) was calculated as follows: M.I. = (mean cpm of antigen-
containing culture − mean cpm of background)/(mean cpm of antigen-free
culture − mean cpm of background)

RESULTS

(1) Identification of s-IgA isolated from human milk:
Following DEAE-cellulose and CM-cellulose chromatography, the s-IgA
fraction was isolated by gel filtration as a single peak (Fig. 1). Both rabbit
anti-human IgA serum and rabbit anti-human SC serum reacted with this fraction, and a single precipitating line was identified between anti-human serum from horses and this fraction in immunodiffusion and immunoelectrophoresis (Photo. 1).

Fig. 1 Gel-filtration on Sephadex-G200
Following DEAE- and CM-cellulose column chromatography, the s-IgA fraction was isolated by gel-filtration as a single peak.

Photo. 1 Immunoelectrophoresis of s-IgA fraction
(2) Lymphocyte transformation to s-IgA fraction:
Table 1 shows the mean cpm of the s-IgA-containing lymphocyte culture and of s-IgA-free lymphocyte culture subtracted from the mean cpm of the background. In these experiments, the mean cpm of the background was 133.3 ± 72.2 (mean ± standard deviation).

Ten out of twenty patients with SjS were associated with RA (five patients), SLE (four patients) and progressive systemic sclerosis (PSS; one patient), and the patients with SjS were subdivided into those with and without these associated diseases (SjS alone).

The mitogenic indices in SjS alone (2.6 ± 1.5) were significantly higher than those in SjS with associated diseases (1.3 ± 0.6; p < 0.02), and were higher than those in normal persons (1.6 ± 0.7; 0.05 < p < 0.1). Among SjS patients with associated diseases, and those with RA and other autoimmune diseases, no significant difference in the mitogenic indices was demonstrated when compared with normal persons (Fig. 2).

Table 1 3H-thymidine incorporation of peripheral blood lymphocytes

<table>
<thead>
<tr>
<th></th>
<th>S-IgA (+) cpm</th>
<th>S-IgA (-) cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>SjS alone</td>
<td>2038.5 ± 2576.5</td>
<td>766.5 ± 614.1</td>
</tr>
<tr>
<td>SjS with associated diseases</td>
<td>832.1 ± 981.5</td>
<td>574.6 ± 434.5</td>
</tr>
<tr>
<td>RA</td>
<td>770.9 ± 370.1</td>
<td>638.3 ± 478.6</td>
</tr>
<tr>
<td>Others</td>
<td>1162.7 ± 994.6</td>
<td>1315.7 ± 1390.9</td>
</tr>
<tr>
<td>Controls</td>
<td>1964.5 ± 1790.9</td>
<td>2067.2 ± 3483.5</td>
</tr>
</tbody>
</table>

*BG 133.3 ± 72.2 (n = 4)

Fig. 2 Mitogenic index (M.I.) of peripheral blood lymphocytes against the s-IgA fraction.
DISCUSSION

Cell-mediated autoimmunity to systemic exocrine glands as a cause of tissue injury has been suggested in SjS by evidence such as the characteristic lymphoid cell infiltration in the glands (11) and the presence of cellular immune responses to the homogenate of salivary glands or to saliva \textit{in vitro} (2, 10, 13, 14). A common antigen associated with the duct cells of exocrine glands was suggested from periductal lymphoid cell infiltration and anti-salivary duct antibody (1, 3, 6, 9) in sera of patients with SjS. However, no additional analysis concerned with duct antigens participating in the pathology of SjS was reported.

We were interested in the secretory component (SC) as a common duct antigen of systemic exocrine glands which was synthesized in the duct cells and then secreted as secretory IgA (s-IgA) following binding with polymer IgA (15) because of the possible analogy between SC in SjS and thyroglobuline in Hashimoto's thyroiditis (12).

We isolated s-IgA as a source of SC from human milk and investigated the lymphocyte response against s-IgA in patients with SjS using the $^3$H-thymidine incorporation method.

The isolated s-IgA fraction was identified as purified s-IgA by immunodiffusion and immunoelectrophoresis with anti-human IgA serum, anti-human SC serum and anti-human serum. Higher mitogenic indices against s-IgA were demonstrated in SjS when compared with RA, other autoimmune diseases and normal persons. In addition, the mitogenic indices in SjS \textit{alone} were significantly higher than those in SjS \textit{with associated deseases}.

Demonstration of cellular immune responses against s-IgA in some patients with SjS, especially in patients with SjS \textit{alone}, suggests not only the role of SC as a common antigen of exocrine glands in SjS, but also the presence of an etiologically different subgroup in patients with SjS, although these cellular immune responses to s-IgA \textit{in vitro} might be derived from a secondary phenomenon following the destruction of exocrine glands.

ACKNOWLEDGEMENTS

This study was supported by a Grant-in-Aid for Research from the Japanese Ministry of Education, Science and Culture (No. 477338), the research fund of the Sjögren's Disease Research Committee of the Japanese Ministry of Health and Welfare, and a Tokai Medical Research Grant.

REFERENCES