Association of serum IgMκ monoclonicity in patients with Sjögren’s syndrome with an increased proportion of κ positive plasma cells infiltrating the labial minor salivary glands

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Abstract

Minor salivary gland biopsy specimens from 11 patients with primary Sjögren’s syndrome with circulating monoclonal IgMκ cryoglobulins, seven without cryoglobulins, and four patients with rheumatoid arthritis and Sjögren’s syndrome (one with monoclonal and three with polyclonal cryoglobulins) were examined by the peroxidase-antiperoxidase bridge technique, using antihuman κ and λ antibodies.

In 6/11 patients with primary Sjögren’s syndrome and in one patient with Sjögren’s syndrome and rheumatoid arthritis with monoclonal cryoglobulins a predominance of plasma cells containing intracytoplasmic κ light chains was found (κ:λ >3:1). Two of those seven patients had immunohistological features of immunocytomas. In the other five patients with circulating monoclonal cryoglobulins the κ:λ ratio of positive cells did not exceed 3:1, while six out of seven patients without cryoglobulins and the patients with rheumatoid arthritis and Sjögren’s syndrome with polyclonal cryoglobulins had almost equal numbers of κ and λ stained cells. One of seven patients with primary Sjögren’s syndrome without cryoglobulins had an increased number of λ light chain positive cells, indicating a non-secretory λ monoclonal population. These findings suggest that the main area of B cell monoclonal expansion in primary Sjögren’s syndrome may be the affected exocrine glands.

During the past 10 years various studies have shown that patients with Sjögren’s syndrome, in addition to the polyclonal B cell activation, as illustrated by autoantibodies, express a monoclonal B cell process. The latter was shown by the presence of serum monoclonal immunoglobulins as well as monoclonal type II cryoglobulins and excretion of monoclonal light chains in the urine.1-5 All these phenomena were evident in patients with the systemic (extraglandular) disease and long before any overt clinical signs of lymphoid malignancy were present.

Previous studies have indicated that the polyclonally activated B cells in patients with primary Sjögren’s syndrome are mainly localised in the affected exocrine glands.6-8 This prompted us to search in the minor salivary gland biopsy specimens of patients with Sjögren’s syndrome, with and without monoclonal cryoglobulins, for evidence of monoclonal plasma cell populations.

Patients and methods

PATIENTS

Eighteen Greek patients with primary Sjögren’s syndrome and four patients with rheumatoid arthritis and Sjögren’s syndrome were selected for the study.

Eleven of the 18 patients presented serum monoclonal cryoglobulins with a total protein content ranging from 16 to 487 mg/l. The presence of a monoclonal IgMκ immunoglobulin component with rheumatoid factor activity (monoclonal type II cryoglobulins)6 in these cryoglobulins was shown by a high resolution agarose electrophoresis technique combined with immunofixation.5 The other seven patients with primary Sjögren’s syndrome did not have circulating cryoglobulins.

The diagnosis of primary Sjögren’s syndrome was based on the presence of at least two of the three following criteria: xerostomia (subjective complaints and decreased stimulated parotid flow <0.5 ml/5 min/gland); keratoconjunctivitis sicca (subjective complaints and Schirmer’s eye test <5 mm/5 min as well as positive slit lamp examination after rose bengal staining); and recurrent or persistent parotid or major gland enlargement. The diagnosis in all cases was

Table 1 Clinical characteristics of the patients (all female) with primary Sjögren’s syndrome

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Age (years)</th>
<th>Disease duration (years)</th>
<th>Extraglandular manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53</td>
<td>13</td>
<td>RF*, vasculitis</td>
</tr>
<tr>
<td>2</td>
<td>74</td>
<td>4</td>
<td>RF, ILD*</td>
</tr>
<tr>
<td>3</td>
<td>42</td>
<td>7</td>
<td>IKD*, purpura</td>
</tr>
<tr>
<td>4</td>
<td>62</td>
<td>17</td>
<td>RF, GMN*</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>10</td>
<td>ILD</td>
</tr>
<tr>
<td>6</td>
<td>58</td>
<td>10</td>
<td>Vascular, RF, IKD</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>10</td>
<td>RF, splenomegaly, GMN</td>
</tr>
<tr>
<td>8</td>
<td>43</td>
<td>12</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>10</td>
<td>RF, ILD</td>
</tr>
<tr>
<td>10</td>
<td>58</td>
<td>9</td>
<td>RF, mononeuritis multiplex, vasculitis</td>
</tr>
<tr>
<td>11</td>
<td>60</td>
<td>50</td>
<td>Chronic active hepatitis</td>
</tr>
<tr>
<td>12</td>
<td>30</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>13</td>
<td>50</td>
<td>3</td>
<td>None</td>
</tr>
<tr>
<td>14</td>
<td>67</td>
<td>5</td>
<td>None</td>
</tr>
<tr>
<td>15</td>
<td>40</td>
<td>7</td>
<td>Splenomegaly, ILD</td>
</tr>
<tr>
<td>16</td>
<td>60</td>
<td>7</td>
<td>ILD</td>
</tr>
<tr>
<td>17</td>
<td>50</td>
<td>2</td>
<td>RF, arthritis</td>
</tr>
<tr>
<td>18</td>
<td>60</td>
<td>2</td>
<td>RF</td>
</tr>
</tbody>
</table>

*RF=Raynaud’s phenomenon; ILD=interstitial lung disease; IKD=interstitial kidney disease; GMN=glomerulonephritis.
confirmed by labial minor salivary gland biopsy, which showed a heavy focal lymphocytic infiltrate (≥2+ according to Tarpley’s criteria). None of these patients had any clinical or laboratory abnormality suggestive of another autoimmune disease. In addition, none of the patients was receiving any form of immunosuppressive treatment.

Of the four patients with rheumatoid arthritis and Sjögren’s syndrome, three had polyclonal cryoglobulins (type III) and one had monoclonal IgMx (type II) cryoglobulin. The diagnosis of rheumatoid arthritis in four patients with secondary Sjögren’s syndrome was based on American Rheumatism Association criteria. Table 1 shows the clinical characteristics of the patients with primary Sjögren’s syndrome.

### IMMUNOHISTOLOGICAL STUDIES

All paraffin embedded labial minor salivary gland biopsy specimens were examined by a modified peroxidase-antiperoxidase method of Sternberger et al. Briefly, 5 μm paraffin sections were pretreated with fresh 0-1% trypsin solution in 0-1% calcium chloride at 37°C for 30 minutes. Rabbit antihuman sera against κ or λ light chains (Dacopatts, Denmark) were then applied at a dilution of 1:200. Subsequently, swine antirabbit IgG and peroxidase-antiperoxidase complexes were added at dilutions of 1:40 and 1:100 respectively. Before immuno-staining the sections were treated with normal swine serum to reduce non-specific staining. Finally, all preparations were counterstained with haematoxylin. The number and proportion of cells positively stained for individual κ or λ light chains were evaluated blind by counting the cells in 10 different non-overlapping fields per section. The plasma cell population containing intracytoplasmic λ light chain was considered monoclonal if the ratio of κ:λ stained cells was greater than 3:1.

### Results

With the peroxidase-antiperoxidase bridge technique it was shown that 6/11 patients with primary Sjögren’s syndrome and IgMx cryoglobulinaemia had in their minor salivary glands more cells stained with anti-κ antisera than with anti-λ antisera and the κ:λ ratio was greater than 3:1. This suggests that the infiltrates contained a monoclonal plasma cell subset (table 2). In two patients (Nos 1 and 2) an intense monoclonal pattern with morphological characteristics of immunocytomas was noted, while in the remaining four cases the κ:λ ratio of stained cells ranged from 3:7 to 4:9 (table 2).

In the other five patients the κ:λ ratio was greater than 2:1 but did not exceed the 3:1 ratio. One patient (No 15) without cryoglobulins had an increased number of λ light chain positive cells (κ:λ=0:8), suggesting a λ monoclonal population, while the remaining six patients had almost equal numbers of κ and λ cells.

The evaluation of the four tissues from patients with secondary Sjögren’s syndrome (Nos 19-22) showed one monoclonal pattern (κ:λ=3:6:1), which corresponded to the patient with the monoclonal cryoglobulins, and three polyclonal patterns (table 2).

### Discussion

Previous reports of sporadic cases have shown that patients with primary Sjögren’s syndrome with circulating monoclonal immunoglobulins have in their minor salivary gland infiltrates monoclonal plasma cells, which contain in their cytoplasm the same monoclonal isotype which was found in the serum. This study showed that over half of the patients with circulating IgMx monoclonal cryoglobulins (type II) have in their minor salivary gland infiltrates an increased proportion of cells expressing λ light chains. In contrast, the minor salivary glands of patients with RA with Sjögren’s syndrome and polyclonal cryoglobulins contained almost equal numbers of λ and κ positive cells. This finding is in agreement with our previous observation that patients with Sjögren’s syndrome usually have polyclonal cryoglobulinaemia.
Some patients with monoclonal cryoglobulins did not show an apparent monoclonal plasma cell population in the minor salivary gland infiltrates, though the $\kappa:\lambda$ ratio was slightly increased. This may be either because there was insufficient tissue for examination to show the monoclonal population or because the monoclonal plasma cell population was located in other organs, such as the bone marrow or the lymph nodes.

A patient with primary Sjögren’s syndrome without cryoglobulins had $\lambda$ light chain cell predominance in the cells infiltrating the labial minor salivary glands. The absence of the cryoglobulins in this case does not exclude the presence of circulating $\lambda$ chains in the serum. On the other hand, this case may represent a non-secreting immunocytoma. In fact, Pavlidis et al (unpublished data) have found that three of six patients with primary Sjögren’s syndrome and immunocytomas of the minor salivary glands did not show circulating monoclonal cryoglobulins. Two of these immunocytomas were of $\lambda$ light chain type.

Fourteen of 18 patients with primary Sjögren’s syndrome had one or more extraglandular manifestations. Although the patients with primary Sjögren’s syndrome and monoclonal cryoglobulins have a higher incidence of extraglandular disease, patients with monoclonal expression in the minor salivary glands did not show a higher incidence of extraglandular manifestations.

Our data, coupled with previous observations that in patients with Sjögren’s syndrome the B cells from minor salivary gland infiltrates have a common idiotype than the monoclonal immunoglobulins from patients with B cell lymphoid malignancies, suggest that neoplastic transformation in primary Sjögren’s syndrome may start in the exocrine glands. In fact, two of these observations have led to the observation of monoclonal light chain pathology in the labial minor salivary glands as we have found in another series of patients with primary Sjögren’s syndrome (unpublished data).

Several immunoregulatory abnormalities noted in the labial minor salivary glands may potentially contribute to the B cell transformation in these tissues. These abnormalities include the inappropriate activation of tissue B and T cells together with the absence of natural killer cells. Furthermore, the increased number of CD3 positive B cells in the affected minor salivary glands of patients with primary Sjögren’s syndrome suggests that the hyperfunction of B cells due to autostimulatory growth factor(s) or other unknown stimuli may have an important role in the B cell monoclonal expansion in primary Sjögren’s syndrome.

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