Nervous system disease, immunological features, and HLA phenotype in Sjögren’s syndrome

Aki Hietaharju, Markku Korpela, Jorma Ilonen, Harry Frey

Abstract
Twenty seven patients with primary or possible Sjögren’s syndrome with neurological manifestations were compared immunologically with 21 patients with Sjögren’s syndrome with an intact nervous system. Patients with Sjögren’s syndrome were divided into seropositive and seronegative subgroups with respect to the occurrence of one or more autoantibodies (antinuclear antibodies, rheumatoid factor, antibodies to SS-B) in their serum samples. This study of 48 patients indicates that the spectrum of nervous system disease in seronegative and seropositive subgroups is almost indistinguishable.

No significant differences were found in the occurrence of circulating immune complexes, the levels of serum complement C3 and C4, or serum IgA, IgM, and β2 microglobulin with respect to the neurological manifestations. The serum IgG level, however, was significantly higher in the patients with Sjögren’s syndrome with intact nervous systems than in those with neurological manifestations.

No significant association was found between the HLA phenotype and nervous system disease. The occurrence of HLA-B8 and DR3 antigens was, however, significantly higher in those patients with antibodies to SS-B than in those without. This finding supports the suggestion that HLA-B8/DR3 may modulate autoantibody responses rather than disease expression in Sjögren’s syndrome.

Sjögren’s syndrome is a systemic lymphoproliferative and autoimmune disease which is characterised by dryness of the eyes, mouth, and other mucous membranes. Extraglandular manifestations of Sjögren’s syndrome may include focal or diffuse lymphocytic and plasma cell infiltration of almost any organ. Extrafolacular lymphoid infiltrates may be found in the kidney, liver, lungs, muscle, and skin.

There is a strong genetic component in Sjögren’s syndrome. It was one of the first autoimmune diseases in which the association with some of the HLA antigens was shown (HLA-B8 and DR3).

Nervous system disease in primary Sjögren’s syndrome has been well reported. Central nervous system disease has been estimated to occur in 25%, and peripheral nervous system disease in approximately 10–20% of patients with primary Sjögren’s syndrome. Some patients have a relapsing course with a cumulative neurological deficit which is indistinguishable from multiple sclerosis. On the other hand, the occurrence of serious and progressive central nervous system disease in patients with Sjögren’s syndrome, as suggested by the Johns Hopkins group, has been questioned in some studies.

There is evidence that nervous system disease in Sjögren’s syndrome, as well as in other collagen vascular disorders, is mediated immunopathologically. It has been shown in Sjögren’s syndrome that inflammatory cells, predominantly mononuclear, can gain access to the nervous system and its blood vessels at multiple levels. Despite increasing knowledge of the different mechanisms taking part in tissue damage in collagen vascular diseases, there are still major questions to be answered.

Neurological manifestations were found in 56% of patients with primary or possible Sjögren’s syndrome evaluated at our institution. In view of the autoimmune nature of Sjögren’s syndrome it seemed of considerable interest to study further the serological and immunogenetic features of those patients with Sjögren’s syndrome with various neurological manifestations and to compare the results with those of patients with Sjögren’s syndrome with intact nervous systems.

Patients and methods
PATIENTS
The sample consisted of 48 patients (45 women and three men) in whom the diagnosis of Sjögren’s syndrome was established on the modified Californian criteria (Table 1). Of these, 27 (56%) fulfilled the criteria of primary Sjögren’s syndrome, and the remaining 21 (44%) the criteria of possible Sjögren’s syndrome. Additional rheumatic disorders (such as systemic lupus erythematosus, rheumatoid arthritis, or progressive systemic sclerosis) were excluded.

The ages of the patients ranged from 20 to 80

Table 1 Criteria used for diagnosis of Sjögren’s syndrome

<table>
<thead>
<tr>
<th>Criteria for inclusion of patients</th>
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<tr>
<td>Keratoconjunctivitis sicca</td>
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<td>Decreased tear flow using Schirmer’s test (&lt;9 mm/5 min)</td>
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<tr>
<td>Increased staining with rose-bengal dye</td>
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<tr>
<td>Symptomatic xerostomia</td>
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<td>Extensive lymphocytic infiltrate on minor salivary gland biopsy (grade 3 or 4 on the Greenspan scale) obtained through normal buccal mucosa</td>
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<tr>
<td>Laboratory evidence of a systemic autoimmune disease</td>
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<tr>
<td>Positive rheumatoid factor</td>
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<tr>
<td>Positive antinuclear antibodies</td>
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<tr>
<td>Positive antibodies to Ro or La (SS-A or SS-B)</td>
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Exclusions
Pre-existent lymphoma, graft v host disease, acquired immune deficiency disease, sarcoidosis
years (median 57). Table 2 shows the neurological manifestations in the patients. Peripheral nervous system symptoms were predominant in this group (78%), the most common manifestations being entrapment neuropathies (19%) and polyneuropathy (15%). The details of the neurological and electrophysiological studies are given elsewhere.18

SEROLOGICAL STUDIES
Blood samples for serological studies were obtained from all patients at the time of the study. Rheumatoid factor was determined by quantitative immunoturbidimetric assay (FS-RF)24 and by the sensitised sheep cell agglutination test (Wa-Ro).25 FS-RF values greater than 25 and Wa-Ro titres of 1/64 or higher were regarded as positive. Antinuclear antibodies were detected by indirect immunofluorescence using rat liver and kidney as substrates26 and staining of serum samples at a titre of 1/100 or higher was considered positive. Circulating immune complexes were assayed by a solid phase Clq enzyme linked immunosorbent assay.27 Serum immunoglobulins and serum complements C3 and C4 were measured by nephelometry.28 Antibodies to SS-B (La) were detected by an immunodiffusion technique using a compound of rabbit thymus and porcine spleen as antigen.29 Serum β2 microglobulin levels were measured by radioimmunoassay (Pharmacia beta-2-micro RIA kit, Pharmacia Diagnostics, Uppsala, Sweden).

HLA TYPING
HLA antigens were determined in 42/48 patients using the standard two stage microlymphocytotoxicity method. B cells for DR typing were separated using either rosetting with sheep red blood cells or immunomagnetic beads. A panel of local and commercial antisera was used for defining various antigens. DR typing was not available in one patient.

STATISTICS
Statistical analysis was by Student's t test and the χ² test with Yates's correction, as appropriate.

Results
ANTINUCLEAR ANTIBODIES AND ANTIBODIES TO SS-B
Antinuclear antibodies were present in 34/48 (71%) patients with Sjögren's syndrome and antibodies to SS-B were detected in 20/47 (43%) patients with Sjögren's syndrome tested. No association could be found between the occurrence of these autoantibodies and nervous system disease.

CIRCULATING IMMUNE COMPLEXES AND SERUM COMPLEMENT COMPONENTS C3 AND C4
There were no significant differences between patients with Sjögren's syndrome with or without nervous system disease with respect to the occurrence of circulating immune complexes, or the mean serum C3 or C4 concentrations.

RHEUMATOID FACTOR
Patients were regarded as seropositive if either the FS-RF or Wa-Ro test was positive. Thirty nine of 48 (81%) patients with Sjögren's syndrome were seropositive. No differences were found in the occurrence of rheumatoid factor between patients with or without neurological manifestations.

SERUM β2 MICROGLOBULIN
Serum β2 microglobulin concentrations were equal (2.91 (1.21) mg/l) in patients with Sjögren's syndrome with and without nervous system disease.

SERUM IMMUNOGLOBULINS
When the 27 patients with neurological manifestations were compared with 21 patients with intact nervous systems, there was a significant difference between the two groups with respect to serum IgG levels (fig). The mean (SD) serum IgG value for the group with neurological complications was 19.1 (8.0) g/l v 26.3 (7.6) g/l for the group with intact nervous systems (p=0.005). The serum IgG value in patients with polyneuropathy (n=7) was 16.9 (6.6) g/l, and in those with entrapment neuropathy (n=9) 15.7 (5.2) g/l. The differences were significant when compared with patients without neurological manifestations (p=0.005 and p=0.0005 respectively).

There were no differences in the levels of serum IgA and IgM between patients with neurological manifestations and those with intact nervous systems.

HLA ANTIGENS
Table 3 gives the occurrence of HLA antigens in patients with Sjögren's syndrome. B8 was present in 52% of all patients with Sjögren's syndrome and in 15% of control subjects (p=0.001). The occurrence of the antigen DR3 was 60% in patients with Sjögren's syndrome and 16% in control subjects (p=0.001). Significant differences between patients with Sjögren's syndrome and control subjects were also found in the occurrence of the antigens DR4 and DRw6. DR4 was present in 7% of patients with Sjögren's syndrome and in 30% of control subjects (p=0.01). The occurrence of...
the antigen DRw6 was also 7% in patients with Sjögren’s syndrome and 26% in control subjects (p = 0.01).

The only significant differences with respect to neurological manifestations were found in antigens B35 and DR1. B35 was present in 46% of patients with neurological manifestations and in 11% with intact nervous systems (p = 0.03). The occurrence of the antigen DR1 was 50% in patients with neurological complications and 11% in those without (p = 0.02). The p values, however, lose their significance when multiplied by the number of antigens studied.

A significant correlation was found between the presence of antibodies to SS-B and HLA-B8/DR3 antigens. B8 was found in 15/18 (83%) of patients with Sjögren’s syndrome with antibodies to SS-B and in 7/24 (29%) of patients without (p ≤ 0.001). The occurrence of the antigen DR3 in patients with Sjögren’s syndrome with antibodies to SS-B was 14/17 (82%) and 11/24 (46%) in patients without antibodies to SS-B (p ≤ 0.02).

Discussion
The immunological and genetic profile of Sjögren’s syndrome has been defined by observation. Although one half to two thirds of patients with Sjögren’s syndrome are seropositive with respect to one or more autoantibodies, a significant proportion are seronegative by standard techniques. Those who are seropositive often have hyperglobulinaemia with numerous autoantibodies, including antinuclear antibodies, rheumatoid factor, antibodies to Ro (SS-A) or La (SS-B), and circulating immune complexes.

It has been suggested that patients with Sjögren’s syndrome with antibodies to SS-A/SS-B tend to have a more serious disease course than those without. On the other hand, Molina et al. have shown that both seronegative and seropositive subgroups of patients with Sjögren’s syndrome develop systemic complications, including vasculitis and nervous system disease.

Our findings agree with the results of Molina et al. with respect to the relationship between neurological manifestations and most indices of seropositivity in Sjögren’s syndrome. The spectrum of nervous system disease in seronegative and seropositive subgroups of patients with Sjögren’s syndrome seems to be almost indistinguishable. We may thus conclude that the clinical outcome of patients with Sjögren’s syndrome cannot be predicted by seropositivity or the occurrence of autoantibodies in serum samples.

The possible protective part played by serum IgG against neurological complications in Sjögren’s syndrome, as suggested by our results, is highly speculative. Immunoglobulin preparations are used to treat a variety of diseases, e.g. autoimmune disorders such as polymyositis and myasthenia gravis. The beneficial effect of immunoglobulin treatment on these diseases has been suggested to be due to antibodies to idotypes. It is possible that patients with Sjögren’s syndrome with higher IgG levels are better provided with suppressive and protective antibodies to idotypes.

Contrary to the findings of Alexander et al., the nervous system disease in our patients with Sjögren’s syndrome was fairly benign. The spectrum of neurological manifestations in our study agreed with the results of studies by
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Binder et al.15 and Andonopoulos et al.14 This makes it difficult to draw the line between patients with completely intact nervous systems and those with neurological manifestations. On the other hand, we tried to avoid biased selection as much as possible. A strong association of Sjögren's syndrome with HLA-B8 and HLA-DR3 phenotypes was clearly confirmed in our study. The differences in the occurrence of DR4 and DRw6 between patients with Sjögren's syndrome and control subjects is partly explained by the increase in DR3. On the other hand, there was no decrease in the occurrence of DR1 and DR2. The occurrence of DR2 in patients with Sjögren's syndrome is actually increased. This agrees with the study of Manthorpe et al.38 in which a significant association was found between Sjögren's syndrome and the DR2 phenotype.

Of 19 patients studied by Fye et al.,7 there was a correlation between the severity of the clinical manifestations and the presence of DR3. On the other hand, Gershwin et al.39 and Clough et al.40 also compared several clinical features in B8 positive and negative patients and found no significant differences between the two groups. In our study, no association could be found between the HLA phenotype and the occurrence of neurological manifestations. It seems that HLA-DR3, or a closely linked genetic locus, may modulate autoimmune responses, rather than disease expression, in Sjögren's syndrome. This suggestion, by Molina et al.,41 is supported by our finding of a significant association between the occurrence of antibodies to SS-B and the presence of HLA-DR3 and HLA-B8 phenotypes.

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