Antiphospholipid antibodies and HLA associations in primary Sjögren’s syndrome

Ronald A Asherson, Hong-Ming Fei, Henrique L Staub, M A Khamashhta, Graham R V Hughes, Robert I Fox

Abstract
Blood samples from 65 patients with primary Sjögren’s syndrome were evaluated for the presence of antiphospholipid antibodies. Increased levels of antiphospholipid antibodies were found in 13 of 65 (20%) of patients. These antiphospholipid antibodies were predominantly of the IgA isotype, in contrast with the IgG isotype antiphospholipid antibodies found in patients with systemic lupus erythematosus (SLE). The presence of IgA antiphospholipid antibodies in the patients with primary Sjögren’s syndrome was not significantly associated with arterial or vascular thrombosis, nor peripheral or central nervous system vasculitis. There was no association with laboratory determined features such as lupus anticoagulant or false positive results of the Venereal Disease Research Laboratory (VDRL) test. Oligonucleotide specific DNA amplification and hybridisation with allele specific probes was used to examine the HLA-D antigens occurring in this group of patients with primary Sjögren’s syndrome. Of 13 patients with antiphospholipid antibodies, seven had the genotype HLA-DR2/DR3. However, compared with the whole group of 65 patients with Sjögren’s syndrome, no increased occurrence of haplotype DR2 or DR3 was noted. These results suggest that gene interaction between DR2 and DR3 may play a part in the production of antiphospholipid antibodies in patients with Sjögren’s syndrome. In contrast with patients with SLE, the IgA antiphospholipid antibodies in patients with Sjögren’s syndrome were more likely to precipitate drug induced lupus such as chlorpromazine or procainamide. The presence of IgA antiphospholipid antibodies in patients with Sjögren’s syndrome probably reflects its production at mucosal sites of inflammation and the absence of vasculopathy may be due to the inability of IgA antibodies to activate complement.

The antiphospholipid antibodies (the lupus anticoagulant) and antibodies to negatively charged phospholipids, particularly cardiolipin, are found in patients with a wide variety of diseases including autoimmune disturbances, infections, malignancies, and in patients receiving drugs known to precipitate drug induced lupus such as chlorpromazine or procainamide. The antiphospholipid syndrome is a term which was introduced in 1987 to cover the association of thrombosis of veins and arteries, recurrent fetal loss and haemocytopenia, particularly thrombocytopenia, accompanied by persistent increases in the number of positive results in anticardiolipin antibodies/lupus anticoagulant tests. The antiphospholipid syndrome is seen equally in patients with systemic lupus erythematosus (SLE), lupus-like disease (probably a variant of SLE where less than four criteria for the classification of SLE are applicable), and in patients with the recently defined primary antiphospholipid syndrome. Thrombotic complications in patients with SLE occur in those in whom the IgG isotype antiphospholipid antibodies are present, usually at high titres. Susceptibility to SLE is determined by the presence of the major histocompatibility complex encoded genes, DR2 or DR3 antigen alleles. In comparison, patients with primary antiphospholipid syndrome do not possess antibodies to extractable nuclear antigens and have an increased occurrence of HLA-DR7.

Sjögren’s syndrome may exist as a primary disorder or in association with other autoimmune diseases. Patients with primary Sjögren’s syndrome have previously been shown to have an increased occurrence of HLA-DR3 and heterozygosity of the HLA-DQ genetic locus, particularly in association with the presence of autoantibodies to the SS-B/La. Patients with Sjögren’s syndrome with antiphospholipid antibodies have been reported, but antiphospholipid antibody associated thromboses are rare. Patients with primary Sjögren’s syndrome may show a peripheral vasculitis in addition to central nervous system and peripheral neuropathy. In this study, a group of patients with primary Sjögren’s syndrome was evaluated for the presence of antiphospholipid antibodies and it was determined whether any of the vasculitic complications in the patients with Sjögren’s syndrome were associated with these autoantibodies.

Patients and methods
PATIENTS
Sixty five white patients (60 women, five men) with definite primary Sjögren’s syndrome were seen at the Scripps Clinic and Research Foundation. These patients, with mean age of 54 years, were classified as having primary Sjögren’s syndrome by the criteria of Fox et al. All patients had objective keratoconjunctivitis sicca, xerostomia, and class IV minor salivary gland biopsy specimens. The autoantibodies determined included antinuclear antibodies using mouse kidney and a Hep-2 substrate; titres of greater than or equal to 1/320 were considered.

Associated genes...

Lupus Arthritis
Research Unit,
The Rayne Institute,
St Thomas’s Hospital,
London SE1 7EH, UK
R A Asherson
H L Staub
M A Khamashhta
G R V Hughes
Scripps Clinic and Research Foundation,
La Jolla,
CA 92037, USA
H-M Fei
R I Fox
Correspondence to:
Dr R A Asherson,
Division of Rheumatology and Connective Tissue Diseases,
St Luke’s/Roosevelt Hospital Centre,
New York,
NY 10019, USA.
Accepted for publication
3 May 1991

Annals of the Rheumatic Diseases 1992; 51: 495-498
significant. Antinuclear antibodies were present in all 65 patients. Antibodies to Ro (SS-A), La (SS-B) were detected by an enzyme linked immunosorbent assay (ELISA). Antibodies to Ro were present in 60 of 65 patients and antibodies to La in 40 of 65 patients. The control population included 150 white women living in San Diego with no history of autoimmune disease, and who were contributors to the San Diego blood bank. The median age of the controls was 46 years.

IgG, IgM and IgA anticardiolipin antibodies were estimated by an ELISA technique according to the standard method of Gharavi et al. As an IgA standard is not yet available, three standard deviations (SD) above the mean of 40 serum samples from healthy blood donors was taken as the upper limit for normal levels of IgA anticardiolipin antibodies. ELISA measurements three to six standard deviations above the mean were considered low positive, values six to ten standard deviations above the mean as moderately positive, and values above ten standard deviations as high positive levels of IgA anticardiolipin antibodies. The lupus anticoagulant was measured by the method of Exner, and the results were expressed as low, moderate, or high according to the accepted standards adopted by the International Workshop of 1986. The treponema immobilization test and rapid plasma reagin test were performed on all patients. Rheumatoid factor was detected in serum samples using a rheumatoid arthritis latex test (Gamma, Houston, TX, USA).

DETECTION OF HLA-DRB1 GENETIC POLYMORPHISM BY POLYMERASE CHAIN REACTION

Genomic DNA was isolated from the peripheral blood of all 65 patients with primary Sjögren’s syndrome and from 150 age and sex matched patients living in the same geographical region. To amplify specific segments of the DRB1 and DRB3 genes, the oligonucleotides GH46 and GH50 were used as polymerase chain reaction primers. These oligonucleotides flank the second exon of the DRB1 genes. The amplified product of the DRB1 gene (272 bp in size) contains allele specific sequences. We used different allele specific oligonucleotide probes to detect HLA-DR1 to DR8 related sequences (Fei HM, unpublished data). These DNA sequences correspond to DRB1 alleles defined by Bodmer et al: DR1 to DRB1*0101; DR2 to DRB5*0201; DR3 to DRB1*0301; DR4 to DRB1*0401; DR5 to DRB1*0501; DR6 to DRB1*0601; and DR8 to DRB1*0801.

These allele specific oligonucleotide probes were synthesised using an Applied Biosystems 380B DNA synthesiser (Foster City, CA, USA). The allele specific oligonucleotides were 5’ end labelled with phosphorus-32 labelled ATP (γ32P-ATP) (Amersham, Arlington, IL, USA) with five units of polynucleotide kinase (New England Bio Labs, Beverly, MA, USA).

Genomic DNA (1 μg) was placed in a tube containing 1·5 mmol/l MgCl2, 200 μmol/l dNTP, 50 μmol/l primers, and 2·5 U Thermus aquaticus (Taq DNA polymerase, Cetus, Emeryville, CA, USA). The samples were subjected to 30 cycles of amplification with denaturation (one minute at 95°C), annealing of primer (one minute at 55°C), and extension of Taq polymerase (1·5 minute at 72°C) by a thermal cycle (Perkin Elmer, Norwalk, CT, USA).

After 30 cycles, 10 μl of the amplified product was analysed by the Southern blot method using a 1·7% agarose gel and Hae III digested φX174 DNA as a molecular weight marker. After transferring the gel to a nylon membrane, prehybridisation for DRB allele specific oligonucleotide probes was performed in 2×SSPE, 5× Denhardt’s, 0·5% sodium dodecyl sulphate for one hour at 42°C. Allele specific oligonucleotide probes end labelled with 32P-ATP (2·5×106 counts per minute) were added to each filter and hybridised overnight. The washing condition was 0·2×SSPE, 0·1% sodium dodecyl sulphate at 42°C for 10 minutes for the DRB allele specific oligonucleotide probes. The autoradiography was performed for one hour.

The occurrence of the genes and antigens in patients with Sjögren’s syndrome were compared with the occurrence in the normal white patients using χ2 statistics with values adjusted by the Bonferroni method for the number of different variables measured (Statview II Computer software, Abacus, Berkeley, CA, USA).

Results

Table 1 shows that 13 of the 65 patients were positive for anticardiolipin antibodies. Eleven of the 13 were positive for IgA anticardiolipin antibodies including six with low titres, three with high titre and two with moderate titre. Only one patient was positive for IgM anticardiolipin antibodies (moderate) and three were positive for IgG anticardiolipin antibodies (two low and one moderate). A false positive Venereal Disease Research Laboratory test (VDRL) was observed in only two patients. The lupus anticoagulant test was negative in all patients.

Among the entire group of 65 patients with Sjögren’s syndrome, antibodies to SS-A and SS-B were present in 92 and 55%, respectively. In the subset of patients with detectable anticardiolipin antibodies, antibodies to SS-A and SS-B were present in 12 of 13 patients (94%). Rheumatoid factor was present in 11 of the 13 patients positive for anticardiolipin antibodies.

**Table 1** Relationship of antiphospholipid antibodies to vasculitis in patients with primary Sjögren’s syndrome

<table>
<thead>
<tr>
<th>Vasculitis (n=6)</th>
<th>No vasculitis (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient No</td>
<td>Anticardiolipin antibody*</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
</tr>
<tr>
<td>1</td>
<td>Moderate</td>
</tr>
<tr>
<td>7</td>
<td>Moderate</td>
</tr>
<tr>
<td>8</td>
<td>High</td>
</tr>
<tr>
<td>9</td>
<td>Low</td>
</tr>
<tr>
<td>11</td>
<td>Low</td>
</tr>
<tr>
<td>12</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

*IgG and IgM anticardiolipin antibodies: low = 5-20; moderate = 20-80; high = >80 U. IgA anticardiolipin antibodies: see text under ‘Patients’.

**Accepted 22 September 1993**
Antiphospholipid antibodies and HLA associations in primary Sjögren’s syndrome

Table 2. HLA-DR analysis of patients with Sjögren’s syndrome with autoantibodies to phospholipids

<table>
<thead>
<tr>
<th>HLA-DR specificity</th>
<th>All patients with primary Sjögren’s syndrome</th>
<th>Antibodies to phospholipids</th>
<th>Antibodies to SS-A (La)</th>
<th>Antibodies to SS-B (Lb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>p Value</td>
<td>No</td>
<td>p Value</td>
</tr>
<tr>
<td>DR2</td>
<td>25</td>
<td>7</td>
<td>&gt;0.23</td>
<td>10</td>
</tr>
<tr>
<td>DR3</td>
<td>35</td>
<td>7</td>
<td>&gt;0.52</td>
<td>24</td>
</tr>
<tr>
<td>DR4</td>
<td>7</td>
<td>1</td>
<td>&gt;0.5</td>
<td>9</td>
</tr>
<tr>
<td>DR5</td>
<td>18</td>
<td>1</td>
<td>&gt;0.5</td>
<td>2</td>
</tr>
<tr>
<td>DR6</td>
<td>6</td>
<td>1</td>
<td>&gt;0.5</td>
<td>2</td>
</tr>
<tr>
<td>DR8</td>
<td>8</td>
<td>4</td>
<td>&gt;0.5</td>
<td>6</td>
</tr>
<tr>
<td>DR2/3</td>
<td>18</td>
<td>5</td>
<td>&lt;0.04</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>13</td>
<td></td>
<td>34</td>
</tr>
</tbody>
</table>

*Patients with Sjögren’s syndrome who show at least one HLA-DR allele as determined by polymerase chain reaction.
†Number of patients with Sjögren’s syndrome who show at least one specific HLA-DR allele and the presence of antiphospholipid antibodies or antibodies to SS-B.
‡p<0.05 by χ² analysis with the Bonferroni correction for number of alleles analysed.

An evaluation of the HLA-D encoded alleles showed a significantly increased occurrence of heterozygotes (HLA-DR2/DR3) among the patients with Sjögren’s syndrome possessing antiphospholipid antibodies (p<0.05) (table 2). However, compared with the whole group of 65 patients with primary Sjögren’s syndrome, the presence of antiphospholipid antibodies was not significantly associated with the single haplotype DR2 and DR3 (using the χ² method with the Bonferroni correction for the number of alleles examined). In comparison, autoantibodies to the SS-B antigen showed a significant association with DR-3 (p<0.01) and with DR2/DR3 heterozygosity (p<0.05) (table 2).

Discussion

Sjögren’s syndrome is a disease characterised by hyperglobulinaemia and an increase of autoantibodies against nuclear antigens, including SS-A (Ro) and SS-B (La). The high occurrence of an association of antibodies to SS-A/SS-B and primary Sjögren’s syndrome in our series probably reflects the allele frequency in diagnosis and the fact that the study group consisted entirely of white patients.17-20 We found an increased incidence (20%) of antiphospholipid antibodies in our patients with primary Sjögren’s syndrome, which was higher than in control subjects in California.27 Several important differences were noted in the antiphospholipid antibodies from patients with SLE and primary Sjögren’s syndrome.

First, the patients with primary Sjögren’s syndrome had IgA antiphospholipid antibodies but relatively few IgG antiphospholipid antibodies, in contrast with patients with SLE where IgG antiphospholipid antibodies predominate. Second, the antiphospholipid antibodies were not associated with nervous system vasculitis (i.e. peripheral neuropathy) or embolic disease in patients with primary Sjögren’s syndrome. This important difference in clinical association may be due to the fact that IgG antibodies can activate complement whereas IgA antibodies cannot. Further, IgG antibody complexes will be aggregated by rheumatoid factor and thus low affinity antibody reactions will be significantly amplified. The specific role of IgA antiphospholipid antibodies in the pathogenesis of Sjögren’s syndrome remains unclear. One possible function of the IgA autoantibodies is to help clear cellular debris derived from normal cell turnover, cells destroyed by the immune system, or from potential pathogens within the gland.28 It is likely that IgA antiphospholipid antibodies made at the salivary gland inflammatory site, as other autoantibodies are produced by lymphocytes eluted from the salivary gland.29 30 As the salivary gland in patients with Sjögren’s syndrome is a mucosal site of inflammation, it is likely that local T helper cells facilitate the isotype switch from IgM to IgA antibodies.

The frequency of antiphospholipid antibodies in primary Sjögren’s syndrome has ranged from 6 to 52% in other reported series.11-34 The difference in the occurrence of antiphospholipid antibodies at different medical centres possibly reflects the variation in criteria applied for the diagnosis of primary Sjögren’s syndrome.20 In this study we have used stringent criteria for the diagnosis of Sjögren’s syndrome, including the presence of characteristic minor salivary gland biopsy specimens and exclusion of patients who fulfilled the American Rheumatism Association criteria for SLE.

We did not find an association of antiphospholipid antibodies with any single HLA allele. In particular, we looked for an association with HLA-DR3, as this haplotype is associated with antibody to SS-B12 35 as well as complement C4 deletions.36 We did find an association with heterozygosity of HLA-DR2/DR3 and antiphospholipid antibodies. This may suggest gene complementation as a factor in pathogenesis. We have also found an increase in the heterozygosity of HLA-DQA4 (linked to DR3) and HLA-DQ1 (linked to DR2) in patients with primary Sjögren’s syndrome with antiphospholipid antibodies (unpublished observation). One mechanism for the association of HLA class II gene heterozygosity and antiphospholipid antibodies is that the α and β chains of the HLA-DQ alleles are rearranged such that new combinations of DQ molecules are expressed on cell surfaces.13

Analysis of HLA-DR antigens and their relationship to antiphospholipid antibodies in patients with SLE have until now provided conflicting information. Savi et al, in 1988, reported a highly significant association between antiphospholipid antibodies and DR7 in their group of 80 SLE patients,37 confirming their earlier observation.38 Canoso et al39 reported on patients treated with chlorpromazine and found an association with B44 and antiphospholipid antibody positivity. As HLA-B44 in linkage disequilibrium with DR7, this implies an association with HLA-DR7.39 However, a study of a group of white patients in the United Kingdom by MacGregor et al.40 could not confirm any relationship with HLA-DR7, B44, or with DR4 alleles. Other reports have suggested an association with HLA-DRB3 allele Dw53 and HLA-DQw7 in patients with SLE.41 In our patients with Sjögren’s syndrome, we did not find an increased occurrence of these alleles. Looking at patients with the primary antiphospholipid syndrome a different picture is emerging. McNeil et al.42 analysing patients
who developed occlusion of coronary bypass grafts, found a decreased occurrence of DR3 and DR53 in their patients with anticardiolipin antibodies, but an increased occurrence of DR4 and DRW53.42 Goldstein et al.43 in a study in Canada, confirmed this increase in DRW52 in their patients with primary antiphospholipid syndrome versus local controls. DR4, DR7, DRW53, and DQW3 were also increased, whereas DR3 was decreased in the patients with primary antiphospholipid syndrome compared with patients with SLE. Our group at St. Thomas's Hospital, in preliminary studies of patients with primary antiphospholipid syndrome, has also confirmed this increase in DRW3 and DR4.44 Alarcon-Segovia et al had previously reported an increase in DR7 in Mexican patients with primary antiphospholipid syndrome.11 Although primary Sjögren's syndrome and SLE are closely related diseases, emerging evidence suggests that the primary antiphospholipid syndrome may well be a separate and genetically distinct group from the disease characteristics of SLE and primary Sjögren's syndrome.

Antiphospholipid antibodies and HLA associations in primary Sjögren's syndrome.

R A Asherson, H M Fei, H L Staub, M A Khamashta, G R Hughes and R I Fox

doi: 10.1136/ard.51.4.495

Updated information and services can be found at:
http://ard.bmj.com/content/51/4/495

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/