The prevalence of antibodies to anionic phospholipids in patients with the primary antiphospholipid syndrome, systemic lupus erythematosus and their relatives and spouses

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Abstract

Objectives. Antiphospholipid antibodies (aPL) have been associated with syndromes involving thrombosis, fetal loss and thrombocytopenia. Genetic and environmental conditions are among the factors attributed to the cause of autoimmune diseases such as the antiphospholipid syndrome (APS) and systemic lupus erythematosus (SLE). The aim of this study was to determine whether these factors determine the prevalence of aPL.

Methods. Three groups of patients were tested for the presence of IgG, IgM and IgA anticardiolipin (aCL), antiphosphatidylinositol (aPI), antiphosphatidylglycerol (aPG) and antiphosphatidylserine (aPS) antibodies: (i) patients with primary APS (PAPS); (ii) patients with SLE and secondary APS; and (iii) patients with SLE without APS. First-degree relatives and spouses of patients with SLE/APS were also tested for circulating aPL.

Results. IgG aPL were particularly prevalent in patients with PAPS. IgG aPI and aCL were more prevalent in patients with PAPS than the IgM equivalents \( (P < 0.0001) \). Notably, none of the patients with PAPS had IgA aPL. A significantly higher number of relatives of patients with SLE/APS possessed IgG aPL than the normal controls. Except for aPG \( (P < 0.03) \), the prevalence of these antibodies in the relatives was not significantly different from patients with SLE/APS. The relatives also had significantly higher prevalence of IgG aPI, aPS and aCL antibodies than IgM aPL antibodies. In contrast, the prevalence of IgG aPL in the spouses was no different than in the healthy controls.

Conclusions. Genetic factors, shared by patients and their relatives, seem to have some effect on the prevalence of aPL in the subjects studied, while environmental factors shared by spouses appear to have no influence.

Key words: Systemic lupus erythematosus, Antiphospholipid syndrome/antibodies, First-degree relatives, Spouses.

The antiphospholipid syndrome (APS) is associated with possession of anticardiolipin antibodies (aCL) and/or lupus anticoagulant (LA) activity, with clinical features such as thrombocytopenia, thromboembolic phenomena and recurrent fetal loss \[1, 2\]. This may occur as a primary syndrome [primary APS (PAPS)] or secondary to an autoimmune condition, such as systemic lupus erythematosus (SLE) \[3, 4\]. aCL are also detected in patients with SLE who do not have secondary APS \[5\].

Antibodies to phospholipids other than cardiolipin have been detected in patients with APS and SLE, some associated with certain clinical features \[6–8\]. Antiphospholipid antibodies (aPL) are also present in the normal population, unassociated with any disease \[9\]. The presence of aPL in the normal population indicates that some trigger factor must be present to induce disease.

Variability in the prevalence of SLE in some ethnic groups living in different parts of the world, and the presence of disease within families, suggest that both environmental and genetic factors may trigger SLE induction in susceptible individuals or induce autoantibody production \[10–12\]. The question may be raised as to whether production of autoantibodies alone is

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(aPI), antiphosphatidylglycerol (aPG), and antiphosphatidylserine (aPS) first, in patients with PAPS, SLE with secondary APS (SLE/APS) and SLE alone, and secondly in the spouses and first-degree relatives of patients with SLE/APS. Our purpose was to determine whether environmental (as shared by the spouses) or genetic (as shared by the relatives) factors influence the prevalence of aPL antibodies.

Patients and methods

Patients

The first part of this study comprised 155 patients from the Lupus Clinic at the Middlesex Hospital, London, UK. Patients were divided into three groups according to their laboratory and clinical records: (i) PAPS patients with aCL and/or LA activity (68% were LA positive), with clinical features such as thrombocytopenia, thromboembolic phenomena and/or recurrent fetal loss; (ii) SLE patients without any clinical features of APS who fulfilled four (or more) of the revised American College of Rheumatology (ACR) criteria for the classification of SLE [13]; (iii) SLE patients with secondary APS (SLE/APS) who had historical records of aCL and/or LA serology (Table 1).

Relatives and spouses

For the second part of the study, 43 healthy individuals were studied, 18 of whom were spouses and 25 were first-degree relatives of 14 patients with SLE/APS. Fifty healthy individuals (samples supplied by the National Blood Service, Colindale, UK) were included as normal controls (Table 1).

Methods

aCL, aPI, aPG and aPS enzyme-linked immunosorbent assay (ELISA). Half the wells of microtitre ELISA plates (Polysorp, Nunc, Life Technologies, Paisley, UK) were coated with each of the following anionic phospholipids at 50 μg/ml: cardiolipin (CL), phosphatidylinositol (PI), phosphatidylglycerol (PG) and phosphatidylserine (PS) (all from Sigma, St. Louis, MO, USA) in ethanol, the other half with ethanol alone, and air dried overnight at 4°C. After blocking with 10% fetal calf serum (Sigma) in phosphate-buffered saline (10% FCS/PBS) for 1 h at 37°C and three washes with PBS, serum diluted 1:100 in 10% FCS/PBS was added in duplicate to both PL-coated and uncoated halves of the plates. After incubation for 1.5 h at 37°C and three washes with PBS, alkaline phosphatase-conjugated goat antihuman IgG, IgM or IgA (Sigma) was added at 1:1000 dilution, for Patients 1 h at 37°C. Colour was developed by adding dinitrophenyl phosphate [1 mg/ml (Sigma) solution containing 1 mM MgCl\(_2\) in bicarbonate buffer (BIC), pH 9.6], after three washes with PBS and two with BIC. The plates were incubated at 37°C and read after 1 h at 405 nm (with reference 490 nm). Background values were obtained from wells containing no antigen, and their absorbances subtracted from all sample readings. Positive [IgG aCL = 126 IgG phospholipid units (GPLu); IgM aCL = 88 IgM phospholipid units (MPLu)] and negative control samples were included on each plate. The results were expressed as a percentage of the positive control: optical density (OD) (sample)/OD (positive control) × 100. Values > 3 standard deviations (s.d.) above the mean of 50 healthy controls were deemed to be positive.

Statistical analysis

Statistical analyses were performed using Biomedical Statistics application software (Italian version). Categorical analysis was performed by the chi-square test.

Results

Patients

Table 2 shows the prevalence (percentage of subjects with aPL levels above the mean + 3 s.d. of 50 normal controls) of IgG/aPI, aCL, aPS and aPG antibodies in the three patient groups; PAPS, SLE/APS, SLE, and normal controls.

Table 1. Details of subjects included in this study

<table>
<thead>
<tr>
<th>Subject groups</th>
<th>n</th>
<th>F:M</th>
<th>Age [mean, (s.d.), range]</th>
<th>Ethnic origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPS</td>
<td>30</td>
<td>23:7</td>
<td>45, (14), 23–72</td>
<td>97% Caucasian</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0% African/Caribbean</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3% Asian (incl. Chinese)</td>
</tr>
<tr>
<td>SLE</td>
<td>75</td>
<td>71:4</td>
<td>48, (12), 28–78</td>
<td>71% Caucasian</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19% African/Caribbean</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10% Asian (incl. Chinese)</td>
</tr>
<tr>
<td>SLE/APS</td>
<td>50</td>
<td>49:1</td>
<td>49, (13), 25–82</td>
<td>72% Caucasian</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14% African/Caribbean</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14% Asian (incl. Chinese)</td>
</tr>
<tr>
<td>Spouses</td>
<td>18</td>
<td>3:15</td>
<td>41, (8), 29–64</td>
<td>83% Caucasian</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6% African/Caribbean</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11% Asian (incl. Chinese)</td>
</tr>
<tr>
<td>Relatives</td>
<td>25</td>
<td>14:11</td>
<td>37, (16), 14–65</td>
<td>72% Caucasian</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4% African/Caribbean</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24% Asian (incl. Chinese)</td>
</tr>
<tr>
<td>Normal controls</td>
<td>50</td>
<td>Age and sex matched</td>
<td></td>
<td>80% Caucasian</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No other details available</td>
</tr>
</tbody>
</table>
There was a significant difference between patients with PAPS and (i) SLE/APS ($P < 0.0001$), (ii) SLE ($P < 0.0001$) and (iii) normal controls ($P < 0.0001$) with regard to IgG aPL (Fig. 1). When considering IgM aPL, there was also a significant difference between PAPS and (i) SLE/APS ($P < 0.0001$, except aPI); (ii) SLE ($P < 0.0001$) and (iii) normal controls ($P < 0.0001$) (Fig. 2).

Figure 3 shows the prevalence of IgA aPL in the three patient groups and normal controls. None of the patients with PAPS had IgA aPL. There was a significant difference between patients with PAPS and SLE/APS ($P < 0.0001$ for aPI, $P = 0.001$ for aPS and $P < 0.005$ for aPG), and no significant difference between the two groups with respect to IgA aCL. There was no significant difference between patients with PAPS and SLE regarding the prevalence of IgA aCL, aPS and aPG. However, a difference occurred in IgA aPI ($P < 0.0001$). Forty-two per cent of patients with SLE/APS and 19% of patients with SLE had IgA aPI. A significant difference between these two groups and patients with PAPS was evident, with respect to IgA aPI ($P < 0.0001$). There was no significant difference in the prevalence of IgA aPL between patients with PAPS and normal controls. When the prevalence of IgG was compared with IgM aPL in the three patient groups, IgG aPL and aCL were more prevalent than their IgM equivalents in the patients with PAPS ($P < 0.0001$). IgG aCL was more prevalent than IgM aCL in patients with SLE.

**Fig. 1.** IgG aPL in patients with PAPS, SLE/APS and SLE. Percentage of patients with aPL above the mean $+ 3$ s.d. of 50 normal controls.

**Fig. 2.** IgM aPL in patients with PAPS, SLE/APS and SLE. Percentage of patients with aPL above the mean $+ 3$ s.d. of 50 normal controls.

**Fig. 3.** IgA aPL in patients with PAPS, SLE/APS and SLE. Percentage of patients with aPL above the mean $+ 3$ s.d. of 50 normal controls.

**Fig. 4.** Prevalence of IgG aPL in patients with PAPS, SLE/APS and SLE. Percentage of patients with aPL above the mean $+ 3$ s.d. of 50 normal controls.

**Relatives and spouses**

Table 2 shows the prevalence (percentage of subjects with aPL levels above the mean $+ 3$ s.d. of 50 normal controls) of IgG/M/A aPL, aCL, aPS and aPG antibodies in the first-degree relatives and spouses of patients with SLE/APS.

Figure 4 illustrates the prevalence of IgG aPL in patients with SLE/APS, their first-degree relatives and normal controls. There was no significant difference between the relatives and patients with SLE/APS regard-

### Table 2. Percentage of subjects with IgG/M/A aPL, aCL, aPS and aPG levels above the upper limit of 50 normal controls (mean $+ 3$ s.d.)

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th>IgM</th>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPS ($n = 30$)</td>
<td>50</td>
<td>27</td>
<td>0</td>
<td>60</td>
<td>33</td>
<td>0</td>
<td>60</td>
<td>77</td>
<td>0</td>
</tr>
<tr>
<td>SLE/APS ($n = 50$)</td>
<td>24</td>
<td>26</td>
<td>42</td>
<td>20</td>
<td>12</td>
<td>4</td>
<td>28</td>
<td>32</td>
<td>12</td>
</tr>
<tr>
<td>SLE ($n = 75$)</td>
<td>8</td>
<td>3</td>
<td>19</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Relatives ($n = 25$)</td>
<td>24</td>
<td>4</td>
<td>8</td>
<td>20</td>
<td>8</td>
<td>4</td>
<td>16</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Spouses ($n = 18$)</td>
<td>11</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Normal controls ($n = 50$)</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>
studied, antibodies against other negatively charged phospholipids have also been detected in human sera. This may be because these phospholipids share some similarities in structure, charge and configuration with CL [14].

We have shown a high prevalence of IgG aPL in patients with PAPS. This is significantly higher than in patients with SLE/APS and SLE (Table 2, Fig. 1). Although some differences were seen, there was no significant difference between the prevalence of IgG and IgM aPL in the patient groups (Table 2). Interestingly, none of the patients with PAPS had IgA aPL.

We have also shown the presence of IgG aPL in the first-degree relatives of patients with SLE/APS. This was significantly different from the prevalence of IgG aPL in the healthy normal controls and generally not significantly different from patients with SLE/APS (Fig. 4). Although these individuals were considered ‘normal’, they have displayed some type of susceptibility to autoantibody production. It is of note that the genetic contribution to autoantibody production may vary between patients with PAPS, SLE/APS and SLE, and only the relatives and spouses of patients with SLE/APS were studied. As reviewed elsewhere, relatives of patients with autoimmune syndromes often have evidence of autoantibody production without evidence of clinical disease [15]. Mackworth-Young et al. found eight out of 101 (7.9%) relatives had aCL [16]. More recently, Goldberg et al. reported IgG aCL in 23% of relatives of aCL-positive patients [17].

The spouses of patients with SLE/APS showed a prevalence of IgG aPL not significantly different from normal controls. The prevalence of IgG aPL in the patients with SLE/APS was significantly different from their spouses (Fig. 5). These data indicate that although sharing environmental conditions with the patients with SLE/APS, the spouses were not predisposed to autoantibody production.

The prevalence of IgG aPL in the first-degree relatives of patients with SLE/APS was higher than in the spouses of patients with SLE/APS, suggesting that genetic predisposition to antibody production is likely to be more important than a shared environmental influence.

It was notable that IgA aPI were present in 42% of patients with SLE/APS, 19% of patients with SLE but 0% of patients with PAPS. In patients with SLE/APS and SLE these figures were the highest incidence of any of the IgG/M/A aPL tested. This observation may be interesting if there is some, as yet undiscovered, link between the presence of IgA aPI and SLE. The question may be raised as to whether SLE alone, or with APS as a secondary syndrome, has some influence on IgA aPI which is non-existent when APS is a primary syndrome. Further work would involve the determination of the clinical significance of IgA aPI in patients with SLE/APS and SLE, compared with patients with PAPS. The results underline the heterogeneity of the aPL detectable in human sera.

There is extensive literature exploring the genetic basis of SLE [15–19]. Amongst the salient features are the
fact that there is a 25% concordance rate for SLE amongst monozygotic twins but only 2–3% amongst dizygotic twins [19]. There have been attempts to link the inheritance of SLE to both major histocompatibility complex (MHC) and non-MHC genes. Genes likely to be involved in the development of SLE include those which regulate immune complex disposal, B cell signaling, regulation of apoptosis, antigen processing, T cell receptor and immunoglobulin structure. These topics have been reviewed in detail recently [20].

Many groups have reported on the prevalence of aPL and their association with clinical manifestations [6, 8, 21, 22]. The major comparison which can be drawn between these and our study is the high incidence of IgG aPL in the groups studied. Bertolaccini et al. reported a prevalence of IgG/M/A aPS and aPI in their patients with SLE/APS (LA/aCL positive) that was very similar to our own findings [21].

Our data show clearly that first-degree relatives of patients with SLE/APS have significantly higher levels of aPL than normal controls, indicating that genetic factors shared by patients and their relatives seem to have some effects on the prevalence of aPL in the subjects studied. However, the presence of autoimmune diseases does not result in overt manifestation of disease, suggesting that environmental triggers are required for disease development. The spouses of patients with SLE/APS do not have significantly higher levels of aPL than normal controls. The environmental factors alone, shared by patients and their spouses, appear to have no influence on the prevalence of aPL.

References