Complement activation by anticardiolipin antibodies

Mittermayer B Santiago, Nelson Gaburo Jr, Ricardo M de Oliveira, Wilson Cossermelli

Abstract
A haemolytic assay was used to test the complement fixation ability of 16 serum samples with high concentrations of anticardiolipin antibodies. Fourteen patients had clinical complications usually associated with these antibodies—namely, recurrent abortions, thrombosis, or thrombocytopenia.

Complement fixation by anticardiolipin antibodies was shown in only four of these patients and was not directly related to the antibody concentration. Because anticardiolipin antibodies in most of these patients did not activate the complement pathway it is unlikely that the complement cascade has an important role in the clinical complications associated with these antibodies.

In the past few years growing attention has been focused on the association between the presence of anticardiolipin antibodies and habitual abortions, thrombosis, and thrombocytopenia, particularly in patients with systemic lupus erythematous. On the other hand, patients with infectious diseases may have anticardiolipin antibodies without these complications.

Little information is available about the pathogenetic role of these antibodies. A decrease in prostacyclin synthesis, binding to platelet membrane with its activation and inhibition of the fibrinolytic process are some of the proposed mechanisms for the clinical complications associated with anticardiolipin antibodies.

Because of the reported statistical association between anticardiolipin antibodies and low complement concentrations we undertook this study to evaluate the complement fixation ability of these antibodies to try to explain their pathogenetic mechanism.

Patients and methods

Patients
We studied 14 patients with clinical complications considered to be associated with anticardiolipin antibodies, such as idiopathic habitual abortions, venous and arterial thrombosis, thrombocytopenia, livedo reticularis, and chorea. One patient with syphilis and one patient with leprosy without such complications were also included in the study. All 16 patients had high concentrations of IgG or IgM anticardiolipin antibodies, or both.

Assay of anticardiolipin antibodies
Both IgG and IgM anticardiolipin antibodies were detected by an enzyme linked immuno-sorbent assay (ELISA).

Complement fixation test for anticardiolipin antibodies
A standardised procedure for complement fixation, developed by the Centers for Disease Control, Atlanta, was carried out with cardiolipin (Sigma) as antigen diluted in saline (100 µg/ml). The appropriate concentration of cardiolipin was determined by block titration comparing negative and positive serum for anticardiolipin antibodies. The test was carried out also in the absence of the patient's serum specimen, and in the absence of test antigen to avoid the anticomplementary activity of the serum or antigen.

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Clinical and serological features in 15 patients with anticardiolipin antibodies (aCL) used in the complement fixation assay (CFA)

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Age (years)</th>
<th>Features*</th>
<th>Treatment</th>
<th>IgG aCL (units)</th>
<th>IgM aCL (units)</th>
<th>CFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31</td>
<td>CVA</td>
<td></td>
<td>101</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>AB</td>
<td></td>
<td>70</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>Haemolytic-uraemic syndrome, AB, TC</td>
<td></td>
<td>150</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>CVA, AB, LA</td>
<td>Aspirin 100 mg/d</td>
<td>151</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>SLE, AB, TC, VT, LA</td>
<td>Prednisone 30 mg/d</td>
<td>178</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>26</td>
<td>SLE, AB, TC, VT, LA</td>
<td>Prednisone 5 mg/d</td>
<td>131</td>
<td>161</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>VT, LA</td>
<td></td>
<td>160</td>
<td>17</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>Chorea, seizures, AB, positive ANA</td>
<td></td>
<td>118</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>32</td>
<td>SLE, CI</td>
<td></td>
<td>Phenobarbital 100 mg/d</td>
<td>96</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>44</td>
<td>CL, LR, Raynaud, LA, positive ANA</td>
<td>Hydrochlorothiazide 50 mg/d</td>
<td>148</td>
<td>8</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>26</td>
<td>CVA, arthritis, positive ANA</td>
<td></td>
<td>98</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>27</td>
<td>AB, VT, positive ANA</td>
<td>Prednisone 20 mg/d</td>
<td>153</td>
<td>24</td>
<td></td>
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<tr>
<td>13</td>
<td>34</td>
<td>Discoid lupus, pulmonary hypertension, VT</td>
<td>Aspirin 100 mg/d</td>
<td>58</td>
<td>0</td>
<td></td>
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<tr>
<td>14</td>
<td>20</td>
<td>Secondary syphilis</td>
<td>Cyclophosphamide 1 g/mo</td>
<td>142</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>49</td>
<td>Leprosy</td>
<td>Dapsone 50 mg/d</td>
<td>98</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

*AB = abortion; ANA = antinuclear antibody; CI = cerebral infarction; CVA = cerebrovascular accident; LA = lupus anticoagulant; LR = livedo reticularis; SLE = systemic lupus erythematosus; TC = thrombocytopathy; VT = venous thrombosis.

ment pathway directly in an antibody independent way as suggested by Kovacsovic et al.8 In our assay, however, we used cardiolipin (100 μg/ml) without serum as control and the test was negative, suggesting that the complement activation was a phenomenon dependent on the complex cardiolipin and its antibody.

Interestingly, anticardiolipin antibodies from patients with leprosy or syphilis did not activate the complement pathway. It is well known that syphilis serum has antibodies against a mixture of phosphatidylcholine, cardiolipin, and cholesterol. Moreover, these antibodies have complement fixing ability as shown by the Wassermann test. This difference may be explained by the specificity of these two groups of antibodies—that is, antibodies to pure cardiolipin do not necessarily bind to the mixture of cardiolipin, phosphatidylcholine, and cholesterol. Similarly, patients with positive serological tests for syphilis have a low prevalence of anticardiolipin antibodies.9

The complement fixation ability did not seem to depend upon the anticardiolipin antibody concentrations as samples with a high anticardiolipin antibody titre did not activate complement.

Because most patients in this study with thrombosis or recurrent abortions, or both, had anticardiolipin antibodies that did not activate complement, we suggest that the complement fixation ability of these antibodies may not be an important factor in explaining their pathogenetic mechanism. We do not exclude the possibility that more sensitive procedures may show other patterns of complement fixation in different groups of patients.

We thank Mrs Yukie Umeki for her secretarial assistance.