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 erythematosus. 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 immunosorbent 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.

If the tube containing serum, complement, and cardiolipin shows no haemolysis, complement has been fixed by antigen-antibody complex, and the result is positive. If the tube shows haemolysis the complement has not been fixed and the result is negative.

Results
The table shows the clinical data, treatment, concentrations of IgG and IgM anticardiolipin antibodies, and results of the complement fixation assay for 15 of the 16 patients. One sample from a patient with systemic lupus erythematosus showed anticomplementary activity and was excluded from the study.

Anticardiolipin antibodies from only four patients activated complement, three of them with predominantly IgG isotype and one with high concentrations of both isotypes. Because no patient had cardioplin antibodies of the IgM class exclusively we were unable to determine whether complement fixation ability is limited to anticardiolipin antibodies of the IgG class.

Discussion
To our knowledge this is the first report showing directly the complement fixation ability of anticardiolipin antibodies. There are a few reports showing complement concentrations in patients with anticardiolipin antibodies. Thus Nörberg et al noted decreased concentrations of complement factor C4 in patients with anticardiolipin antibodies. Similar results were obtained by Cheng and Yap. These last authors suggested that the association between low C4 and high anticardiolipin antibody concentrations was due to the ability of cardiolipin (diphosphatidylglycerol) to activate the comple-
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>12</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, LR, 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>Phenobarbital 100 mg/d</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 10 mg/d</td>
<td>96</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>44</td>
<td>CI, 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>Prednisone 20 mg/d</td>
<td>98</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>27</td>
<td>AB, VT, positive ANA</td>
<td>Aspirin 100 mg/d</td>
<td>153</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>34</td>
<td>Discoid lupus, pulmonary hypertension, VT</td>
<td>Cyclophosphamide 1 g/mo</td>
<td>58</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>20</td>
<td>Secondary syphilis</td>
<td>Dapsone 50 mg/d</td>
<td>142</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>49</td>
<td>Leprosy</td>
<td></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=thrombocytopenia; VT=venous thrombosis.

We thank Mrs Yukie Umeki for her secretarial assistance.

Complement activation by anticardiolipin antibodies.

M B Santiago, N Gaburo, Jr, R M de Oliveira and W Cossermelli

doi: 10.1136/ard.50.4.249

Updated information and services can be found at:
http://ard.bmj.com/content/50/4/249

These include:
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/