Recurrent Cerebral Infarctions in Two Brothers with Antiphospholipid Antibodies That Block Coagulation Reactions

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SUMMARY Inhibitors blocking coagulation reactions, often called lupus anticoagulants, are readily identifiable but rarely considered as risk factors for cerebral infarction. These inhibitors are inconsistently found in a number of diseases (often autoimmune) and after treatment with certain drugs and appear to be closely associated with, or identical to, antibodies to certain phospholipids. We have observed two brothers with these inhibitors who both experienced recurrent cerebral infarctions. Such familial occurrence has rarely been reported. In addition, some other family members were found to have depressed factor XII levels. Using the technique of double immunodiffusion, we found that the serum from these brothers formed precipitin lines against certain phospholipid substrates, lending further support to the antiphospholipid nature of this inhibitor.

INHIBITORS which block coagulation reactions, often called lupus anticoagulants (LA), are a heterogeneous group of immunoglobulins that may act by interfering with the activation of prothrombin by the prothrombinase complex (factor Xa, factor V, calcium, phospholipid). Some of these inhibitors bind to the phosphate esters of a variety of biological molecules and have been termed antiphospholipid antibodies.

In this report we use the term "blocking inhibitors" (BI) rather than LA because they are more often than not associated with disorders other than systemic lupus erythematosus (SLE) and because of their ability to block coagulation reactions. Initially described in two patients with SLE by Conley and Hartman in 1952,6 these inhibitors have since been associated with a variety of immunological disorders, malignancies, drugs (including phenothiazines, penicillin derivatives, phenytoin, hydralazine, procainamide, isoniazid),6,9 and pregnancy.10,11 Their occurrence in SLE has been estimated to be 5-10%. Of all blocking inhibitors (about 300 cases) reviewed in this laboratory, less than 20% were found in patients with documented SLE (unpublished data).

BI are usually recognized by their effects upon the activated partial thromboplastin time (APTT) — prolongation that does not fully correct when mixed 1:1 with normal plasma — and by their frequent association with biological false-positive tests for syphilis11,12 and occasional association with thrombocytopenia.2,13 About 15% of sera containing these inhibitors forms a precipitin line against phosphatidylserine using double immunodiffusion techniques (J.H.L., personal communication).14

Despite abnormal coagulation tests, patients with the inhibitor usually do not have abnormal bleeding tendencies. Those few patients with this inhibition who do have abnormal bleeding often have an associated hypohomocystinemia and/or thrombocytopenia.1,2,16,17 Paradoxically, blocking inhibitors have been associated with thrombotic events.6,9,10,15,18,22 Most of the published reports have described venous thrombosis; however, several have included patients with cerebral infarcts6-15,16,20,22 and transient neurological deficits.10,16,23

In this report we describe the presence of BIs in the blood of two brothers who suffered recurrent cerebral ischemic events. Familial occurrence of this inhibitor has rarely been reported.6 Using double immunodiffusion, the sera from these two cases were found to contain autoantibodies to certain phospholipid substrates. In addition, one of the brothers and three other family members were found to have abnormally low factor XII levels.

Methods

Coagulation methods have been detailed elsewhere.2 The reagent used for the APTT was General Diagnostics Automated APTT. The dilute tissue thromboplastin assay, called the LA test, was performed according to the method described by Schleider, et al.2

Double immunodiffusions were carried out in 100 x 15 mm disposable petri dishes (Scientific Products). Fifteen ml of warm Agarose Indubiose A 45 (Accurate Chemical & Scientific Corp.), 1% in pH 8.6 barbitol buffer, was poured into each plate and allowed to cool and gel. Four equally spaced groups of six wells (3 mm) surrounding a center well (3 mm) and separated from each other and the center well by 4 mm were cut in the gel with a punching template. Each of the outside wells was numbered (1-24). Even numbered wells were filled with 20 μl of 5% human serum albumin in saline. This was done to prevent "bending" which is sometimes seen with strong antibodies. Odd wells were filled with patient or control plasma samples which had been heated to 56°C for 5 min. and then centrifuged. The center wells were filled with one of the agents listed in table 1. The first three were obtained from Sigma Chemical Company and were pre-
pared by dissolving or suspending 5 mg in 1 ml barbital buffer (pH 8.6). Platelet extract and brain suspension were used because they contain, among other things, complex phospholipid mixtures. Platelet extract was prepared by washing one unit of platelets in 250 ml citrate-saline 3 times. After the final centrifugation the supernatant was used. Both platelet extract and brain suspension have been stored frozen for four years and continue to react with our circulating inhibitor with antiphospholipid activity was identified (table 1). The LA test was positive. Other abnormal findings in the coagulation tests are summarized in table 2. Dipyridamole was discontinued four days before the coagulation tests were performed. He had received two units of fresh frozen plasma and 20 mg of parenteral vitamin K three days before testing.

Cerebrospinal fluid (CSF) assessment revealed 1 RBC/cu mm, 6 lymphocytes/cu mm, total protein 40.5 mg/dl, gamma fraction 4.9 mg/dl (normal = less than 10% total protein), calculated IgG index 0.29, glucose

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Demonstration of Antiphospholipid Antibodies by Double Immunodiffusion</th>
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<tbody>
<tr>
<td>Reagent</td>
<td>Positive control</td>
</tr>
<tr>
<td>L-o-Phosphatidyl-L-Serine (5 mg/ml)</td>
<td>+</td>
</tr>
<tr>
<td>Phosphatidyl ethanolamine (5 mg/ml)</td>
<td>+</td>
</tr>
<tr>
<td>L-o-Phosphatidyl ethanolamine (5 mg/ml)</td>
<td>+</td>
</tr>
<tr>
<td>Platelet extract</td>
<td>+</td>
</tr>
<tr>
<td>Human brain</td>
<td>+</td>
</tr>
</tbody>
</table>

Case Reports

Case 1

A 39-year-old right-handed man was hospitalized in January 1984 following the acute onset of right-sided weakness and slurred speech. He had been well until 1976 when he suffered an anterior wall myocardial infarction. His rapid plasma reagin (RPR), a screening test for syphilis, was reactive at 1:2; coagulation tests were not performed. In 1977 cardiac catheterization revealed complete occlusion of the left anterior descending artery. All other arteries were widely patent. The procedure was complicated by brachial artery thrombosis for which he was treated for about a year with warfarin. In July 1983 he had a stroke which was thrombosis for which he was treated for about a year with warfarin. In July 1983 he had a stroke which was thrombosis for which he was treated for about a year with warfarin. In July 1983 he had a stroke which was thrombosis for which he was treated for about a year with warfarin. In July 1983 he had a stroke which was thrombosis for which he was treated for about a year with warfarin. His general examination revealed a blood pressure of 110/70 mm Hg, telangiectasias of the oral mucosa, and absent right radial, right brachial and right and left dorsalis pedis pulses. His speech was non-fluent with pauses and incorrect attempts for word insertions. He was able to repeat short phrases but had mild difficulty with object naming. Comprehension of verbal but not written commands was intact. He was able to spell short words but was unable to write them. Finger agnosia, acalculia and right-sided agraphesthesia were present. Other findings included moderate right lower facial weakness, right homonymous hemianopsia, increased tone with weakness of the right arm (and to a lesser degree of the leg), hyperactive muscle stretch reflexes (especially on the right), bilateral extensor plantar responses and mildly impaired pin-prick sensation on the left side of his body.

Pertinent laboratory results included: WBC, 3,900/cu mm; hematocrit, 43.9%; serum creatinine, 1.5 mg/dl; erythrocyte sedimentation rate (ESR), 49 mm/hr.; antinuclear antibody (ANA), positive at 1:40 with speckled pattern; anti-DNA, negative; gamma globulin level, 1.96 gm/dl (normal = 0.7-1.6 gm/dl); immune complexes, positive; RPR, positive at 1:4; FTA-ABS, negative; direct and indirect antiglobulin test, negative; lipoprotein electrophoresis, normal. A circulating inhibitor with antiphospholipid activity was identified (table 1). The LA test was positive. Other abnormal findings in the coagulation tests are summarized in table 2. Dipyridamole was discontinued four days before the coagulation tests were performed. He had received two units of fresh frozen plasma and 20 mg of parenteral vitamin K three days before testing.

Cerebrospinal fluid (CSF) assessment revealed 1 RBC/cu mm, 6 lymphocytes/cu mm, total protein 40.5 mg/dl, gamma fraction 4.9 mg/dl (normal = less than 10% total protein), calculated IgG index 0.29, glucose

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Coagulation Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulation tests</td>
<td>Normals</td>
</tr>
<tr>
<td>Bleeding time</td>
<td>3.0-9.5 min</td>
</tr>
<tr>
<td>Prothrombin time</td>
<td>10.0-13.0 sec</td>
</tr>
<tr>
<td>Partial thromboplastin time</td>
<td>24.0-34.0 sec</td>
</tr>
<tr>
<td>1:1 mix</td>
<td>&lt;39.5 sec</td>
</tr>
<tr>
<td>Factor XII</td>
<td>0.5-1.5 U/ml</td>
</tr>
<tr>
<td>Platelet count</td>
<td>150-450 × 10^3/μl</td>
</tr>
<tr>
<td>Platelet aggregations*</td>
<td></td>
</tr>
<tr>
<td>ADP (2 × 10^{-4} M)</td>
<td>&gt;60%</td>
</tr>
<tr>
<td>collagen (1.9 mg/ml)</td>
<td>&gt;60%</td>
</tr>
<tr>
<td>ristocetin (15 mg/ml)</td>
<td>&gt;60%</td>
</tr>
<tr>
<td>thrombin (10 U/ml)</td>
<td>&gt;60%</td>
</tr>
<tr>
<td>arachidonic acid (5 mg/ml)</td>
<td>&gt;60%</td>
</tr>
</tbody>
</table>

*The concentrations of working solutions which were diluted 1:10 in the tests are listed in parentheses.
61 mg/dl (simultaneous serum glucose 96 mg/dl), negative VDRL, and negative immune complexes.

The ECG showed evidence of an old anteroseptal myocardial infarction. Cardiac rhythm monitoring for 24 hours revealed rare premature atrial contractions. The echocardiogram demonstrated localized hypokineticis of the septum and apex and dense apical echoes suggestive of fibrosis or thrombus; a left ventricular aneurysm was not detected. Oculoplethysmography was normal, bilaterally. Computerized tomography (CT) of the brain showed areas of decreased attenuation in the left temporal-occipital and right posterior parietal regions without abnormal enhancement and a large area of decreased attenuation high in the left parietal region with gyroform enhancement compatible with three separate areas of infarction at different stages of evolution.

**Case 2**

A 41-year-old, right-handed brother of the first patient was admitted in January 1984 for re-evaluation of aphasia and right-sided weakness. He had been well until January 1980 when he experienced a three hour episode of dysarthria and right arm weakness. Four vessel cerebral angiography at that time was normal. He was treated with aspirin and dipyridamole and did well until January 1982 when he was admitted for evaluation of intermittent claudication. Angiography revealed stenosis of the distal aorta and he underwent an uncomplicated aortic endarterectomy. The APTT in the hospital laboratory at that time was 37.6 secs with a control of 26.6 secs. At discharge all medications were stopped. He was re-admitted two months later because of expressive aphasia and right-sided weakness. His APTT was still prolonged at 32.0 secs with a control of 27.0 secs. A left cervical bruise was heard and four vessel cerebral angiography revealed a minimal smooth plaque of the left common carotid artery bifurcation and occlusion of major left posterior Sylvian branches. CT of the brain revealed a lucency in the left posterior Sylvian region. His RPR was positive at 1:4 and his FTA-ABS was negative. He had been treated with warfarin until four weeks prior to the current admission. His past medical history was otherwise significant for an uncomplicated tonsillectomy, bilateral distribution of the distal left middle cerebral artery and the posterior right frontal region, the latter with gyral enhancement, compatible with two separate areas of infarction at different stages of evolution.

**Results**

The plasma from both cases demonstrated a prolonged APTT without full correction when mixed 1:1 with normal plasma. This suggested the presence of a circulating inhibitor. By double immunodiffusion, the serum from Case 1 formed precipitin lines with phosphatidylserine and phosphatidylinositol while that from Case 2 precipitated only with phosphatidylserine (table 1). Factor assays of both cases were normal except for the abnormal factor XII level demonstrated in Case 2.

The abnormal bleeding time and platelet aggregation studies found in Case 2 were consistent with the effects of aspirin. Abnormal platelet aggregation with collagen and arachidonic acid were also found in Case 1.

The family pedigree is shown in figure 1. Of those family members tested, seven (including Case 2) were found to have factor XII levels below 0.62 U/ml, four of which were below 0.50 U/ml. Table 3 summarizes the coagulation data from those members. Only one relative (III 6) had a prolonged APTT, but this corrected fully when mixed 1:1 with normal plasma. No family member’s serum formed precipitin lines with phospholipid antigens. The LA test was negative in all relatives tested.

**Discussion**

The two cases presented illustrate features frequently associated with blocking inhibitors (of the lupus anticoagulant type): an abnormal APTT with incomplete correction when mixed with normal plasma, abnormal LA test (dilute thromboplastin), thrombocytopenia, biological false-positive tests for syphilis, and lack of a bleeding tendency. It should be noted that commercial reagents used in the APTT vary in their gamma globulin level, 1.57 gm/dl; immune complexes, positive; RPR, positive at 1:1; FTA-ABS, negative; direct and indirect antiglobulin test, negative; lipoprotein electrophoresis, Type IV hyperlipidemia. A circulating inhibitor with antiphospholipid activity was identified (table 1). The LA test was positive. Other abnormal findings in the coagulation tests are summarized in table 2. Aspirin 10 grains daily was started three days before the coagulation tests were performed.

CSF assessment revealed 2 RBC/cu mm, 2 lymphocytes/cu mm, total protein 26.5 mg/dl, gamma fraction 1.6 mg/dl, glucose 55 mg/dl (simultaneous serum glucose 130 mg/dl), negative VDRL, and negative immune complexes.

The ECG and echocardiogram were normal. Four vessel cerebral angiography revealed minimal atherosclerotic changes of the distal common carotid and proximal internal carotid arteries, bilaterally. CT of the brain demonstrated areas of low attenuation in the distribution of the distal left middle cerebral artery and in the posterior right frontal region, the latter with gyral enhancement, compatible with two separate areas of infarction at different stages of evolution.
ability to detect these inhibitors. Therefore, weak inhibitors may be missed if only the APTT is used for screening their detection.

The echocardiographic findings in Case 1 raise the possibility that cardiac embolization might have been the etiology of his cerebral infarcts. However, the seven-year interval between his myocardial infarction and first stroke suggest that this association would be highly unlikely, especially since a left ventricular aneurysm was not detected. The screening test for syphilis was reactive at the time of his cardiac catheterization. Although a more specific test was not performed, subsequent evaluation revealed that this was a biological false-positive result, suggesting that a BI may have been present when he experienced the myocardial infarction. Case 2 had laboratory evidence (prolonged APTT) suggestive of the existence of a BI two years prior to the current investigation. Since other laboratory abnormalities often associated with thrombotic diseases (antithrombin III, antiplasmin, plasminogen) were normal, we concluded that a BI was the predisposing factor for thrombotic disease in these two brothers.

Screening of all available members of the same family failed to detect others with BI or antiphospholipid antibodies. However, Case 2 and six family members were found to have abnormally low or low-normal factor XII levels. The relationship, if any, between our two cases with BI and their family members with abnormal factor XII levels is not clear.

These blocking inhibitors appear to be autoantibodies directed towards the phosphate esters of a variety of biological molecules including the prothrombinase complex. The strong association between these inhibitors and biological false-positive tests for syphilis, the assay for which uses a cardiolipin (phospholipid) antigen, supports the role of phospholipid as the active site. Moreover, the fact that platelets supply the phospholipid framework for the intrinsic coagulation pathway, and the frequent occurrence of thrombocytopenia with BI, further strengthens this hypothesis. Consistent with these observations, the serum (56°C treated plasma) from both of our two cases with BI formed precipitin lines against certain phospholipid antigens.

The pathophysiologic mechanism by which BI predispose to arterial thrombosis has not been clearly defined but may involve inhibition of arterial prostacyclin (PGI\textsubscript{2}). The interaction of BI with various phospholipid substrates suggests that inhibition of arterial PGI\textsubscript{2} might result from interference with membrane phospholipid conversion to arachidonic acid, the precursor of PGI\textsubscript{2}. Using a more refined technique for the detection of antiphospholipid antibodies, Harris, et al\textsuperscript{18} demonstrated a significant correlation between patients with antiphospholipid antibodies, thrombocytopenia, biological false-positive tests for syphilis and thrombotic events. Furthermore, of nine patients with SLE and cerebral infarctions, eight had high levels of antiphospholipid antibodies suggesting that this might be a useful marker for those patients with BI at risk for cerebral infarction.

In management of patients with antiphospholipid coagulation inhibitors and thrombotic complications, the first step is to search for and treat underlying disease (particularly collagen-vascular) or to discontinue ongoing drug therapy with one of the implicated drugs. Prophylactic treatment of thrombotic manifestations is probably best accomplished with antiplatelet therapy. Aspirin is of possible use but the optimal antithrombotic dose has not been determined due to the known differential effects of aspirin on platelet thromboxane A\textsubscript{2} and vascular wall PGI\textsubscript{2} production. Other antiplatelet drugs might be considered. Steroids, cytotoxic agents and plasmapheresis — used either to treat the primary disease or the inhibitor’s effects — have been reported to correct

### Table 3

<table>
<thead>
<tr>
<th>Family member*</th>
<th>Factor XII (U/ml)&lt;sup&gt;†&lt;/sup&gt;</th>
<th>Partial thromboplastin time (sec)&lt;sup&gt;‡&lt;/sup&gt;</th>
<th>1:1 mix (sec)&lt;sup&gt;§&lt;/sup&gt;</th>
<th>Antiphospholipid activity&lt;sup&gt;¶&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>II 6</td>
<td>0.23</td>
<td>41.1</td>
<td>32.9</td>
<td>0</td>
</tr>
<tr>
<td>II 7</td>
<td>0.34</td>
<td>32.9</td>
<td>32.5</td>
<td>0</td>
</tr>
<tr>
<td>II 2 (propositus)</td>
<td>0.46</td>
<td>57.6</td>
<td>43.4</td>
<td>+</td>
</tr>
<tr>
<td>III 1</td>
<td>0.48</td>
<td>31.7</td>
<td>30.5</td>
<td>0</td>
</tr>
<tr>
<td>II 6</td>
<td>0.54</td>
<td>32.6</td>
<td>32.2</td>
<td>0</td>
</tr>
<tr>
<td>II 4</td>
<td>0.57</td>
<td>30.1</td>
<td>29.3</td>
<td>0</td>
</tr>
<tr>
<td>II 1</td>
<td>0.62</td>
<td>32.4</td>
<td>30.5</td>
<td>0</td>
</tr>
</tbody>
</table>

*Identified by nomenclature used in fig. 1.
†See table 2 for the normal values.
‡As determined by the ability of serum to form precipitin lines with phospholipid reagents.
the coagulation test abnormalities in isolated cases or brief series, however, their use must be decided on an individual case basis.

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Special thanks is given to Benjamin Eidelman, M.D. who brought the patients to our attention; Ruth Romm, R.N., Michael Snyder, and Carol Church for technical assistance; Beverly Caputo for preparation of the manuscript; and Christopher Terrence, M.D., who reviewed the manuscript.

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