Immunological Specificity and Mechanism of Action of IgG Lupus Anticoagulants

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Although observations have implied that lupus anticoagulants have immunologic specificity toward anionic phospholipids, this assumption has been directly demonstrated in only one patient with a monoclonal IgM paraprotein. We tested the generality of this hypothesis directly by isolating five IgG lupus anticoagulants from patients with lupuslike syndromes and/or thrombosis. IgG lupus anticoagulant fractions were isolated free of other plasma proteins and free of contaminating phospholipid by adsorption to and elution from cardiolipin-cholesterol-dicetyl phosphate liposomes, followed by chromatography on protein A-Sepharose. Cardiolipin liposomes, but not phosphatidylcholine liposomes, were capable of removing all, or nearly all, lupus anticoagulant activity from patient plasma. The affinity-purified IgG preparations reacted with cardiolipin, phosphatidylserine, phosphatidylinositol, and phosphatic acid, but not with phosphatidylcholine or phosphatidylethanolamine, and inhibited calcium-dependent binding of prothrombin and of factor X to phosphatidylserine-coated and to cardiolipin-coated surfaces. F(ab')2 fragments retained lupus anticoagulant activity and bound to cardiolipin in an enzyme-linked immunosorbent assay (ELISA). Anticardiolipin and lupus anticoagulant activity were both present in acidic fractions on isoelectric focusing. These data strongly suggest that most, if not all, lupus anticoagulants are antibodies that have immunologic specificity toward anionic phospholipids, thereby blocking the calcium-mediated binding of vitamin K-dependent coagulation factors to coagulation-active phospholipid surfaces.

Lupus Anticoagulants are acquired inhibitors of coagulation tests that occur in systemic lupus erythematosus (SLE), in various other clinical settings, and in apparently normal persons.1

Because the lupus anticoagulant effect is noted in all phospholipid-dependent coagulation tests2-6 and because of the correlation between the presence of lupus anticoagulants and reactivity against cardiolipin in solid-phase immunoassays,7-11 lupus anticoagulants have been widely assumed to be antibodies reactive against anionic phospholipids. Actually, the immunologic nature and mechanism of action of the lupus anticoagulant effect have only been demonstrated in one patient.12 In 1980, our laboratory12 clarified the mechanism of action of an IgM λ paraprotein with lupus anticoagulant activity. The purified paraprotein showed immunologic reactivity against the anionic phospholipids phosphatidylserine, phosphatidylinositol, and phosphatic acid, but did not react with the neutral phospholipids phosphatidylcholine or phosphatidylethanolamine. This protein inhibited the binding of prothrombin and factor X to phospholipid micelles, thereby accounting for the prolongation of phospholipid-dependent coagulation tests.

Since this initial work, it has been widely assumed, although not rigorously demonstrated, that all lupus anticoagulant activity is due to the same mechanism. However, other authors have stressed the heterogeneity of the lupus anticoagulant patient population, as well as the apparent variability in the response of lupus anticoagulant tests to the presence of platelets,13 to argue against a common mechanism of action. Interestingly, neither the original patient, nor four other IgM lupus anticoagulants we subsequently studied, nor IgM lupus anticoagulants reported by others,14,15 have been associated with thrombosis. Most lupus anticoagulants, on the other hand, occur in a setting of polyclonal antibodies reactive against cardiolipin in solid-phase immunoassays.13-21 To assess whether there is indeed a general mechanism for the lupus anticoagulant effect, a technique for isolating lupus anticoagulants from plasma of patients without monoclonal paraproteins is necessary. The preparation must be a pure immunoglobulin free of contaminating phospholipid. We developed a technique that uses affinity purification on cardiolipin liposomes followed by protein A-Sepharose chromatography to prepare phospholipid-free purified lupus anticoagulants. We isolated five IgG lupus anticoagulants, four from patients with a history of thrombosis, and examined the immunologic nature of their interaction with phospholipids and the mechanism of their inhibition of phospholipid-dependent coagulation tests.

Materials and Methods

Human thrombin was a gift from Dr John Fenton, New York State Department of Health Laboratories, Albany. Pepsin was purchased from Worthington Biochemical, Freehold, NJ. Protein A-Sepharose, Sephracyl G-75 (Superfine) and Sephacyl 5-300 were obtained from Pharmacia Fine Chemicals, Piscataway, NJ. Ampholines were purchased from LKB, Bromma, Sweden. Phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine, phosphatic acid, and phosphatidylcholine were obtained from Supelco, Bellafonte, PA. Cardiolipin, dicetyl phosphate, cholesterol, alkaline phosphatase-labeled goat antibodies to human IgG, human IgM, and rabbit IgG, as well as p-nitrophenyl phosphate and bovine serum albumin (BSA) (fraction V) were purchased from Sigma Chemical, St Louis. The purity of phospholipids was checked by thin-layer chromatography.21 Russell viper venom was obtained from Burroughs Wellcome, Raleigh, NC, and Thrombomax was purchased from Ortho Diagnostic Systems, Raritan, NJ. Antiserum to human IgG, IgA, and IgM were obtained from Meloy Laboratories.

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Submitted December 5, 1986; accepted February 11, 1987.

Supported in part by Grants No. HL-09163, AG-04861 (S.S.S.), HL-27278 (P. T.), and T32-AM-07084 (M.J.H.) from the National Institutes of Health; an Established Investigator Award (84-190); a grant-in-aid from the American Heart Association (P.T.); and the Sheryl N. Hirsch Award of the Lupus Foundation of Philadelphia (S.S.S.). V.P. is a Visiting Research Fellow from the Cardiology Department, University of Padua, Italy.

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0006-4971/87/7001-0008$3.00/0
IgG Purification

Lupus anticoagulant plasma was clotted by incubation with 2 U/mL of human thrombin for 2 hours at 37°C. Fibrin was removed by centrifugation at 4°C for 10 minutes at 14,000 g. γ-Globulins were precipitated from the supernatant serum by addition at 4°C of an equal volume of saturated ammonium sulphate. After 25 minutes of stirring, the precipitate was recovered by centrifugation in the cold for 10 minutes at 14,000 g. The precipitate was dissolved in approximately one-seventh the original plasma volume, using Tris-buffered saline (TBS: 0.02 mol/L of Tris, 0.15 mol/L of NaCl, pH 7.4), and applied to a 2.5 × 90-cm column of Sephacryl S-300 equilibrated with TBS. Fractions of 2.5 mL were collected and tested qualitatively for IgG, IgM, and IgA by Ouchterlony double diffusion and for lupus anticoagulant activity by their ability to prolong the dilute Russell viper venom time (RVVT) of normal plasma.23-24 Fractions containing lupus anticoagulant activity were pooled and applied to a 1 × 5-cm column of protein A-Sepharose equilibrated with TBS. The lupus anticoagulant activity, in every case, was retained on the column and was eluted using a buffer consisting of 0.1 mol/L of glycine, 0.5 mol/L of NaCl, pH 3, and immediately neutralized with 1 mol/L of Tris buffer, pH 8.4. Eluates were dialyzed against TBS before being tested for lupus anticoagulant activity. Control IgG was prepared from normal plasma in an identical manner. IgG prepared in this manner is free of other immunoglobulins and shows no bands other than IgG on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) after staining with Coomassie blue.

F(ab')2 Preparation

After dialysis against acetate buffer (0.2 mol/L of Na acetate, 0.2 mol/L of NaCl, pH 4), purified IgG (2 to 3 mg/mL) was incubated at 37°C for 20 hours with freshly prepared pepsin at a weight ratio of 50:1.25 At the end of the 20-hour incubation, no intact IgG was detectable by SDS-PAGE. The reaction was stopped by adding solid Tris (5 to 10 mg). The digested material was dialyzed against TBS and applied to a 1 × 5-cm protein A-Sepharose column. The flowthrough, containing the F(ab')2 fragments, was concentrated by Amicon ultrafiltration using PM-10 membranes (Amicon, Lexington, MA) and tested for lupus anticoagulant and anticondolipin activities. The protein A-Sepharose flowthrough was free of whole IgG and Fc-fragments, as judged by SDS-PAGE and by double diffusion against antisera to human IgG (Fc-specific).

ELISA for Anticardiolipin Antibodies

One microgram of cardiolipin in 25 µL of ethanol was added to each well of a 96-well microtiter plate (Falcon #3911, Becton Dickinson, Oxnard, CA) and evaporated under a stream of deionized water. To the Sephadex was added: 2.5 mL of Ampholine, pH 5 to 7; 2.5 mL of Ampholine, pH 7 to 9; and 5 mL of purified patient IgG (10 mg/mL in 1% glycine). After gentle stirring, the mixture was poured into a 10 × 20-cm glass tray, and excess water was evaporated under a stream of air. Focusing was performed at 10°C for 10 hours at 5 W constant power. The gel was then cut into six fractions. A small amount of each fraction was resuspended in deionized water for pH measurement; the remainder of each fraction was resuspended in 5 mL of TBS in a small column, and the proteins were eluted by washing the column with 5 mL of TBS. Column eluates were dialyzed against TBS and tested for lupus anticoagulant and anticondolipin activities. Analytical isoelectric focusing was performed in an LKB Multiphor apparatus on 5% polyacrylamide gels containing 2.2% (wt/vol) Ampholine, pH 5.5 to 8.5. Purified IgG or liposome eluates were dialyzed against deionized water, applied to the gel, and run at 50 mA with a constant power of 25 W. The gels were fixed for 1 hour in a solution of 5% trichloroacetic acid and 5% sulfosalicylic acid and stained using the Bio-Rad silver-staining kit (Bio-Rad Laboratories, Richmond, CA).

Immunofinity Purification of Anticardiolipin Antibodies

Liposomes were prepared essentially according to the method of Kinsky.26 A mixture of cardiolipin, cholesterol and dicetyl phosphate (molar ratio 10:15:2) in chloroform was dried in a stream of nitrogen in a 25-mL glass tube. The lipids were resuspended in 1 mL of 0.15 mol/L of NaCl by vigorous agitation, using a vortex mixer. After resuspension, the concentration of phospholipids was 5.3 to 5.9 mg/mL. Ten milliliters each of saline and plasma were added to 1 mL of liposome suspension, and the mixture was incubated at 4°C for 30 minutes. After centrifugation at 27,000 g for 10 minutes at 4°C, the precipitate was washed three times with 0.15 mol/L of NaCl. The precipitate was resuspended by vigorous agitation in 2 mL of 3 mol/L of NaCl, following which the suspension was added to a separatory funnel containing 10 mL of ethyl ether.27 After settling, the clear aqueous layer was removed from the bottom of the funnel, centrifuged, and dialyzed in the cold against three changes of TBS. The dialyzed eluate was tested for lupus anticoagulant activity by the dilute RVVT, for immunoglobulin class by Ouchterlony double diffusion, and for reactivity against phospholipids by enzyme-linked immunosorbent assay (ELISA). The eluate was further purified by protein A-Sepharose column chromatography, as previously described, and retested for purity and for biological activities.
Labeling of Cardiolipin with $^{125}\text{I}$

Cardiolipin was radiiodinated directly by the chloramine-T method, as described by Antonov and colleagues. Thin-layer chromatography demonstrated that ~60% of the added radioactivity had been incorporated into cardiolipin. Most of the remainder traveled with the solvent front. Labeled cardiolipin had the same mobility as standard cardiolipin. The spot containing the radioactive cardiolipin was extracted from the silica gel plate as described previously. When re-run on thin-layer chromatography, this extracted labeled material showed a single spot with the mobility of standard cardiolipin; no radioactivity was present in the solvent front. On the basis of phosphorus content, the specific radioactivity of $^{125}\text{I}$-cardiolipin was calculated as 26 mCi/mmol.

Coagulation Studies and Assessment of Lupus Anticoagulant Activity

Venous blood was collected in one-tenth volume of 3.8% trisodium citrate, and plasma was obtained by centrifugation in the cold at 2,300 g for 15 minutes. Routine coagulation studies were performed as previously described. Lupus anticoagulant activity of patient plasma was assessed by the dilute RVVT, as previously described. In our laboratory, the dilute RVVT of normal plasma is ~25.6 ± 2.6 seconds (~2 SD). Patients were not considered to have lupus anticoagulants unless their dilute RVVT was >30 seconds and was not corrected by addition of an equal volume of normal plasma. The anticoagulant activity of IgG or of F(ab')$_2$ was measured in the dilute RVVT by adding 200 L of the dialyzed test material, instead of the usual 100 L, to this system. Addition of 200 L of TBS alone to normal plasma gives a dilute RVVT of 27 to 30 seconds.

Prothrombin and Factor X Binding to Phospholipid

Prothrombin was purified from normal plasma by a slight modification of the method of Morrison and Esnouf, and labeled with $^{125}\text{I}$ by the iodogen method to a specific activity of 231 Ci/mmoll. Factor X was purified according to the method of Miletich and co-workers, with some modifications described previously, and labeled with $^{125}\text{I}$ by the iodogen method to a specific activity of 60 Ci/mmoll. Two micrograms of phospholipid were added to each well of a microtiter plate and dried and washed as described above. Two hundred microliters of TBS containing 1 mg/mL of BSA was then added, to block nonspecific binding, and the plates were incubated for 1 hour. After plates were washed once with TBS, 150 L of TBS containing 1 mg/mL of BSA and 2 mmol/L of calcium was added, followed immediately by 50 L of immunoaffinity-purified IgG (134 Lg/mL) or control IgG (150 Lg/mL) in TBS. The plates were incubated for 1 hour at room temperature, after which 10 L of labeled prothrombin (1.4 × 10$^6$ cpm) or factor X (1.7 × 10$^6$ cpm) was added. After 10-minute incubation at room temperature, the wells were aspirated dry, and the bottoms of the wells were punched out and counted in an Isodyne y-counter (Model 1 185, GD. Searle, Chicago). Maximum binding of prothrombin was determined by addition of TBS-albumin-calcium buffer alone, in the absence of any IgG. As a control, prothrombin and factor X binding were measured in the presence of 2 mmol/L of EDTA and were 3% and 11% of maximum binding, respectively. Residual binding, measured in the presence of a 50-fold excess of cold prothrombin or factor X, was subtracted from total binding, to give "specific" binding.

Patients

Patient no. 1. The patient was a 35-year-old woman with a 10-year history of a manic-depressive illness for which she had been treated with lithium, chlorpromazine, and other tricyclic antidepressants. He had not received any medication except lithium in the 6 months prior to study. He had had several episodes of deep vein thrombosis as well as at least one probable pulmonary embolism. He had a normal platelet count, and his serological test for syphilis (RPR) was negative. At the time of the study, he was receiving warfarin.

Patient no. 2. The patient was a 39-year-old woman with SLE of 8-year duration who had end-stage renal disease and was on hemodialysis. She had had a cerebrovascular accident at the age of 35, and also had a history of possible pulmonary emboli. She had occlusion of arteriovenous shunts in the forearm on at least two occasions. Her platelet count was in the low-normal range. She had a false-positive serological test for syphilis (RPR) at the time of diagnosis, but not at the time of study, when she was receiving 20 mg of prednisone every other day.

Patient no. 3. The patient was a 32-year-old woman with a history of three spontaneous abortions, after which she delivered a full-term live baby while on steroid therapy. She had a normal platelet count. She had had a false-positive serology (RPR) since her teens, but had no other signs of SLE.

Patient no. 4. The patient was a 49-year-old man with a 10-year history of SLE. He had an acute episode of right iliofemoral arterial occlusion 1 year earlier, at which time a lupus anticoagulant was discovered. He had a normal platelet count and a negative serological test for syphilis (RPR).

Patient no. 5. The patient was a 58-year-old woman with renal disease and possible SLE. She had no history of thromboembolic phenomena. She had a normal platelet count and a false-positive serology (RPR). At the time of the study, she was receiving 20 mg of prednisone daily.

As shown in Table I, all five patients had a prolonged dilute RVVT, which did not normalize on addition of an equal volume of normal plasma. Four of the five patients had a prolonged activated partial thromboplastin time (APTT), whereas only one (patient no. 1, receiving warfarin) had a prolonged prothrombin time (PT).

RESULTS

Immunoglobulin Nature of the Lupus Anticoagulant and Anticardiolipin Activities

Lupus anticoagulant activity in all five patients gel-filtered on Sepharcl S-300 in the IgG peak. Three patients (patients no. 1, 3, and 5) had, in addition, a very small amount of inhibitory activity associated with the IgM peak. The maximum prolongation of the dilute RVVT in IgM fractions was 2 seconds, whereas the maximum prolongation in the IgG peak was 13 to 14 seconds. No prolongation of the RVVT was observed in any of the Sepharcl S-300 fractions of control plasma. The IgG peaks were further purified by chromatography on protein A-Sepharose. Glycine eluates of the protein A-Sepharose columns contained the inhibitory activity, whereas the flowthrough fractions had no lupus anticoagulant activity.

IgG anticardiolipin activity was detected in all five patients.

<table>
<thead>
<tr>
<th>Table 1. Screening Coagulation Tests</th>
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<tbody>
<tr>
<td>Patient</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>APTT (s)</td>
</tr>
<tr>
<td>PT (s)</td>
</tr>
<tr>
<td>Dilute RVVT (s)</td>
</tr>
<tr>
<td>Patient</td>
</tr>
<tr>
<td>Patient + normal (1:1)</td>
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</table>
plasmas; in addition, patients 1, 3, and 5 had weak IgM anticardiolipin activity.

F(ab')2 fragments prepared from patient IgG prolonged the dilute RVVT of normal plasma in a dose-dependent manner; normal F(ab')2 had only a minimal effect on this test (Fig 1A). Patient F(ab')2 fragments also bound in a dose-dependent manner to cardiolipin in the ELISA, whereas control F(ab')2 showed only minimal binding (Fig 1B).

Preparative isoelectric focusing of purified IgG from patient 1 (Fig 2) demonstrated a peak of lupus anticoagulant and anticardiolipin activity in the first fraction, pH 5.8; in contrast, IgG was distributed throughout all six fractions relatively equally.

**Immunofinity Purification and Properties of Lupus Anticoagulant and Anticardiolipin Antibodies**

As shown in Table 2, when IgG-containing fractions from a Sephacryl S-300 column (patient no. 5) were incubated with cardiolipin liposomes, nearly all lupus anticoagulant activity was adsorbed. When phosphatidylcholine liposomes were used instead, no lupus anticoagulant activity was adsorbed. The cardiolipin liposome eluate contained lupus anticoagulant activity, whereas the phosphatidylcholine liposome eluate contained none.

Cardiolipin liposome eluates were prepared from patient and normal plasmas (Table 3). Eluates from patient plasma, in contrast to eluates from normal plasma, possessed lupus anticoagulant activity. When tested by Ouchterlony double diffusion, patient eluates contained IgG and albumin, whereas control eluates contained only albumin. Patient and control eluates were negative for IgM, IgA, and lipoproteins by the same method. Final purification of patient liposome eluates was achieved by chromatography on protein A-Sepharose. Eluates from this column were pure IgG preparations, as judged by double diffusion and by silver staining of SDS-PAGE gels. To estimate the cardiolipin contamination of the affinity-purified IgG, we purified one patient plasma with liposomes containing [125I]cardiolipin. The starting liposome preparation contained 216,250 cpm. The centrifuged, dialyzed liposome eluate contained 219 cpm. After protein A-Sepharose chromatography, the eluted IgG contained only 42 cpm, representing <1 mol of cardiolipin/10^19 mol of IgG.

The affinity-purified antibodies prolonged the dilute RVVT of normal plasma in a dose-dependent manner, as shown in Fig 3. A final concentration of as little as 3.3 μg/mL of this purified antibody produced a measurable increase in the clotting time. Control IgG had no effect. Based on the anticoagulant activity of the affinity-purified IgG, we estimate that lupus anticoagulants in these patients account for no more than 0.2% of the total plasma IgG.

Table 2. Specificity of Liposome Purification

<table>
<thead>
<tr>
<th>Test Material</th>
<th>Dilute RVVT (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sephacryl S-300 IgG pool</td>
<td>38.5</td>
</tr>
<tr>
<td>Liposome eluate*</td>
<td>42.3</td>
</tr>
<tr>
<td>Cardiolipin</td>
<td>29.9</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>31.7</td>
</tr>
<tr>
<td>Liposome supernatant*</td>
<td>41.1</td>
</tr>
<tr>
<td>Cardiolipin</td>
<td>28.8</td>
</tr>
</tbody>
</table>

*After protein A-Sepharose chromatography.
MECHANISM OF LUPUS ANTICOAGULANTS

Table 3. Lupus Anticoagulant Activity of Liposome Eluates From Patient and Normal Plasma

<table>
<thead>
<tr>
<th>Patient</th>
<th>A_{200} Eluate</th>
<th>Dilute RVVT (Eluate/Buffer, s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.237</td>
<td>34.4/27.9</td>
</tr>
<tr>
<td>2</td>
<td>0.991</td>
<td>38.8/27.9</td>
</tr>
<tr>
<td>3</td>
<td>0.291</td>
<td>36.9/28.4</td>
</tr>
<tr>
<td>A</td>
<td>0.044</td>
<td>26.9/27.8</td>
</tr>
<tr>
<td>B</td>
<td>0.114</td>
<td>29.4/28.4</td>
</tr>
</tbody>
</table>

With the ELISA test system, the cardiolipin liposome eluates reacted with cardiolipin and, as shown for patient 1 in Fig 4, also reacted with phosphatidylserine, phosphatidylinositol, and phosphatic acid, but not with phosphatidyethanolamine, phosphatidylcholine, or dicetyl phosphate. Identical results were obtained in all four patients tested (patients no. 1, 2, 3, and 5). Normal IgG, at approximately the same concentration as in patient eluates, showed no reactivity against any phospholipids. In addition, liposome eluates from normal plasma contained only trace amounts of protein and showed little or no reactivity.

Analytical isoelectric focusing of affinity-purified, phospholipid-free IgG from patients no. 2 and 3 (Fig 5) showed several bands of acidic pI; in contrast, the bulk of normal IgG focused in the alkaline range.

Inhibition of Prothrombin and Factor X Binding to Phosphatidylserine-coated Surfaces

The calcium ion-dependent binding of ^{125}I-prothrombin to phospholipid-coated surfaces was studied in microtiter plates. As expected, prothrombin bound to phosphatidylserine–coated wells (136 to 205 fmol/well, in eight experiments), and, to a much lesser extent, to cardiolipin-coated wells (27 and 44 fmol/well, in two experiments). In contrast, prothrombin binding to phosphatidylcholine-coated wells was only 6.2 and 7.0 fmol/well in two experiments, a figure similar to the value obtained for nonspecific binding to phosphatidylserine–coated wells in the presence of EDTA (6 to 9 fmol/well). As shown in Fig 6A, immunoaffinity-purified patient IgG inhibited the binding of prothrombin to phosphatidylserine–coated wells by 73% and 66%, respectively. Purified IgG from patient no. 1 (not shown in Fig 6), tested only at a much lower protein concentration (23 μg/mL), inhibited prothrombin binding by 25.5%. Binding of factor X was also inhibited by patient IgG, although to a slightly lesser extent than prothrombin (Fig 6B). Three control IgG preparations showed no inhibition of prothrombin or factor X binding. Binding to cardiolipin-coated wells was also inhibited by patient eluates.

DISCUSSION

Although earlier observations implied that lupus anticoagulants have as their locus of action the negatively charged phospholipids, this assumption has been directly demonstrated in only one patient. On the basis of this case, the correlation that has been observed between the presence of anticardiolipin activity and lupus anticoagulant activity, as well as some more recently described absorption studies, all lupus anticoagulants have been widely assumed to operate by the same mechanism. We tested the generality of this hypothesis directly in five patients with lupuslike syndromes and/or thrombosis, whose lupus anticoagulants were largely IgG.

Fig 4. Antiphospholipid activity (ELISA) of cardiolipin liposome eluates. Because control eluates contained essentially no IgG, reactivity of purified normal IgG is also shown.

Fig 5. Analytical isoelectric focusing of normal IgG (lane 1) and immunoaffinity-purified IgG from patients no. 2 and 5 (lanes 2 and 3).
cardiolipin liposome eluates invariably contain significant amounts of albumin, a further purification step by protein A-Sepharose chromatography was added.

Using these preparations, we were able to obtain a good dose–response relationship between lupus anticoagulant activity and IgG concentration and to detect lupus anticoagulant activity at IgG concentrations as low as 3 μg/mL. Thus, the dilute RVVT, as normally performed on plasma, can detect lupus anticoagulants at plasma concentrations of ≥10 μg/mL.

The mechanism of prolongation of phospholipid-dependent coagulation tests in the patients we studied is similar to that of the patient previously reported. Our data clearly show inhibition by patient affinity-purified IgG of prothrombin binding to phosphatidylserine surfaces, and, to a lesser extent, a similar inhibition of factor X binding. The reason for this difference in inhibition is not presently known. Our data further indicate that prothrombin can bind to cardiolipin surfaces, although to a much lesser extent than to phosphatidylserine surfaces, and that this binding is also inhibited by the affinity-purified IgG.

Although all six lupus anticoagulants we studied in this and the previous report are clearly antibodies with anionic phospholipid specificity, these anticoagulants probably represent only a subpopulation of antibodies to anionic phospholipids. Several studies suggest that there may be more than one type of anticardiolipin antibody, and we have studied patients with true-positive and false-positive serologies whose anticardiolipin antibodies did not possess lupus anticoagulant activity. Furthermore, the presence of several distinct bands on isoelectric focusing of the affinity-purified IgG preparations from our patients raises the possibility that not all affinity-purified IgG anticardiolipin antibodies possess lupus anticoagulant activity.

In addition to anticardiolipin antibodies, other phenomena have been correlated with the presence of a lupus anticoagulant, including biologic false-positive serology, thrombocytopenia, a history of recurrent abortions, and a high incidence of thromboembolic phenomena. Three of our patients had false-positive serologies, and only 1 may have had thrombocytopenia, whereas 4 of the 5 had either a history of thrombosis or of recurrent abortion. Because this is a highly selected group, no conclusions can be drawn concerning incidence of these clinical findings. Because all patients had anticardiolipin antibodies, it is surprising that two had negative serologies. Even a high concentration (56 μg/mL) of affinity-purified IgG from one patient did not produce a positive RPR serology, suggesting that antibody concentration alone may not explain this observation. In this connection, it is of interest that the two patients with positive serologies at the time of our study had some IgM lupus anticoagulant and IgM anticardiolipin activity. Because many flocculation tests are more sensitive to IgM than to IgG antibodies, a biologic false-positive serology may correlate better with levels of IgM than of IgG anticardiolipin antibodies.

The high correlation between lupus anticoagulants and thrombocytopenia raises the question of the interaction of lupus anticoagulants with platelets. The strong correlation
with thromboembolic phenomena and the possible effect of lupus anticoagulants on endothelial cell prostacyclin production raise similar questions concerning the interaction of these antibodies with endothelial cells. It will be of interest to determine whether purified lupus anticoagulants interact with platelets or endothelial cells, or whether the clinical phenomena observed depend on other factors.

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Immunological specificity and mechanism of action of IgG lupus anticoagulants

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