A Mechanism for the Hypoprothrombinemia of the Acquired Hypoprothrombinemia-Lupus Anticoagulant Syndrome

By S. P. Bajaj, S. I. Rapaport, D. S. Fierer, K. D. Herbst, and D. B. Schwartz

Antibodies that bind prothrombin without neutralizing its coagulant activity were demonstrated in the plasma of two patients with the acquired hypoprothrombinemia-lupus anticoagulant syndrome. The first patient’s plasma contained <1% prothrombin activity and no detectable prothrombin antigen. The second patient’s plasma contained about 6% of both prothrombin activity and antigen. Neither patient’s plasma neutralized the prothrombin coagulant activity of normal plasma or of purified prothrombin added in vitro. Nevertheless, double immunodiffusion studies and binding experiments utilizing 125I-prothrombin demonstrated the presence of prothrombin antibodies in each patient’s plasma. A Scatchard analysis of the binding data obtained with different concentrations of 125I-prothrombin and the first patient’s plasma indicated the presence of a high affinity antibody, apparent Kd ~ 10^{-10} M, and a lower affinity antibody, apparent Kd ~ 10^{-6} M. Studies utilizing purified cleavage products of prothrombin suggested that the antibodies were directed against an epitope or epitopes located on the carboxyl-terminal, latent thrombin segment of the prothrombin molecule. We postulate that the acquired hypoprothrombinemia in these two patients and in other reported patients with the acquired hypoprothrombinemia-lupus anticoagulant syndrome stems from rapid clearance from the circulation of prothrombin antigen–antibody complexes.

However, acquired hypoprothrombinemia has been described in a handful of additional patients with systemic lupus erythematosus and abnormal bleeding. In most, the lupus inhibitor was also demonstrated; in two patients the data are insufficient to decide if the inhibitor was present. In no patient was an anticoagulant demonstrated that neutralized the coagulant activity of prothrombin added to the plasma in vitro. Thus, the cause for the hypoprothrombinemia has remained a mystery.

We recently saw two patients with severe hypoprothrombinemia and the lupus inhibitor. One patient had established systemic lupus erythematosus; the other patient had an acute illness with features suggestive of systemic lupus erythematosus. Studies of plasma from these patients have clarified the mechanism of hypoprothrombinemia in this syndrome. Antibodies were demonstrated in each patient’s plasma that bind prothrombin yet fail to neutralize its coagulant activity in vitro.

MATERIALS AND METHODS

Reagents

Tissue factor was a saline extract of human brain tissue. Automated APTT reagent was purchased from General Diagnostics, Morris Plains, N.J. A buffer containing 0.05 M Tris-HCl, 0.15 M NaCl, and 1 mg/ml bovine serum albumin, pH 7.5 (Tris/NaCl/Alb) was used for dilutions of radiolabeled proteins and antibodies. Hereditary clotting-factor-deficient plasmas were obtained from patients seen in this laboratory or from George King Biomedical, Overland Park, Kansas. A reference plasma, prepared by pooling plasma from 10 healthy donors, was defined as containing 1 U/ml of all clotting factors. All other reagents were of the best grade available from commercial sources.

Coagulation Assays

The prothrombin time and APTT were performed by standard methods. Factors XII, XI, and VIII were measured in one-stage activated partial thromboplastin time assay systems utilizing heredi-
tary clotting-factor-deficient plasmas as substrates. Factors V, VII, and X were measured in assays based on the ability of a diluted test plasma to shorten the clotting time of recalibrated hereditary specific-deficient plasma in the presence of tissue factor. Prothrombin was measured in a one-stage assay described in detail previously.\textsuperscript{5}

**Purified Coagulation Proteins**

Human prothrombin, factor IX, and factor X were purified as described earlier.\textsuperscript{11} Prothrombin fragment I and prothrombin I were made by treating prothrombin with thrombin and were separated by chromatographic techniques.\textsuperscript{14} Human α-thrombin was prepared\textsuperscript{13} and purified by chromatography on SP-Sephadex C-50 by a method similar to that described by Lundblad.\textsuperscript{13} Human prothrombin fragment 2 was kindly provided by Dr. K.G. Mann, Mayo Clinic, Rochester, Minn. All preparations were greater than 95% homogeneous upon examination by sodium dodecylsulfate gel electrophoresis. Protein concentrations were determined from their known extinction coefficients.

**Goat Anti-Prothrombin Antiserum**

An immunoglobulin fraction containing antibodies to human prothrombin was prepared, as described elsewhere for factor VII,\textsuperscript{14} by immunizing a goat with purified human prothrombin. A 14-fold dilution of the antiserum neutralized 50% of the prothrombin activity of normal plasma.

**Immunodiffusion**

Immunodiffusion was carried out in 1.5% agarose in 0.06M sodium barbital buffer, pH 8.6, on microscope slides. The goat anti-prothrombin antiserum was used undiluted. The patients' plasmas required concentration before use as antisera. In the first patient, this was accomplished by adsorbing the plasma with BaSO₄, heating the adsorbed plasma for 30 min at 56°C, followed by lyophilization and reconstitution in distilled water in one-fourth the volume of the starting plasma. In the second patient, the adsorbed heated plasma was made 33% in ammonium sulfate, centrifuged, and the resultant pellet was dissolved in Tris/NaCl and dialyzed extensively against distilled water in one-tenth the volume of the starting plasma.

**Labeling of Coagulation Proteins With \textsuperscript{125}I**

Enzymobead reagent (BioRad Laboratories, Richmond, Calif.) was utilized to label prothrombin, factor IX, factor X, and prothrombin cleavage products with \textsuperscript{125}I as described in detail earlier.\textsuperscript{12} Radioactive activities were as follows: prothrombin, 0.7 x 10⁶ cpm/mg; factor IX, 4.2 x 10⁵ cpm/mg; factor X, 4.9 x 10⁵ cpm/mg; prothrombin fragment 1, 1.08 x 10⁶ cpm/mg; prothrombin fragment 2, 1.1 x 10⁶ cpm/mg; and α-thrombin, 1.1 x 10⁶ cpm/mg. All proteins were structurally intact after radiolabeling as analyzed by sodium dodecylsulfate gel electrophoresis. Prothrombin and α-thrombin retained greater than 85% of the biologic activity of the unlabelled materials. The labeled proteins were stored frozen in plastic tubes at -60°C for up to 2 wk.

**Preparation of Disopropylphosphate (DIP) α-Thrombin**

α-thrombin or \textsuperscript{125}I-α-thrombin, at a concentration of 1 mg/ml in Tris/NaCl buffer, was incubated with disopropylphosphorophate at a final concentration of 5mM for 20 min at room temperature. Excess disopropylphosphorophate was removed by dialysis against Tris/NaCl buffer. These DIP-inactivated reagents were used in place of active α-thrombin in immunologic experiments to prevent clotting of the patient’s plasma.

**Binding of \textsuperscript{125}I-Prothrombin and \textsuperscript{125}I-Prothrombin Cleavage Products to Anti-Prothrombin Antibodies**

The binding properties of the anti-prothrombin antibodies in the first patient's plasma were studied extensively. One-tenth milliliter of \textsuperscript{125}I-prothrombin (5μg/ml) was added to duplicate 12 x 75 mm glass test tubes containing either 0.1 ml of various dilutions of the first patient's plasma of July 7 as a source of antibody, or 0.1 ml of various dilutions of goat anti-prothrombin antisera. After incubation for 16 hr at 4°C, 0.1 ml of saturated ammonium sulfate was added to each tube. After mixing and further incubation at 4°C for 30 min, the samples were centrifuged at 2000 x g for 30 min at 4°C. The supernatants were decanted and the tubes blotted on absorbent towels to remove drops remaining on the edge of the tubes. Radioactivity of the pellets was determined in a Searle γ Counter. Controls were prepared, in duplicate, for each test sample by substituting dilutions of normal plasma for the patient's plasma and by substituting dilutions of a goat immunoglobulin reagent, prepared from blood drawn prior to immunization, for the goat anti-prothrombin antisera. Counts in the control tube pellets, which were less than 10% of the total counts added to the control tubes, were subtracted from the counts in the pellets of the corresponding test sample tubes.

In further experiments, a fixed dilution of the first patient's plasma of July 7 and a fixed dilution of goat anti-prothrombin antisera were incubated with a series of dilutions of \textsuperscript{125}I-prothrombin. Control data were obtained and treated as described above. The resultant experimental data were plotted according to Scatchard\textsuperscript{15} to obtain the binding affinities of the antibodies.

Additional experiments were carried out in which 0.1 ml of an \textsuperscript{125}I-labeled specific prothrombin cleavage product at a concentration of 8 x 10⁻⁶ M in Tris/NaCl/Alb buffer was incubated for 16 hr at 4°C with 0.1 ml of either a twofold dilution of the first patient's plasma of July 7 or a 40-fold dilution of goat anti-prothrombin antisera. Controls were performed with a twofold dilution of normal plasma and a 40-fold dilution of nonimmune goat immunoglobulin reagent. Binding was determined as described above.

The ability of the antibodies in the second patient’s plasma to bind prothrombin was evaluated by two techniques: ammonium sulfate precipitation as described above and binding to Staphylococcal protein A. In the latter, after incubation of the plasma with \textsuperscript{125}I-prothrombin overnight, an equal volume of 10% staphylococcus A (Pansorbin, Calbiochem., San Diego, Calif.) was added to the tube. After 1 hr of incubation at 4°C, the suspension was centrifuged and a measured volume of the supernatant was counted for radioactivity. The percent binding to antibody of \textsuperscript{125}I-prothrombin was calculated by subtracting the counts in the supernatant from the total radioactivity present in the incubation mixture.

**CASE DESCRIPTIONS**

**First Patient**

A 4-year-old girl was hospitalized on July 6, 1980 because of bruising that had begun 2 days earlier. Nine days earlier she had experienced diarrhea, which had stopped after the administration of Donnagel\textsuperscript{6}. Four days before admission she had developed skin lesions, described as urticarial erythema, which subsided after she was given diphenhydramine. She had experienced intermittent headaches for 1 mo and also had been given aspirin 4 days prior to admission.

Skin ecchymoses and a temperature of 100°F were the only notable physical findings. The hematocrit was 35.3%; the white-cell count was 7200 with 29% neutrophils, 65% lymphocytes, 5% mono-
cytes, and 1% eosinophils; the platelet count was 261,000. Values for the prothrombin time and APTT of the patient's plasma and of a mixture of patient's plasma and normal plasma are listed in the upper portion of Table 1. Values for specific coagulation factor assays are given in Table 2. The Ivy bleeding time was 4.5 min. The thrombin time was 8 sec (normal 8–10 sec), and the fibrinogen concentration was 220 mg/dl.

The urine was normal. The blood urea nitrogen (BUN) was 9 mg/dl; the serum albumin was 4.6 g/dl; the serum alanine aminotransferase activity (SGPT) was 27 U/ml. The chest film was normal. A screening test for infectious mononucleosis was normal. Administration of 10 mg of vitamin K, failed to shorten the prothrombin time or APTT. Complement studies, drawn on July 9, revealed a CH₅₀ of less than 8 U/ml (normal 75–180 U/ml), a C₃ of 95 mg/dl (normal 90–230 mg/dl), and a C₄ or 5 mg/dl (normal 10–35 mg/dl). The ANA test, the Venereal Disease Research Laboratory (VDRL) test for syphilis, the Coombs test, and tests for antithyroid antibodies were all normal.

The bruising subsided rapidly without specific therapy. The prothrombin time fell to 17 sec by the 7th day and to 14 sec by the 18th day. Serologic studies on the 18th day for DNA binding antibodies, anti-Sm antibodies, and antiribonucleoprotein antibodies were all negative. A physical examination on the 32nd day was normal. The APTT was still prolonged to 56 sec on the 51st day and was 47 sec on the 81st day. A moderate neutropenia persisted through the 81st day.

**Second Patient**

A 12-year-old girl was hospitalized in May 1982 with fever, proteinuria, and hematuria. Serologic studies revealed a positive antinuclear antibody test at a 1:160 dilution and markedly reduced levels of C₄ and CH₅₀. A biopsy of the kidney demonstrated membranoproliferative glomerulonephritis. A diagnosis of systemic lupus erythematosus was made, and the patient was treated with a high dose of oral prednisone, which was gradually tapered.

In January 1982, the patient developed symptoms of a viral respiratory tract infection accompanied by epistaxis and bleeding gums. Values for the prothrombin time and APTT of the patient's plasma and of a mixture of patient's plasma and normal plasma are listed in the lower portion of Table 1. Values for specific coagulation factor assays are given in Table 2. The patient was treated with increasing doses of corticosteroids and with Imuran, 125 mg/day. Seven days later, the prothrombin time was 14 sec and the APTT was 62 sec; after 5 more days, the prothrombin time was 10 sec and the APTT was 30 sec.

### RESULTS

**Coagulation Studies**

A prolonged prothrombin time and a prolonged APTT were found on examination of the plasma samples from both patients. The prothrombin time of these plasmas was shortened substantially when each was mixed with an equal volume of normal plasma (Table 1). In contrast, the APTT was not substantially shortened. Indeed, in the first patient, the APTT of the mixture exceeded the APTT of the patient's plasma alone (Table 1), a finding that has been referred to as the "lupus cofactor phenomenon."²⁹,³⁰

Specific factor assays based on correction of the prolonged APTT of hereditary clotting-factor-defici-ency plasmas revealed low levels for factors VIII, IX, XI, and XII (Table 2). This pattern is typical of what may be found with the lupus anticoagulant;² it presumably reflects nonspecific interference by the anticoagulant in the assay systems. Evidence for such nonspecific interference was obtained in the first patient, whose plasma gave increased values for factors VIII, IX, and XI when assayed at a 1:40 dilution (Table 2).

The prolonged prothrombin times reflected the markedly reduced prothrombin activity in the plasma samples: no measureable prothrombin in the first patient, and 6% prothrombin activity in the second patient. The values for the other factors influencing the prothrombin time (factor V, factor VII, and factor X) were not notably reduced (Table 2).

Neither patient's plasma neutralized the coagulant activity of prothrombin added in vitro. For example, when 1 part of normal plasma was incubated for 1 hr with 4 parts of either control buffer or the first patient's plasma, each mixture yielded a value for prothrombin activity of 0.2 U/ml. When 1 part of normal plasma was incubated for 1 hr with 9 parts of the second patient's plasma, the mixture yielded a value of 0.19 U/ml as compared with a value of 0.10 U/ml for a mixture with control buffer. (The difference reflects the contribution of the residual prothrom-

### Table 1. Screening Coagulation Test Results on Hospital Admission

<table>
<thead>
<tr>
<th>Clotting Time (sec)</th>
<th>Prothrombin Time</th>
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<tr>
<td>Plasma</td>
<td>APTT</td>
</tr>
<tr>
<td>Patient 1</td>
<td>117</td>
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<tr>
<td>Patient (Pt)</td>
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</tr>
<tr>
<td>Normal (N)</td>
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</tr>
<tr>
<td>Pt/N (1/1)</td>
<td>162</td>
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<tr>
<td>Patient 2</td>
<td>79</td>
</tr>
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<td>Patient</td>
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</tr>
<tr>
<td>Normal</td>
<td>61</td>
</tr>
<tr>
<td>Pt/N (1/1)</td>
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### Table 2. Specific Clotting Factor Assays

<table>
<thead>
<tr>
<th>Clotting Factor</th>
<th>Percent Normal Activity</th>
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<tbody>
<tr>
<td>Patient 1</td>
<td>Patient 2</td>
</tr>
<tr>
<td>Factor affecting the prothrombin time</td>
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</tr>
<tr>
<td>Prothrombin</td>
<td>&lt;1</td>
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<tr>
<td>Factor V</td>
<td>92</td>
</tr>
<tr>
<td>Factor VII</td>
<td>51</td>
</tr>
<tr>
<td>Factor X</td>
<td>83</td>
</tr>
<tr>
<td>Factor affecting only the APTT</td>
<td></td>
</tr>
<tr>
<td>Factor VIII (1/10, 1/40 dil.)</td>
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</tr>
<tr>
<td>Factor IX</td>
<td>14.64</td>
</tr>
<tr>
<td>Factor XI</td>
<td>10.56</td>
</tr>
<tr>
<td>Factor XII</td>
<td>10.32</td>
</tr>
</tbody>
</table>
bin in the 9 parts of the second patient’s plasma.) In additional experiments, purified prothrombin was incubated with the first patient’s plasma. When 10 μl of purified prothrombin, 7.5 U/ml, was incubated for 1 hr at room temperature with 65 μl of the first patient’s plasma, the prothrombin activity of the incubation mixture was found to be between 1.0 and 1.2 U/ml.

**Measurement of Prothrombin Antigen**

Prothrombin antigen could not be demonstrated in the initial plasma sample (July 7) from the first patient. In the double immunodiffusion experiment illustrated in Fig. 1A, the center well contained goat anti-human prothrombin antiserum. A precipitin line was obtained against normal plasma (right well, labeled NP) and against purified prothrombin (lower well, labeled II). A precipitin line was not obtained against the patient’s plasma of July 7 (left well, labeled PP). A precipitin line was obtained against a second sample of the patient’s plasma, obtained on July 23, at which time her prothrombin activity had returned to 60% of normal (upper well, labeled PP2). Absence of prothrombin antigen in the patient’s plasma of July 7 was also confirmed by the technique of electroimmunoassay (data not shown).

Prothrombin antigen also could not be demonstrated by double immunodiffusion in the plasma from the second patient. However, a small amount of prothrombin antigen, approximately 6%–8%, was demonstrable by the technique of electroimmunoassay (data not shown).

**Demonstration by Immunodiffusion of Antibody Against Prothrombin in the Patients’ Plasmas**

The first patient’s plasma of July 7, concentrated fourfold, was used as the source of antibody in double immunodiffusion studies, such as that of Fig. 1B, in which the center well contained the patient’s concentrated plasma. The similarity is readily apparent between the findings of Fig. 1B and the findings of Fig. 1A, in which goat anti-human prothrombin antiserum was the source of antibody. As is shown in Fig. 1B, the patient’s plasma contained antibody that gave a precipitin line against normal plasma (right well), against purified prothrombin (bottom well), against the patient’s own plasma after recovery (top well), but not against a sample of the patient’s plasma of July 7 (left well). When the patient’s plasma after recovery (sample of July 23) was concentrated fourfold and used as the source of antibody in similar double immunodiffusion studies, precipitin lines were not observed.

Since these data provide direct evidence that the first patient’s plasma of July 7 contained a precipitating antibody against prothrombin, the question may be asked as to why a precipitin line was not visible between the left outer well (PP,) of Fig. 1B as a source of antibody, and the upper outer well (PP,) of Fig. 1B as a source of antigen. Two possible reasons come to mind. First, the distance between these two wells is 1.5 cm, in contrast to a 1.0-cm distance between the center well and the outer wells. Second, the concentration of the antibody in the center well is four-fold higher than the concentration of the antibody in the left outer well (PP,).

It was not possible to demonstrate unequivocally the presence of an antibody in the second patient’s plasma by the double immunodiffusion technique, presumably because of the lower concentration of the antibody (see below). When the patient’s plasma, concentrated tenfold, was used as the source of antibody in the center well (Fig. 2), a faint precipitin line was visible after 48 hr against purified prothrombin in a concentration of 1 mg/ml (right well), but not against prothrombin in lesser concentrations. The interpretation of this line is complicated by the presence of a large amount of precipitated material around the central well.
Antibody Concentration (%)

Fig. 3. Binding of \(^{125}\text{I}\)-prothrombin to antibodies in the first patient’s plasma of July 7 (A) and to antibodies in goat anti-prothrombin antiserum (B). One-hundred percent antibody concentration in A represents undiluted patient’s serum and in B represents a five-fold dilution of the antiserum. The total counts per minute in each tube were approximately 30,000. \(B/B_0\) represents radioactivity in the pellet divided by the total radioactivity in the tube. Conditions of incubation and precipitation of counts are described in Methods.

Binding of \(^{125}\text{I}\)-Prothrombin to Antibodies in the Patients’ Plasmas

Binding of \(^{125}\text{I}\)-prothrombin to antibodies in dilutions of the first patient’s plasma of July 7 (Fig. 3A) was compared with binding of \(^{125}\text{I}\)-prothrombin to antibodies in dilutions of goat anti-human prothrombin antiserum (Fig. 3B). Antigen–antibody complexes were precipitated with ammonium sulfate (see Methods). With both sources of antibody, the amount of labeled prothrombin bound increased linearly as the concentration of antibody was increased. Nonspecific binding was insignificant. At high concentrations of the patient’s plasma (100% antibody in Fig. 3A represents undiluted patient’s plasma), approximately 90% of the labeled prothrombin was bound; at high concentrations of the goat antiserum (100% antibody in Fig. 3B represents a fivefold dilution of the antiserum), 100% of the labeled prothrombin was bound. In further experiments, binding studies were carried out with a 1:1 mixture and with a 1:2 mixture of labeled and unlabeled prothrombin. The binding curves were superimposable, which suggests that radioiodination of prothrombin did not alter the affinity for prothrombin of the antibodies in either the patient’s plasma or the goat antiserum.

Comparable experiments, utilizing ammonium sulfate to precipitate antigen–antibody complex, were carried out at three concentrations of the second patient’s plasma. The following values were obtained for the fraction of \(^{125}\text{I}\)-prothrombin bound (\(B/B_0\)): undiluted plasma, 0.53; a twofold dilution of plasma, 0.32; a fourfold dilution of plasma, 0.14. When these values are compared with the values obtained with the first patient’s plasma (Fig. 3A), one notes that the \(B/B_0\) of 0.53 for the undiluted second patient’s plasma corresponds to the \(B/B_0\) obtained with an approximately fivefold dilution of the first patient’s plasma. A similar approximately fivefold difference is also seen for the other two dilutions.

A further experiment was carried out in which Staphylococcal protein A was used to quantitate the binding of \(^{125}\text{I}\)-prothrombin to the second patient’s antibody. A dilution of the second patient’s plasma that gave a \(B/B_0\) of 0.36 with ammonium sulfate precipitation gave a value of 0.26 with the Staphylococcal protein A binding method. When, as a control, normal plasma was used instead of the patient’s plasma, the value for \(B/B_0\) was 0.03. When \(^{125}\text{I}\)-factor-IX or \(^{125}\text{I}\)-factor-X was substituted for \(^{125}\text{I}\)-prothrombin in mixtures containing either the second patient’s plasma or normal control plasma, the values for \(B/B_0\) were less than 0.04.

To obtain data on the affinities of the antibodies in the first patient’s plasma, we titrated a constant concentration of the patient’s plasma of July 7 (fourfold final dilution) with increasing concentrations of \(^{125}\text{I}\)-prothrombin from 3 nM to 384 nM. A Scatchard analysis of the data is shown in Fig. 4A. For comparison, a Scatchard analysis of similar data obtained with the goat anti-human prothrombin antiserum is shown in Fig. 4B. From the plot of Fig. 4A, it appears that the patient’s plasma contained at least two types of antibody: higher affinity antibody with an apparent \(K_d\) of \(-10^{-10}\) M and lower affinity antibody with an apparent \(K_d\) of \(-10^{-7}\) M. Extrapolation of the curve to the X-axis intercept yielded a value of 210 nM prothrombin as an
Fig. 4. Scatchard plots of the binding of 125I-prothrombin to antibodies in the first patient’s plasma of July 7 (A) and to antibodies in goat anti-prothrombin antiserum (B). A constant final concentration of the patient’s plasma (four-fold dilution) and of goat anti-prothrombin antiserum (80-fold dilution) was titrated with a series of 125I-prothrombin solutions between 3 nM and 64 nM, as described in Methods. Specifically bound 125I-prothrombin/free 125I-prothrombin (B/F) is plotted against specifically bound 125I-prothrombin.

apparent minimum concentration of total binding sites of the antibodies in the assay mixture. Since the plasma was diluted fourfold in the assay mixtures, the concentration of binding sites in the undiluted first patient’s plasma of July 7 was at least 850 nM prothrombin (60 μg/ml). It is recognized that the values for $K_d$ and for number of binding sites represent crude approximations, but they seem to us nevertheless useful in providing some estimate of the affinities and quantity of the patient’s antibodies against prothrombin. The goat anti-prothrombin antiserum appeared to be highly heterogeneous with an apparent average $K_d$ of $5 \times 10^{-10}$ M.

Activity of the Antibodies in the First Patient’s Plasma Against Prothrombin Cleavage Products

Experiments were carried out to identify the location on prothrombin of antigenic sites for antibodies that could bind to prothrombin without interfering with its coagulant activity. Double immunodiffusion studies utilizing prothrombin cleavage products are shown in Fig. 1C in which the center well contained goat anti-human prothrombin antiserum, and in Fig. 1D, in which the center well contained concentrated first patient’s plasma of July 7. As can be seen from Fig. 1C, the antibodies in the goat antiserum reacted with all of the prothrombin cleavage products studied: prethrombin I (right well, labeled P-I); prothrombin fragment 2 (lower well, labeled F-2); prothrombin fragment 1 (left well, labeled F-1) and DIP-α-thrombin (upper well, labeled II). In contrast, the antibodies in the patient’s plasma gave a precipitin line only against prethrombin 1, the COOH-terminal segment of prothrombin with a mol wt of 51,000, and against DIP-α-thrombin, the COOH-segment of prethrombin 1, mol wt 40,000. A precipitin line was not visible against fragment 1, the NH₂-terminal segment of prothrombin, mol wt 23,000, or against fragment 2, the NH₂-terminal segment of prethrombin 1, mol wt 12,000.

Binding studies were also carried out in which 125I-labeled purified cleavage products of prothrombin were incubated either with the first patient’s plasma of July 7 or with goat anti-human prothrombin antiserum. The results (average of 4 determinations), summarized in Table 3, reveal that the antibodies in the patient’s plasma bound to the whole prothrombin molecule and to prethrombin 1 but did not bind to fragment 1 or fragment 2. These data confirm the observations made with the double immunodiffusion technique. The binding data obtained with DIP-α-thrombin were equivocal. For an unknown reason, the background counts obtained in the pellets of control tubes (125I-DIP-α-thrombin incubated with normal plasma) ranged from 20% to 30% of the total control tube counts. Subtraction of this high background from the pellet counts in the sample tubes may have resulted in a falsely low binding number for α-thrombin. This could account for the apparent difference between the data obtained for α-thrombin by the double immunodiffusion technique and by the binding studies.

DISCUSSION

Two important points about the hypoprothrombinemia of the acquired hypoprothrombinemia-lupus anticoagulant syndrome were known prior to the present report. First, it had been clearly established that the plasma does not contain anticoagulant material capable of neutralizing the coagulant activity of prothrombin added in vitro. Second, it had been shown that plasma prothrombin antigen is decreased to the same extent as prothrombin activity. The data presented herein from two new patients with this syndrome now provide additional information key to the understanding of the mechanism of the hypopro-

Table 3. Binding of Prothrombin and Its Cleavage Products to First Patient’s Anti-Prothrombin Antibodies and to Goat Anti-Prothrombin Antibodies

<table>
<thead>
<tr>
<th>Component</th>
<th>Antibodies in First Patient’s Plasma</th>
<th>Antibodies in Goat Antiserum</th>
</tr>
</thead>
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<tr>
<td>$^{125}$I-prothrombin</td>
<td>0.91</td>
<td>1.0</td>
</tr>
<tr>
<td>$^{125}$I-prethrombin 1</td>
<td>0.50</td>
<td>0.85</td>
</tr>
<tr>
<td>$^{125}$I-fragment 1</td>
<td>0.08</td>
<td>0.76</td>
</tr>
<tr>
<td>$^{125}$I-fragment 2</td>
<td>0.002</td>
<td>0.48</td>
</tr>
<tr>
<td>$^{125}$I-DIP-α-thrombin</td>
<td>0.05 - 0.2</td>
<td>0.79</td>
</tr>
</tbody>
</table>
thrombinemia. They establish that the plasma of these patients with the acquired hypoprothrombinemia-lupus anticoagulant syndrome contained antibodies that bind prothrombin without neutralizing its in vitro coagulant activity.

We infer from these new findings that the hypoprothrombinemia in this syndrome stems from the binding of prothrombin to prothrombin antibodies in vivo. The clearance of immune complexes from the circulation depends on their quantity and size, \[^{23}\] and indeed, persistence of circulating factor IX antigen–antibody complexes has been demonstrated in a patient with hemophilia B. \[^{24}\] Nevertheless, we strongly suspect that the decreased concentration of the prothrombin molecule in the plasma of patients with the acquired hypoprothrombinemia-lupus anticoagulant syndrome results from a rapid clearance of prothrombin antigen–antibody complexes.

Quantitative considerations are consistent with this assumption. Thus, at a time when prothrombin antigen was not measurable in the first patient’s plasma, it contained antibodies capable of binding at least 60 \(\mu g/ml\) of prothrombin added in vitro. Normal plasma contains about 150 \(\mu g/ml\) of prothrombin with an estimated intravascular T/2 of 2–5 days. \[^{25}\] Therefore, 60 \(\mu g/ml\) of prothrombin represents more prothrombin than would normally be added to plasma in vivo within a 24-hr period. Similar quantitative data could not be obtained for the second patient because of a limited amount of plasma. However, it is interesting to note that this patient, who had a substantially reduced apparent concentration of antibodies as compared with the first patient, had about 6% residual prothrombin antigen in the plasma.

Studies with prothrombin cleavage products revealed that the first patient’s antibodies bound to prethrombin I and to \(\alpha\)-thrombin but not to fragment 1 or to fragment 2 (Fig. 1D, Table 3). Fragment 1 contains the phospholipid-binding region of prothrombin, \[^{26}\] and fragment 2 contains the factor V\(_e\)-binding region of prothrombin. \[^{26}\] Since binding to both phospholipid and to factor V\(_e\) is essential for the physiologic activation of prothrombin, the inability of the antibodies to bind to fragment 1 or fragment 2 is consistent with the inability of the antibodies to impair the coagulant activity of prothrombin added to the patient’s plasma in vitro. Furthermore, since the first patient’s antibodies bind to \(\alpha\)-thrombin yet do not interfere with its coagulant activity, one may conclude that the antigen–antibody interaction does not impair the function of either the active site or the binding pocket of \(\alpha\)-thrombin.

In all \[^{1-5,11,22-27}\] but two \[^{6,22}\] prior reports of patients with acquired specific hypoprothrombinemia without demonstrable plasma prothrombin neutralizing activity, the patients have had or have developed systemic lupus erythematosus. One exception was a patient with hypertension and no evidence of systemic illness 1 yr later; \[^{6}\] the other exception was a patient with a lymphoma in whom systemic lupus erythematosus was carefully searched for and not found over a 4-yr period of observation. \[^{22}\] One of our two patients had an established diagnosis of systemic lupus erythematosus. The first patient, only 4 yr of age, had an acute illness with features suggestive of systemic lupus erythematosus. Although not diagnostic of systemic lupus erythematosus, the discovery of an acquired specific prothrombin deficiency clearly serves as a strong warning of the possibility of systemic lupus erythematosus or of its future development.

It is reasonable to assume that the same type of antibodies as those demonstrated in our two patients were responsible for the other reported instances of acquired specific hypoprothrombinemia without plasma prothrombin neutralizing activity. However, studies of further patients are needed to confirm this generalization. In some patients, as in our second patient, it may prove difficult to detect plasma antibodies by the conventional double immunodiffusion technique. Demonstration of prothrombin-binding antibodies may require more sensitive techniques in which complexes of radiolabeled prothrombin and antibody are detected.

The reason is unknown for the association between the acquired hypoprothrombinemia and the lupus anticoagulant. Their occurrence together must be more than coincidence. Of 14 documented instances of acute, specific hypoprothrombinemia, the lupus anticoagulant has been demonstrated in 12 patients and might have been detected in the other 2 patients had further studies been carried out. \[^{10,31}\] Yet, it is difficult to formulate a hypothesis as to why generation of antibodies reacting with prothrombin should be linked to generation of the antibodies responsible for the lupus anticoagulant phenomenon—antibodies that impair the activation of prothrombin by the prothrombin activator complex \[^{1-2}\] possibly by reacting with its phospholipid component. \[^{22}\] Moreover, it is important to reiterate that the lupus anticoagulant is a common phenomenon seen in a variety of patients, whereas its association with acquired hypoprothrombinemia is uncommon and very probably confined to patients who have or will develop lupus erythematosus.

The prothrombin antibodies in our patients had to be of polyclonal origin, since they gave a precipitin line against prothrombin on double immunodiffusion and, in our first patient, Scatchard analysis of binding to \(^{125}\)I-prothrombin showed a curvilinear plot. Because of
the very limited quantity of plasma available for study, it was not possible for us to determine definitely the immunoglobulin class or classes of the antibodies. However, in the second patient, about 70% of the antibodies appeared to be of the IgG class, because a plasma sample that gave a value for B/BO of 0.36 by the ammonium sulfate precipitation method gave a value of B/BO of 0.26 by the Staphylococcal protein A binding technique. Since binding to Staphylococcal protein A appears primarily to be a property of human IgG subclasses 1, 2, and 4, about 30% of the antibodies in the second patient’s plasma could have been of IgG subclass 3 or of other immunoglobulin classes.

The demonstration in the patients reported here of antibodies that could deplete plasma of prothrombin without inhibiting the coagulant function of prothrombin added to the plasma in vitro has general implications for the laboratory diagnosis of hemorrhagic disease. It means that the standard screening test for antibodies in the clinical laboratory—inhibition of function of a given coagulation protein in incubation with a patient’s plasma—is not foolproof. It is not known whether antibodies that bind without neutralizing in vitro coagulant function will be found against coagulation proteins other than prothrombin. It behooves physicians caring for patients with hemorrhagic disease to be mindful of this possibility.

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A mechanism for the hypoprothrombinemia of the acquired hypoprothrombinemia-lupus anticoagulant syndrome

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