A Unique Precipitating Autoantibody Against Plasma Thromboplastin Antecedent Associated With Multiple Apparent Plasma Clotting Factor Deficiencies in a Patient With Systemic Lupus Erythematosus

By Man-Chiu Poon, Hidehiko Saito, and William J. Koopman

A 42-yr-old woman with systemic lupus erythematosus without bleeding diathesis developed a prolonged activated partial thromboplastin time that was not corrected by normal plasma. An inhibitor that acted rapidly and inactivated 0.5 U/ml plasma thromboplastin antecedent (PTA, factor XI) at a 1:200 plasma dilution was demonstrated. In addition to a low titer of PTA (<0.01 U/ml), plasma assayed at 20-fold dilution also showed low titers of Hageman (factor XII, 0.02 U/ml), Fletcher (plasma prekallikrein, 0.02 U/ml), and Fitzgerald (high molecular weight kininogen, <0.01 U/ml) factors. The titer of these factors, except PTA, returned to normal upon further plasma dilution or upon removal of the inhibitor by protein A adsorption. Thus, the inhibitor appeared to interfere with these clotting factor assays, possibly by inactivating PTA in the substrate plasmas in the test system. Its specificity was further confirmed. The inhibitor did not interfere with surface-induced proteolytic cleavage of Hageman factor.

Surface-induced generation of plasma kallikrein activity (amidolysis of H-D-val-leu-lys-pNa) and cold-promoted factor VII activity enhancement) requires only Hageman, Fletcher, and Fitzgerald factors and was normal. Reactions requiring all 4 contact phase factors, including PTA, such as surface-induced generation of plasmin activity (amidolysis of H-D-val-leu-lys-pNa) and activated Christmas factor (factor IXa) activity, were defective. Furthermore, the inhibitor bound to agarose-protein A inactivated and removed PTA selectively from normal plasma. The inhibitor was an IgG-λ autoantibody that precipitated PTA. The inhibitor inactivated activated PTA (factor Xla) without the requirement for an additional cofactor. Furthermore, it inhibited surface-induced activation of PTA by interfering with its proteolytic cleavage upon glass surface exposure and with its binding onto the reactive surfaces.

The occurrence of acquired inhibitors against contact phase clotting factors, including plasma thromboplastin antecedent (PTA, factor XI), is rare. Only two patients with congenital PTA deficiency complicated by the development of PTA inhibitor have been reported. Acquired PTA inhibitors complicating other disorders have been reported in only nine other patients, with eight having systemic lupus erythematosus (SLE). Hageman factor (HF, factor XII) level was frequently low in these patients, and it has been postulated that the inhibitor acted against both PTA and Hageman factor. The mechanism of action of these inhibitors has been incompletely determined in most instances. In the majority of cases, inactivation of activated PTA (factor Xla) has been shown. One inhibitor has been shown to interfere with the binding of PTA to high molecular weight (HMW) kininogen-coated glass surfaces and has inhibited proteolytic cleavage of PTA by activated Hageman factor. In another case, the inhibitor interfered with the adsorption of PTA to a reactive surface without preventing its proteolytic cleavage by trypsin. The inhibitor in this case did not inactivate activated PTA activity. Although one inhibitor was shown to bind to PTA, none has been shown to be precipitating.

We describe an acquired PTA inhibitor, complicating SLE, that is a precipitating IgG-λ autoantibody. The inhibitor interfered with the binding of PTA onto reactive surfaces. Furthermore, it inhibited surface-mediated proteolytic cleavage of PTA, as well as purified activated PTA and activated PTA generated by surface contact of normal plasma. Our study also suggests an explanation for the frequent observation that Hageman factor titer is often low in plasma containing PTA inhibitors.

MATERIALS AND METHODS

Plasma and Serum Preparation

Non-glass-contacted plasmas from the patient, normal volunteers, and patients with congenital clotting factor deficiencies, as well as a
standard pool of 24 normal plasmas, were prepared as previously described. Additional citrated plasmas congenitally deficient in Fitzgerald factor (HMW kininogen) and plasma thromboplastin antecedent (PTA, factor XI) were obtained from George King Biomedicals (Salem, NH). Sera were separated from whole blood clotted in glass tubes at 37°C for 4 hr and then further incubated at 37°C for 16 hr.

Clotting Factor Preparation

Hageman factor (HF, factor XII, 70 U/mg protein) and PTA (134 U/mg) were purified and 125I-radiolabeled by the chloramine T method, as previously described. In some experiments, PTA was a generous gift of Dr. Allen P. Kaplan, State University of New York at Stony Brook, Stony Brook, NY. All these preparations were free of known clotting factors or IgG. Activated PTA (factor XIa) was prepared by incubation of partially purified PTA with agarose-bound trypsin. This PTA preparation contained some IgG, such that the percent cleavage during activation is unclear.

Immunoglobulin Fractionation

Immunoglobulin fractions of plasmas were prepared from citrated plasmas that had been sequentially adsorbed with celite 512 (a generous gift of Johns Manville, Denver, CO), 40 mg/ml plasma) and 1/10 volume of alumina Cγ-gel (aged aluminum hydroxide gel, Calbiochem, San Diego, CA) to remove the contact phase clotting factors, including PTA and vitamin K-dependent clotting factors, and heated at 56°C for 45 min to inactivate the procoagulant activities of the residual clotting factors. The separation methods used included gel filtration through ultragel AcA22 (LKB-Producker AB, Bromma, Sweden), anion exchange chromatography through diethylaminoethyl 52 (DE 52) cellulose (Whatman, Maidstone, Kent, England), and protein A affinity chromatography with Sepharose 4B-protein A (Sigma, St. Louis, MO), as described previously. The immunoglobulin-containing fractions were dialyzed against barbital-saline buffer (BSB) containing 0.02 g/dl sodium azide, assayed for IgA, IgG, and IgM by solid phase radioimmunonassay (RIA) or immunodiffusion, and for concentrations of total proteins before clotting studies.

Kaolin (Surface) Induced Generation of Plasmin and Kallikrein Activities

This was assayed, respectively, by the amidolysis of H-D-val-\[\delta\]-lys-p-nitroanilide (S-2251, Kabi Diagnostica, Stockholm, Sweden) and of H-D-pro-\[\delta\]-phe-arg-p-nitroanilide (PPAN, S-2302, Kabi), as described.

Kaolin-Induced Enhancement of Factor VII Activity in the Cold

This was performed by reported method.

Glass Surface-Induced Activation of Hageman Factor (HF)

This was assessed by following the cleavage of 125I-HF on 10% SDS-PAGE and was performed essentially as described for PTA activation, substituting 125I-HF for 125I-PTA.

IgG Preparations and Generation of Activated PTA Activity

The effect of IgG preparations on glass surface-induced activation of PTA activity and on the generated activated PTA activity was measured by a modification of earlier methods. Twelve-hundredth milliliter of Christmas factor (factor IX) deficient plasma and 0.12 ml normal or patient IgG preparation in BSB was incubated in a 12 x 75 mm polystyrene tube at 37°C for 15 min. A 0.2-ml aliquot of the mixture was then incubated at 37°C in a 10 x 75 mm glass tube. At time intervals, 0.02-ml aliquots were removed and mixed in a 10 x 75 mm polystyrene tube with 0.08 ml BSB and 0.02 ml normal or patient IgG preparations. After 1-min incubation at 37°C, 0.1 ml of 0.1% soybean phosphatide (Centrolex R, 0.1% in 0.15 M NaCl, a generous gift of Central Soya Chemergy, Wayne, IN) and 0.1 ml PTA-deficient plasma were added. After an additional 1-min incubation at 37°C, 0.1 ml 0.025 M CaCl₂ was added and the clotting time measured. In this experiment, IgG was present in the initial stage to assess its effect on glass surface-induced activation of PTA. Following the activation step, IgG was again added to assess its effect on the activated PTA that might have been generated.

IgG Preparations and Generation of Activated Christmas Factor Activity

The effect of IgG preparation on glass surface-induced activation of Christmas factor (factor IXa) activity and on the generated activated Christmas factor (factor IXa) activity was determined by a modification of an earlier method. The design was similar to that for the PTA activation experiment (see above). Twelve-hundredth milliliter of classic hemophilic factor (factor VIII-deficient) plasma was incubated with 0.12 ml normal or patient IgG preparation in BSB in a 12 x 75 mm polystyrene tube at 37°C for 15 min. A 0.2-ml aliquot of the mixture was then incubated at 37°C with 0.05 ml 0.05 M CaCl₂ in a 10 x 75 mm glass tube. At time intervals, a 0.02-ml aliquot was removed and mixed in a 10 x 75 mm polystyrene tube with 0.08 ml citrated BSB (85 parts BSB mixed with 15 parts 0.13 M trisodium citrate) and 0.02 ml normal or patient IgG. After 1-min incubation at 37°C, 0.1 ml 0.1% soybean phosphatide and 0.1 ml Christmas factor-deficient plasma were added. After an additional 1-min incubation at 37°C, 0.1 ml 0.05 M CaCl₂ was added, and the clotting time measured.

Surface Binding of PTA to Glass Tubes

Surface binding of PTA to glass tubes was determined as described by Schiffman et al., with minor modifications. Five-hundredth milliliter of test plasma undiluted, or diluted 10-fold or 100-fold in citrated BSB, was incubated with 0.01 ml 125I-PTA (about 4,000 cpm, 60,000 cpm/ng protein) at room temperature for 30 min in a polystyrene tube. Then, 0.5 ml of the mixture was incubated in a 12 x 75 mm glass tube at room temperature for 1 hr, during which time, the original radioactivity of the sample was measured. The sample was then aspirated, and the tube washed twice with citrated BSB (2 ml each). The radioactivity bound was measured by counting the radioactivity of the washed tube in a Packard Auto-Gamma Spectrometer (Packard Instrument Co., Rockville, MD).
Clotting Assays

Routine clotting assays, including kaolin-activated partial thromboplastin time (aPTT) and one-stage prothrombin time (PT), were performed as previously described. One-stage prothrombin time was also performed using tissue factor (Permaplastin, Alban and Co., St. Louis, MO) that had been diluted 100-fold in BSB (diluted PT).

The procoagulant activities of Hageman factor, Fletcher factor, Fitzgerald factor, PTA, Christmas factor, and antihemophilic factor (AHF, factor VIII) were assayed by the modified one-stage aPTT systems. Assays for factor VII, Stuart factor (factor X), procofactor (factor V), and prothrombin (factor II) were performed by the modified one-stage prothrombin time method. The incubation period between test plasma dilution and the substrate plasma prior to the addition of 0.025 M CaCl₂ to initiate clotting for each assay is indicated in Table I.

Clotting Factor Antigen Assay

Radioimmunoassays for Hageman factor, Fletcher factor, Fitzgerald factor, PTA, Christmas factor, and antihemophilic factor (AHF, factor VIII) were assayed by the modified one-stage aPTT systems. Assays for factor VII, Stuart factor (factor X), procofactor (factor V), and prothrombin (factor II) were performed by the modified one-stage prothrombin time method. The incubation period between test plasma dilution and the substrate plasma prior to the addition of 0.025 M CaCl₂ to initiate clotting for each assay is indicated in Table I.

Clotting Factor Inhibition Assay

Clotting factor inhibition assay was performed as previously described by assaying for the residual factor activity of standard pooled plasma incubated at 37°C with an equal volume of the test sample. The inhibitor titer is defined as the dilution of the test sample that will inactivate 0.5 U/ml of standard pooled plasma following a specified period of incubation at 37°C. Unless otherwise specified, the incubation time period was 30 min.

Effect of Activated PTA on Plasma

The effect of activated PTA on various plasmas was performed as follows: 0.1 ml plasma to be tested and 0.1 ml 0.1% soybean phosphatase was incubated in 10 × 75 mm polystyrene tube at 37°C for 1 min; 0.1 ml activated PTA was then added, and immediately followed by the addition of 0.025 M CaCl₂ to initiate clotting.

Effect of Patient and Normal IgG on Activated PTA

The effect of patient and normal IgG preparation on activated PTA was assayed by incubating equal volumes of the test IgG preparation and activated PTA at 37°C for 10 min. The residual activated PTA activity on PTA-deficient plasma was then measured.

Antibody Neutralization Assay

Neutralization of PTA inhibitory activities of adsorbed and heated plasma, as well as isolated PTA preparations, by rabbit heavy chain specific antisera against human IgM, IgG, IgA, as well as κ and λ light chains (Calbiochem-Behring Corp., San Diego, CA), were performed as previously described, substituting PTA assay for AHF assay. All the antisera and control normal rabbit serum used had been sequentially adsorbed with kaolin (100 mg/ml) and

### Table 1. Clotting Factor Activities in Patient’s Plasma

<table>
<thead>
<tr>
<th>Clotting Factor</th>
<th>Time Sample Incubated With Substrate Plasma*</th>
<th>Normal Range</th>
<th>Plasma Before Protein A Adsorption†</th>
<th>Plasma After Protein A Adsorption‡</th>
<th>Antigen§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(min)</td>
<td>(U/ml)</td>
<td>1/20</td>
<td>1/200</td>
<td>1/1,000</td>
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<tr>
<td>Hageman factor (factor XII)</td>
<td>8</td>
<td>0.35–1.83</td>
<td>0.02</td>
<td>0.75</td>
<td>1.05</td>
</tr>
<tr>
<td>Fletcher factor (plasma pre-kallikrein)</td>
<td>1</td>
<td>0.50–1.50</td>
<td>0.02</td>
<td>0.68</td>
<td>1.18</td>
</tr>
<tr>
<td>Fitzgerald factor (HMM-W-kininogen)</td>
<td>8</td>
<td>0.46–1.32</td>
<td>&lt;0.01</td>
<td>0.40</td>
<td>0.95</td>
</tr>
<tr>
<td>PTA (factor XI)</td>
<td>8</td>
<td>0.50–1.56</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Christmas factor (factor IX)</td>
<td>0</td>
<td>0.65–1.85</td>
<td>0.70</td>
<td>1.75</td>
<td>1.75</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>0</td>
<td>0.46–1.75</td>
<td>1.86</td>
<td>2.50</td>
<td>3.10</td>
</tr>
<tr>
<td>AHA (factor V)</td>
<td>1</td>
<td>0.45–1.65</td>
<td>1.40</td>
<td>1.40</td>
<td>1.29</td>
</tr>
<tr>
<td>Prothrombin factor II</td>
<td>1</td>
<td>0.65–1.50</td>
<td>1.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor VII</td>
<td>1</td>
<td>0.55–0.85</td>
<td>1.43</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*This represents the length of time patient’s plasma dilution was incubated with the substrate plasma at 37°C before clotting was initiated by the addition of 0.1 ml 0.025 M CaCl₂ for the modified aPTT system or 0.2 ml 0.0125M CaCl₂-tissue factor for the modified PT system.
†Procoagulant activity, measured by a modification of the activated partial thromboplastin time or one-stage prothrombin time, as appropriate. The patient’s plasma was diluted 20-, 200-, or 1,000-fold before addition to the substrate mixture; its concentration in the final assay mixture was 1/80, 1/800, or 1/4,000, respectively.
‡Agarose-protein A (0.3 ml, 2 mg protein A/ml gel) was incubated with 3 ml patient plasma diluted 1/20 for 1 hr at RT and overnight at 4°C. The supernatant contained no PTA inhibitor activity and was assayed for the various clotting factor activities without further dilution. The inhibitor and clotting factor activities of patient’s plasma similarly adsorbed with agarose-human serum albumin (2.2 mg HSA/ml gel) were similar to those of unadsorbed plasma. None of these gels changed the clotting factor activities of normal plasma following adsorption.
§AHF-like antigen (by radioimmunoassay) as well as HF-like and Fletcher factor-like antigens (by RIA) were assayed on plasma before protein A adsorption. PTA-like antigen was assayed by RIA on plasma dilutions (1/20, see ‡ above) after protein A adsorption. RIA on plasma before protein A adsorption gave uninterpretable results on PTA-like antigen, as the plasma inhibitor bound and precipitated the 125I-PTA in the assay system. HF-like and Fletcher factor-like antigen titers before and after protein A adsorption were comparable.
calcium phosphate (20 mg/ml) and heated at 56°C for 45 min. The treated antisera contained no clot-promoting activity and did not interfere with the clotting assays. In immunodiffusion with 0.009 ml reagent in each well, each of the adsorbed and heated antisera formed single precipitin lines with patient plasma at antiserum dilutions of 1/8 for anti-IgG, IgA, IgM, and λ and 1/16 for anti-κ.

Protein A Adsorption

The effect of protein A adsorption on plasma PTA inhibitory and contact phase clotting factor activity was assessed as follows. The washed and pelleted gels from 0.3 ml agarose-protein A (2 mg protein A/ml gel, Sigma) or agarose-human serum albumin (agarose-HSA, 2.2 mg HSA/ml gel) was mixed with 0.75 ml or 3 ml of patient or control plasma diluted 5- or 20-fold, respectively, in BSB containing 0.05 g/dl sodium azide (BSB-azide). The mixture was rotated end-to-end at room temperature for 1 hr and at 4°C overnight, and then centrifuged at 2,000 g for 10 min. The supernatant was tested for PTA inhibitory activity and for titers of the four contact phase clotting factors.

Effect of Protein A-Bound Inhibitor on Plasma Clotting Factors

This was assessed as follows: the agarose-protein A and agarose-HSA, which had been incubated with the plasmas in the above experiment, was washed 5 times with BSB-azide (4.5 ml each) and incubated further with 2 ml of normal pooled plasma, diluted 20-fold in BSB-azide, for 15 min at room temperature and then overnight at 4°C. The supernatant obtained by centrifugation at 2,000 g for 10 min was assayed for titers of the contact phase clotting factors and for PTA inhibitory activity.

Immunodiffusion

Immunodiffusion was performed in 2-mm thick 1% agarose (BioRad Laboratories, Richmond, CA) on plastic film (0.2-mm thick, GelBond Film, Marine Colloids, Rockland, ME). The wells, 2.5 mm in diameter and 7 mm apart (center-to-center), were filled with 0.009 ml of preparations to be tested, or 0.009 ml patient 121H-IgG preparation, and allowed to diffuse at 4°C in a humid chamber for 36 hr. For radioautography, Kodak X-omat AR film was exposed to the washed and dried gel film for 24 hr at −70°C and then developed.

Radiolabeling of IgG

Radiolabeling of patient IgG preparations was performed by the chloramine T method, as previously described. The final preparation had radioactivity of 1.8 × 106 cpm/0.01 ml, with a specific activity of 2,000 cpm/ng labeled IgG.

PAGE

Polyacrylamide gel electrophoresis was carried out on 10% tube gels, in the presence of SDS, essentially according to the method of Weber and Osborn. The electrophoresed gels were sliced into 2-mm sections for radioactivity measurement.

Agarose-Protein Binding

Proteins were covalently bound to Sepharose 4B (4% agarose) activated by cyanogen bromide from Pharmacia Fine Chemicals (Piscataway, NJ), according to the manufacturer’s instructions.

Barbital-saline buffer (BSB) contained 0.025 M sodium barbital and 0.125 M NaCl, pH 7.35.

Protein concentration was determined by the method of Lowry et al.

CASE HISTORY

A 42-yr-old black female with a 15-yr history of documented systemic lupus erythematosus was admitted because of proteinuria (4.56 g/24 hr) and hypertension (210/110 mm Hg). She has had episodes of hair loss, myalgia, symmetrical arthralgias of knee, ankle, and finger joints, as well as a photosensitive malar rash. There was no personal or family history of bleeding diathesis. Medication on admission included oral propanolol (10 mg, 4 times daily), prednisone (15 mg every other day), sulindac (15 mg twice daily), and folic acid (1 mg daily). Examination revealed swollen deformity of fingers, and painless swelling of the left ankle without restriction of motion. There was no evidence of bleeding. She had a hematocrit of 26 ml/dl (hemoglobin 9.4 g/dl) and a WBC of 2.9 × 109/liter. Her serum blood urea nitrogen (BUN) was 29 mg/dl, with a serum creatinine of 1.7 mg/dl and a creatinine clearance of 36 ml/min. Serologic studies showed negative Veneral Disease Research Laboratory (VDRL) test and rheumatoid factor, but positive antinuclear antibody (homogeneous pattern, titer >1:1,280) and anti-native DNA (87% bound, normal up to 10%). Serum complements (β1C-β1A) were 32 mg/dl (normal 108–208 mg/dl) and CH50 was 66 U/ml (normal >170 U/ml). Coagulation screen was performed for anticipated renal biopsy. Her platelet count was 245 × 109/liter, and she had normal PT (patient 12.1 sec, control 12.2 sec) and diluted PT (patient 39.1 sec, control 40.7 sec). Her aPTT was prolonged to 88.8 sec (control 41.2 sec) and was not corrected with an equal volume of normal control plasma (assyed without prior incubation, 95.2 sec). Further investigation confirmed the presence of a PTA inhibitor (see Results). Because of the uncertainty about the possible in vivo hematostatic defects associated with the inhibitor, a renal biopsy was not performed. Her blood pressure and renal function improved upon additional antihypertensive agents as well as prednisone and immunosuppressive agents.

RESULTS

Specificity and Titer of the Inhibitor

Routine coagulation studies revealed a prolonged aPTT, which was not corrected with an equal volume of normal plasma (see Case History), indicating the presence of a plasma inhibitor interfering with the intrinsic coagulation pathway. Lupus-type inhibitor was excluded by the normal diluted PT test.

As indicated in Table 1, specific clotting factor assays revealed low titers of all four contact phase factors, i.e., PTA (<0.01 U/ml), Hageman factor (0.02 U/ml), Fletcher factor (0.02 U/ml), and Fitzgerald factor (<0.01 U/ml). Upon further dilution, the titers of Hageman, Fletcher, and Fitzgerald factors progressively increased to normal, but that of PTA remained at <0.01 U/ml, suggesting that the plasma inhibitor was directed against PTA. In the routine assays for Christmas factor and AHF, in which the patient’s plasma dilution was added immediately before the addition of CaCl2 to initiate clotting, the titers obtained were within the normal range. There was, nonetheless, a slight rise in their titers upon further dilution. When the patient’s plasma dilution was incubated with the substrate plasmas for 5 min before the addition of CaCl2, both Christmas factor
and AHF assays yielded extremely low values (<0.01 U/ml). Thus, the inhibitor was sufficiently potent to interfere with the factor assays, probably by inhibiting the PTA in the substrates within 1–8 min incubation. The four extrinsic pathway clotting factors (factor VII, Stuart factor, proaccelerin, and prothrombin) determined by the modified PT system were all normal, with no change in titers upon further dilution (Table 1). The inhibitor activity was completely removed by adsorption with agarose-bound protein A. Following protein A adsorption, the activities of Hageman, Fletcher, and Fitzgerald factors, assayed at a plasma dilution of 1:20, returned to normal (Table 1). The agarose-bound protein A, upon exposure to the patient’s plasma, presumably bound the inhibitor (see below). When this complex was added to normal diluted plasma, PTA activity and antigen were completely removed, with the activity and antigen titers of the other three contact phase factors remaining relatively unchanged. PTA inhibitor activity was not present in the supernatant plasma. These experiments further confirm that the inhibitor acts specifically against PTA.

The PTA inhibitor titer was examined by incubating equal volumes of patient plasma dilution and pooled plasma at 37°C. The titer was 1/200 without incubation, and increased only slightly, to 1/350, after 4-hr incubation. It should be noted that, during the assay for residual PTA activity of the mixture, the patient plasma dilution and the pooled plasma would have 8 min reaction at 37°C during incubation with the substrate plasma (see Table 1). This observation, together with that of the clotting factor assays, suggests that the inhibitor on native PTA is rapid acting, although not instantaneous.

Activity of the Inhibitor on Various Surface-Mediated Reactions

The following experiments provided further evidence for the PTA specificity of the inhibitor.

Following exposure of normal or PTA-deficient plasma to a glass surface, 125I-Hageman factor, present in the plasma as a probe, became activated and cleaved. Before glass contact, a single peak, representing a native molecule of mol wt 80,000, was seen. After exposure to glass, the 80,000 peak decreased and new peaks appeared at mol wt 52,000 and 29,000, indicating cleavage of HF during contact activation, as previously reported. The pattern of cleavage of 125I-HF in the patient’s plasma was identical to that in normal plasma (data not shown).

Surface (kaolin) induced generation of plasma kallikrein activity, as assessed by amidolysis of H-D-pro-phe-arg-pNA (PPAN) and enhancement of factor VII activity, requires the participation of Hageman, Fletcher, and Fitzgerald factors. These activities were normal in the patient’s plasma (Table 2). On the contrary, kaolin (surface) induced generation of plasmin activity, as assessed by the amidolysis of H-D-val-leu-lys-pNA (S-2251), requires PTA and the other three contact phase factors. This activity was defective in the patient’s plasma (Table 2). In addition, the same activity in normal plasma became defective in the presence of 5% patient plasma, but was normal in the presence of 5% PTA-deficient plasma. The falling OD405 in each case following prolonged incubation is due to the natural plasma inhibitor activities against the fibrinolytic enzymes.

Glass surface-induced generation of activated Christmas factor activity also requires all four contact phase factors. Activated Christmas factor generated by glass surface activation of classic hemophilic plasma in the presence of CaCl2 shortens the clotting time of Christmas factor-deficient plasma. In the presence of patient IgG, generation of activated Christmas factor was defective (Fig. 1). The patient IgG, however, has no effect on the activated Christmas factor activity generated in the absence of the inhibitor.

Immunoglobulin and Precipitating Nature of the PTA Inhibitor

The inhibitor was present both in the plasma and in the serum with equivalent titers. It was not removed following adsorption of plasma or serum with aluminum hydroxide (1/10 volume), tricalcium phosphate (20 mg/ml), kaolin (100 mg/ml), or celite 512 (40 mg/ml) and was not inactivated during incubation at 56°C for 1 hr. The inhibitor activity was recovered only in the IgG-containing fractions during DE-52 cellulose ion-exchange chromatography, AcA22 gel filtration, and agarose-protein A adsorption-elution.

In the antibody neutralization experiments, the inhibitory activity of the patient’s adsorbed and heated plasma or IgG fraction could be neutralized only by antisera against IgG and light chains (data not shown), suggesting that the inhibitor was a restricted IgG autoantibody.

The ability of the PTA inhibitor (125I-IgG fraction) to precipitate normal PTA is shown in Fig. 2. Normal plasma, serum, and partially purified PTA all formed precipitin lines of identity with the inhibitor. A precipitin line was not formed with a panel of seven different human PTA-deficient plasmas (only one shown) or with the patient’s own plasma. Unlike PTA-deficient plasma, the patient’s plasma interfered with the formation of a full precipitin line by normal plasma in the adjacent well.
Mechanism of Action of the PTA Inhibitor

Trypsin-activated partially purified PTA shortened the clotting time in both normal plasma and PTA-deficient plasma, but failed to do so with Christmas factor-deficient plasma or the patient's plasma (Table 3). The clotting time of the patient's plasma with undiluted activated PTA was equivalent to that obtained in PTA-deficient plasma with activated PTA diluted 40-fold, indicating that 97.5% of the activated PTA had been inactivated instantaneously in the patient's plasma. The activated PTA was also inactivated by the IgG fraction of the patient's, but not control, plasma (data not shown), suggesting that another plasma cofactor(s) was not required for the inhibitory activity.

**Table 2. Kaolin (Surface) Induced Plasma Enzymatic Activities**

<table>
<thead>
<tr>
<th>Kaolin-Induced Activities</th>
<th>Patient Plasma</th>
<th>Normal Plasma</th>
<th>Normal Plus Patient Plasma (95:5)</th>
<th>Normal Plus PTA-Deficient Plasma (95:5)</th>
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<tbody>
<tr>
<td>Plasma kallikrein generation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPAN amidolysis (%) activity*</td>
<td>104</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold-promoted factor VII activity enhancement†</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Factor VII (U/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-hr Incubation (baseline)</td>
<td>1.36</td>
<td>1.12</td>
<td>1.08</td>
<td>1.04</td>
</tr>
<tr>
<td>3-hr Incubation</td>
<td>2.40</td>
<td>2.05</td>
<td>2.18</td>
<td>2.01</td>
</tr>
<tr>
<td>Plasma plasmin generation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-2251 Amidolysis (OD₄₀₅)‡</td>
<td></td>
<td></td>
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<tr>
<td>0-min Incubation</td>
<td>0.221</td>
<td>0.336</td>
<td>0.198</td>
<td>0.301</td>
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<tr>
<td>1-min Incubation</td>
<td>0.251</td>
<td>0.395</td>
<td>0.184</td>
<td>0.304</td>
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<tr>
<td>5-min Incubation</td>
<td>0.176</td>
<td>0.363</td>
<td>0.119</td>
<td>0.320</td>
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<tr>
<td>10-min Incubation</td>
<td>0.166</td>
<td>0.428</td>
<td>0.124</td>
<td>0.320</td>
</tr>
<tr>
<td>20-min Incubulation</td>
<td>0.125</td>
<td>0.386</td>
<td>0.106</td>
<td>0.289</td>
</tr>
<tr>
<td>30-min Incubulation</td>
<td>0.123</td>
<td>0.317</td>
<td>0.095</td>
<td>0.240</td>
</tr>
</tbody>
</table>

*Test plasma (0.1 ml) diluted in 0.1 M Na phosphate, 0.15 M NaCl, pH 7.5 (PBS), was incubated with 0.1 ml kaolin suspension (10 mg/ml PBS) with shaking for 3 min at RT. Then 0.5 ml n-pro-phe-arg-pNA (PPAN), 0.0005 M in PBS, was added at 37°C for 30 min. The reaction was terminated by the addition of 0.3 ml glacial acetic acid. After centrifugation (1,400 g for 5 min), the optical density of the supernatant solution was measured at 405 nm. The amidolytic activity was compared to those obtained for serial dilutions of normal pooled plasma and expressed as percentage of activity of normal pooled plasma.

†Test plasma (0.2 ml) was incubated with 0.06 ml kaolin suspension (2.5 mg/ml BSB) in a melting ice bath for the indicated time period. The supernatant plasma was then assayed for factor VII activity. Control experiments in which plasmas were incubated with BSB instead of kaolin showed no appreciable increase in factor VII activity.

‡Test plasma (0.12 ml) was incubated with 0.04 ml kaolin suspension (50 mg in 4.2 ml BSB) with shaking in a 12 × 75 mm polystyrene tube for the indicated time period. A quantity of 2.3 ml 0.01 M Na acetate buffer (pH 4.7) was added and further incubated at 37°C for 1 hr. The resulting euglobulin-kaolin precipitate obtained by centrifugation (1,400 g for 5 min) was resuspended in 0.12 ml BSB containing 27% glycerol, 0.02 M lysine, and 0.001 M EDTA. The 0.05-ml aliquots were tested for plasmin activity by addition to 1 ml 0.6 mM H-D-val-leu-lys-pNA (S-2251) at 37°C for 10 min. The reaction was stopped by addition of 0.1 ml glacial acetic acid. After centrifugation, the optical density of the supernatant solution was measured at 405 nm. The mixtures of normal plasma with patient plasma or PTA-deficient plasma had been incubated at RT for 10 min before incubation with kaolin.

**Fig. 1.** Effect of IgG preparations on glass surface-induced generation of activated Christmas factor activity in classic hemophilic plasma. Classic hemophilic plasma was preincubated with normal (open symbols) or patient (closed symbols) IgG preparations in polystyrene tubes and then activated by incubation in glass tubes in the presence of CaCl₂, as described in Materials and Methods. At time intervals, aliquots were further incubated with normal (circles) or patient (triangles) IgG before assay for clot-promoting activity on Christmas factor-deficient plasma. Generation of activated Christmas factor will result in shortening of the clotting times.

**Fig. 2.** Immunodiffusion pattern of ¹²⁵I-IgG fraction of patient plasma against various plasma fractions. The patient ¹²⁵I-IgG preparation was placed in the center well and was allowed to immunodiffuse against normal plasma (NP), purified PTA (XI, 38 ug/ml, specific activity 134 U/mg protein), normal serum (NS), patient’s own plasma (PP), and PTA-deficient plasma (DP) in the surrounding wells.
Glass surface-induced generation of activated PTA activity could be generated in Christmas factor-deficient plasma. In the presence of control IgG (1.068 mg protein/ml) the clotting time for activated PTA assay shortened from 405 sec, after 10-min incubation, to 258 sec, after 15-min incubation; in the presence of patient IgG (1.475 mg protein/ml, inhibitor titer 1:12.5), the clotting times were 640 sec and 664 sec, respectively. Christmas factor-deficient plasma mixed with normal IgG preparation in a polystyrene tube and immediately assayed (0-sec incubation) for activated PTA activity gave a clotting time of 650 sec. The surface-induced activated PTA activity in the presence of control IgG could be completely abolished by the addition of patient, but not control, IgG.

Surface-induced 125I-PTA activation and cleavage were also inhibited by the inhibitor. Native 125I-PTA (mol wt 78,000) exposed to glass surface in normal or PTA-deficient plasma was cleaved into two radiolabeled bands with mol wt 51,000, and 32,000 in the reduced SDS-PAGE system (Fig. 3). Only one uncleaved 78,000 band was detected when 125I-PTA was exposed to glass surface in the patient’s plasma and similarly analyzed.

The inhibitor interfered with the binding of PTA to glass surfaces (Table 4). The binding to glass surface of 125I-PTA in normal and PTA-deficient plasmas increased with increasing plasma dilutions and was similar at each plasma dilution. In contrast, the binding to glass surface of 125I-PTA in patient’s plasma was significantly lower than that in normal plasma, especially at plasma dilutions of 1/10 and 1/100.

**DISCUSSION**

The PTA inhibitor in this SLE patient is a unique, potent, and rapidly acting precipitating IgG-λ autoantibody.

The antibody inhibited activated PTA (factor Xla) instantaneously, without the requirement for additional cofactors. In addition, it also inhibited the surface-mediated generation of plasma PTA activity and proteolytic cleavage of PTA. The latter could, at least in part, be accounted for by its interference with the binding of PTA onto the reactive surface, a step essential for the interaction of PTA with other contact phase clotting factors for its subsequent activation. Although Schifman et al.9 have described a plasma inhibitor of PTA adsorption to surface, their inhibitor did not block activated PTA activity. The mechanism of action of another PTA inhibitor, described recently by Stern et al.2 in a patient with congenital PTA deficiency, was quite similar to that of ours. Their PTA inhibitor, which also prevented HF-mediated 125I-PTA activation and 125I-PTA binding to surface-bound HMW-kinogen, was a polyclonal IgG-κ/IgG-λ antibody. Although antibodies in both patients bound PTA, our autoantibody of restricted diversity (IgG-λ) is unique in its ability to form precipitates with plasma and serum, as well as purified PTA.

The rapid action of the inhibitor is also unique among PTA inhibitors and appears to account for the interesting finding of apparent concomitant multiple intrinsic pathway clotting factor deficiencies in the

**Table 3. Inhibition of Activated PTA by Patient’s Plasma**

<table>
<thead>
<tr>
<th>Test Condition</th>
<th>Clotting Time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted Activated PTA in</td>
<td></td>
</tr>
<tr>
<td>Normal plasma</td>
<td>58</td>
</tr>
<tr>
<td>PTA-deficient plasma</td>
<td>63</td>
</tr>
<tr>
<td>Christmas factor-deficient plasma</td>
<td>300</td>
</tr>
<tr>
<td>Patient plasma</td>
<td>162</td>
</tr>
<tr>
<td>Diluted (1/40) activated PTA in</td>
<td>163</td>
</tr>
<tr>
<td>PTA-deficient plasma</td>
<td></td>
</tr>
</tbody>
</table>

Plasma to be tested (0.1 ml) and 0.1 ml soybean phosphatide (0.1% in 0.15 M NaCl) was incubated in 10 × 75 mm polystyrene tube at 37°C for 1 min; 0.1 ml activated PTA was then added, immediately followed by the addition of 0.025 M CaCl₂, and the clotting time measured. The activated PTA used was obtained by activation of partially purified PTA with agarose-bound trypsin and had a procoagulant activity of 0.40 U/ml.

**Table 4. Binding of 125I-PTA to Glass Surface**

<table>
<thead>
<tr>
<th>Test Plasma</th>
<th>Undiluted</th>
<th>1/10 Diluted</th>
<th>1/100 Diluted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal plasma</td>
<td>2.14 ± 0.01</td>
<td>5.51 ± 0.30</td>
<td>12.58 ± 0.63</td>
</tr>
<tr>
<td>PTA-deficient plasma</td>
<td>2.10 ± 0.33</td>
<td>5.01 ± 0.47</td>
<td>11.45 ± 1.45</td>
</tr>
<tr>
<td>Patient plasma</td>
<td>0.44 ± 0.29</td>
<td>1.16 ± 0.25</td>
<td>2.75 ± 0.14</td>
</tr>
</tbody>
</table>

Details of the binding experiment are outlined in Materials and Methods. The results represent an average of three experiments.
patient's plasma. When clotting factors were assayed in the patient's plasma diluted 20-fold, titers of Hageman, Fletcher, and Fitzgerald factors were found to be unexpectedly low (<0.01-0.02 U/ml). When the plasma was further diluted, the titers of the three contact phase clotting factors progressively increased to normal. It was obvious that the patient's plasma inhibitor interfered with the various factor assays by inhibiting the PTA in each substrate plasma, creating additional clotting defects in addition to the original substrate factor deficiency. Following removal of the inhibitor by protein A adsorption, these clotting factors, assayed at plasma dilution of 1:20, became normal, further indicating that the inhibitor had no specific activity on these factors. It is noted that the inhibitor interfered less with Christmas factor and AHF assays, in which the plasma inhibitor was in contact with the substrate plasma only immediately prior to the initiation of clotting. In the assays for the contact phase factors, the plasma inhibitor was in contact with the substrate plasma for 1 min or longer. The apparently low titers of Christmas factor and AHF obtained in a modified assay system in which the inhibitor plasma was incubated with the substrate for 5 min, as is practiced in many clinical laboratories, confirmed this conclusion. Thus, the action of the inhibitor on native PTA is rapid, although not instantaneous. We have previously documented an AHF inhibitor with similar multiple apparent intrinsic pathway clotting factor deficiencies. At least three of the cases in the literature with an inhibitor against PTA were reported to have low Hageman factor level, and an inhibitor active against both clotting factors has been postulated. We believe that similar interference of clotting factor assays by an inhibitor specific for only one clotting factor may have also been operative in these patients.

The specificity of this inhibitor has been further confirmed in this study. Agarose-protein A-bound inhibitor removed plasma PTA activity and antigen selectively. The inhibitor did not interfere with surface-mediated proteolytic cleavage of Hageman factor. The surface-mediated plasma reaction requiring contact phase factors other than PTA, including the generation of kallikrein activity as measured by synthetic substrate amidolysis and factor VII activity enhancement in the cold, were completely normal. In addition to inhibition of PTA activation, surface-mediated reactions that require the participation of PTA were defective. These included the generation of plasmin activity and the generation of activated Christmas factor activity. However, the activity of the activated Christmas factor that has been generated by surface activation was not influenced by the inhibitor.

Patients with congenital PTA deficiency have variable bleeding symptoms. Of the patients reported with PTA inhibitor, only one patient had postoperative bleeding that could be attributed to the inhibitor. The two patients with congenital PTA deficiency with PTA inhibitor had bleeding diathesis prior to the development of the inhibitors, which certainly complicated the management of hemorrhage. Our patient had no symptoms of spontaneous bleeding diathesis. Because a surgical procedure was not performed in this patient, the effect of the inhibitor, and therefore the role of PTA, on postoperative hemostasis could not be evaluated.

ACKNOWLEDGMENT

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REFERENCES

A unique precipitating autoantibody against plasma thromboplastin antecedent associated with multiple apparent plasma clotting factor deficiencies in a patient with systemic lupus erythematosus

MC Poon, H Saito and WJ Koopman