Immune Complexes Containing Factor V in a Patient With an Acquired Neutralizing Antibody

By Hui Chong Chiu, A. Koneti Rao, Cheryl Beckett, and Robert W. Colman

An 82 year-old woman presented with extensive hematomas and melena associated with markedly decreased plasma factor V coagulant activity (FV:C). Using a competitive enzyme-linked immunosorbent assay developed in our laboratory, we made serial measurements of factor V antigen (FV:Ag) in plasma and found it to be normal or elevated. The patient’s plasma was demonstrated to contain an IgG antibody that could neutralize FV:C in normal plasma. The antibody was of restricted heterogeneity (IgG1, IgG2K). Circulating immune complexes containing antibody to factor V and FV:Ag were demonstrated directly in the plasma by immunoelectrophoresis with polyclonal monospecific antibody and with a monoclonal antibody using an enzyme-linked immunosorbent assay. Presence of neutralizing antibody could be demonstrated in vitro even at times when FV:C was within normal limits by heat inactivation of FV:C. Treatment with plasma and platelet transfusions as well as plasmapheresis induced definite but transient elevation of FV:C. Steroid therapy lowered the neutralizing antibody concentration and produced a rapid and persistent elevation of FV:C during two separate hospitalizations. This report describes a patient in whom levels of FV:Ag have been serially measured, and the presence of circulating immune complexes consisting of factor V and a neutralizing antibody have been directly demonstrated.

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MATERIALS AND METHODS

Clinical Information

The patient was an 82-year-old white female admitted to the Temple University Hospital with a one-week history of spontaneous development of extensive ecchymoses and melena. At the time of admission, she had frank hematuria as well. She denied previous history suggestive of a bleeding diathesis, and any recent symptoms of fever, chills, respiratory infections, or weight loss. She had been in good health except for previous episodes of pain in the small joints of the hands and the wrists without recent exacerbation. The past and family history were unremarkable. She had been taking aspirin, vitamin C (500 mg), and vitamin E (ten drops of an unknown preparation) daily prior to the admission. The physical examination revealed extensive hematomas extending from the low back area to the lateral aspect of her thighs, with numerous other smaller ones on her abdomen and extremities. Petechiae were noted on her feet. No hepatosplenomegaly or lymphadenopathy was noted. At admission, the hemoglobin was 6.1 g/dL; the total WBC count was 9,100/μL with a normal differential. The peripheral smear showed no microangiopathic changes in RBC morphology. There was no biochemical evidence of underlying hepatic or renal disease. Other pertinent laboratory results included an elevated serum rheumatoid factor titer (1:1,250) and normal serum antinuclear antibody titer (1:20).

The results of the initial coagulation studies are summarized in Table I and the subsequent course is shown in Figs 1 and 2. The factor V activity was 0.03 U/mL on admission (normal, 0.50 to 1.50 U/mL). The patient was treated with transfusions of fresh-frozen plasma, packed RBCs, and platelets, which resulted in a rise in FV:C to 0.19 U/mL on day 3 and control of the bleeding (Fig 1). On the fifth day, she was subjected to plasmapheresis. A total of 2,200 mL of plasma was removed and replaced with seven units of fresh-frozen plasma. Despite a further rise in FV:C to 0.30 U/mL, the coagulant activity declined to less than 0.01 U/mL by day 10. On the 12th day, exchange transfusions were performed; two units of whole blood were exchanged with two units of packed RBCs and four units of fresh-frozen plasma. She was started on prednisone 60 mg/d that night. On the 17th day, the FV:C was 0.30 U/mL. She was discharged on the 20th day with FV:C in the normal range (0.75 U/mL). The steroids were gradually tapered over a period of the next four weeks.

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Laboratory Studies at the Time of the First Hospital Admission

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin time</td>
<td>42 s (10–14 s)</td>
</tr>
<tr>
<td>Activated partial</td>
<td></td>
</tr>
<tr>
<td>thromboplastin time</td>
<td>&gt;100 s (25–40 s)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>285 mg/dL (200–400 mg/dL)</td>
</tr>
<tr>
<td>Prothrombin</td>
<td>1.20 U/mL (0.50–1.50 U/mL)</td>
</tr>
<tr>
<td>Factor V</td>
<td>0.03 U/mL (0.50–1.50 U/mL)</td>
</tr>
<tr>
<td>Factor VII</td>
<td>2.00 U/mL (0.50–1.50 U/mL)</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>1.70 U/mL (0.50–1.50 U/mL)</td>
</tr>
<tr>
<td>Factor IX</td>
<td>2.10 U/mL (0.50–1.50 U/mL)</td>
</tr>
<tr>
<td>Factor X</td>
<td>1.00 U/mL (0.50–1.50 U/mL)</td>
</tr>
<tr>
<td>Platelet count</td>
<td>250,000/μL (140,000–400,000/μL)</td>
</tr>
</tbody>
</table>

Six weeks after the steroids were discontinued, the patient was found to have new petechiae on the lower extremities but was asymptomatic. The prothrombin time (PT) was 32 seconds and the activated partial thromboplastin time (APTT) was >100 seconds; the FV:C was less than 0.01 U/mL. Five days later, she was admitted to the hospital with gross hematuria; the APTT and PT were 94 and 27 seconds, respectively, with FV:C of less than 0.01 U/mL (Fig 2). She was transfused with two units of fresh-frozen plasma and was administered one intravenous injection of methylprednisolone (40 mg). The laboratory studies a few hours later revealed an APTT and PT of 81 and 27 seconds, respectively, with FV:C unchanged. She received a transfusion of six units of platelet concentrates. Six hours later, the APTT was 50 seconds and the PT was 24 seconds without appreciable change in FV:C. By the third hospital day, there was further shortening of APTT and PT to 35 and 19 seconds, respectively, with a transient rise in FV:C to 0.04 U/mL. Over the next four days, there was persistence of the relative shortening in APTT (35 to 46 seconds) and PT (19 to 23 seconds), even though the FV:C showed no appreciable rise. Because of persistently low levels of FV:C and subsequent prolongation of APTT and PT, she was started on prednisone 80 mg/d on the ninth day. The FV:C levels rose promptly, 0.30 U/mL on the morning of the 12th day, and she was discharged from the hospital on prednisone (60 mg/d). Over the next five weeks, the prednisone was tapered to a dose of 30 mg, administered on alternate days and subsequently maintained on low doses (10 to 20 mg) on alternate days for the next five months, during which time she remained in remission.

General Methods

Blood was collected from the patient and normal controls in 1:10 volume of 3.8% sodium citrate and centrifuged at 2,000 g for 15 minutes at 4 °C. The platelet-poor plasma was stored at −70 °C. Platelet-riche plasma (PRP) was prepared by centrifuging blood collected in 3.8% sodium citrate for ten minutes at 200 g at room temperature. The bleeding time was performed by the template method (Simplate II, General Diagnostics, Morris Plains, NJ). Platelet aggregation was studied using a Payton (Buffalo) aggregometer during stimulation of PRP with ADP (8 μmol/L), epinephrine (4 to 8 μmol/L), collagen (2.5 μg/mL), and platelet-activating factor (2 μmol/L). Secretion from the dense granules was examined by measuring the release of 14C-serotonin from prelabeled platelets during stimulation with the above agonists as described elsewhere. All chemicals used were reagent grade or better.

Plasma and Antibodies

Plasma selectively deficient in factor V was prepared for the specific substrate FV:C assay by incubating plasma with 10 mmol/L EDTA, pH 7.5, at 37 °C for five hours according to the methods of

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**Fig 1.** Serial changes in factor V coagulant activity (●●●) and antigen (○○○; mean ± SE) during first hospitalization. Also shown are the therapeutic interventions. The assay for the antigen was performed in triplicate. FFP, fresh-frozen plasma.

**Fig 2.** Serial changes in factor V coagulation activity (●●●) and antigen (○○○) during the second hospitalization. The first sample (−5 days) was obtained five days prior to admission to the hospital. Also shown are the therapeutic interventions.
Nesheim et al. The artificially deficient plasma had less than 0.01 U/mL FV:C.

Pooled normal plasma and congenital FV:C-deficient plasma were purchased from George King Biomedical, Overland Park, Kan. Paranitrophenyl phosphate and goat-antirabbit IgG conjugated with alkaline phosphatase were supplied by Sigma Chemical Co, St Louis. Human immunoglobulin subtyping kits were purchased from Miles Laboratories, Elkhart, Ind, and Boehringer Mannheim Biochemicals, Indianapolis. Monospecific rabbit–anti-human factor V antibodies and purified human factor V were prepared as described previously.4

A mouse monoclonal antibody against human factor V was produced. BALB/c mice 3 to 4 months old were immunized with purified human factor V (75 μg) intraperitoneally with complete Freund's adjuvant and were boosted with 50 μg in saline 14 days later. Three days later, the mice spleens were aseptically removed and the cells (10^9/mL) were fused with P3-X63/Ag 8.653 mouse myeloma cells (10^9/mL) in 38% polyethylene glycol (mol wt 1,450) for one minute at 37 °C. After centrifugation for five minutes at 200 g, 3 mL of serum-free Dulbecco's modified Eagle's medium was added. The cells were then washed and reseeded in a medium containing hypoxanthine (13.6 mg/L), aminopterin (200 μL/L), and thymidine (3.87 mg/L), along with 15% fetal calf serum, gentamycin (50 mg/L), penicillin (25,000 U/L), streptomycin (25 mg/L), and L-glutamine (2 mmol/L). They were cloned by limiting dilution, distributed in 2 mL of Linbro tissue culture plates, and cultured for two weeks. The culture fluids were tested for antibody by adding 100 μL to polystyrene cuvettes precoated with factor V. After washing, the bound antibody was detected using the ELISA. A factor V non-neutralizing antibody (B10.27.3) was detected, recloned, and propagated in the ascites of pristane-prepared mice. A mouse monoclonal (IgG,κ) antibody was purified by precipitation with 33% saturated ammonium sulfate solution, followed by affinity chromatography on protein A-Sepharose CL-4B or on diethylaminoethanol (DEAE) Affi-gel blue. Immunoblotting indicated that the antibody recognized intact factor V and one of the activation peptides (mol wt 150,000) after thrombin cleavage of factor V (A. Gerwitz, H.C. Chiu, A. Annamali, K. Dosai, and R.W. Colman, submitted for publication, 1985).

**Assay of Factor V Coagulant Activity and Antigen**

Factor V coagulant activity was assayed by the one-stage clotting method of Ware et al, using artificially deficient factor V plasma and an automatic fibrometer. (BBL Fibro System, Fisher Scientific Co, Pittsburgh). One unit of FV:C is defined as the amount of factor V coagulant activity in 1 mL of normal human plasma. Factor V antigen was measured using purified human factor V and monospecific rabbit anti-human factor V antibodies as reagents in a competitive ELISA method described previously.5 All determinations of factor V:Ag were assayed for at least three dilutions and only those falling in the linear range were selected for calculation. The level of residual FV:C in the mixtures of normal and patient plasma, after neutralization, were expressed as percentages of the activity of normal plasma incubated with assay buffer (0.02 mol/L Na barbiturate, pH 7.2, containing 0.12 mol/L NaCl) as control.

**Immunoechemical Characterization of the Inhibitor to Factor V**

The nature of the inhibitor in patient plasma was demonstrated in a neutralization assay by preincubation of the heat-inactivated patient plasma with anti-human immunoglobulin of each type (IgG, IgA, IgM, IgD, and IgE) for two hours at room temperature. The mixtures were then assayed by their ability to neutralize FV:C in human normal plasma by incubating overnight equal volumes of each at 4 °C, as described above. Controls with mixtures of heat-inactivated patient plasma and normal human plasma, anti-human immunoglobulin antisera and normal plasma, and normal plasma and buffer were included in each experiment.

The factor V inhibitors were also characterized as to their heavy-chain subclass and light-chain type by an ELISA method. Purified factor V was coated onto the polystyrene cuvettes and incubated with preheated patient plasma or normal human plasma as a control. The tubes were incubated subsequently with rabbit antibody to human immunoglobulin subtypes and then with alkaline phosphatase-conjugated goat-antirabbit IgG. Cuvettes were thoroughly washed after each step. Following incubation with the chromogenic substrate solution, p-nitrophenyl phosphate, the absorbance was measured at 405 nm at a fixed time interval after addition of the substrate. The difference of absorbance at 405 nm between patient and normal plasma was used to determine the immunoglobulin class or heavy- and light-chain type of antibody tested.

**Demonstration of Antibody–Antigen Complexes by Immunoelectrophoresis**

The antibody present in patient plasma was characterized as to whether it was free or complexed with factor V by immunoelectrophoresis. Patient plasma was electrophoresed in a 2% agarose gel in the first dimension and then diffused in the second dimension against the monospecific rabbit–anti-human factor V and rabbit–anti-human IgG antibodies to form the specific precipitation lines. The gel was washed, dried, and then stained with Coomassie brilliant blue.

**Detection of Specific Immune Complexes by a Modified ELISA Using a Monoclonal Antibody**

A "sandwich" ELISA method was developed to detect the presence of specific immune complexes containing factor V and anti-factor V antibody. The mouse monoclonal, non-neutralizing anti-human factor V antibody (B10.27.3) directed against the 150,000-dalton fragment of factor V, which is not part of factor Va, was chosen to coat the plastic surface and serve as a solid phase to bind both factor V and factor V-antifactor V immune complexes in the patient plasma. Since the antibody in the patient plasma neutralizes the FV:C, it is presumably directed against the portion of factor V containing coagulant activity (factor Va), which is distinct from the epitope recognized by the mouse antibody. Thus, these two antibodies would not compete with each other and the immune complexes could bind to the immobilized mouse antibody. A sample of the patient’s plasma obtained on the first day of the second admission was studied; this sample showed no free FV:C, despite the presence of greater than normal FV:Ag level. Since this plasma could further neutralize FV:C in normal plasma, it was hypothesized that all factor V molecules existed in the plasma as immune complexes with...
a slight excess of the antibody. The plasma was compared with normal plasma as a control. To detect the human antibody to the factor V associated with the bound plasma factor V, an alkaline phosphatase conjugated IgG of goat-anti-human IgG was next incubated followed by p-nitrophenyl phosphate. This “sandwich” technique would be positive only if factor V-antibody complexes were present in the plasma.

**Measurement of Factor V in Platelets After Release by Thrombin**

PRP was subjected to gel filtration as described elsewhere. Platelet suspensions (1 mL) were incubated with saline or bovine thrombin (Parke-Davis, Morris Plains, NJ) at a concentration of 5 U/mL for five minutes at 37°C and centrifuged at 12,000 g for two minutes, and the supernatants were frozen. Levels of FV:Ag were measured as described above in the supernates.

**Effect of Platelets in Correcting the Defect in Patient Plasma**

Because of a previous report that platelet transfusions may be beneficial in at least partial correction of the coagulopathy in patients with factor V inhibitors and because of the shortening of the PT noted following platelet transfusions during the second hospitalization of our patient, we examined the effect of normal platelets in correcting the abnormality in the patient's plasma in the following experiments. PRP from normal donors who denied ingesting any drugs for at least ten days was washed twice by the albumin density gradient separation method as described by Walsh. Different volumes (0.25, 0.5, and 1.0 mL) of the final platelet suspension (3 to 7 x 10^5 platelets per milliliter) were pelleted by centrifugation at 1,000 g for 15 minutes at 4°C and the plasma was discarded. The pellets were resuspended by gentle agitation and incubated for 60 minutes at 37°C in either patient or normal plasma (1 mL) that had been heat-inactivated (at 56°C for 30 minutes). The samples were then centrifuged at 2,500 g for 20 minutes at 4°C and APTT, PT, and FV:C were measured in the plasma. Experiments were also performed using washed aspirin-treated (8.8 mmol/L) normal platelets and using plasma from a patient congenitally deficient in FV:C. Lastly, the effect of incubating the patient’s platelets (obtained while in remission) with her plasma obtained during relapse was also studied. Separate experiments were performed to determine if incubation of the patient's plasma with normal washed platelets resulted in a decrease in its ability to neutralize FV:C in normal plasma in mixing experiments. In these experiments, the patient's plasma was preheated at 56°C for 30 minutes before incubation with normal washed platelets. The resultant plasma was tested for its inhibitory effect as described already.

**RESULTS**

**General Laboratory Evaluation of Hemostatic Function**

Results of the studies obtained at the time of the first hospital admission are shown in Table 1. The abnormalities are those expected for an isolated low FV:C, ie, a prolonged APTT and PT. The bleeding time measurements were made on several occasions. It was not measured at the time of her first admission, since she had recently ingested aspirin. However, on the 11th day, it was found to be eight and nine minutes in duplicate (normal, two to eight minutes). Interestingly, one of the earliest manifestations of the relapse following the discontinuation of steroid therapy was the development of petechiae, predominantly on her extremities. Duplicate bleeding time measurements performed five days prior to the second admission were nine and 11 minutes, respectively. Platelet aggregation and 14C-serotonin secretion during stimulation with adenosine diphosphate (ADP) (4 and 8 μmol/L), epinephrine (4 and 8 μmol/L), collagen (2.5 μg/mL), and platelet-activating factor (2 μmol/L) were within normal limits. The repeat bleeding times on the 11th day following institution of steroid therapy were five and six minutes when FV:C level was 0.30 U/mL.

**Serial Changes in Factor V Coagulant Activity and Antigen: Effect of Therapy**

The FV:C in the patient plasma at time of admission to the hospital was only 0.03 U/mL. However, the FV:Ag level was normal (12.5 μg/mL), as determined by the competitive ELISA. The FV:C and the corresponding FV:Ag levels in the patient samples during the first hospitalization are shown in Fig 1. Despite low levels of FV:C, FV:Ag levels were consistently elevated in all patient samples. Plasma and platelet transfusions as well as plasmapheresis induced a transient elevation of both FV:C and FV:Ag levels, followed by a decrease to admission levels. Exchange transfusions with normal plasma concomitant with administration of prednisone (60 mg/d) led to a rapid progressive rise in FV:C and FV:Ag, which persisted as long as steroids were continued. The ratio of FV:C to FV:Ag was 0.03 on admission and rose to normal levels (0.82) during treatment with steroids. After discharge, steroids were tapered and discontinued. On the second admission (Fig 2), FV:C was again less than 0.01 U/mL and remained low despite normal FV:Ag. On administration of prednisone, a prompt rise in FV:C occurred.

**Neutralization of Factor V Coagulant Activity by the Patient Plasma**

Table 2 presents the results of a representative neutralization experiment, performed with the plasma sample obtained on the first day of admission, to demonstrate the presence of inhibitors to factor V in patient plasma. At higher relative amounts of patient to normal plasma, the percentage of neutralization increased until at 16 parts patient to one part normal, 95% of the FV:C was neutralized. When plasma samples from the patient used in the neutralization assay were heated at 56°C for 30 to 60 minutes, no FV:C was detected prior to mixing with normal plasma. These heat-inactivated samples showed greater extents of factor V neutralization at all antibody concentrations than the same samples that were not
heat-treated (Fig 3). Thus, inactivation of endogenous FV:C allowed more complete expression of the ability of the antibody to neutralize exogenous factor V. This result was noted whether or not a substantial amount of FV:C was present in the patient’s plasma, suggesting that part of the effect was due to dissociation of factor V–antibody complexes. Heat-treated plasma samples, obtained before the steroid treatment (second admission, day 7), showed greater ability to neutralize FV:C in mixing experiments than two samples following steroid treatment (days 29 and 43) at all plasma concentrations (Fig 4). Thus, the neutralizing antibody titer correlated inversely with FV:C. Further, heat treatment permitted the demonstration of the presence of the antibody even at times when the FV:C was within normal limits; this was not observed in nonheated samples.

**Immunochemical Characterization of the Factor V Inhibitors**

The immunoglobulin class was demonstrated to be IgG by neutralization of factor V inhibitor activity by an antiserum against human IgG. The inhibitory activity of factor V antibody in two different plasmas was neutralized by a rabbit–anti-human IgG antiserum in a dose-dependent manner (Fig 5). The antibody titer in the plasma prior to steroid therapy was higher than that after steroids and neutralized to a lesser degree by the same concentration of anti-IgG antiserum. No other immunoglobulin classes were represented in the

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**Table 2. Neutralization of Factor V Coagulant Activity in Normal Plasma by Patient Plasma**

<table>
<thead>
<tr>
<th>Ratio of Buffer or Patient Plasma to Normal Plasma</th>
<th>Buffer Control Coagulation</th>
<th>Patient Plasma Coagulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (s) U/mL</td>
<td>Time (s) U/mL</td>
<td>Neutralization (%)</td>
</tr>
<tr>
<td>2</td>
<td>39.0 0.500</td>
<td>49.5 0.200 60</td>
</tr>
<tr>
<td>4</td>
<td>46.2 0.250</td>
<td>65.4 0.074 70</td>
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<tr>
<td>8</td>
<td>57.7 0.125</td>
<td>91.5 0.023 82</td>
</tr>
<tr>
<td>16</td>
<td>66.5 0.062</td>
<td>116.5 0.003 95</td>
</tr>
</tbody>
</table>

Ten microliters of patient plasma was incubated with 10 µL of the serially diluted pooled normal plasma and 80 µL of assay buffer for 18 hours at 4 °C. The mixtures were then assayed for the residual FV activity by clotting assay. Normal plasma dilutions incubated with buffer instead of the patient’s plasma were used as controls.

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**Fig 3.** The effect of heat treatment on the factor V-inhibitory activity of the patient plasma. The neutralizing activity of unheated (●●●●) and heat-treated (56 °C for 30 minutes; ○○○○) patient plasma was tested by incubating a constant amount of normal plasma and 80 µL of assay buffer for 18 hours at 4 °C. The mixtures were then assayed for the residual FV activity by clotting assay. Normal plasma dilutions incubated with buffer instead of the patient’s plasma were used as controls.

**Fig 4.** The effect of steroid treatment on the factor V-inhibitory activity of patient plasma. The factor V-inhibitory activity of heat-treated (56 °C for 30 minutes) patient plasma samples obtained during the second hospitalization before (day 7, ●●●●) and while on (day 29, x-x-x, and day 43, □-□-□) treatment with prednisone was tested by incubating a constant amount of normal plasma with different amounts of patient plasma. The residual FV:C in the incubation mixtures was measured by coagulation assay and expressed as a percentage of the control, in which the assay buffer was used in place of patient plasma.

**Fig 5.** Neutralization of the factor V-inhibitory activity of the patient plasma by anti-human IgG antiserum. Heat-treated (56 °C for 30 minutes) patient plasma (20 µL) was preincubated with different amounts of anti-human IgG antiserum at room temperature for two hours. Ten microliters of human normal plasma was added (1:8 dilution) and incubated at 4 °C for 18 hours. The total volume of the incubation mixture was made up to 100 µL, with factor V substrate and the FV:C in the resulting incubation mixture measured by clotting assay. The percentage of inhibition was calculated from the measured FV:C as follows: % inhibition = (normal plasma alone – incubation mixture/normal plasma alone) x 100. The FV:C in the mixture of heat-inactivated patient plasma and anti-human IgG antiserum was negligible. Two samples were taken for the assay: ●●●●, plasma collected five days before the second admission to the hospital (before steroid therapy), and ○○○○, plasma collected 85 days following admission (on steroid therapy).
antibody against factor V (Fig 6), since in an ELISA assay, IgA, IgM, IgD, and IgE antibodies that could bind to factor V were not detected. Only antibodies to human IgG, IgG, and κ-light chains reacted with the factor V antibodies in patient plasma (Fig 6).

**Demonstration of Immune Complexes Composed of Factor V and an IgG Antibody to Factor V**

Immunoelectrophoresis of concentrated patient plasma showed a single broad long precipitin arc against anti-human–IgG antiserum (Fig 7). Two arcs merging to display a reaction of identity were observed when patient plasma was diffused against monospecific rabbit–anti-human factor V antiserum. Normal plasma showed a single precipitin arc against anti-human IgG and a single precipitin arc against rabbit–anti-human factor V antiserum, each corresponding in position to one of the arcs seen with patient plasma (Fig 7). The fact that a precipitin arc was observed against each antiserum with patient plasma and that they fused in a line of identity suggested the presence of free factor V as well as a factor V–antibody immune complexes in her plasma. Further confirmation of this observation was that following adsorption of factor V from the patient plasma to the non-neutralizing monoclonal antibody coated on a polystyrene surface, a heterologous antibody to human IgG also adsorbed, indicating the existence of an IgG linked to factor V in the patient’s plasma (Fig 8). Normal plasma showed a much lesser degree of absorbency at 405 nm. Addition of purified factor V to patient’s plasma reduced the absorbance due to competition of the added free factor V with the factor V in immune complexes in the patient’s plasma for the solid-phase coated antibody. However, due to the presence of free antibody in patient plasma that would react with solid-phase factor V, only partially reduced absorbency was observed. Thus, we have directly demonstrated an IgG–factor V complex by two distinct methods.

**Platelet Factor V in the Patient With Inhibitor**

Following gel filtration of platelets from the patient or normal individual, factor V was released by incubating platelets with bovine thrombin (5 U/mL) under conditions known to release >80% of factor V. The platelets were centrifuged at 12,000 g for two minutes and FV:Ag was assayed in the supernatant. The normal platelets released 1.13 μg FV:Ag/10⁶ platelets, while the patient’s platelets released 0.97 μg FV:Ag/10⁶ platelets. Therefore, no substantial differences were observed for the FV:Ag levels present in the patient’s platelets as compared with normal plasma.

**Effect of Platelets in Correcting the Defect in Patient Plasma**

Incubation of patient’s plasma with washed normal platelets resulted in a shortening of the APTT without
an appreciable effect on the PT or FV:C levels in the harvested plasma (Fig 9A); this was dependent on the platelet concentration. Pretreatment of the normal platelets with aspirin did not prevent the shortening of the APTT (data not shown). When the patient’s plasma containing the inhibitor was incubated with her own platelets obtained while in remission, a similar shortening was noted in the APTT, but without salutary effect on PT or FV:C (Fig 9B). Further, normal washed platelets induced shortening of APTT of plasma from a patient with congenital deficiency of FV:C (Fig 9C). We next investigated the possibility that normal intact platelets may be instrumental in decreasing the inhibitor levels in patient plasma. Neutralizing experiments involving mixing of normal plasma with heated patient plasma preincubated with normal platelets revealed that the platelets did not decrease the FV:C-inhibitory capacity of the patient plasma (Table 3).

**DISCUSSION**

The clinical presentation of the patient described was typical for individuals with acquired factor V inhibitors. With one exception occurring in a patient with a hereditary factor V deficiency, all inhibitors have occurred in normal individuals in an older age group. The prolonged APTT and PT are typical and the hemorrhage can vary from mild to severe, as in this case. The inhibitor can disappear spontaneously and frequently lasts less than ten weeks. In this individual, the inhibitor persisted for at least 25 weeks.

In most cases, it has been difficult to assess the result of therapy. In the present case, it has been possible to clearly document the clinical and laboratory effects of therapy. A response to corticosteroids (Fig 1) was followed by a relapse off steroid therapy. A second response on reinstitution of prednisone (Fig 2) strongly suggests that this therapy did influence the course of the disease. Moreover, steroid therapy not only increased FV:C but decreased the titer of the neutralizing antibody (Fig 4), suggesting a direct effect on antibody synthesis.

A second unique feature of this report was the demonstration of circulating immune complexes containing FV:Ag. Thus, on initial presentation and subsequent relapse, the FV:C was less than 0.05 U/mL, while the FV:Ag (12.5 μg/mL) was greater than

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**Table 3. Inability of Normal Platelets to Adsorb the Factor V Antibody in Patient Plasma**

<table>
<thead>
<tr>
<th>Plasma Clotting Time (s)</th>
<th>Normal</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>No platelets</td>
<td>48</td>
<td>96</td>
</tr>
<tr>
<td>1.75 x 10⁸ Platelets/mL</td>
<td>48</td>
<td>114</td>
</tr>
<tr>
<td>3.49 x 10⁸ Platelets/mL</td>
<td>48</td>
<td>120</td>
</tr>
<tr>
<td>6.98 x 10⁸ Platelets/mL</td>
<td>48</td>
<td>104</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial, nonheated PPP</td>
<td>35</td>
<td>140</td>
</tr>
<tr>
<td>Heat-treated PPP</td>
<td>122</td>
<td>142</td>
</tr>
<tr>
<td>Heat-treated PPP (80 μL) plus PNP (20 μL)</td>
<td>48 128</td>
<td></td>
</tr>
<tr>
<td>Buffer (80 μL) plus PNP (20 μL)</td>
<td>—</td>
<td>—</td>
</tr>
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Heat-inactivated (56 °C for 30 minutes) patient or normal platelet-poor plasma (PPP) was incubated with an increasing number of washed normal platelets at 37 °C for 60 minutes and centrifuged to harvest the plasma. The supernatant plasma (80 μL) was incubated with pooled normal plasma (PNP) 20 μL for 18 hours at 4 °C. A 10-μL aliquot of the incubation mixture was then used to assay factor V:C.
normal (11.1 μg/mL). Two explanations could account for the marked excess of FV:Ag over coagulant activity. First, the patient might have a congenital antigen-positive variant of factor V deficiency similar to four cases recently reported from this laboratory. This possibility was ruled out by mixing experiments in which normal plasma failed to correct the deficiency of FV:C in patient plasma. The second explanation is the presence of circulating immune complexes composed of inactive factor V (FV:Ag) and an antibody directed against it.

Several lines of evidence support the latter hypothesis. The ability of patient plasma to neutralize FV:C in normal plasma was blocked by a rabbit antiserum to human IgG in a dose-dependent manner (Fig 5), indicating that the anticoagulant was an immunoglobulin. In addition, the antibody was characterized using the ELISA technique. The typing was negative for IgA, IgM, IgG3, IgG4 and λ but positive for IgG1, IgG2, and κ-light chains (Fig 6). This is the first antibody against human factor V to be typed by an ELISA binding assay. Most previous factor V inhibitors have been identified by immune neutralization as IgG. Two had both IgM and IgG antibodies and one had both IgA and IgG antibodies. Since most of these antibodies had both κ- and λ-light chains, they were polyclonal. One monoclonal antibody (IgGκ, λ) has been described. The present antibody, with only one type of light chain (κ) and two types of heavy chains, γ1 and γ2, is either biclonal or of restricted heterogeneity. Similar restricted heterogeneity (IgGκ, κ, λ) has been previously described against factor VIII:C. Direct evidence of a circulating immune complex was obtained by immunoelectrophoresis, which showed an elongated precipitin arc with components against both a monospecific polyclonal antibody to human factor V and an antibody to human IgG. Compared with normal plasma, one component was in the position of free factor V, while the other migrated more cathodally as expected for IgG. In addition, a “sandwich”-type assay allowed demonstration of factor V linked to an anti-factor V antibody in the patient’s plasma (Fig 8).

Prior to steroid therapy, the immune complexes were present in equilibrium with free antibody that was detectable by the ability of unheated patient plasma to neutralize FV:C in normal plasma. After steroid therapy, immune complexes were present in reduced amount in equilibrium with free FV:C. No free antibody was present. However, following heat treatment, the neutralizing antibody could be detected in reduced concentration. These findings explain two clinical observations. First, the ability of transfusions (Fig 1) to produce a transient but measurable rise in FV:C was due to infusion of excess FV:Ag that transiently exceeded the neutralizing capability of the antibody. Second, an eightfold decrease in the titer of the neutralizing antibody (Fig 4) allowed a rise in FV:C to normal levels after corticosteroid therapy.

When the plasma containing the putative antibody–antigen complexes was heated at 56 °C for 30 minutes, the titer of the free neutralizing antibody increased (Fig 4). This observation is attributed to thermal denaturation of factor V protein with consequent dissociation of the factor V antigen–antibody complex, allowing full expression of the antibody-neutralizing activity. Using this method, it was possible to demonstrate persistence of the inhibitory antibody in the patient plasma even when the FV:C levels were normal (Fig 4). At times during the patient’s course, the free antibody was at a sufficiently low titer and was detected only at high ratios of patient plasma to normal plasma until the patient plasma was preheated to allow it to combine with exogenous factor V in normal plasma. As a corollary, these observations suggest that normalization of FV:C levels in plasma does not necessarily imply complete remission of the disease and the absence of the inhibitor.

Borchgrevink and Owen have reported shortening of the secondary bleeding time following platelet transfusions in a patient with congenital deficiency of factor V. Chediak et al have reported partial correction of PTT and a rise in plasma FV:C levels following platelet transfusions in a patient with acquired factor V inhibitor. Our patient, who exhibited a prolonged bleeding time, received platelet transfusions during both admissions. However, because of the other simultaneous therapeutic interventions, it is difficult to discern clearly the role of platelets. During the second hospital admission, the patient received two units of FFP, a single injection of methylprednisolone (40 mg), and six units of platelets in the first 24 hours. A shortening of the APTT was noted six hours following the platelet transfusions and it persisted for several days. The changes in PTT resemble those reported previously by Chediak et al and might reflect the platelet contribution. In contrast to their findings, we noted no increase in FV:C (Fig 2).

We examined the effect of platelets on the coagulation defect in vitro by incubating patient plasma with washed normal platelets and found a partial correction of the APTT in the supernatant plasma (Fig 9). A similar shortening of the APTT was noted following incubation of plasma congenitally deficient in FV:C with normal platelets. In experiments with both inhibitor plasma and congenitally deficient plasma, convincing elevations in FV:C were not noted, in contrast to the findings by Chediak et al, who noted small but consistent increases in FV:C. Thus, the contribution of FV:C by intact platelets to plasma may not be the
primary mechanism to explain the partial correction of APTT. Alternatively, since activated platelets have been shown to express factor Va on their surface, the effect on the APTT could have been due to binding of the antibody to the platelet surface and its removal, with subsequent expression of small amounts of plasma FV:C. However, when heated patient plasma was pre-adsorbed with platelets, it failed to show a decrease in its FV:C-neutralizing capacity (Table 3). Thus, the mechanisms by which platelets shorten the APTT remain to be elucidated. A possible mechanism may involve a nonspecific contribution of platelet membrane fragments containing phospholipid remaining in the plasma even after centrifugation to remove the normal platelets.

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Immune complexes containing factor V in a patient with an acquired neutralizing antibody

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