Comparison of Inhibitory and Binding Characteristics of an Antibody Causing Acquired von Willebrand Syndrome: An Assay for von Willebrand Factor Binding by Antibody

By William A. Fricke, K.M. Brinkhous, J.B. Garris, and Harold R. Roberts

An acquired inhibitor of von Willebrand factor (vWF) activity occurring in a patient with benign gammopathy and von Willebrand syndrome (vWS) has been partially characterized. The inhibitor-induced syndrome resulted in low to undetectable plasma levels of vWF/ristocetin, vWF/botrocetin, FVIIIIR:Ag, and FVIII:C with a normal to slightly prolonged bleeding time. Platelet vWF was normal. Intensive and continuous infusion of a heat-treated factor VIII concentrate (Hemofil-T, Hyland, Glendale, Calif.) elevated the FVIII:C plasma levels to about 100%, with an increase in FVIIIIR:Ag levels to about 340% and vWF/ristocetin levels to about 40%, much lower than expected based on the dose of Hemofil-T and its content of vWF and FVIII:C activities. The inhibitor bound to staphylococcal protein A (SpA) with high affinity, indicating an IgG antibody (Ab). An assay for the vWF-binding capacity was developed on the basis of absorption of the Ab from serially diluted plasma by SpA and removal of vWF and FVIII:C activities from normal plasma by the SpA-Ab complex. The Ab-binding site was on the vWF component of the factor VIII complex. The Ab was unable to bind isolated FVIII:C. The combined use of the new vWF-binding assay and a battery of tests for inhibition of vWF-dependent platelet aggregation with ristocetin (which detects high molecular weight vWF), with botrocetin (which detects high and low molecular weight vWF), and with platelet-aggregating factor (which detects high molecular weight vWF) provided a means of analysis of Ab effect on in vitro vWF function. Using these tests, a comparison was made of the effects of the vWS Ab with those of an Ab inhibitor occurring in homozygous von Willebrand’s disease. The Ab of the vWS patient had weak inhibitory action on vWF/ristocetin without having an effect on vWF/botrocetin and platelet-aggregating factor, a high titer vWF-binding capacity, and no anamnestic response following concentrate therapy. These findings contrasted with those of the Ab occurring in inhibitor von Willebrand’s disease in which vWF inhibitor and binding values were similar, with a strong anamnestic response. The findings indicate that the vWS Ab binds to an epitope on the molecular vWF in such a way that causes only limited inhibition of vWF/ristocetin function and no inhibition of vWF/botrocetin function, suggesting that these two functional domains are at separate sites.

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a comparison is made of the inhibitory and binding activities of the vWS Ab, with an Ab occurring in homozygous vWD; observations are also included on the anamnestic response after factor VIII concentrate therapy.

**MATERIALS AND METHODS**

*VWF and FVIII:C assays.* Plasma vWF activities were determined by macroscopic aggregation in the botrocetin and ristocetin assays20 developed in this laboratory, using lyophylized platelets.20 Bovine PAggF was determined by a modification of the method of Griggs et al,25 also using lyophylized platelets. Canine vWF assays were performed with botrocetin as described by Read et al.7

Aggregometric tracings of vWF-dependent platelet aggregation of platelet-rich plasma (PRP) with ristocetin (final concentration, 1 mg/mL) were obtained as previously described.19 Procedures for study of FVIIIR:Ag by the Laurell method and by crossed immuno-electrophoresis as an index of vWF multimer size were modified from those described by Lamb et al.24 For the latter procedure, plasma samples with low FVIIIR:Ag were concentrated to about 10 mg/mL of CaCl2 for dissociation, as described by Owen and Wagner.14

Platelet vWF was determined by the macroscopic procedures used for plasma vWF. The platelet vWF was harvested as follows: Venous blood from the patient and from controls was drawn into a syringe containing 7.5 mL of acid-citrate-dextrose (ACD) up to a final volume of 50 mL. PRP was prepared by centrifugation at 340 g for eight minutes. An aliquot of PRP was diluted 1:4 with citrated saline (1 vol. 0.11 mol/L sodium citrate plus 19 vol. 0.154 mol/L NaCl) and the platelets were pelleted by centrifugation at 640 g for ten minutes. The platelet pellet was then resuspended in citrated saline to a final concentration of 1.2 x 10^10 platelets per liter. The platelets in the washed suspension were lysed by freezing (−70 °C) and thawing (37 °C) five times.25 The platelet lysates were sedimented by centrifugation at 10,000 g for 30 minutes and the supernate vWF activities were measured. Results are expressed as units per 10^10 platelets.

FVIII:C levels were determined by a modified one-stage assay using human hemophilic plasma with kaolin as the substrate.29 For plasma samples, results are expressed as a percentage of normal human reference plasma, for therapeutic concentrates as units, with one unit being that present in 1 mL of normal plasma. FVIII:C was measured by the method of Kasper et al, and the results are expressed as Bethesda units. The FVIII:C preparation devoid of vWF used for testing direct FVIII:C-binding capacity by Ab was a gift from Dr Helena Sandberg (KabiVitrum AB, Stockholm).

*vWF inhibitor assays.* The macroscopic test for inhibition of vWF-dependent platelet aggregation2 was performed as follows: To a mixture of 0.025 mL of human reference plasma diluted 1:4 with citrated saline and 0.025 mL of serially diluted inhibitor plasma or of imidazole (84 mmol/L)-buffered saline were added 0.025 mL of lyophylized platelet suspension (800,000 platelets per cubic millimeter)19 and then 0.025 mL activator, either ristocetin (final concentration, 0.6 mg/mL) or botrocetin (12.5 U/mL). The onset of platelet aggregation was determined in seconds.31 The titer of the inhibitor is the maximum dilution of the test plasma causing at least three to four seconds’ lengthening of the onset of aggregation compared to that in the control mixture. For inhibition of bovine vWF-dependent platelet aggregation, the PAggF test17 was used in which 0.025 mL of bovine plasma diluted 1:32 was substituted for normal human plasma, and 0.025 mL of buffer was substituted for the activator in the above procedure. Citrated plasma from a severe homozygous vWD plasma with a circulating inhibitor was obtained from a previously described patient.1

*Assay for vWF-binding Ab using SpA.* A suspension of staphylococci with protein A on their surface (SpA) (Pansorbin, Calbiochem-Behring, La Jolla, Calif.) was used to adsorb vWF antibody from the patient’s plasma.27,28 An aliquot of SpA suspension (0.2 mL) was diluted with 0.5 mL of buffered saline, pH 7.35 (0.84 mol/L of imidazole, 0.154 mol/L of NaCl), and the SpA was sedimented by centrifugation (1,620 g for eight minutes). The sedimented SpA was then resuspended with a vortex mixer in 0.1 mL of serially diluted patient’s plasma and incubated at 23 °C for 20 minutes. This suspension was diluted with 0.5 mL of the above buffer and the SpA was sedimented as above. The pellet was resuspended in 0.2 mL of normal reference plasma or other FVIII-containing material and incubated for an additional 20 minutes at 23 °C. The SpA-antibody complex was removed by centrifugation as above and the supernate plasma was assayed for residual FVIII complex activities. Controls were run in parallel by substituting normal reference plasma for patient’s plasma. The titer of antibody was chosen to be the greatest dilution showing removal of at least 25% of a FVIII complex activity from the normal plasma in comparison to the control.

**Pharmacokinetics of infused Hemofil-T.** Values for expected steady-state concentrations of plasma FVIII:C and FVIIIR:Ag were calculated using pharmacokinetic analysis during continuous infusion of Hemofil-T.30 Using this method, the steady-state concentration equals the total units infused per hour divided by the product of the volume of distribution times the elimination constant. The volume of distribution was assumed to be equal to the plasma volume (45 mL/kg body weight), and the elimination constant was 0.693 divided by the half-life. It was also assumed that steady-state conditions were reached after five half-lives.31 Values of 12 hours for the half-life of FVIII:C and 20 hours for the half-life of FVIIIR:Ag were used.30,31 FVIIIR:Ag was assumed to be present in the same proportion to FVIII:C in the infusate as in reconstituted Hemofil-T used for analysis of factor VIII complex activities (Table 1). Hemofil-T for the laboratory analysis was provided by Hyland, Glendale, Calif, and each vial was reconstituted in 20 mL of sterile water.

**Other procedures.** Platelet aggregation studies of PRP with adenosine diphosphate (ADP) and thrombin were performed in a Payton dual-channel platelet aggregometer. The PRP (0.4 mL with 200,000 platelets per microliter) was mixed with 0.04 mL of aggregating agent. The concentrations of ADP (Sigma Chemical Corp, St Louis) were 1.0 mol/L and 0.5 mol/L, and of thrombin (Parke-Davis, Morris Plains, NJ) 0.5 U/mL, 1.0 U/mL, and 5.0 U/mL. Bleeding times were determined using the template method (Sim-
diffusion for precipitin reaction was performed by a modified Ouchterlony method\textsuperscript{35} with the peripheral wells containing serially diluted test Ab plasma, with normal or homologous vWD plasma in the central well, or with the Ab plasma in the central well and serially diluted normal, vWD, or void volume of normal plasma in the peripheral wells.

**PATIENT HISTORY**

The patient is a 73-year-old man who was first admitted to North Carolina Memorial Hospital (NCMH) in April 1976 with gastrointestinal (GI) bleeding. He had suffered from frequent epistaxis for several months and easy bruising for several years. However, he had had no bleeding complications during previous surgeries, which included a tonsillectomy and adenoidectomy, an appendectomy, and a right herniorrhaphy. The physical examination was unremarkable. Laboratory values included: nonactivated partial thromboplastin time (PTT), 94.2 seconds (control, 59.8); prothrombin time (PT), 12.5 seconds (control, 12.6); thrombin clotting time (TCT), 13.0 seconds (control, 13.8); bleeding time, 3.0 minutes; platelet count, 300,000 per microliter; and FVIII:C, 52%. GI evaluation revealed diverticulosis, but no specific bleeding sites were identified. There was no family history of bleeding tendencies.

During the ensuing eight years, the patient was seen frequently in the NCMH clinic and was admitted to NCMH eight times. Five of the eight admissions were for GI bleeding, one was for a left herniorrhaphy, and one, the most recent, was for repair of a hydrocele. A total colectomy was performed in 1979 in an effort to control the GI bleeding.

The coagulation tests performed between 1976 and 1984 are summarized in Table 2. Of note is that the PTT was prolonged each time, but was corrected after mixing with normal plasma. FVIII:C ranged from 3% to 52%, FVIII:Ag values were always very low, vWF/ristocetin and vWF/botrocetin were not detectable, the platelet counts were essentially normal, and the bleeding times were usually normal. Assays for inhibitors of FVIII:C were negative. Studies of five sisters and several nieces and nephews of the patient revealed no coagulation abnormalities.

Hemostasis was maintained during the patient’s three operations by infusion of cryoprecipitate and FVIII concentrates. Prior to the first operation (1979), 20 bags of cryoprecipitate given every 12 hours raised the plasma FVIII:C level to 30%, 4,000 units of Koate (Cutter, Emeryville, Calif) every 12 hours raised the FVIII:C to 40% to 50%, and 6,000 units of Koate every eight hours was required to maintain FVIII:C levels of 60% to 100%. During the most recent surgery, continuous infusion of 800 units of Hemofil-T every hour (8.9 U/kg/h) were required to maintain FVIII:C levels of about 100% during the repair of the hydrocele.

Serum immunoglobulin electrophoresis revealed an IgGκ monoclonal protein band, although total protein and albumin levels have always been normal. Serum aspartate aminotransferase, alanine aminotransferase, and lactic dehydrogenase levels became elevated after the patient received FVIII concentrates. This was believed to be due to hepatitis, although the HbSAb and HbSAg have always been negative.

**RESULTS**

**Infusion studies of the vWS patient.** The search for a circulating inhibitor in the vWS patient was stimulated by the relatively poor response of the subject to infusions of a commercial high-potency factor VIII concentrate (Hemofil-T). The observations made following continuous infusion of the concentrate for 11 days are presented in Fig 1. Levels of FVIII:C, FVIII:Ag, and vWF/ristocetin were determined daily. The infusion raised plasma levels of these three activities—FVIII:C, FVIII:Ag, and vWF/ristocetin—to about 100%, 340%, and 40%, respectively. These levels were sufficient to provide hemostasis during the perioperative period. However, considerable discrepancy was noted between the observed and the expected plasma values for FVIII:C (Table 1). The expected FVIII:C level should have been 340%,\textsuperscript{35} whereas it was only 100%, assuming a half-life for FVIII:C of 12 hours.\textsuperscript{23} The disparity between observed and expected levels of FVIII:C prompted us to analyze Hemofil-T. When reconstituted according to the manufacturer’s instructions, each vial was found to contain about 40 U/mL or approximately 800 units of FVIII:C (Table 1). Our analysis also revealed 172 U/mL of FVIII:Ag (about 3,440 units per vial) and 64 U/mL of vWF/ristocetin (about 12,800 units per vial). Crossed immunoelectrophoresis studies were done on Hemofil-T, diluted to contain approx.

<table>
<thead>
<tr>
<th>Test</th>
<th>No. of Tests</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
<th>Normal* Range</th>
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</thead>
<tbody>
<tr>
<td>FVIII:C (%)</td>
<td>12</td>
<td>13</td>
<td>10</td>
<td>3-52</td>
<td>60-150</td>
</tr>
<tr>
<td>FVIII:Ag (%)</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>0-12</td>
<td>60-150</td>
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<tr>
<td>vWF/ristocetin (%)</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>60-150</td>
</tr>
<tr>
<td>vWF/botrocetin (%)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>70-150</td>
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<tr>
<td>Bleeding time (min)</td>
<td>10</td>
<td>6.3</td>
<td>5</td>
<td>2.5-13.5</td>
<td>2.5-8.0</td>
</tr>
<tr>
<td>Platelets (no./µL)</td>
<td>7</td>
<td>253,000</td>
<td>250,000</td>
<td>147,000-390,000</td>
<td>150,000-440,000</td>
</tr>
<tr>
<td>PT (s)</td>
<td>5</td>
<td>10.9</td>
<td>10.4</td>
<td>10-12.5</td>
<td>11.6-13.7</td>
</tr>
<tr>
<td>PTT, nonactivated (s)</td>
<td>7</td>
<td>91.2</td>
<td>93.1</td>
<td>74.0-100.9</td>
<td>45-67</td>
</tr>
<tr>
<td>Thrombin time (s)</td>
<td>5</td>
<td>12.9</td>
<td>12.3</td>
<td>11.3-15.2</td>
<td>9.6-12.8</td>
</tr>
<tr>
<td>FVIII:C inhibition ( Bethesda titer)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

*Range of normal values used by the Coagulation Laboratory, North Carolina Memorial Hospital.
testing with ristocetin did not increase the vWF/ristocetin inhibitory activity. It is of interest that the inhibitor plasma did not affect the vWF/botrocetin test. FVIIIIR:Ag likewise was not inhibited by the patient’s plasma. The Ag values were the same for patient’s plasma diluted with normal plasma and for normal plasma diluted with buffer. No precipitin bands were observed in the double immunodiffusion tests. There was no inhibition of either human or canine FVIII:C. Thus, the Ab partially inhibited only one form of functional vWF activity, the high molecular weight form reactive with ristocetin.

Evidence for a vWF-binding antibody. The striking discrepancy between the expected and observed levels of plasma FVIII:C and FVIIIIR:Ag during treatment with Hemofil-T, in the absence of a corresponding inhibitor to either of these activities in the vWS plasma, suggested the presence of an antibody that binds to the factor VIII complex. In preliminary studies, it was found that treatment of patient’s plasma with SpA removed the vWF/ristocetin-neutralizing activity, suggesting that a SpA–Ab combination was formed. A series of experiments were thus conducted to determine if the SpA–Ab would bind the different FVIII complex activities and remove them from normal plasma, using the protocol indicated in Materials and Methods. In a typical experiment, the normal plasma after treatment with SpA–Ab contained no residual FVIII:C, vWF/ristocetin, or vWF/botrocetin, and only about 10% FVIIIIR:Ag. These data obtained with SpA indicate that the Ab binds the FVIII complex and that it belongs to IgG subclasses, 1, 2, or 4, each of which have high-affinity binding to SpA.37

In another series of experiments, a void-volume concentrate of vWF free of factor VIII:C was used in place of normal plasma. The vWF preparation had 100% vWF/botrocetin activity and 120% FVIIIIR:Ag, and only 1% FVIII:C activity. The vWF/botrocetin activity was completely removed by the SpA–antibody complex. The residual FVIIIIR:Ag was 18%. Also, a preparation of FVIII:C devoid of vWF was treated with SpA–Ab. The FVIII:C preparation had 230% FVIII:C activity, but no vWF activity or FVIIIIR:Ag. It was treated with the SpA–Ab complex prepared with patient plasma, as in line 2 of Table 4.
Table 4. Binding Assay of Antibody: Residual FVIII Activities in Normal Plasma After Exposure to Antibody Bound to Staphyloccocal Protein A

<table>
<thead>
<tr>
<th>Plasma Dilution</th>
<th>FVIII:C (%)</th>
<th>vWF/Botrocetin (%)</th>
<th>vWF/Ristocetin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:32</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1:64</td>
<td>31</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1:128</td>
<td>38</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>1:256</td>
<td>72</td>
<td>28</td>
<td>32</td>
</tr>
<tr>
<td>1:512</td>
<td>100</td>
<td>70</td>
<td>63</td>
</tr>
<tr>
<td>1:1.024</td>
<td>—</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*Values are expressed as a percentage of control plasma treated with SpA alone.

and was compared to the control consisting of FVIII:C treated with protein A, which had been incubated with normal reference plasma. The residual VIII:C value was 109% of the control, indicating that no specific binding of FVIII:C by Ab had occurred. This group of studies suggests that the antibody recognized an epitope on the vWF molecule and that FVIII:C was passively removed from normal plasma along with vWF because it was bound to vWF.

**Assay of vWF-binding antibody.** A quantitative assay of Ab binding to vWF was developed. Serially diluted antibody plasma was added to a constant amount of SPA, followed by mixing the SpA-Ab with a constant amount of normal plasma. Residual levels of FVIII activities in the adsorbed normal plasma were determined. These levels gradually increased as higher dilutions of antibody plasma were used. The results of these studies with regard to FVIII:C, vWF/botrocetin, and vWF/ristocetin are listed in Table 4. The titer of the Ab was thus 1:256 for FVIII:C and 1:512 for vWF/botrocetin and vWF/ristocetin binding. Similar binding studies were performed using bovine and canine plasma to determine whether the human antibody recognized animal FVIII complexes. The results were negative. The antibody neither inhibited nor recognized bovine PAggF or canine FVIII:C or vWF/botrocetin.

**Ab recognition of platelet vWF and bleeding time.** Platelet vWF values determined in lysates of the vWS patient platelets were comparable to those obtained for normal platelets (Table 5). The vWF/botrocetin and FVIII:Ag values were of the same general order, whereas the vWF/ristocetin values were considerably greater. These findings suggest that both types of platelets contained a preponderance of high-molecular weight forms of vWF. Adsorption of both patient and control lysates with the SpA–Ab complex completely removed their vWF activities and FVIII:Ag. These results indicate that the vWF and FVIII:Ag in the circulating platelet are protected from the antibody, but that the platelet vWF contains the epitope recognized by the Ab. Several screening tests for platelet function, clot retraction, and platelet aggregation of patient PRP with ADP and thrombin were normal. PRP aggregated poorly with ristocetin. The normal platelet vWF and functional values may have contributed to the maintenance of a normal or nearly normal bleeding time.

**Comparison of vWF inhibitor and binding titers of the vWS Ab with an Ab occurring in vWD with circulating inhibitor.** A battery of vWF inhibitor tests along with the new procedure for determining the extent of vWF binding by Ab was used to characterize further the circulating inhibitors that developed in the vWS patient and a vWD patient. The results are displayed in Table 6. Each patient was treated with factor VIII concentrate. In the preinfusion period the pattern of results are very different in the two patients. All of the inhibitor tests were positive in the vWD patient. Postinfusion in the vWS patient, there was no change in the Ab titer in either the ristocetin inhibitor test or in the vWF-binding assay, indicating that no anamnestic response occurred. On the other hand, in the vWD inhibitor patient postinfusion, all three inhibitor tests and the vWF-binding test reacted in a similar fashion, with a great increase in the Ab titer regardless of the method of testing. In this multitransfused patient, the antigen recognized by the Ab was believed to be the vWF molecule, which would be in accord with the equivalence in Ab titers as determined in the neutralizing and binding procedures. The lack of an anamnestic response in the vWS patient suggests that the vWF molecule is not serving as the antigen and that the antibody is not being produced in response to it.

**DISCUSSION**

In this study, a circulating antibody inhibitor occurring in a patient with severe vWS and benign gammopathy was partially characterized. Of the several activities of the factor VIII macromolecular complex, only vWF-dependent aggregation of platelets with ristocetin was affected. Inhibitory activity was weak (Table 3). Other vWF-dependent aggregation tests of platelets, the botrocetin or venom coagglutinin tests and the platelet-aggregating factor test using bovine plasma as a source of vWF, were unaffected by the antibody. Likewise, neither FVIII:Ag nor FVIII:C were inhibited. Yet the patient had the laboratory findings of severe vWD, with little or no vWF or FVIII:C detectable in the circulating plasma (Table 2). However, the bleeding time, characteristically greatly prolonged in severe vWD, was normal or nearly so. There was an IgG light-chain monoclonal antibody in the serum, as has been reported in several other cases of vWS with benign gammopathy. The antibody inhibitor could be removed from the patient’s plasma with SpA, indicating it belonged to IgG subclass 1, 2, or 4. The protein A–bound Ab added to normal plasma depleted it of its factor VIII complex activities (Table 4), providing an in vitro counterpart of the patient’s plasma. If instead of normal plasma either a vWF preparation devoid of FVIII:C activity or a FVIII:C preparation devoid of vWF was exposed to Ab, it was found that the Ab bound the vWF but not the FVIII:C, indicating the
Ab-binding site was on the vWF component of the factor VIII complex. This site appears not to be the domain(s) related to vWF-dependent platelet aggregation, in view of the weak inhibitory capacity and the strong binding capacity of the Ab (Tables 3 and 4). The limited inhibition of ristocetin-induced aggregation could be due to steric hindrance, should the Ab binding site be topographically situated near the functional domain that is related to the ristocetin effect. Botrocetin is believed to cause vWF-dependent platelet aggregation by a mechanism different from ristocetin42 and may utilize a separate domain on the vWF molecule. At any rate, the Ab completely lacked an inhibitory capacity and the strong binding capacity related to vWF-dependent platelet aggregation, in view of the weak inhibitory capacity and the strong binding capacity of the Ab for vWF.

The effect of the Ab in causing the vWS was overcome by intensive and continuous replacement therapy with Hemofil-T, a heat-treated factor VIII concentrate (Fig 1). Hemostasis was normalized, as judged by the lack of bleeding at surgery and postoperatively. Hemofil-T contained amounts of vWF and FVIII:C activities similar to that of heatsensitive concentrates,4,44 with almost four times as much FVIIIIR:Ag as FVIII:C and about 1½ times more vWF/ristocetin activity than FVIII:C (Table 1). On crossed immunoelectrophoresis, the Hemofil-T revealed few of the larger multimers, as was true of the original Hemofil.4,44 The observed levels of FVIIIIR:Ag and FVIII:C during treatment were much less than expected with the doses of concentrate administered (Table 1). In spite of the comparatively large amounts of vWF/ristocetin infused, its plasma level was considerably less than that of FVIII:C, reflecting perhaps the great avidity of the Ab for vWF.

Cellular vWF, as judged by the normal platelet vWF content in this patient (Table 5), is protected from the action of the Ab. Analysis of normal and patient platelet lysates showed comparable values for vWF/botrocetin and FVIIIIR:Ag, with higher values for ristocetin in both subjects. Platelet vWF has been studied by several groups,5,45-46 and was found to contain a preponderance of larger multimers, the multimeric distribution of which would be indicated by the high ristocetin vWF values.47 vWF/botrocetin values reflect a broad spectrum of vWF multimers, as do values for FVIIIIR:Ag.5,48 It is known that factor VIII concentrates largely deficient in high-molecular weight forms of vWF are ineffective in correcting the long bleeding time in vWD,49 suggesting that these multimeric forms are needed to normalize hemostasis. The maintenance of a predominance of higher molecular weight forms of vWF in platelets in this patient suggests that the availability of platelet vWF at the bleeding site could be responsible for the short bleeding times observed.

The development of a procedure for estimating the titer of the binding Ab depended on the use of SpA to absorb the Ab from serially diluted patient plasma. The bound Ab removed vWF and FVIII:C from normal plasma in an SpA–Ab concentration-dependent fashion (Table 4). We have used as the endpoint the removal by Ab of at least 25% of the vWF or FVIII:C activity from normal plasma. The combined use of the vWF-binding assay and the inhibitor assays using the ristocetin, botrocetin, and PAggF tests appears to provide a basis for more detailed analysis of the action of vWF inhibitors, whether in vWS or in vWD with a complicating inhibitor (Table 6). In the latter condition, the binding and neutralization assays demonstrate concordance, presumably because of a diverse Ab population that reacts with many different antigenic determinants on vWF, thus effectively blocking the functional domains related to vWF activity. In this patient with vWS due to an autoantibody, the discordance between the two types of assays is probably due to the restricted Ab population, which reacts with few epitopes. Regardless of the binding sites, it appears that both types of Abs remove the factor VIII-related activities from normal plasma. Use of the binding assay could be especially advantageous in subjects with low-titer vWF inhibitors, as this assay appears to provide a good indicator of the full Ab potential in interfering with factor VIII complex biological function. The titer of Ab, as determined by both the inhibitor and binding assays, was determined after concentrate therapy in both the vWS and the inhibitor vWD patients (Table 6). In the vWS patient, there was no anamnestic response. In the vWD patient, there was a marked antibody response, with a remarkable similarity in values of the inhibitor and binding titers. The pathogenetic mechanism causing acquired vWS as observed in this patient, in which an autoantibody with limited or no inhibitory function binds to the procoagulant protein and rapidly clears it from the circulation, may cause other acquired coagulopathies such as...
acquired prothrombin deficiency. It is suggested that the use of binding assay for prothrombin or other procoagulants, similar to that reported here for factor VIII complex, could be of value in assessing other types of acquired bleeding diatheses with low-titer inhibitors.

REFERENCES


ACKNOWLEDGMENT

We thank Dr Howard Reisner for performing the immunoradiometric assays for FVIIIIR:Ag and the FVIII:C:Ag assays. We also thank Dr M.S. Read for her technical help and advice during the course of this study.
35. Budtz-Olsen OE: Clot Retraction. Springfield, Ill, Thomas, 1951, p 2
Comparison of inhibitory and binding characteristics of an antibody causing acquired von Willebrand syndrome: an assay for von Willebrand factor binding by antibody

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