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 with 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.

Circulating inhibitors of the factor VIII complex have been reported both in severe inherited von Willebrand’s disease (vWD) and in the acquired bleeding disorder known as von Willebrand’s syndrome (vWS) or acquired vWD. The occurrence of these antibody inhibitors was first recognized in a patient with homozygous vWD in 1974. Although vWS had been recognized earlier, it was not until 1976 that a circulating antibody inhibitor was described in this syndrome, occurring in a patient who had a lymphosarcoma. Since these original descriptions, circulating inhibitors have been described in several other patients with homozygous vWD and with vWS. The antibodies react primarily with the component of the factor VIII macromolecular complex possessing the von Willebrand factor (vWF) activities. In some patients the antibody also neutralized the coagulant component of the factor VIII complex (factor VIII:C) or the antihemophilic factor. The vWF component of the complex can be measured both immunologically as factor VIIIIR:Ag and functionally in platelet aggregation or agglutination tests. Three functional tests used to identify vWF are (1) the ristocetin platelet aggregation test, which detects the high-molecular weight forms of vWF in human plasma, designated ristocetin cofactor (FVIIIIR:RCoF) or vWF/ristocetin; (2) the platelet-aggregating factor (PAggF) test in which bovine or porcine vWF aggregates human platelets and, like ristocetin, detects high-molecular-weight forms of vWF; and (3) the botrocetin or venom coagglutinin test, which detects a broad spectrum of molecular sizes of vWF, designated vWF/botrocetin.

The circulating antibodies occurring in inhibitor vWD and in vWS have been identified most frequently by inhibition of vWF/ristocetin platelet aggregation. The Abs from different patients exhibit varying patterns of inhibitory capacity. The Abs from some patients inhibit vWF/ristocetin but not FVIIIIR:Ag or FVIIIIR:Ag. In others, the Abs inhibit vWF/ristocetin and/or FVIIIIR:Ag and/or FVIIIIR:C. Inhibition of bovine PAggF and/or vWF/botrocetin was demonstrated in a few cases. In addition, an Ab that binds but does not inhibit the factor VIII macromolecular complex has been identified in a patient with vWS. In those cases in which the Abs have been classified, most were of the IgG class and light chain.

We describe an acquired antibody against the vWF component of the factor VIII macromolecular complex, occurring in a patient with benign gammopathy and vWS. The Ab had strong binding capacity for the factor VIII complex, but limited inhibitory activity. A new procedure for measuring the titer of the Ab-binding capacity was reported, utilizing staphylococcal protein A (SpA). Our study includes the results of the use of this Ab-binding assay plus the assays for inhibition of vWF/ristocetin, PAggF, and vWF/botrocetin platelet aggregation in further characterizing the Ab, as well as the use of these tests in diagnosis. Using these procedures,
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 anamnetic 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 assays developed in this laboratory, using lyophilized platelets. Bovine PAggF was determined by a modification of the method of Griggs et al, also using lyophilized platelets. Canine vWF assays were performed with botrocetin as described by Read et al. Aggregometric tracings of vWF-dependent platelet aggregation of platelet-rich plasma (PRP) with ristocetin (final concentration, 1 mg/mL) were obtained as previously described. Procedures for study of FVIIIR:Ag by the Laurell method and by crossed immunoelectrophoresis as an index of vWF multimer size were modified from those described by Lamb et al. For the latter procedure, plasma samples with low FVIIIR:Ag were concentrated to about 100% values by ultrafiltration. Immunoradiometric assay for FVIIIR:Ag was performed as described. Chromatographic preparation of isolated vWF devoid of FVIII:C was made using 0.25 mol/L of CaCl₂ for dissociation, as described by Owen and Wagner. 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 of 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 10⁹ platelets per liter. The platelets in the washed suspension were lysed by freezing (−70 °C) and thawing (37 °C) five times. 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 10⁷ platelets.

FVIII:C levels were determined by a modified one-stage assay using human hemophilic plasma with kaolin as the substrate. 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 preparation devoid of vWF used for testing direct FVIII:C-binding capacity by Ab was a gift from Dr Helena Sandberg Kabi Vitrum AB, Stockholm.

vWF inhibitor assays. The macroscopic test for inhibition of vWF-dependent platelet aggregation 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 lyophilized platelet suspension (800,000 platelets per cubic millimeter) 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. The inhibitor of the test platelet aggregation 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 test 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.

Assays for vWF-binding Ab using Sp.A. A suspension of staphylococci with protein A on their surface (Sp.A) (Pansorbin, Calbiochem-Behring, La Jolla, Calif.) was used to adsorb vWF antibody from the patient's plasma. 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. Using this method, the steady-state concentration equals 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. Values of 12 hours for the half-life of FVIII:C and 20 hours for the half-life of FVIIIR:Ag were used.

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 platelet 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-
Plate II, General Diagnostics, Morris Plains, NJ). Clot retraction was measured by a modified Hayem procedure. Double immunodiffusion for precipitin reaction was performed by a modified Ouchterlony method with the peripheral wells containing serially diluted test Ab plasma, with normal or homozygous vWD plasma in diffusion for precipitin reaction was performed by a modified Ouchterlony method, with the peripheral wells containing serially diluted normal, vWD, or void volume of normal plasma in the central well.

**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 vWF/botrocetin 2000-70-150.

**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, FVIIIIR:Ag, and vWF/ristocetin were determined daily. The infusion raised plasma levels of these three activities—FVIII:C, FVIIIIR: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%, whereas it was only 100%, assuming a half-life for FVIII:C of 12 hours. 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 FVIIIIR: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 approximate-...
VON WILLEBRAND SYNDROME

Continuous infusion of Hemofil-T. The values of FVIII:C, C, and vWF/ristocetin are expressed as a percentage of the normal reference plasma. Determinations were made daily during the treatment period and hourly during the first four hours after discontinuation of Hemofil-T infusion. Duration of therapy is indicated by the horizontal line at the top. Dosage of Hemofil-T was 800 FVIII:C U/h, the amount that maintained plasma FVIII:C levels at approximately 100% with normal hemostasis.

Fig 1. Response of the patient with severe von Willebrand’s syndrome to continuous infusion of Hemofil-T. The values of plasma FVIII:Ag, FVIII:C, and vWF/ristocetin are expressed as a percentage of the normal reference plasma. Determinations were made daily during the treatment period and hourly during the first four hours after discontinuation of Hemofil-T infusion. Duration of therapy is indicated by the horizontal line at the top. Dosage of Hemofil-T was 800 FVIII:C U/h, the amount that maintained plasma FVIII:C levels at approximately 100% with normal hemostasis.

Table 3. Tests for Inhibition of Factor VIII Complex Activities by vWS Plasma

<table>
<thead>
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<th>Activity</th>
<th>Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of vWF</td>
<td></td>
</tr>
<tr>
<td>vWF/ristocetin</td>
<td>1:4–1:16*</td>
</tr>
<tr>
<td>vWF/botrocetin</td>
<td>Negative</td>
</tr>
<tr>
<td>FVIII:Ag</td>
<td>Negative</td>
</tr>
<tr>
<td>Bovine PAggF</td>
<td>Negative</td>
</tr>
<tr>
<td>Canine vWF/botrocetin</td>
<td>Negative</td>
</tr>
<tr>
<td>Inhibition of FVIII:C</td>
<td></td>
</tr>
<tr>
<td>Human FVIII:C</td>
<td>Negative</td>
</tr>
<tr>
<td>Canine FVIII:C</td>
<td>Negative</td>
</tr>
</tbody>
</table>

*Ranges of values of three separate plasma samples collected at different nontherapeutic periods.

Evidence for a vWF-binding antibody. The striking discrepancy between the expected and observed levels of plasma FVIII:C and FVIII: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% FVIII: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.

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 only 120% FVIII:Ag, and no FVIII:C activity. The vWF/botrocetin activity was completely removed by the SpA–antibody complex. The residual FVIII: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 FVIII:Ag. It was treated with the SpA–Ab complex prepared with patient plasma, as in line 2 of Table 4.
patient platelets were comparable to those obtained for FVIIIR:Ag. These results indicate that the vWF and Adsorption of both patient and control lysates with the normal platelets (Table 5). The vWF/botrocetin and vWF/nistocetin binding. Similar binding studies were performed using bovine and canine plasma to determine whether the human 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 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/nistocetin 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/nistocetin 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 FVIIIIR:Ag values were of the same general order, whereas the vWF/nistocetin 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 FVIIIIR:Ag. These results indicate that the vWF and FVIIIIR: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 responsible for Ab production is 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 FVIIIIR: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

| Table 4. Binding Assay of Antibody: Residual FVIII Activities in Normal Plasma After Exposure to Antibody Bound to Staphylococcal Protein A |
|--------------------------|--------------------------|--------------------------|--------------------------|
| Plasma Dilution | FVIII:C (%) | vWF/Botrocetin (%) | vWF/Ristocetin (%) |
| 1:32 | 13 | 0 | 0 |
| 1:64 | 31 | 0 | 0 |
| 1:128 | 38 | 9 | 0 |
| 1:256 | 72 | 28 | 32 |
| 1:512 | 100 | 70 | 63 |
| 1:1,024 | — | 100 | 100 |

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

| Table 5. vWF Activities and FVIIIIR:Ag in Patient and Control Platelets |
|--------------------------|--------------------------|--------------------------|--------------------------|
| Platelet Lysate | vWF/Botrocetin | vWF/Ristocetin | FVIIIIR:Ag |
| Patient | 2.83 | 4.42 | 2.25 |
| Normal control | 2.16 | 3.58 | 2.00 |

Values are expressed as units per 10^9 platelets.
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 effect on botrocetin action, whereas ristocetin action was partially blocked. The severity of the syndrome with loss of plasma vWF and FVIII:C activities indicates that the Ag–Ab complex is rapidly cleared from the plasma.

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 unheated concentrates,43,44 with almost four times as much FVIIIIR:Ag as FVIII:C and about 1 1/2 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.43,44 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 groups45,46 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.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

<table>
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<tr>
<th>Patient Plasmas</th>
<th>vWF-Inhibitor Ab Titer</th>
<th>vWF/Botrocetin</th>
<th>PAggF</th>
<th>vWF-Binding Ab Titer</th>
</tr>
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<tbody>
<tr>
<td>von Willebrand's syndrome</td>
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<td>Preinfusion</td>
<td>1:8</td>
<td>Negative</td>
<td>Negative</td>
<td>1:512</td>
</tr>
<tr>
<td>3 wk</td>
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<td>Negative</td>
<td>Negative</td>
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<tr>
<td>6 mo</td>
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<td>Negative</td>
<td>Negative</td>
<td>1:512</td>
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<tr>
<td>Homozygous von Willebrand's disease with inhibitor</td>
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</tr>
<tr>
<td>Preinfusion</td>
<td>1:8</td>
<td>1:16</td>
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<tr>
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Table 6. Comparison of Inhibitor and Binding Titers for Various vWF Activities in vWS and Homozygous vWD in Relation to Concentrate Therapy
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

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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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