New Variant of von Willebrand Disease With Defective Binding to Factor VIII

By Masato Nishino, Jean-Pierre Girma, Chantal Rothschild, Edith Fressinaud, and Dominique Meyer

A new variant of von Willebrand disease (vWD) was identified by a new analytic method which characterizes the ability of plasma von Willebrand Factor (vWF) to bind to purified factor VIII (F.VIII). vWF was isolated from small amounts of plasma by immunoadsorption with a selected monoclonal antibody to vWF previously coated onto wells of microtitration plates. Plasma F.VIII was removed from immobilized vWF by washing with 0.4 mol/L CaCl₂; purified F.VIII was then added to the well. The amount of bound F.VIII was estimated directly in the wells by a chromogenic assay and immobilized vWF was estimated by an immunologic assay. Binding of plasma vWF to F.VIII was analyzed in a pool of normal plasma, ten control individuals, 13 with hemophilia A and five with type I vWD. In all cases, the dose-response curves were linear and the slopes of the regression lines were essentially the same. The method was then applied to investigate the binding of vWF to F.VIII in two vWD patients (sister and brother) who demonstrated significantly lower activity of F.VIII than of vWF. The first patient, with a long history of epistaxis, bruising, and hematomas, showed a slightly prolonged bleeding time (10 minutes); 15% F.VIII:C and 39% of vWF:Ag and vWFRCO. Her brother, who has a bleeding syndrome but no hematomas, showed similar data (bleeding time 9 minutes, 20% F.VIII:C, 53% vWF:Ag and vWFRCO). Similar levels of F.VIII were observed in the two propositi by four different methods (one- and two-stage clotting and chromogenic and immunologic assays). Sodium dodecyl sulfate (SDS) 1.4% agarose gel electrophoresis showed that all multimers of vWF were present in both patients. vWF binding to F.VIII was markedly decreased in the two propositi. The abnormal binding of vWF to F.VIII was not corrected during pregnancy or after infusion of 1-deamino (8-D-arginine) vasopressin despite an increase in vWF levels. The qualitative abnormality of vWF in both patients was associated with a subtle alteration of the multimeric structure by SDS 3% agarose gel electrophoresis in which the two central subbands of the quintuplet of individual oligomers were undetectable or poorly visible. SDS-polyacrylamide gel electrophoresis under reducing conditions demonstrated a single band of 275 Kd in the plasma of both patients, and there was no evidence of a second band corresponding to pro-vWF, the precursor of the mature vWF subunit, suggesting that proteolytic processing of vWF was normal. In conclusion, a new variant of vWD has been identified in two siblings with a defective binding of vWF to F.VIII, leading to lower levels of F.VIII than vWF in plasma.

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HUMAN von Willebrand factor (vWF) is a large multimeric glycoprotein which plays two major roles. First, it is essential for the initial attachment of platelets to the subendothelium in vessels where blood circulates at high shear rates. Second, it serves as carrier for factor VIII (F.VIII), stabilizing its coagulant activity. The multimeric structure of vWF, which is required for optimal biologic activity, can be visualized by electrophoresis in sodium dodecyl sulfate (SDS)-agarose gels. With a low agarose concentration, vWF appears as a set of bands with apparent molecular weight (mol wt) ranging from 0.5 to greater than 15 million. With high agarose concentrations, each multimer can be resolved into a triplet or a quintuplet with a major central band and two or four satellite subbands. By polyacrylamide gel electrophoresis under reducing conditions, vWF appears as a predominately 275-Kd subunit. There is a single type of subunit that consists of a linear arrangement of 2,050 amino acids residues. In recent years, data dealing with the structure-function relationship of vWF have shown that the major information for vWF function resides on discrete sequences of the basic monomer. Thus, a domain for the attachment of F.VIII on vWF has been localized on the N-terminal part of the subunit between residues 1 and 272.

von Willebrand disease (vWD) results from quantitative or qualitative defects of vWF and, at present, patients are phenotypically classified among three major types including several subtypes. Type I is characterized by a quantitative decrease of an apparently normal vWF. Type II shows evidence of intrinsic functional defects of vWF with a discrepancy between the levels of vWF antigen (vWF:Ag), which are normal or moderately decreased, and a generally reduced activity estimated by its ristocetin cofactor activity (vWFRCO). This group exhibits an abnormal multimeric structure associated with an absence of the largest multimers. The third type of vWD refers to the severe form of the disease. In all three types, the levels of F.VIII are usually proportional to those of vWF:Ag because F.VIII coagulant activity (VIII:C) is labile in the absence of vWF. However, some patients exhibit decreased or subnormal levels of an apparently normal vWF, associated with low levels of F.VIII.

To understand the impairment of F.VIII and/or vWF responsible for the latter discrepancy better, we reinvestigated plasmas from such patients previously classified among type I vWD by setting up a solid-phase system to allow immunoisolation of vWF free of F.VIII from minute amounts of plasma and to study the binding of exogeneous purified normal F.VIII to vWF. In two patients belonging to the same kindred, we found clear evidence that a molecular abnormality of vWF resulted in a decreased ability of vWF to bind to normal F.VIII and was responsible for the discrepancy between levels of vWF and F.VIII.

From INSERM U.143 and Laboratoire Central d'Hématologie, Hôpital de Bicêtre, Paris, France.

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Address reprint requests to J. P. Girma, PhD, INSERM U. 143, Hôpital de Bicêtre, 94275 Bicêtre Cedex, France.

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MATERIALS AND METHODS

Reagents. Carrier-free Na\(^{25}\)I and nitrocellulose paper (Hybond-C extra) were from Amersham International, Amersham, England; Iodo-Gen was from Pierce Chemical, Rockford, IL; F.VIII-deficient plasma was from Diagnostica Stago, Asnières, France; and chromogenic kits for measurement of VIII:C were from KabiVitrum (Coast factor VIII), Stockholm. Sepharose 2B and CL-4B and Sephadex G 25 were from Pharmacia Fine Chemicals, Uppsala, Sweden; polystyrene 96-well microtitration plates were from Becton Dickinson, Oxnard, CA; films for autoradiography (Kodak X-Omat films) and cassettes (Kodak X-Omatic) were from Eastman Kodak, Rochester, NY; agarose (HGT, Seakem) and Sephadex G 25 were from Pharmacia Fine Chemicals, England; Iodo-Gen was from Pierce Chemical, Roxeboro, IL; plasmas were obtained from 10 unrelated healthy subjects, and a normal plasma pool was prepared from 20 control donors.

Patients. The present study was performed with the informed consent of the patients in accordance with the Declaration of Helsinki and under the control of the Human Rights Committee of Hôpital de Bicêtre.

Two patients (sister and brother) with decreased or subnormal vWF:Ag and a discrepancy between VIII:C and vWF:Ag levels were selected from a family previously classified as type I vWD (Table 1). The sister has epistaxis, bruising, and hematomas. Her brother also has a bleeding disorder but no hematomas. The other family members, in particular the parents, were unavailable for study. One patient with type III vWD (vWF:Ag <0.01 U/dL) and five patients with type I vWD (vWF:Ag between 6 and 34 U/dL) were tested for comparison in this study. Thirteen patients with hemophilia A previously characterized in our laboratory were also selected, four with severe hemophilia A (VIII:C <1 U/dL) and nine with moderate hemophilia A (VIII:C between 1 and 36 U/dL). Individual plasmas were obtained from 10 unrelated healthy subjects, and a normal plasma pool was prepared from 20 control donors.

Infusion study of 1-deamino-(8-D-arginine) vasopressin (DDAVP). DDAVP (Minirin) was from Ferring Laboratories, Malmö, Sweden. DDAVP was infused into patient 2 after informed consent was obtained (Table 1) at a dose of 0.3 μg/kg body weight in 30 minutes. Blood samples were obtained before and 30 minutes and one and four hours after the end of the infusion.

Preparation of plasma. Nine parts blood were collected in one part 3.8% trisodium citrate, pH 7.4. Platelet-poor plasma was isolated by centrifugation at 2,500 g for 15 minutes at 4°C and immediately used or stored in aliquots at –80°C.

Antibodies to vWF or F.VIII. A polyclonal antibody to vWF, rendered monospecific by immunopurification, was raised in rabbits and used in this study. Two monoclonal antibodies (MoAbs) to vWF and a MoAb to F.VIII were produced and characterized as previously described. MoAb 9 to vWF inhibits binding of vWF to thrombin-stimulated platelets and recognizes an epitope on the C-terminal part of the vWF subunit. MoAb D4H1 is directed against F.VIII and partially inhibits VIII:C.

Radiolabeling of antibodies. vWF and IgG were labeled with carrier-free Na\(^{25}\)I according to the method of Fraker and Speck, using Iodo-Gen. Specific radioactivity was 4 and 8 to 10 μCi/μg, respectively.

Bleeding time and assays of F.VIII and vWF. Bleeding time was measured according to the method of Mielke et al. VIII:C was estimated by a one-stage activated partial thromboplastin time as described by Langdell et al with F.VIII-deficient plasma, kaolin, and cephalin; by a two-stage assay as described by Biggs et al, and by a chromogenic assay performed as described by the manufacturer. VIII:Ag was estimated by a two-site immunoradiometric assay as described, with an oligoclonal antibody to F.VIII from a multi-transfused hemophilia A patient. vWFRCO was estimated with formalin-fixed platelets and 1 mg/mL ristocetin according to the method of McFarlane et al. vWF:Ag was analyzed by immunoradiometric assay using the polyclonal antibody to vWF or MoAb 9.

Purification of human F.VIII. Human F.VIII was isolated from lyophilized F.VIII/vWF concentrates (donated by the Centre National de Transfusion Sanguine, France) dissolved in imidazole buffer (0.02 mol/L imidazole, 0.15 mol/L NaCl, 0.1 mol/L lysine-HCl), pH 6.8, as described by Fulcher and Zimmerman. The immunoadsorbent was prepared by coupling MoAb 9 to Sepharose 2B (1.5 mg IgG/mL). The final preparation had a specific activity of 450 to 600 U VIII:C/mg and contained <0.01 U vWF:Ag/mg.

Table 1. Laboratory Data of the Two Propositi

<table>
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<tr>
<th>Patient</th>
<th>Sex</th>
<th>Bleeding Time (min)</th>
<th>APTT (s)</th>
<th>VIII:C</th>
<th>VIII:Ag</th>
<th>vWF:Ag</th>
<th>vWFRCO</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>One-stage</td>
<td>Two-stage</td>
<td>VIII:Ag</td>
<td>vWF:Ag</td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>8-10</td>
<td>64-68</td>
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<td>16 (14-20)</td>
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<td>2</td>
<td>M</td>
<td>7-9</td>
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<td>20 (12-28)</td>
<td>19</td>
<td>15 (12-23)</td>
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</tr>
<tr>
<td>Normal</td>
<td></td>
<td>4-8</td>
<td>50-52</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>

Abbreviations: APTT, activated partial thromboplastin time; VIII:Ag, chromogenic assay of F.VIII.

*Results are the mean of four determinations; range is shown in parentheses.
†Two determinations.
50% acetic acid. F.VIII activity was estimated at 405 nm with a Titertek Multiskan MCC/340 (EFLAB oy, Helsinki).

After F.VIII activity was measured by chromogenic assay, the wells were washed and the levels of vWF bound to coated MoAb 9 were controlled by immunoradiometric assay using 125I-MoAb 9. In each experiment, the least-squares regression lines for test plasmas and normal plasma pool were calculated. The slopes of the regression lines were compared by the sum of squares of deviations to estimate the normality of binding of F.VIII to vWF.

In control experiments, the amount of MoAb associated with immobilized vWF in the final step of the assay was compared with that obtained in parallel experiments after each step of the binding assay. Results indicated that no loss of bound vWF occurred through the various steps of the procedure.

The amount of immobilized vWF on the wells was quantified by adding purified labeled vWF (1 U vWF:Ag, 6 μg) to 1 mL plasma from a patient with severe type III vWD, and serial dilutions of this mixture, containing from 2 × 10⁻⁵ to 1 U/mL, were incubated instead of normal pool plasma. After the wells were washed, fixed vWF was estimated by counting. In parallel experiments, the F.VIII associated with the various amounts of bound vWF was estimated in the presence of a constant amount of F.VIII (1 U/mL) with the chromogenic assay by comparison with control curves using serial dilutions of purified F.VIII and uncoated wells.

**Gel electrophoresis.** SDS-gel electrophoresis for multimer analysis was performed with either 1.4% agarose and short running gels (90 mm) according to the method of Ruggieri and Zimmerman or 3% agarose as described by Kinoshita et al and long running gels (200 mm). In both cases, the stacking gel contained 0.8% agarose. Plasma was diluted to 1/20 with 10 mmol/L Tris-HCl buffer, pH 8.8 containing 2% SDS for 1.4% agarose gels and to one fifth with the same buffer but containing 2% SDS and 6 mol/L urea for 3% agarose gels. Electrophoresis was run horizontally using an LKB multiphor 2117 (LKB Instruments AB, Bromma, Sweden) cooled at 10°C. It was started at constant voltage of 200 and 330 V for the 1.4% and 3% agarose gels, respectively. After the samples (25 μL) had completely entered the gels, wells (2 × 10 mm) were filled with the agarose solution prepared for the stacking gels, and the power was decreased to 100 and 300 V. After electrophoresis, gels were fixed, washed, and reacted with 125I-MoAb 9 or polyclonal antibody and vWF was visualized by autoradiography as described. Scanning of selected autoradiographs was performed with a laser densitometer (LKB Ultrascan XL, LKB Instruments AB, Bromma, Sweden).

Electrophoresis on SDS-polyacrylamide gels was performed according to the method of Laemmli with 5% running gels and a vertical system. Plasma diluted to one half in 0.125 mol/L Tris-HCl buffer, pH 7.4, containing 4% SDS and 6 mol/L urea (final concentration) was reduced with dithiothreitol at a final concentration of 100 mmol/L at 60°C for 20 minutes. Immunoblotting of the gel was performed according to the method of Towbin et al, followed by staining with 125I-polyclonal antibody to vWF and autoradiography. As control, recombinant vWF expressed in baby hamster kidney (BHK 21) cells was used, containing a mixture of mature vWF subunit (275 Kd) and of pro-vWF including vWA G II (370 Kd).

**RESULTS**

**Binding of purified F.VIII to vWF from normal pool plasma.** The specificity of the binding of purified F.VIII to immobilized vWF was examined using serial dilutions (1/1 to 1/512) of a normal plasma pool and a constant amount (1 U/mL) of purified F.VIII (Fig 1). Comparison of the dose–response curves obtained when F.VIII activity was measured by a chromogenic assay (Fig 1A), VIII:C by a one-stage assay (not shown), and VIII:Ag by immunoradiometric assay with 125I-MoAb D4H1 (Fig 1B) established that F.VIII bound to immobilized vWF and that binding varied as a function of the amount of vWF. Plasma from a patient with severe vWD served as a negative control and demonstrated that no other plasma protein bound F.VIII in the assay. Comparison of the curves indicated that the chromogenic assay was the most sensitive method. The maximal F.VIII binding was observed when normal pool plasma was used undiluted (1 U/mL vWF:Ag). It also corresponded to the saturation of coated MoAb by vWF. This was indicated by the plateau of the dose–response curve showing the bound vWF, estimated using labeled MoAb 9, as a function of the plasma dilutions (Fig 1C). Results using labeled vWF confirmed that immobilization of vWF on coated MoAb was dose dependent and that saturation of 4 × 10⁻³ U vWF:Ag occurred when 0.5 to 1 U/mL vWF:Ag was incubated (not shown). Figure 1D shows that immobilized vWF was the limiting factor for F.VIII binding and demonstrated that a ratio of ~1/1 (U F.VIII/U vWF:Ag), similar to that observed in normal plasma, was consistently obtained.
Binding of F.VIII to plasma vWF from normal individuals, patients with type I vWD, and hemophilia A. Figure 2 compares the binding of F.VIII to immobilized plasma vWF from 10 normal individuals (Fig 2A), five patients with type I vWD (Fig 2B), and 13 patients with hemophilia A (Fig 2C) with that of vWF from normal pool plasma. The experiments were performed with a constant amount of F.VIII (1 U/mL) and serial dilutions of plasma. With the different types of plasmas (Fig 2), there was a consistent linear relationship between the amount of immobilized vWF and that of bound F.VIII, as demonstrated by the high values \( r \geq 0.96 \) repeatedly obtained for the correlation coefficient of the least-squares regression lines. In addition, F.VIII binding to immobilized vWF was not dependent on the type of plasma because the slopes of the regression lines were not significantly different when normal pool plasma was compared with plasma from control subjects (Fig 2A), patients with type I vWD (Fig 2B), or patients with hemophilia A (Fig 2C).

Binding of F.VIII to plasma vWF from the two propositi. F.VIII failed to bind normally to vWF from the two propositi (Fig 3). A significant difference in the slopes of the regression lines was consistently observed for control plasmas and plasmas from the two propositi. Figure 3A shows the results of a typical experiment in which four different samples of plasma from patient 1 were compared with normal pool plasma. These plasmas were collected in a 4-year period and included a sample obtained at 7 months of pregnancy. Even though there was in the latter an increase of the level of vWF from 35 to 71 U/dL and of F.VIII level from 10 to 31 U/dL, specific binding of F.VIII per unit of vWF was not significantly improved. All patient samples were characterized by regression lines not significantly different from one another, with slopes between 2.4 and 3.1 \( \times 10^{-5} \). These were markedly nonparallel to that of control plasma (8.4 \( \times 10^{-5} \)). Similar results were consistently observed when four plasma samples from patient 2 were tested (Fig 3B). The slopes for the patient's plasmas were between 2.4 and 2.8 \( \times 10^{-5} \), whereas the control was 8.7 \( \times 10^{-5} \).

The abnormality of F.VIII binding to vWF from patients 1 and 2 was confirmed using a constant amount of vWF and increasing concentrations (0 to 1 U/mL) of purified F.VIII. Binding curves using undiluted plasma from both patients were compared with those using various dilutions of normal pool plasma (Fig 4). In all cases, purified F.VIII specifically bound to vWF in a dose-dependent and saturable manner. The maximum of F.VIII bound to normal vWF varied linearly from \( A_{405nm} = 0.2 \) to 0.62 as a function of the amount of immobilized vWF (from 7,960 to 20,550 cpm). All curves reached a half-maximum binding value for a similar concentration of purified F.VIII \( \approx 0.11 \) U/mL. In contrast, vWF from patients 1 and 2 exhibited a decreased capacity to bind to F.VIII. Strikingly low values for the maximum of F.VIII binding (\( A_{405nm} = 0.20 \) and 0.29, respectively) were observed for an amount of immobilized vWF (21,500 and 20,900 cpm) similar to that observed using one-half to one-third dilutions of normal plasma.

After infusion of DDAVP in patient 2, the bleeding time was shortened from nine to five minutes for four hours (Table 2). The increase of vWFRCo, vWF:Ag, and F.VIII reached a maximum between 30 minutes and one hour and remained at high levels after four hours. Figure 5 shows the binding of purified F.VIII to immobilized vWF from samples obtained before infusion or 30 minutes, one hour, and four hours afterward, as compared with control plasma. Even though DDAVP induced a marked increase of vWF levels, the released vWF did not have an improved capacity to bind.
Fig 3. Binding of purified F.VIII to plasma vWF from the two propositi. Purified F.VIII (1 U/mL) was incubated with immobilized vWF from serial dilutions of normal pool plasma (□), four different samples of plasma (●, ○, ▲, △) from patient 1 collected in a 4-year period including one (●) obtained at 7 months of pregnancy (A) and of three different samples of plasma (●, ○, ▲) from patient 2 collected in a 1-year period (B). Bound F.VIII and immobilized vWF were estimated as described in the Materials and Methods section. For the test plasmas, the least-squares regression line corresponds to the grouped data from four (A) or three (B) different determinations, but the equations are given for the individual determinations. The equation for the control plasma is that of the regression line.

to F.VIII, as demonstrated by much lower slopes of the regression lines (2 to 2.4 × 10⁻⁵) than for the control plasma (10.5 × 10⁻⁵). There was no significant variation of the slopes at different times after DDAVP in this patient.

Electrophoretic analysis of vWF from the two propositi. The multimeric structure of vWF from patients 1 and 2 was compared with that from normal plasma by electrophoresis with 1.4% and 3% agarose gels (Fig 6). On a 1.4% agarose gel, the multimeric pattern of vWF from both patients was similar to that of control vWF (Fig 6A). On a 3% agarose gel, each oligomer of control vWF appears as a quintuplet with a main central band, two fast-moving, and two slow-moving satellite bands. In vWF from patients 1 and 2, the nearest satellite bands from the main band were absent or present in only trace amounts (Fig 6B). This slight but consistent abnormality was confirmed by scanning the autoradiographs (Fig 7). When patient 2 plasma was compared with a similar level of normal vWF:Ag, the scans demonstrated that the absence or marked decrease of subbands b and c was not related simply to decreased levels of vWF.

After SDS-polyacrylamide gel electrophoresis of reduced plasma, blotting and immunostaining, a single band with a Table 2. Laboratory Data of Patient 2 Before and After DDAVP Infusion

Table 2. Laboratory Data of Patient 2 Before and After DDAVP Infusion

<table>
<thead>
<tr>
<th>Variable</th>
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<td>Bleeding time (min)</td>
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<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>vWFRCO (U/dL)</td>
<td>52</td>
<td>149</td>
<td>118</td>
<td>95</td>
</tr>
<tr>
<td>vWF:Ag (U/dL)</td>
<td>56</td>
<td>165</td>
<td>122</td>
<td>102</td>
</tr>
<tr>
<td>VIII:C (U/dL)</td>
<td>28</td>
<td>88</td>
<td>90</td>
<td>54</td>
</tr>
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</table>

Fig 4. Effect of varying amount of purified F.VIII (0 to 1 U/dL) on its binding to immobilized vWF from patients 1 and 2. Dilutions of normal pool plasma were □ (●), △ (○), ▲ (△), ▲ (△), △ (□); plasma from patient 1 (●) and patient 2 (△). Right: Amount of immobilized vWF:Ag (cpm of MoAb 9) for each dilution of plasmas used in the experiment. Plasma from type III vWD was used as a negative control.

<table>
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<th>SAMPLE</th>
<th>vWF Ag (cpm)</th>
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<tr>
<td>NP 1/2</td>
<td>20550</td>
</tr>
<tr>
<td>NP 1/3</td>
<td>20150</td>
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<td>7960</td>
</tr>
<tr>
<td>TYPE III 1/1</td>
<td>1220</td>
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</table>
Fig 5. Binding of F.VIII to plasma vWF from patient 2 after infusion of DDAVP. Serial dilutions of normal pool plasma (□) and of plasma samples of patient 2 obtained before (●) or 30 minutes (▲), one hour (△), and 4 hours (■) after infusion of DDAVP (0.3 μg/kg body weight). Results are expressed as indicated in the legend to Fig 3.

DISCUSSION

vWF plays two major roles in hemostasis: it is essential for the initial attachment of platelets to the subendothelium at high shear rates and serves as carrier for F.VIII, stabilizing its coagulant activity. Previous studies using purified vWF and F.VIII established that an F.VIII binding domain is located on the extreme N-terminal portion of vWF between amino acid residues 1 and 272. We describe a new analytic method developed to evaluate the ability of plasma vWF to bind to exogenous purified F.VIII. The method is based on the immunoisolation of vWF from plasma onto microtitration wells coated with a selected MoAb to vWF, previously shown not to interfere with the binding of F.VIII to vWF. Plasma F.VIII was eliminated from the system by washing wells with calcium added, and binding of purified F.VIII to immobilized vWF was estimated by a chromogenic assay performed directly in the wells. Purified F.VIII bound in a dose-dependent and saturable manner to a constant amount of vWF immobilized from normal plasma, and F.VIII binding increased linearly as a function of the concentration of immobilized vWF. The specificity of the method was assessed by the lack of binding using plasma from a patient with severe vWD. The minimal amount of bound F.VIII (absorbance twice that of the nonspecific binding) was observed with plasma samples diluted to ~1 U/dL vWF:Ag. The reproducibility of the method was satisfactory when the F.VIII binding ability of plasma vWF from normal individuals or patients with hemophilia A or type I vWD was analyzed as compared with normal pool plasma. Within-assay variation was not signifi-
DEFECTIVE vWF BINDING TO F.VIII IN vWD VARIANT

Mr x 10^{-3}

440 —

340 —

160 —

vWF

Fig 8. SDS-5% polyacrylamide gel electrophoresis of reduced plasma from the two propositi. NP, normal pool plasma; 1 and 2, plasma from patients 1 and 2; rvWF, recombinant vWF expressed in BHK cells. Position of the molecular weight markers is indicated (left). Position of pro-vWF and mature vWF subunit (arrows).

cantly different in all cases, and between-assay variation was minor provided that immobilized vWF was measured with the same preparation of labeled MoAb.

vWD is a heterogeneous set of disorders resulting from quantitative or qualitative defects of vWF. It is phenotypically classified, mainly by the pattern of vWF multimers, into three main types, including various subtypes. In type I, the amount of vWF is decreased, resulting in similarly reduced levels of vWFRCO and vWF:Ag and consequently of F.VIII. A similar pattern is observed in severe, presumably homozygous, vWD (type III), in which the total absence of vWF protein results in the presence of only trace amounts of F.VIII. In type II, levels of vWFRCO are lower than levels of vWF:Ag, indicating a modified ability of vWF to interact with the platelet membrane glycoprotein Ib. However, the ratio of F.VIII to vWF:Ag remains essentially the same as in normal persons, suggesting that the various abnormalities of vWF in this type do not modify its ability to bind to and to stabilize F.VIII.

We report a new variant of vWD in two patients from the same kindred in whom the defect of vWF leads to an abnormal interaction with F.VIII. The propositi exhibited similar abnormalities of hemostasis tests, with a slightly prolonged bleeding time, prolonged activated partial thromboplastin time, decreased or subnormal levels of vWF:Ag and vWFRCO, and lower levels of F.VIII as measured by four different techniques. The discrepancy between the levels of vWF and F.VIII in both patients was not corrected either during pregnancy (patient 1) or after infusion of DDAVP (patient 2).

The plasmatic abnormality of the two propositi was investigated using the method described to determine if it resulted from the presence in plasma of a modified vWF or F.VIII molecule. Binding of purified F.VIII to immobilized vWF from the two propositi was markedly decreased, confirming the existence of a functional abnormality of their vWF. The defect of vWF appeared similar in both patients and stable with time, indicating the presence of a "true" variant. Additional evidence was obtained during pregnancy or after infusion of DDAVP (ie, when levels of plasma vWF, raised by increased synthesis or release, were not accompanied by a correction of F.VIII binding). This strongly suggested that the vWF defect was inherited and occurred during synthesis or processing of vWF. Comparison of the binding of purified F.VIII with similar amounts of normal vWF or patients' vWF suggested that the vWF abnormality did not modify the affinity of vWF for F.VIII; however, the lower plateau of the binding curves for the patient samples clearly indicated that the abnormal vWF contained a decreased number of binding sites for F.VIII as compared with normal vWF.

The defect of vWF which leads to an impaired interaction with F.VIII did not appear to modify the other functions of the vWF. This was first indicated by the hemostatic values from the propositi. These data, with the exception of the lower levels of F.VIII than of vWF, fitted with the pattern of type I vWD: slightly prolonged bleeding time; moderately low or subnormal plasma levels of vWF:Ag and vWFRCO, which increased after DDAVP infusion or during pregnancy; and the full range of vWF multimers by SDS-agarose gel electrophoresis. In addition, the ability of our patients' vWF to bind to platelet glycoprotein Ib was normal because the same displacement curves were consistently observed when serial dilutions of normal plasma or patients' plasma were used to inhibit the binding of labeled purified vWF to platelets with ristocetin added (not shown).

Attempts were made to associate the functional abnormality of the patients' vWF with structural modifications of its multimeric banding pattern. The patients' vWF is characterized by the presence of all multimers in plasma, as shown by a low-resolution electrophoretic system. However, each individual multimer shows a mild although consistent aberrant structure, with either absence or marked decrease of the two central satellite subbands.

Among the various factors that may explain the association of an aberrant multimeric banding pattern of the abnormal vWF and a decreased number of binding sites for F.VIII, despite an apparent normal affinity, would be the existence of a steric effect resulting from the presence of the pro-peptide vWAg II on part of the subunits in the vWF multimers. This would lead to the presence of two species of vWF, with a molecular weight of 270 and 370 Kd, respectively. Alternatively, a shortening of the N-terminal...
sequence may lead to a defective binding to F.VIII in association with a decreased mol wt of the subunit. However, the size of the subunit from the patient's vWF was shown to be normal by gel electrophoresis, and there was no evidence of the existence of a complex (pro-vWF) between vWAg II and the mature vWF subunit. Therefore, the abnormalities of vWF in the propositi probably result from a point mutation of its subunit.

Our patients appear to have a unique variant of vWD. Montgomery et al. described eight patients with apparent type I vWD characterized by similar levels of vWF:Ag and of VIII:C when measured by a one-stage method but consistently decreased levels of VIII:C when measured by a two-stage assay. This discrepancy resulted from an increased susceptibility of F.VIII to Al(OH)₃ adsorption and was corrected when vWF from a patient with severe hemophilia A was added. Our patients are clearly different in that they show identical levels of F.VIII by one-stage or two-stage clotting assays and chromogenic and immunoradiometric assays. In addition, our patients exhibit VIII:C levels consistently lower than those of vWF:Ag.

Thus, the present findings indicate that vWD is more heterogeneous than was previously believed. Numerous variants have been described with a decreased or increased reactivity toward platelets with ristocetin added (ie, presumably an abnormality of the GP Ib-binding domain of vWF). Our patients clearly correspond to a new variant of vWD with a unique abnormality of vWF which prevents it from binding to F.VIII.

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New variant of von Willebrand disease with defective binding to factor VIII

M Nishino, JP Girma, C Rothschild, E Fressinaud and D Meyer