Antibody-induced von Willebrand’s Disease: 
A Newly Defined Inhibitor Syndrome

By Robert I. Handin, Vera Martin, and William C. Moloney

A patient is described with clinical and laboratory evidence of von Willebrand’s disease (VWD) in association with lymphosarcoma. He consistently had a bleeding time of over 20 min; factor VIII on, VIIIAGN, and VIIIWF under 20%; and severe, diffuse hemorrhage. Following transfusion with cryoprecipitate, the patient had the expected rise in VIIIAGN and VIIIWF, but no secondary increment in VIIIWF. The patient’s plasma contained an inhibitor which prevented aggregation of normal platelets by ristocetin, but which did not interfere with the measurement of VIIIAGN or inactivate VIIIWF activity. The inhibitor was present in serum heated at 56°C for 30 min, was present in a purified IgG fraction of serum, and was precipitated by anti-human IgG. The antibody did not interact directly with washed platelets, but appeared to interact with that portion of the factor VIII protein that supports ristocetin aggregation (VIIIWF). The data provide the first explanation for the pathophysiology of the acquired von Willebrand’s syndrome.

VON WILLEBRAND’S DISEASE (VWD) is usually inherited as an autosomal dominant trait and is characterized by a prolonged bleeding time and a moderate deficiency of factor VIII coagulant activity (VIIIAHF)*. Unlike patients with hemophilia, patients with VWD also have a decreased level of the factor VIII-associated antigen (VIIIAGN), low platelet retention in glass bead columns, abnormal ristocetin-induced platelet aggregation, and poor adhesion of platelets to vascular subendothelium. These abnormalities in platelet function can usually be corrected in vitro by adding factor VIII-rich plasma fractions to the patient’s blood. This property of the factor VIII molecule has been called the von Willebrand factor (VIIIWF). To date, the precise molecular nature and interrelation between the coagulant activity, the von Willebrand factor activity, and the antigen recognized by heterologous antisera have not been determined with certainty.

A number of patients have also been described who have an acquired bleeding disorder that has similar clinical and laboratory features to inherited VWD. In these patients, the levels of VIIIAGN were decreased and, in one

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*The factor VIII nomenclature proposed by Weiss and co-workers will be used in this paper. Factor VIII refers to a high-molecular-weight plasma glycoprotein which has three functional characteristics: (1) VIII coagulant activity (VIIIAHF), the activity which corrects the coagulation abnormality of hemophiliac plasma; (2) factor VIII antigen (VIIIAGN), the antigen precipitated by heterologous antiserum produced by immunization with purified VIII, present in normal amounts in hemophiliac plasma and decreased in the patients with VWD; (3) von Willebrand factor (VIIIWF), the activity deficient in VWD which causes abnormalities in platelet function such as low platelet retention and defective ristocetin aggregation.

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Submitted January 9, 1976; accepted May 20, 1976

Supported in part by grants from the Massachusetts Division, American Cancer Society, and NIH Grants HL-17513 and 5-MO1-RR-00031-14. Dr. Handin is a Cancer Research Scholar of the American Cancer Society.

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case, the low platelet retention was corrected by the addition of cryoprecipitate to the patient’s blood. The patients with acquired VWD differed from other patients as they did not have a sustained rise in $\text{VIII}_{\text{AHF}}$ after a plasma infusion. While there has been no direct evidence for an immune etiology for acquired VWD, many of the patients have had immunologic or lymphoproliferative disorders, and some have had other demonstrable autoantibodies.

This report describes, in detail, a patient with lymphosarcoma and acquired VWD whose serum contained antibody that selectively destroyed the $\text{VIII}_{\text{WF}}$ activity of normal plasma. Brinkhous and associates have recently characterized serum with similar specificity obtained from a patient with inherited VWD who had received multiple blood transfusions. The studies reported here relate the development of the specific defects in platelet function seen in VWD to the presence of antibody specifically directed against that portion of the VIII molecule that interacts with circulating blood platelets.

**MATERIALS AND METHODS**

**Case History**

M.E., a 62-yr-old man with no prior history of bleeding, developed a neck mass, night sweats, easy bruising, recurrent epistaxis, and gum bleeding. In addition, there was no family history of bleeding. On physical examination, he had diffuse lymphadenopathy, hepatosplenomegaly, and scattered ecchymoses. When first seen, his white blood count was 35,000 x 10^9/liter with 90% lymphocytes. his platelet count was 235,000 x 10^9/liter, and his hematocrit was 39%. Bone marrow aspirate and biopsy showed infiltration with immature, fissured lymphocytes, and a cervical lymph node had its normal architecture obliterated by nodular poorly differentiated lymphocytic lymphoma. The patient subsequently became anemic and developed reticulocytosis and a positive antiglobulin test. He had no detectable antinuclear antibody or LE cells. His serum immunoelectrophoretic pattern was normal, although his level of IgM was decreased to 10 mg/100 ml.

The patient was treated with a combination of cyclophosphamide (Cytoxan, Mead & Johnson, Evanston, Ill.), vincristine (Oncovin, Eli Lilly, Indianapolis, Ind.), and prednisone (Deltasone, Upjohn, Kalamazoo, Mich.), and later received bleomycin (Bleocin, Bristol Myers, Syracuse, N.Y.) and doxorubicin (Adriamycin, Farmitalia, Milan, Italy). Following therapy, adenopathy and lymphocytosis decreased, and there was a fall in the number of circulating lymphocytes. However, his bleeding persisted and during the course of his illness he was hospitalized several times with gastrointestinal bleeding. He was treated with transfusions of packed red cells, platelet concentrates, and cryoprecipitate without any significant effect and eventually died of pneumonia and intractable gastrointestinal bleeding. Postmortem examination showed widespread, poorly differentiated lymphocytic lymphoma and diffuse hemorrhage. There were also several small ulcerations noted in the ileum in areas of lymphomatous infiltration.

**Coagulation Tests**

The prothrombin time, partial thromboplastin time (PTT), thrombin time, and fibrinogen level were measured by standard techniques. Factors VIII and IX, XI and XIII were assayed by a one-stage method using the activated PTT. The bleeding time was measured by the modified Ivy technique using plastic templates. Platelet retention was measured on native blood by the Salzman technique, using commercially packed glass bead columns containing 1.3 g of glass beads (Becton-Dickinson, Co., Fairfield, N.J.). Fibrin degradation products were detected with a micromodification of the staphylococcal-clumping technique. The presence of an inhibitor to $\text{VIII}_{\text{AHF}}$ activity was measured by incubating mixtures of test and control plasma at 37°C for 60 min and assaying the residual $\text{VIII}_{\text{AHF}}$ activity. One unit of inhibitor was defined as the amount of antibody that destroyed 0.75 units of $\text{VIII}_{\text{AHF}}$ activity after 1 hr of incubation.

**Purification of Factor VIII and Preparation of Factor VIII Antiserum**

Cryoprecipitate was prepared from fresh ACD plasma as described by Pool. Purified factor VIII was prepared from a commercial factor VIII concentrate (Hyland Method IV, Travenol Lab-
oratories. Costa Mesa, Calif.) by adsorption with 10 mg/ml bentonite, precipitation with 30\(^\circ\), polyethylene glycol (MW 3700, Matheson, Coleman and Bell, Cincinnati, Ohio), and chromatography on Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N.J.).\(^2\) The void volume fraction, which contained VIII\(_{AHF}\) activity and VIII\(_{AGN}\), was then used to immunize rabbits as described by Zimmerman et al.\(^2\) This serum, after absorption with VIII\(_{AHF}\)-poor plasma, produced a single precipitin line upon immunoelectrophoresis against human plasma or cryoprecipitate. Absorption was carried out with the supernatant obtained after precipitation of plasma with 8\(^\circ\), ethanol as described by Zimmerman et al.\(^2\) The rabbit anti-factor VIII serum neutralized 50\(^\circ\) of VIII\(_{AHF}\) activity of normal plasma at a dilution of 1:16 and neutralized 50\(^\circ\), of VIII\(_{AHF}\) activity of normal plasma at a dilution of 1:16 and neutralized 50\(^\circ\), of VIII\(_{AHF}\) in the ristocetin assay at a 1:32 dilution. Similar results were obtained with a commercial anti-factor VIII serum obtained from Behring Diagnostics, Woodbury, N.J.

**Measurement of VIII\(_{AGN}\)**

VIII\(_{AGN}\) was measured by the Laurell technique of quantitative immunoelectrophoresis.\(^31\) The system, as described by Zimmerman et al.\(^2\), was modified so that plasma samples were electrophoresed directly into 1\(^\circ\), agarose (Indubiose, Lyon, France) containing 0.5\(^\circ\), rabbit anti-human factor VIII serum. Electrophoresis was performed for 16 hr at 4\(^\circ\)C with an applied current of 25 mA/gel. The plate was then soaked for 24 hr in 0.9\(^\circ\), sodium chloride, rinsed for 30 min in distilled water, dried, and stained with 0.025\(^\circ\), Coomassie blue. Standards were prepared by diluting pooled normal plasma prepared by collecting blood from 10 to 20 normal volunteers into 0.38\(^\circ\), sodium citrate and centrifugation at 2000 \(g\) for 10 min at 4\(^\circ\)C. The platelet-poor plasmas were pooled, divided into 1-ml aliquots and frozen at -40\(^\circ\)C until used. One unit VIII\(_{AGN}\) was arbitrarily defined as the amount of antigen found in 1 ml of this plasma pool. As little as 0.030 unit VIII\(_{AGN}\) could be detected, which represented 10 pI of a 1:32 dilution of plasma.

In some studies, 100 \(\mu\)l patient's plasma or purified IgG which had been adjusted to 10 mg/ml protein concentration was incubated with the pooled plasma for 2 hr at 37\(^\circ\)C or 12 hr at 4\(^\circ\)C. The samples were then centrifuged at 12,300 \(g\) for 30 min at 4\(^\circ\)C, and the supernatant was then applied to the gel.

**Platelet Studies**

Platelets were separated from plasma by gel filtration.\(^32\) The platelet-rich plasma was layered onto a 1.5 x 8-cm siliconized column packed with Sepharose 2B and eluted with a modified Tyrode's albumin buffer containing 0.15 \(M\) NaCl, 5 \(x\) \(10^{-3}\) \(M\) KCl, 5 \(x\) \(10^{-5}\) \(M\) CaCl\(_2\), 2.9 \(x\) \(10^{-4}\) MgCl\(_2\), 1.1 \(x\) \(10^{-3}\) \(M\) Tris HCl, pH 7.4, 10 mg/ml albumin, and 10 mg/ml glucose. Following filtration, the platelet count was adjusted to 200,000 \(\times\) 10\(^9\)/liter with platelet-poor plasma or the Tyrode's albumin buffer. The release of \(^{14}\)C-serotonin from the platelets and the effect of platelets on the Russell's viper venom time of plasma were assayed by previously described techniques.\(^33\),\(^34\) Platelet aggregation was performed with a turbidometric technique.\(^35\) Aggregating agents included adenosine diphosphate (ADP, Sigma Chemical Co., St. Louis, Mo.), epinephrine bitartrate, and a crude collagen extract prepared from desiccated bovine Achilles tendon.

**Detection of Antiplatelet Antibody**

As previously described, antibody was measured by mixing platelets that had been incubated in test serum with autologous granulocytes in the presence of 0.2\(^\circ\), nitroblue tetrazolium dye.\(^36\) Antibody-induced release of \(^{14}\)C-serotonin was measured by incubating radiolabeled platelets in Tyrode's albumin buffer containing antisera for 30 min at 37\(^\circ\)C.\(^37\) Release of \(^{51}\)Cr from platelets was measured by incubating labeled platelets with heated serum or IgG for 30 min at 37\(^\circ\)C, as described by Aster and Enright.\(^38\)

**Preparation of Patient’s Serum and IgG**

The patient’s plasma was prepared by anticoagulation with 0.38\(^\circ\), sodium citrate and centrifugation at 2000 \(g\) for 15 min at 4\(^\circ\)C. Serum was prepared by letting blood incubate at 4\(^\circ\)C overnight. It was then aspirated and heated at 56\(^\circ\)C for 30 min.

IgG was purified from the heated serum by precipitation of the globulins in 50\(^\circ\), saturated

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ammonium sulfate. After extensive dialysis against 0.01 M phosphate buffer, pH 7.8, the soluble globulin fraction was chromatographed on DEAE-cellulose equilibrated with the same buffer. The flowthrough volume, which contained IgG, gave a single precipitin line when tested by immunoelectrophoresis against anti-human serum. Material was then stored at −40°C until used. In some experiments involving whole serum, the IgG fraction was precipitated with an excess of rabbit anti-human IgG (Hyland Laboratories, Costa Mesa, Calif.), followed by centrifugation at 12,000 g for 30 min at 4°C.

**Ristocetin Assay**

The quantity of VIIIıwF in plasma was measured with a modification of the visual technique described by Sarji et al. The platelets were gel filtered through Sepharose 2B equilibrated with Tris-HCl 10⁻² M, 0.15 M NaCl, 1 mM EDTA, pH 7.4, centrifuged at 1000 g for 10 min, and then resuspended in Tyrode’s albumin buffer at a concentration of 250,000 x 10⁹/liter. Aliquots of washed platelets were then mixed with various dilutions of plasma or test material and incubated at 37°C for 5 min, then 1.5 mg/ml final concentration of ristocetin was added and the time to visible aggregation recorded. In the absence of plasma, platelets did not aggregate after 120 sec of incubation with ristocetin. When ten samples were assayed for VIIIıwF activity on three different occasions, the coefficient of variation was 8%.

**RESULTS**

The patient had a number of laboratory abnormalities that are usually associated with VWD. As shown in Table 1, the patient’s bleeding time was prolonged, and there was decreased platelet retention when measured by the Salzman technique. In addition, the patient had a prolonged partial thromboplastin

<table>
<thead>
<tr>
<th>Table 1. Platelet Function Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient</strong></td>
</tr>
<tr>
<td>Platelet count</td>
</tr>
<tr>
<td>Bleeding time (min)</td>
</tr>
<tr>
<td>Platelet retention (%)</td>
</tr>
<tr>
<td>RVV time (sec)</td>
</tr>
<tr>
<td>¹⁴C-serotonin released (%) with</td>
</tr>
<tr>
<td>ADP (10 µM)</td>
</tr>
<tr>
<td>Epinephrine (5 µM)</td>
</tr>
<tr>
<td>Collagen (70 µg/ml)</td>
</tr>
</tbody>
</table>

⁺*Mean ± 1 SD

⁺Range.

Table 2. Plasma Coagulation Studies

<table>
<thead>
<tr>
<th><strong>Patient</strong></th>
<th><strong>Son A</strong></th>
<th><strong>Son B</strong></th>
<th><strong>Normal</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin time (sec)</td>
<td>10.9</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Partial thromboplastin time (sec)</td>
<td>43</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>Fibrinogen (g/liter)</td>
<td>3.9</td>
<td>4.0</td>
<td>2.9</td>
</tr>
<tr>
<td>Fibrin degradation products (mg/liter)</td>
<td>&lt;3</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Factor VIII’h</td>
<td>14, 18, 25</td>
<td>96</td>
<td>150</td>
</tr>
<tr>
<td>Factor VIII’k</td>
<td>10, 15, 20</td>
<td>90</td>
<td>125</td>
</tr>
<tr>
<td>Factor VIII’h</td>
<td>13, 15</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Factor IX (%)</td>
<td>90</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Factor XI (%)</td>
<td>110</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Factor XII (%)</td>
<td>78</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

⁺Mean ± SD.

⁺Range.
time, a low \(\text{VIII}_{\text{AHF}}\) as measured by coagulation assay, a decreased level of \(\text{VIII}_{\text{AGN}}\) as measured by quantitative immunoelectrophoresis, and a low \(\text{VIII}_{\text{VWF}}\) as measured by the ristocetin assay (Tables 1 and 2, Fig. 1). No coagulation abnormalities were noted in either son.

The patient’s platelets aggregated normally when incubated with standard concentrations of ADP, collagen extract, and epinephrine (Fig. 2). After incubation with these aggregating agents, the platelets released normal quantities of radiolabeled serotonin (Table 1). Following kaolin activation, the patient’s platelets also shortened the Russell’s viper venom (RVV) time of platelet-poor plasma as well as normal platelets.

However, the patient’s platelets did not aggregate when incubated with 1.5 mg/ml of ristocetin (Fig. 2). For comparison, ten normal individuals all had full aggregation with this dose of ristocetin, while six patients with severe inherited VWD did not aggregate. In contrast to the usual patient with VWD, the defect in ristocetin aggregation was not corrected by the addition of purified factor VIII concentrate (Fig. 3).

In order to determine if this lack of aggregation was due to an intrinsic platelet defect, the patient’s platelet-rich plasma was chromatographed on Sepharose...
2B to separate the platelets from the majority of plasma proteins. Following gel filtration, the platelets, which were now suspended in Tyrode's albumin buffer, were mixed with plasma from the patient under study, normal plasma, or plasma from a patient with inherited VWD, and their response to ristocetin tested (Fig. 4). After gel filtration, normal platelets retained the ability to aggregate with ristocetin because high-molecular-weight proteins like factor VIII were excluded from the gel and eluted in the void volume of the column along with the platelets. However, as shown in Fig. 4, normal gel-filtered platelets lost the ability to aggregate when mixed with plasma from the patient under study. Conversely, following gel filtration, the patient's platelets aggregated when mixed with normal plasma. As expected, normal plasma or plasma from patients with inherited VWD which did not contain any inhibitor would not affect the platelet response to ristocetin. These data suggested that the platelets from this patient with acquired VWD were capable of responding normally to ristocetin following separation, by gel filtration, from plasma proteins and with the provision of a source of VIII<sub>VWF</sub>.

The presence of an inhibitor which might interact with factor VIII or with the patient's platelets to produce some of the above laboratory abnormalities was studied by several different techniques. As shown in Table 3, the patient's serum did not destroy the VIII<sub>AHF</sub> activity of normal plasma after incubation for up
Fig. 4. In these experiments, platelets from a normal subject and from the patient were gel filtered into Tyrode's albumin buffer and then mixed with an equal volume of test plasma prior to the addition of 1.2 mg/ml ristocetin.

to 3 hr at 37°C, or 12 hr at 4°C, followed by centrifugation at 12,000 g to remove antigen antibody complexes. Moreover, incubation of plasma from this patient with normal plasma did not decrease the amount of VIII_AGN detected in the normal plasma by the quantitative immunoelectrophoretic technique, as shown in Fig. 1. However, the patient's serum or purified IgG inhibited the expression of VIII_VWF activity in normal plasma. As shown in Table 4, incubation of the patient's serum or IgG with normal plasma destroyed its ability to support the ristocetin-dependent aggregation of washed normal platelets. The VIII_VWF activity, in this assay, was decreased to less than 10% of normal following 1 hr of incubation at 37°C.

The inhibitory effect of the patient's serum was abolished by prior incubation with rabbit anti-IgG. The effect was maximal when whole serum was undiluted but was detected after a 1:4 dilution with buffer. In contrast, the inhibitory effect of rabbit anti-factor VIII serum was detected after a 1:32 dilution.

The presence of an inhibitor to the ristocetin response was also demonstrated by incubating heat-inactivated serum or purified IgG with normal gel-filtered platelets. As shown in Fig. 5, both these plasma fractions inhibited ristocetin aggregation. The inhibitory effect of the patient's IgG could be partially reversed by the addition of purified factor VIII to the incubation mixtures prior to the addition of ristocetin. In contrast, serum from hemophiliacs that contained antibody of VIII_AHF activity did not inhibit aggregation with ristocetin. The rabbit anti-factor VIII serum inhibited both VIII_AHF activity and ristocetin aggregation.

The patient's serum was then tested for antiplatelet antibody activity, using several techniques. The serum would not opsonize platelets for subsequent

| Table 3. Effect of Various Samples on VIII_AHF Activity |
|-----------------------------------|------|------|------|------|
|        | 1 hr | 2 hr | 3 hr | 12 hr |
| Soline                          | 100  | 98   | 90   | 85   |
| Human hemophilic anti-factor VIII plasma | 9    | 8    | 7    | 9    |
| ME's plasma                      | 87   | 85   | 80   | 79   |
| Rabbit anti-factor VIII serum    | 1    | 0    | 0    | 0    |

*An equal volume of the addition and of normal plasma were mixed and incubated at 37°C for 1-3 hr and at 4°C for 12 hr. The sample was assayed for residual VIII_AHF activity and compared to the 1-hr saline control.
phagocytosis by autologous granulocytes (Table 5). In addition, the serum did not stimulate release of $^{14}$C-serotonin and did not induce complement-dependent platelet lysis, as measured by leakage of intraplatelet $^{51}$Cr (Table 5). Within the limits of these three assays, there was no detectable interaction between the patient’s serum and washed platelets.

Because of severe epistaxis and gastrointestinal hemorrhage, the patient was treated with cryoprecipitate and blood on many occasions. A typical response to transfusion is depicted in Fig. 6. Following the infusion of 1760 units of cryoprecipitate, the plasma $\text{VIII}_{\text{AHF}}$ rose to 0.55 U/ml, expected rise 0.63 U/ml, and there was a concomitant rise in $\text{VIII}_{\text{AGN}}$ concentration. Over the next 24 hr, the $\text{VIII}_{\text{AHF}}$ and $\text{VIII}_{\text{AGN}}$ levels returned to pretransfusion values, and there was no secondary rise in either $\text{VIII}_{\text{AHF}}$ or $\text{VIII}_{\text{AGN}}$. There was also no change in the patient’s bleeding time, platelet retention, or response to ristocetin when tested 2 hr and 18 hr after the infusion. There was no evidence of any sustained rise in $\text{VIII}_{\text{AHF}}$ concentration following any of his other cryoprecipitate infusions.

### Table 4. Effect of Serum and IgG on $\text{VIII}_{\text{VWF}}$

<table>
<thead>
<tr>
<th>Addition*</th>
<th>Time (sec)</th>
<th>$\text{VWF Activity (%)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>Human hemophiliac anti-factor VIII</td>
<td>13</td>
<td>90</td>
</tr>
<tr>
<td>Rabbit anti-factor VIII</td>
<td>$&gt;25$</td>
<td>$&lt;1$</td>
</tr>
<tr>
<td>M.E.’s serum</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>1.2</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>1.4</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>1.8</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>1.16</td>
<td>13</td>
<td>90</td>
</tr>
<tr>
<td>M.E.’s IgG (10 mg/ml)</td>
<td>23</td>
<td>5</td>
</tr>
<tr>
<td>M.E.’s serum and anti-IgG</td>
<td>12.5</td>
<td>95</td>
</tr>
</tbody>
</table>

*Equal volumes of addition and normal plasma were incubated at 37°C for 30 min before testing. The incubation mixture consisted of 0.1 washed platelets in Tyrode’s albumin buffer, 0.1 ml ristocetin (1.2 mg/ml final conc.), and 0.1 ml test material.

†Per cent activity calculated from a standard curve relating dilutions of pooled normal plasma to the time to visible aggregation after the addition of ristocetin.

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**Fig. 5.** Gel-filtered platelets (0.4 cc) from a normal subject were incubated with 0.1 ml of the indicated antiserum or purified immunoglobulin for 5 min at 37°C prior to the addition of 1.2 mg/ml ristocetin. In one series of experiments, various concentrations of purified factor VIII were added along with the immunoglobulin preparation.
Table 5. Measurement of Antiplatelet Antibody

<table>
<thead>
<tr>
<th>Addition</th>
<th>Release of $^{14}$C 5-HT (%)</th>
<th>Release of $^{51}$Cr (%)</th>
<th>Increase in Rate of NBT Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal serum</td>
<td>5 ± 3*</td>
<td>15 ± 8*</td>
<td>12 ± 4*</td>
</tr>
<tr>
<td>M.E.'s serum</td>
<td>8</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Rabbit antiplatelet serum</td>
<td>85</td>
<td>74</td>
<td>220</td>
</tr>
<tr>
<td>ITP serum</td>
<td>6</td>
<td>8</td>
<td>135</td>
</tr>
</tbody>
</table>

*Mean ± SD for 35 normal subjects.
†In this assay, the rate of NBT reduction by granulocytes incubated with platelets exposed to the various serums is compared to the rate of reduction by washed platelets and granulocytes.

DISCUSSION

The onset of bleeding late in life in the absence of any family history and in association with the development of a lymphosarcoma makes an acquired etiology seem most likely. In addition, most of the previously reported cases of acquired VWD have occurred in association with lymphoproliferative or autoimmune disorders. Several patients have had systemic lupus erythematosus, and one patient had a monoclonal immunoglobulin, although this monoclonal protein did not have detectable anti-factor VIII activity. All patients tested have had a low level of $\text{VIII}_\text{AGN}$, including the present case, and all have lacked the secondary increase in $\text{VIII}_\text{AHF}$ usually seen after infusion of plasma into patients with inherited VWD.

The patient reported here may be unique in that his plasma, in addition to having a low level of circulating $\text{VIII}_\text{AHF}$ and $\text{VIII}_\text{AGN}$, could inhibit ristocetin-induced platelet aggregation when added to platelet suspensions obtained from normal donors. The inhibitory activity was present in heated serum, was precipitated by anti-IgG and co-chromatographed with purified IgG, suggesting that it was an antibody.

The in vivo consequences of this antibody were quite dramatic. The patient consistently had an infinitely prolonged bleeding time, and often oozed from minor cuts for several days. The patient's hospital admissions for severe hemorrhage required multiple units of cryoprecipitate and red cell replacement with no objective evidence of response. It was also of interest that the antibody

Fig. 6. The patient received the 1760 units of factor VIII in the form of cryoprecipitate and had samples withdrawn for measurement of $\text{VIII}_\text{AHF}$ (---) and $\text{VIII}_\text{AGN}$ (---) as indicated.
activity and the hemostatic defect persisted despite intensive combination chemotherapy, which included immunosuppressive drugs such as cyclophosphamide, prednisone, and vincristine, which have been used to treat other patients with factor VIII inhibitors.\textsuperscript{40,42}

Patients with malignancy, particularly lymphosarcoma, may develop various immunologic aberrations, including deficiency in cellular immune function, hypogammaglobulinemia, monoclonal immunoglobulins, and specific antibodies directed against platelets and red cells causing secondary thrombocytopenia or hemolytic anemia. \textit{VIII}_{\text{AHF}} inhibitors are seen in elderly patients, postpartum females, and after allergic reactions to drugs like penicillin.\textsuperscript{43} Although there are limited data, there is no strikingly increased incidence of \textit{VIII}_{\text{AHF}} antibody associated with malignancy.\textsuperscript{43} It seems very likely that the malignant lymphocytes in this patient were responsible for production of the antibody detected in this patient. In addition to being an interesting paraneoplastic syndrome, the presence of this antibody complicated the patient’s therapy with myelosuppressive medication, caused profound hemorrhage, and directly contributed to his death.

Although the antibody prevented certain platelet reactions dependent on \textit{VIII}_{\text{VWF}}, others were spared. Thus, the patient’s platelets aggregated normally in response to ADP, epinephrine, and collagen extract. His platelets, when stimulated with aggregating agents, released normal quantities of \textsuperscript{14}C-serotonin, and shortened the RVV time of normal plasma. These observations eliminated an intrinsic platelet release defect as the cause of the prolonged bleeding time. There was also no evidence of antibody that could directly interact with the platelet surface as measured by three different tests. In addition, the patient was not thrombocytopenic at the onset of his bleeding disorder. This fact is particularly important since antibody, in addition to causing thrombocytopenia, might lead to aberrant function by inhibiting aggregation or inducing an acquired abnormality in storage pool nucleotide content.\textsuperscript{44,45} Presence of the specific platelet abnormalities, which are dependent on a plasma protein, factor VIII, and the restoration of function following gel filtration of the patient’s platelets, also confirm the lack of an intrinsic platelet defect.

Plasma factor VIII has several functions that could be altered by the presence of an antibody. A large number of patients have previously been described to have acquired “hemophilia” due to an antibody that destroyed \textit{VIII}_{\text{AHF}} activity.\textsuperscript{43} These patients have had typical hemophilic bleeding into joints and soft tissues and no apparent platelet abnormalities. The patient reported here was clinically different from other patients with acquired factor VIII inhibitors in that he had the typical clinical features of VWD, with severe mucosal and gastrointestinal bleeding. He also had the laboratory evidence of abnormal platelet function mentioned previously.

The patient’s antibody was selective in that it did not inhibit \textit{VIII}_{\text{AHF}} or interfere with the precipitin reaction between normal plasma and the rabbit antifactor VIII serum. The immunochemistry of factor VIII is complex, and a variety of antisera have been produced which can react with this protein (Fig. 7). The rabbit serum has the broadest specificity and can neutralize \textit{VIII}_{\text{VWF}} and \textit{VIII}_{\text{AHF}} as well as precipitate \textit{VIII}_{\text{AGN}}. According to Weiss and co-workers, the serum from hemophiliacs who have circulating inhibitors will
Fig. 7. Schematic representation of the interaction of various antisera and the factor VIII molecular complex.

not inhibit expression of \( \text{VIII}_{\text{VWF}} \) activity.\(^{39} \) There are, however, two subsequent reports that some inhibitor sera obtained from hemophiliacs may interfere with platelet aggregation by ristocetin.\(^{46,47} \) None of the human inhibitor sera precipitates \( \text{VIII}_{\text{AGN}} \). Hoyer has speculated that this might relate to the low binding affinity of the human antibody or to the low molecular weight of the postulated \( \text{VIII}_{\text{AHF}} \) subunit.\(^{48} \)

Serums with some selectivity toward \( \text{VIII}_{\text{VWF}} \) activity have been produced by immunizing rabbits with the factor VIII-like protein synthesized by cultured endothelial cells or by immunizing with the void volume fraction from hemophiliac plasma.\(^{49,50} \) However, Bennett and co-workers subsequently found that the hemophiliac factor VIII molecule produced antibody which could neutralize normal \( \text{VIII}_{\text{AHF}} \).\(^{51} \) The serum described here is of special interest because it does not interfere with measurement of \( \text{VIII}_{\text{AGN}} \) or \( \text{VIII}_{\text{AHF}} \) but can selectively neutralize \( \text{VIII}_{\text{VWF}} \) activity.

The fact that the patient had a low level of plasma factor VIII measured by immunoassay as well as by coagulation assay suggests that the antibody may affect the production or the intravascular life span of factor VIII. Therefore, the transfusion study depicted in Fig. 6 is quite pertinent. Although there are limited data on the fate of transfused factor VIII in patients with VWD, using an immunoassay, the half-life of \( \text{VIII}_{\text{AGN}} \) is reported to be 24–48 hr.\(^{52,54} \) In the patient reported here, the transfused material disappeared from the circulation much more rapidly. It is possible that the presence of antibody in the circulation altered the survival of transfused \( \text{VIII}_{\text{AGN}} \). The circulating antibody may also account for the lack of secondary increase in \( \text{VIII}_{\text{AHF}} \) by preventing release of factor VIII from synthetic sites or by impairing the interaction of transfused factor VIII protein with precursor material destined to become the complete factor VIII molecular complex.

ACKNOWLEDGMENT

The authors thank Eric Chun Lian, M.D., Veterans' Administration Hospital, Miami, Fla., for the initial measurements of \( \text{VIII}_{\text{AGN}} \) and for a gift of rabbit anti-factor VIII serum; Sherwin Kevy, M.D., Children's Hospital Medical Center, for samples of blood from his patients with factor VIII inhibitors; and Irma Szynanski, M.D., Massachusetts Division, American Red Cross, who assisted in providing cryoprecipitate for the transfusion studies and plasmapheresed the patient.

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