Selective Absence of Large Forms of Factor VIII/von Willebrand Factor in Acquired von Willebrand’s Syndrome. Response to Transfusion

By D. Meyer, D. Frommel, M. J. Larrieu, and T. S. Zimmerman

A previously healthy elderly man with mucocutaneous bleeding was found to have a benign monoclonal IgG gammapathy associated with criteria for severe von Willebrand disease (Factor VIII procoagulant activity, Factor-VIII-related antigen, and ristocetin cofactor activity, <10% of normal). Associated qualitative abnormalities of factor VIII/von Willebrand factor were demonstrated by radiocrossed immunoelectrophoresis and immunoradiometric assay. The late clinical onset and negative family history are in favor of an acquired form of vWD. The monoclonal gammapathy and abnormalities of factor VIII/von Willebrand factor have been stable over a 10-yr period. No inhibitor to Factor VIII procoagulant activity, ristocetin cofactor activity, or Factor-VIII-related antigen could be demonstrated. Following transfusion of cryoprecipitate (with a normal cross immunoelectrophoretic pattern), there was a rapid removal of the large forms of Factor-VIII-related antigen, paralleled by a decay of ristocetin cofactor activity. The transfusion study of this patient with acquired von Willebrand disease type II (variant of von Willebrand disease) serves to emphasize the relationship between polydispersity of Factor VIII/von Willebrand Factor and functional heterogeneity.

VON WILLEBRAND’s DISEASE (vWD) is a common congenital hemorrhagic disorder characterized by a prolonged bleeding time resulting from quantitative and/or qualitative abnormalities of factor VIII/von Willebrand factor (FVIII/vWF). FVIII/vWF exists in normal plasma in multiple molecular forms, with the largest possessing ristocetin cofactor activity and the smaller forms lacking this property. The clinical forms of vWD can be divided into two main groups based on the nature of the abnormality of FVIII/vWF observed in the plasma of the affected patients. In type I vWD, all forms of FVIII/vWF are decreased, with factor VIII procoagulant activity (VIII:C) and ristocetin cofactor activity (VIIIIR:RCO) diminished to a similar degree. In type II vWD, the larger forms of FVIII/vWF are missing, but the smaller forms are present in normal, increased, or decreased amounts. In this form of the disease, VIII:C may be normal but VIIIIR:RCO is always decreased disproportionately to VIII:C and factor-VIII-related antigen (VIIIIR:Ag).

In addition to congenital vWD, some cases of acquired von Willebrand syndrome have been reported, often associated with autoimmune or lymphoproliferative disorders. In this article we describe a patient with a benign monoclonal IgG...
gammapathia in whom a bleeding disorder occurred, characterized by FVIII/vWF abnormalities similar to those described in type II congenital vWD. Following transfusion with cryoprecipitate, preferential disappearance of the large forms of FVIII/vWF was observed.

MATERIALS AND METHODS

Bleeding time (BT) was determined according to Borchgrevink; platelet retention to glass beads (PR) according to Bowie; ristocetin-induced platelet aggregation as described elsewhere. VIII:C was measured by a one-stage assay based on the correction of a kaolin-activated partial thromboplastin time using diluted test plasma and plasma from a patient with severe hemophilia A. VIIIR:RCo was measured using formalin-fixed washed platelets, test plasma, and ristocetin; factor-VIII-related antigen (VIIIR:Ag) was measured by quantitative radioimmunoelectrophoresis. In this technique, affinity-purified rabbit anti-VIIIR:Ag antibody, which had been labeled with $^{125}$I, was mixed with unlabeled rabbit anti-human VIIIR:Ag antiserum. Autoradiography of the plates was performed using a 15-hr exposure time. VIIIR:Ag was also measured by immunoradiometric assay (IRMA) as previously described. Radiocrossed immunoelectrophoresis of VIIIR:Ag was carried out as described elsewhere, using a mixture of unlabeled and labeled anti-VIIIR:Ag. Isolation of IgG was performed by precipitation with 40% ammonium sulphate and n-octanoic acid and concentration by pressure dialysis to a protein content of 1 mg/ml. The presence of an inhibitor to VIII:C and VIIIR:RCo was investigated by incubating mixtures of test and control plasma or IgG at 37°C for periods of time ranging from 0 to 2 hr and assaying residual VIII:C and VIIIR:RCo as previously described. The presence of a precipitating antibody to VIIIR:Ag was investigated by reacting patient’s plasma or IgG against normal plasma, cryoprecipitate, or purified FVIII/vWF in immunodiffusion and electroimmunodiffusion systems.

Case Report and Laboratory Studies

In 1967, a 58-year-old retired engine driver presented with the sudden onset of a bleeding diathesis characterized by easy bruising upon minor trauma, epistaxis, and prolonged bleeding following minor cuts. There was no prior history of excessive bleeding, even after two major surgical procedures. Laboratory tests revealed a normal platelet count and decreased VIII:C (0.04 U/ml). The films of peripheral blood and bone marrow were normal. Serum electrophoresis and immunoelectrophoresis revealed a moderate IgG-Kappa M component; the concentrations of IgG, IgM, and IgA were within normal limits; screening for autoantibodies was negative; proteinuria was absent. More extensive hemostasis investigations were performed in 1972, 1974, and 1978: bleeding time, 12–15 min (normal range, 4–8); platelet retention to glass beads, 10%–15% (normal range, 70%–100%); ristocetin (1 and 2 mg/ml) induced aggregation of platelets in platelet-rich plasma, nil. Correction of ristocetin-induced platelet aggregation was achieved using normal plasma, but not plasma from four patients with severe vWD. Platelet aggregation induced by adenosine diphosphate (ADP) and collagen was normal. Factor VIII procoagulant activity (VIII:C) was 0.06–0.09 U/ml (normal range, 0.5–1.5); factor-VIII-related antigen (VIIIR:Ag) 0.04–0.06 U/ml by radio-Laurell and 0.02 U/ml by IRMA (normal range, 0.5–1.6); ristocetin cofactor activity (VIIIR:RCo), below 0.03 U/ml (normal range, 0.5–1.5). No significant differences were observed between the results of the plasma samples in 1972, 1974, and 1978. The IgG-Kappa M component remained unchanged between 1967 and 1978. No inhibitor to VIII:C or VIIIR:RCo was demonstrated, and no precipitin line was observed when the patient’s plasma or IgG were reacted by immunodiffusion or electroimmunodiffusion with normal plasma, cryoprecipitate, or purified human FVIII/vWF.

Radiocrossed immunoelectrophoretic analysis of patient plasma detected none of the large, less anodic forms of normal FVIII/vWF (Fig. 1). This was a consistent finding on 6 separate occasions from 3 different dates (1972, 1974, and 1978). The results of similar studies performed on the patient’s asymptomatic daughter were normal. The dose-response curve obtained by IRMA with the patient’s plasma was different from that of normal, showing a decreased maximal antibody-binding capacity. This pattern was similar to those observed in congenital vWD type II.
Fig. 1. Autoradiograph of crossed immunoelectrophoresis plate using $^{125}$I-labeled anti-VIII:Ag antibody. NP, Control pool plasma (upper plate); PT, patient plasma (lower plate). The vertical black bar indicates the point of origin in the first dimension. The anode is to the right in the first dimension and to the top in the second. The individual autoradiographs have been partially superimposed for comparison.

Table 1. Hemostasis Data in a Case of Acquired von Willebrand Syndrome: Response to Infusion of Cryoprecipitate (30 U/kg) Containing 3.4 U/ml VIII:C, 9.6 U/ml VIII:Ag, and 7.0 U/ml VIII:Co

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>BT (min)</th>
<th>PR Bowie (%)</th>
<th>VIII:C (U/ml)</th>
<th>VIII:Ag* (U/ml)</th>
<th>VIII:RCo (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>15</td>
<td>10–15</td>
<td>0.09</td>
<td>0.06</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>After 0.5</td>
<td>9</td>
<td>27</td>
<td>0.24</td>
<td>0.26</td>
<td>0.15</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>61–69</td>
<td>0.48</td>
<td>0.75</td>
<td>0.30</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>17</td>
<td>0.44</td>
<td>0.30</td>
<td>0.10</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>12</td>
<td>0.31</td>
<td>0.25</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>24</td>
<td>—</td>
<td>—</td>
<td>0.11</td>
<td>0.07</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Normal range</td>
<td>4–8</td>
<td>70–100</td>
<td>0.5–1.5</td>
<td>0.5–1.6</td>
<td>0.5–1.5</td>
</tr>
</tbody>
</table>

*Radio quantitative immunoelectrophoresis.
Fig. 2. Autoradiograph of crossed immunoelectrophoresis plate using $^{125}$I-labeled anti-VIII:Ag antibody. NP, Control pool plasma; Cryo, cryoprecipitate prior to infusion. The individual autoradiographs have been superimposed.

Transfusion Studies

Alterations of bleeding time and levels of VIII:C, VIIIIR:Ag, and VIIIIR:RCo following infusion of lyophilized cryoprecipitate (C.N.T.S., Paris) (30 U/kg) are indicated in Table 1. Radiocrossed immunoelectrophoresis demonstrated that the cryoprecipitate infused had a normal pattern (Fig. 2) and, in particular, contained the slower, less anodic forms of VIIIIR:Ag present in normal plasma. Different patterns were obtained by radiocrossed immunoelectrophoresis at various times following infusion of cryoprecipitate (Fig. 3). After 1 hr, larger less anodic forms of FVIII/vWF were present, but in a slightly smaller amount than in normal plasma or infused cryoprecipitate (Fig. 3). After 6 hr, the less anodic forms were totally absent (Fig. 3). After 4 hr, an intermediate pattern was obtained (Fig. 4). The abnormalities found by radiocrossed immunoelectrophoresis correlated with the results of bleeding time. Correction was seen after 1 hr when the less anodic forms were present, but not after 6 hr when they were absent.

DISCUSSION

The most striking finding in this case of acquired von Willebrand syndrome is the highly abnormal crossed immunoelectrophoretic pattern of VIIIIR:Ag, with the larger less anodic forms missing. Qualitative abnormalities of FVIII/vWF were also demonstrated by IRMA in this patient, with a different dose–response curve from normal, i.e., a decreased maximal antibody-binding capacity. Such findings by IRMA, as well as the abnormal immunoelectrophoretic pattern, are similar to the abnormalities described in congenital vWD type II (variants of vWD).3,5,6,27 Among the other cases of acquired von Willebrand syndrome reported in the
Fig. 3. Autoradiography of crossed immunoelectrophoresis plate using $^{125}$I-labeled anti-VIII:Ag antibody. Cryo. Cryoprecipitate; 1 hr, patient’s plasma 1 hr following infusion of cryoprecipitate (30 U/kg); 6 hr, patient’s plasma 6 hr following infusion of cryoprecipitate (30 U/kg). The individual autoradiographs have been superimposed.

Fig. 4. Autoradiography of crossed immunoelectrophoresis plate using $^{125}$I-labeled anti-VIII:Ag antibody. 4 hr, Patient’s plasma 4 hr following infusion of cryoprecipitate; 6 hr, patient’s plasma 6 hr following infusion of cryoprecipitate. The individual autoradiographs have been superimposed.
literature, only one has been analyzed by crossed immunoelectrophoresis. The larger, less anodic forms of VIIIIR:Ag were also missing in that case. In the patient reported here, the quantitative abnormality of FVIII/vWF was more severe, VIIIIR:Ag being only 0.04–0.06 U/ml by radio quantitative immunoelectrophoresis and 0.02 U/ml by IRMA, with less than 0.03 U/ml VIIIIR:RCO.

The response to transfusion of cryoprecipitate demonstrated a rapid initial disappearance of the large slow moving forms of VIIIIR:Ag, with an apparent persistence of the small forms. Only forms already present in the infused material could be detected. The normal immunoelectrophoretic pattern of the infused cryoprecipitate contrasts with that of some factor VIII concentrates in which the less anodal forms are missing.

There seems to be a good correlation in vivo between the size and function of FVIII/vWF. One hour following transfusion, bleeding time was normal and VIIIIR:RCO was increased to 30%; at that time, large forms of VIIIIR:Ag were present, although already to a lesser extent than normal. Six hours after transfusion, only small, fast moving forms of VIIIIR:Ag were present, and VIIIIR:RCO was absent. At that time, bleeding time was no longer corrected.

We cannot rule out the possibility that transfused normal, less anodal forms of VIIIIR:Ag were converted into more anodal ones, as reported in one study of inherited vWD type II. However, it seems likely that, in this case, there was a preferential removal of the large forms of FVIII/vWF. A sequence of metabolism of infused FVIII/vWF similar to that reported here has been documented by another method in normals and hemophiliacs.

Acquired von Willebrand’s syndrome, in the absence of an inhibitor, may result from the decreased production of the functional, less anodic forms of FVIII/vWF or accelerated clearance of these forms. Accelerated clearance may have resulted from the binding of the large forms of FVIII/vWF to the lymphoid cell population responsible for the patient’s gammapathy. Such binding to abnormal lymphoid cells has been previously demonstrated.

ACKNOWLEDGMENT

We wish to thank F. Josso for referring the patient to us and N. Ardaillou and J. P. Girma for performing the IRMA of VIIIIR:Ag.

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