Platelet-Type von Willebrand's Disease: Characterization of a New Bleeding Disorder

By Jonathan L. Miller and Antonio Castella

An autosomally transmitted bleeding diathesis sharing some, but not all, features previously described in von Willebrand's disease (vWD) was studied in five patients representing three generations of a single family. Bleeding times in the upper normal range in conjunction with low-normal platelet counts, normal factor VIII coagulant activity and VIII-related antigen, decreased VIII-ristocetin cofactor activity, selective decrease of the higher molecular weight factor VIII/von Willebrand factor (VIII/vWF) multimers, and increased ristocetin-induced platelet agglutination at low ristocetin concentrations were characteristic. Binding of patient VIII/vWF to washed normal platelets was within normal limits, whereas binding of normal VIII/vWF to patient platelets was significantly increased (p < 0.001 at 0.6 mg/ml ristocetin). This disorder accordingly appears to involve an intrinsic platelet abnormality affecting platelet-VIII/vWF interactions. It is proposed that the concept of vWD be broadened to include patients with this abnormality, which may appropriately be called "Platelet-type von Willebrand's disease."

A UTOSOMALLY TRANSMITTED bleeding disorders involving quantitative and/or qualitative abnormalities of the factor VIII/von Willebrand factor (VIII/vWF) complex comprise the family of disorders known as von Willebrand's disease (vWD). Recently, a new vWD variant (type IIB) has been described, in which plasma VIII/vWF lacked the higher molecular weight multimers, ristocetin-induced platelet agglutination (RIPA) was heightened, patient VIII/vWF could be shown to bind in increased amount to normal platelets, but patient platelets revealed no abnormalities in their interactions with VIII/vWF from normal plasma. Patients sharing some similarities with the type IIB variant, but manifesting quite different platelet-VIII/vWF interactions, have subsequently been encountered both by ourselves and other workers. Evidence for an intrinsic platelet defect in such patients is presented and discussed in this article.

MATERIALS AND METHODS

Five patients representing three generations of a single family were studied. All had significant histories of bleeding following minor trauma or in association with surgical procedures. Five healthy adult volunteers (three male and two female) served as normal controls.

Bleeding times were performed by the Simplate II technique (General Diagnostics, Morris Plains, N.J.). Blood was collected by a two-syringe technique and anticoagulated with 0.38% sodium citrate. Platelet counts were performed on a Coulter S Plus (Coulter Electronics, Hialeah, Fl). The activated partial thromboplastin time (aPTT), prothrombin time (PT), and thrombin time (TT) were measured by standard methods. Factor-VIII-coagulant activity (VIII-C) was measured by a one-stage PTT assay using severely deficient VIII-C substrate (Dade). Factor-VIII-related antigen (VIII-R:Ag) was quantitated by the Laurell rocket electrophoresis technique using rabbit anti-human factor VIII antibody. Factor-VIII-ristocetin cofactor (VIII-R:Co) was assayed by aggregometry using formalin-fixed normal platelets. Measurements of platelet aggregation were made in a Payton aggregometer using platelet-rich plasma adjusted to 300,000 platelets/μl. Multimer composition of VIII/vWF measured radioautographically following SDS agarose gel electrophoresis was performed by Scripps-Miles Immunology Reference Laboratory by the method of Ruggeri and Zimmerman. VIII/vWF binding studies were performed by the following method. Blood was drawn into ACD and platelets washed by the method of Miller et al., with the first wash containing 0.2 mM EGTA. Washed platelets were suspended at 450,000/μl in buffer containing 137 mM NaCl, 2.7 mM KCl, 0.2% (w/v) dextrose, and buffered to pH 7.5 with 25 mM Tris-HCl. Pooled normal or patient's platelet-poor plasma (PPP) was adjusted with barbital buffer to contain 70% VIII-R:Ag, and 0.5 ml was then incubated with an equal volume of washed platelets. Incubations were performed for 30 min at 37°C with increasing concentrations of ristocetin (0–1.8 mg/ml), following which the samples were centrifuged at 2500 g for 15 min. Supernatants were then assayed for unbound residual VIII/vWF assayed as VIII-R:Ag by the Laurell technique.

Platelet glycoproteins from patient II-1 and from two normal controls were kindly analyzed by Dr. David Phillips using lactoperoxidase-catalyzed iodination and additionally by periodate oxidation followed by reduction with tritiated sodium borohydride.

RESULTS

The aPTT, PT, TT, and VIII-C levels were normal in four of the five patients, as shown in Table 1. The prolonged aPTT and markedly reduced VIII-C level in patient III-1 were associated with a potent inhibitor to VIII-C that developed in this patient following prior cryoprecipitate therapy; his VIII/vWF parameters and platelet binding findings were otherwise indistinguishable from those of other family members.

The level of VIII-R:Co was significantly decreased in all patients, but VIII-R:Ag was consistently normal (or slightly increased). Multimer studies of VIII/vWF (Fig. 1) showed absence of the larger multimers in each patient, with normal multimer patterns in both

From the Division of Clinical Pathology, SUNY Upstate Medical Center, Syracuse, N.Y.

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Address reprint requests to Jonathan L. Miller, M.D., Ph.D., Division of Clinical Pathology, SUNY Upstate Medical Center, 750 East Adams Street, Syracuse, N.Y. 13210.

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Table 1. Coagulation Studies

<table>
<thead>
<tr>
<th>Patients</th>
<th>aPTT (17.5–27.5 sec)</th>
<th>PT (9.2–11.2 sec)</th>
<th>TT (&lt;29 sec)</th>
<th>Bleeding Time (3–8 min)</th>
<th>VIII-C (50%–150%)</th>
<th>VIII-R:Ag (46%–154%)</th>
<th>VIII-R:Co (50%–194%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient I-1 (mother)</td>
<td>20.6</td>
<td>9.4</td>
<td>18.5</td>
<td>5</td>
<td>97%</td>
<td>200%</td>
<td>&lt;12.5%</td>
</tr>
<tr>
<td>Patient II-1 (son)</td>
<td>24.0</td>
<td>9.8</td>
<td>19.0</td>
<td>7.5</td>
<td>64%</td>
<td>88%</td>
<td>30%</td>
</tr>
<tr>
<td>Patient II-2 (son)</td>
<td>22.3</td>
<td>9.4</td>
<td>24.6</td>
<td>7.5</td>
<td>85%</td>
<td>108%</td>
<td>29%</td>
</tr>
<tr>
<td>Patient II-3 (son)</td>
<td>20.7</td>
<td>9.4</td>
<td>23.4</td>
<td>8</td>
<td>64%</td>
<td>52%</td>
<td>14%</td>
</tr>
<tr>
<td>Patient III-1 (son of patient II-1)</td>
<td>51.1</td>
<td>10.6</td>
<td>21.1</td>
<td>8.5</td>
<td>&lt;1%</td>
<td>86%</td>
<td>18%</td>
</tr>
</tbody>
</table>

Individual and pooled normal controls processed in parallel. Bleeding times were quite close to the upper limit of normal for four of the five patients. Although marked thrombocytopenia was not encountered, borderline thrombocytopenic values (140,000–170,000) were consistently found in all patients, except for thrombocytosis occurring in patient III-1 in association with severe bleeding. Additionally, platelet glycoproteins in the one patient studied (patient II-1) were not distin-

Fig. 1. SDS agarose gel electrophoresis of VIII/vWF from plasmas: (A) patient III-1, (B) normal control, (C) patient I-1, (D) normal control 1:2, (E) mother of patient III-1 (normal pattern), (F) normal control 1:4, (G) patient II-1. The point of application is at the top and the anode is at the bottom.
Fig. 2. Ristocetin-induced platelet agglutination. Ristocetin at the final concentrations indicated (mg/ml) was added to platelet-rich plasma (PRP) from patient II-3 in a Payton aggregometer. Normal PRP under similar conditions shows little or no agglutination until ristocetin concentrations exceed 0.5 mg/ml. PPP, platelet-poor plasma. Platelet aggregation with collagen, ADP, and epinephrine was studied in three patients, with normal aggregation seen in each case. Ristocetin-induced platelet agglutination (RIPA) was studied in all five patients, with supranormal responses to low concentrations of ristocetin noted in each case (Fig. 2).

Binding of VIII/vWF from pooled normal plasma was studied with washed platelets from five normal volunteers. In each case there was increased binding as the concentration of ristocetin was increased, with peak slopes occurring between 0.75 and 1.5 mg/ml ristocetin (Fig. 3). In contrast, binding of this same VIII/vWF by washed patient platelets was enhanced, with peak slopes occurring between 0 and 0.6 mg/ml ristocetin. This difference became highly significant ($p < 0.001$ by $t$ test of means) at a ristocetin concentration of 0.6 mg/ml, with $37.6\% \pm 6.8\%$ (mean $\pm$ SD) residual VIII/vWF present following incubation of normal plasma with patient platelets, versus $96.4\% \pm 3.5\%$ for normal plasma incubated with control platelets. Patient VIII/vWF incubated with washed normal platelets consistently produced binding curves falling within the normal range, examples of which are also shown in Fig. 3.

DISCUSSION

The most common inherited bleeding diathesis encountered in clinical practice is increasingly believed to be von Willebrand's disease or some constituent of the "von Willebrand syndrome." Of prime importance in differentiating these abnormalities of the factor VIII complex from classic hemophilia A is their autosomal inheritance, although a number of specific distinguishing aspects from the clinical to the molecular levels have additionally been described. The present work poses the possibility that an autosomally transmitted bleeding disorder manifesting qualitative as well as quantitative abnormalities of plasma factor VIII/vWF may be attributable, at least in part, to an intrinsic platelet abnormality.

Through the use of crossed-immunoelectrophoresis...
and subsequently SDS agarose gel electrophoresis autoradiography of the factor VIII/vWF complex, Ruggeri, Zimmerman, and coworkers have in recent years devised a classification of von Willebrand's disease emphasizing the qualitative changes in distribution of factor VIII/vWF multimers of varying molecular weights. Accordingly, in type I, all multimers are decreased, while in type II, the larger multimers are selectively absent. Type II is further subdivided into A and B subtypes based largely (although not solely) on the increased agglutination of patient platelets by ristocetin in IIB, but abnormally low agglutination in IIA.

In all five members from three generations of the family we have studied, there was a history of significant clinical bleeding episodes, decreased VIII-R:Co activity, selective decrease of the higher molecular weight VIII/vWF multimers, and increased platelet agglutination at low concentrations of ristocetin added to platelet-rich plasma. However, in contrast to the IIB patients described by Ruggeri et al., the patients in our study showed no enhanced binding of their VIII/vWF by normal platelets. The platelets from our patients, furthermore, showed a highly significant increase in their ability to bind factor VIII/vWF from normal plasma. Weiss et al. have also recently reported a family with findings that appear similar to those of our patients.

The present studies thus suggest an abnormality at a different point of the platelet-factor-VIII/vWF binding axis—at the surface of the platelet itself. It may accordingly be that decreases in plasma levels of the higher VIII/vWF multimers may result from their enhanced binding to the patients' own platelets in vivo. Although we cannot at present eliminate the possibility that the bleeding disorder occurring in this family involves abnormalities in the synthesis and/or release of factor VIII/vWF components in addition to enhanced binding by platelets, the existence of an intrinsic platelet abnormality appears very likely in this family. A similar explanation may underlie the related findings in patients recently reported by several other groups.

The methodology for the binding studies in the present investigation is largely similar to that employed by Ruggeri et al. Of possible significance, however, is the inclusion of EGTA in our platelet washing procedure. As shown by Kao et al., this divalent cation chelator inhibits binding of 125I-factor-VIII/vWF to normal platelets. To the extent that such binding is reversible, EGTA would accordingly be expected to promote the dissociation from platelets of factor VIII/vWF previously bound in vivo. If the factor VIII/vWF binding sites approached saturation in vivo in the IIB patients described by Ruggeri et al., then it appears conceivable that less vigorous removal of the bound VIII/vWF during washing of the platelets could result in decreased uptake of exogenously added VIII/vWF in subsequent binding studies.

Further study of the platelets from patients with this subtype of the von Willebrand syndrome will be important for the continuing elucidation of platelet receptors for factor VIII/vWF as well as for the cellular pathways of plasma factor VIII/vWF metabolism. A better understanding of these processes should also be expected to contribute to the development of appropriate therapy for patients with this disorder.

REFERENCES

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