Thrombocytopenia Associated With Pregnancy in a Patient With Type IIIB von Willebrand’s Disease

By Margaret E. Rick, Sybil B. Williams, Ronald A. Sacher, and Laurie P. McKeown

Thrombocytopenia may accompany variant (type IIIB) von Willebrand’s disease (vWD) and is thought to result from binding of the abnormal von Willebrand factor (vWF) to the patient’s platelets with subsequent platelet aggregate formation and clearance. We have studied a patient with type IIIB vWD who became thrombocytopenic during two pregnancies. During the third trimester of pregnancy, her platelet counts dropped to 20,000 to 30,000/µL, and an increase in the intermediate-sized vWF multimers was seen on agarose gel electrophoresis. During this time her platelet-rich plasma showed spontaneous platelet aggregation, and her plasma caused spontaneous aggregation of normal washed platelets. Antibody to platelet glycoprotein IIb completely blocked the spontaneous platelet aggregation, while antibody to platelet glycoprotein IIb/IIIa did not block the response at the concentrations used. Inhibitors of platelet function that elevate platelet cyclic AMP also blocked the response, but aspirin had no effect on the spontaneous platelet aggregation. The patient illustrates that the platelet counts in one individual can vary greatly in type IIIB vWD and that the thrombocytopenia that occurs can appear under physiologic conditions that stimulate the endogenous production of the patient’s abnormal vWF. The mechanisms leading to spontaneous platelet aggregation and thrombocytopenia appear to be similar to those described for other patients with type IIIB vWD.

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CASE REPORT

This 33-year-old white female was initially referred to the National Institutes of Health in 1981 during the ninth month of her pregnancy because of recently discovered thrombocytopenia and a life-long history of bruising. She had recurrent epistaxis during both childhood and adult years, bled with loss of deciduous teeth, and had heavy menstrual bleeding. Her father and paternal grandfather also had a history of life-long epistaxis and easy bruising. The patient’s obstetrician had noted thrombocytopenia during the sixth month of her pregnancy but referred her to a hematologist only when the platelet count was noted to drop to <50,000 during the eighth month. A bone marrow aspiration showed normal numbers of megakaryocytes, a presumptive diagnosis of idiopathic thrombocytopenic purpura was made, and the patient was placed on prednisone, 60 mg/d when her platelets dropped to 20,000 to 30,000/µL. The patient was referred to NIH for further evaluation because of the lack of response in her platelet count while on prednisone.

Physical examination showed fading ecchymoses on all extremities and findings compatible with a normal pregnancy in the ninth month. The patient’s laboratory data are shown in Fig 1 (12/23/81). Spontaneous labor began within one week of the expected date of delivery; although no fetal heart sounds were detected on admission to her private hospital, a prolonged labor was allowed to proceed terminating with the vaginal delivery of a dead fetus on 1/5/82. The
obtained by a two-syringe technique using a 19-gauge needle and frozen in aliquots at -70°C. Centrifugation of citrated whole blood at 750 g for three minutes at room temperature; the platelet count was adjusted to 200,000/L or inhibitors were used in the platelet aggregation studies, they were added to the platelet-rich plasma three minutes before addition of the stir bar (except P-PACK, which was added ten minutes before). Two monoclonal antibodies (MoAbs) were used, both the gift of Dr Barry Coller: 6D1 directed against platelet glycoprotein Ib (GP Ib) (9.8 μg/mL final concentration), and 10E5 directed against platelet glycoprotein Ib/IIa (GP Ib/IIa) (1 to 16 μg/mL final concentration). The inhibitors included PGI2, 24 μg/mL (Sigma Chemical); db cAMP, 9.1 mmol/L (Sigma); acetyl salicylic acid (ASA), 0.45 μg/mL (Aldrich Chemical, Milwaukee); and D-phenylalanyl-L-phenylalanyl-L-arginine chloromethyl ketone (P-PACK), 10^{-8} mol/L (Calbiochem-Behring, LaJolla, CA), all given as final concentrations.

Mixing studies were performed using platelets that were separated from plasma proteins by arabinogalactan gradient centrifugation as previously reported. Platelets from a normal subject who had not taken medications for two weeks and platelets from the patient were prepared simultaneously. In these experiments the separated platelets and platelet-poor plasma were mixed in the cuvette to a final concentration of 200,000 platelets/μL in a total of 0.4 mL volume. Spontaneous platelet aggregation was assessed as noted above. In instances where an inhibitor or antibody was added, the separated platelets and platelet-poor plasma were placed in the cuvette and the inhibitor added immediately and incubated for three minutes before stirring and platelet aggregation.

VIII:C, vWF antigen (vWF:Ag) and ristocetin cofactor assays were performed as previously reported. One unit of activity or antigen is that present in 1 mL of pooled normal plasma for each assay. Glyoxyl agarose gel electrophoresis was performed according to the method of Hoyer and Shainoff.

Bleeding times were performed by the Mielke et al template method.

RESULTS

Coagulation studies. When initially seen during the ninth month of her first pregnancy, the patient was thrombocytopenic and had a prolonged bleeding time of 19 minutes (Fig 1). Her factor VIII and vWF values were within the normal laboratory ranges (nonpregnant population), but her vWF multimer pattern showed a decrease in the largest multimers (Fig 1: 12/23/81). After recovery from the traumatic delivery and perineal infection, the patient’s platelet count rose to the normal range and her factor VIII and vWF values fell to abnormal levels; the vWF multimer pattern became more distinctly abnormal, with an overall decrease in quantity and fewer large forms present (Fig 1: 8/82 to 8/83).

During her second pregnancy, decreased platelet counts, increased factor VIII and vWF values, and vWF multimer patterns similar to those seen in the first pregnancy were observed (Fig 1: 1/29/85 to 4/14/85); these values returned to the patient’s baseline levels within approximately one month after delivery.

Platelet aggregation studies. Routine platelet aggregation studies performed when the patient was not pregnant and when her platelet count was in the normal range showed normal aggregation responses and release of ATP to epinephrine, ADP, and collagen. Ristocetin-induced platelet aggregation revealed that the patient’s platelets responded to 0.34 mg/mL of ristocetin, while control platelet-rich plasma required >0.87 mg/mL to elicit an aggregation response (Table 1). No spontaneous platelet aggregation was observed when the patient was not pregnant.

<table>
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<tr>
<th>Date</th>
<th>vWF:Ag (%)</th>
<th>Ristocetin Coeff (%)</th>
<th>VIII:C (%)</th>
<th>Platelet Count (x10^4/μL)</th>
<th>Bleeding Time (min)</th>
<th>vWF Multimer Pattern</th>
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<tr>
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<td>95</td>
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<td>14</td>
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<td>7</td>
<td>56</td>
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<td>80</td>
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Fig 1. Patient's laboratory data: the vWF multimer gels are shown on the right with top of gel at left, bottom at right.

delivery was complicated by hypertension, a third-degree perineal laceration and subsequent infection, and marked blood loss requiring 65 units of blood products, including RBCs, whole blood, platelets, and cryoprecipitate. The prednisone was slowly discontinued over the next four months, and the patient’s platelets gradually returned to normal during that time.

The patient remained stable with normal platelet counts on multiple samples during the next two years (Fig 1). Studies of her vWF and factor VIII (VIII:C) were performed, which confirmed the diagnosis of type IIB vWD (Fig 1). She became pregnant again in the fall of 1984 and had a normal pregnancy except that thrombocytopenia was noted to develop during the second trimester of pregnancy and persisted through the third trimester (Fig 1); spontaneous labor began on April 14, 1985, and a healthy boy was delivered at Georgetown University Hospital by a planned cesarean section. The patient was given both cryoprecipitate and platelets prior to and following delivery but had increased blood loss of approximately 1,800 cc. She recovered uneventfully.

METHODS

The patient gave informed consent for the studies. Blood was obtained by a two-syringe technique using a 19-gauge needle and was placed in 1/100 volume of sodium citrate (10.9 mL/L final concentration). Platelet-poor plasma was obtained by centrifugation at 2,000 g for ten minutes at 4 °C; the plasma was used immediately or was frozen in aliquots at − 70 °C.

Platelet-rich plasma for aggregation studies was obtained by centrifugation of citrated whole blood at 750 g for three minutes at room temperature; the platelet count was adjusted to 200,000/μL with platelet-poor plasma (or less as noted in each experiment when the patient was thrombocytopenic; in that case the normal control was adjusted to the same platelet count). Platelet aggregation was completed within two hours using a Chrono-Log Lumi Aggregometer (Chrono-Log, Havertown, PA). Baseline values for light transmission in platelet-rich plasma were adjusted to 5% to 10% on the recorder. Agonists for routine platelet aggregation studies included epinephrine, 7.5 μmol/L (Parke Davis, Morris Plains, NJ); ADP, 3.8 μmol/L (Sigma, St. Louis); collagen, 225 μmol/L (Millipore, Freehold, NJ); and ristocetin, (Lenau, Copenhagen, Denmark). Ristocetin-induced platelet aggregation (RIPA) was tested by adding ristocetin at final concentrations varying from 0.34 to 2.0 mg/mL to 400 μL of platelet-rich plasma; a normal control was run alternately with the patient sample.

Spontaneous platelet aggregation was assessed by placing 400 μL platelet-rich plasma in an aggregometer cuvette, stirring at 900 rpm and following the change in light transmission for 15 minutes; control platelet-rich plasma prepared in parallel was alternated with the patient’s sample during the testing. When antibodies (purified IgG) or inhibitors were used in the platelet aggregation studies, they were added to the platelet-rich plasma three minutes before addition of the stir bar (except P-PACK, which was added ten minutes before).
Table 1. RIPA: Initial Slopes of Patient and Control at Varying Ristocetin Concentrations

<table>
<thead>
<tr>
<th>Ristocetin (mg/mL)</th>
<th>Patient (mm/min)</th>
<th>Control (mm/min)</th>
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</thead>
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<td>1.30</td>
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<td>47</td>
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<td>0.87</td>
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Platelet-rich plasma from patient and control (200,000/μL) were run alternately using the concentrations of ristocetin noted.

*Average for PRP from six normal subjects: 0.34 mg/mL, 0 mm/min; 0.5 mg/mL, 4 mm/min; 1.0 mg/mL, 10 mm/min; 2.0 mg/mL, 62 mm/min.

**Special platelet aggregation studies.** Studies performed during the third trimester of the patient's second pregnancy demonstrated that the patient's platelets aggregated spontaneously (Fig 2, panel A). This spontaneous aggregation was inhibited by the MoAb to GP Ib; however, the antibody to GP IIB/IIIA did not inhibit aggregation (Fig 2, panels B and C).

Mixing studies using platelet-poor plasma and platelets separated from plasma proteins indicated that the patient's plasma rather than her platelets was responsible for the spontaneous platelet aggregation. Patient plasma added to normal platelets showed a spontaneous aggregation response (Fig 2, panel D), while normal plasma added to patient platelets showed no spontaneous aggregation. The aggregation response observed with patient plasma and normal platelets could be inhibited by the antibody to GP Ib but could not be inhibited by the antibody to GP IIB/IIIA, similar to that seen with patient platelet-rich plasma.

The addition of various inhibitors to the patient's platelet-rich plasma revealed no inhibitory action of ASA; however, PG12 caused disaggregation of an early small aggregation response, and the thrombin inhibitor P-PACK abolished the spontaneous aggregation. Prostaglandin I2 and dibutyryl cyclic AMP also inhibited the spontaneous aggregation in the mixture of patient plasma and normal platelets (data not shown).

Studies performed four months after the successful termination of her second pregnancy showed that the patient no longer demonstrated spontaneous platelet aggregation. Ristocetin-induced platelet aggregation (1.3 mg/mL) of patient platelet-rich plasma was completely abolished by preincubation with the anti-GP Ib but not by the anti-GP IIB/IIIA, similar to that seen with normal platelet-rich plasma. The patient, but not the control, aggregated when ristocetin was added at a final concentration of 0.87 mg/mL; the anti-GP IIB/IIIA abolished this aggregation response. This antibody also prevented aggregation of patient platelets when ADP (5, 10, and 25 μmol/L) was added.

**DISCUSSION**

Recent studies have indicated that the abnormal vWF of patients with type IIB vWD binds to the platelet membrane receptor GP Ib in the absence of ristocetin. This binding apparently stimulates exposure of the platelet membrane receptor for fibrinogen, GP IIb/IIIA, making it available to plasma fibrinogen, which binds and initiates aggregation.

The platelet aggregates are then thought to be cleared in vivo by normal clearance processes. Although the majority of patients with type IIB vWD do not have thrombocytopenia, a number of patients have been reported with low platelet counts. Thrombocytopenia has also occurred in type IIB patients after therapeutic infusion of desmopressin (DDAVP), which transiently elevates levels of vWF in the circulation, including the largest multimers, making them available to bind to platelets.

The production of an abnormal vWF in our patient was physiologically stimulated during pregnancy, and the levels rose into the "normal" range; larger multimers than had been previously noted were also observed. Both the qualitative and quantitative changes in the abnormal vWF led to spontaneous platelet aggregation and thrombocytopenia, likely by the mechanism outlined above. Although this same phenomenon may occur when the patient is not pregnant, it appears that the concentration of abnormal vWF is not sufficient to lead to enough platelet aggregation in vivo to cause thrombocytopenia. The levels of abnormal vWF and the larger multimers in particular are clearly important in the clinical manifestation of thrombocytopenia.

Antibodies to GP Ib completely inhibited the spontaneous aggregation in our patient, similar to previous studies. The anti-GP IIB/IIIA did not inhibit the aggregation, however. Since this concentration of anti-GP IIB/IIIA did prevent the exaggerated response to ristocetin (0.87 mg/mL) and pre-
vented ADP-induced platelet aggregation four months after delivery, there may have been another mechanism (not related to GP IIb/IIIa) mediating part of the spontaneous platelet aggregation during pregnancy. Alternatively, the lack of inhibition by anti-GP IIb/IIIa may be related to the quantity of the antibody required to prevent exposure of the fibrinogen binding site during pregnancy in this patient despite the fact that this quantity of lOE5 has prevented spontaneous platelet aggregation in several other (nonpregnant) type IIB patients.5

The spontaneous platelet aggregation in this patient was inhibited by agents that increase platelet levels of cyclic AMP (PGI3 and db cAMP) but was not inhibited by aspirin, indicating that the aggregation is not dependent on prostaglandin metabolism. This pattern of inhibition has been observed previously in patients with type IIB vWd.5 The inhibition by P-PACK suggests that generation of small amounts of thrombin may have contributed to the exposure of fibrinogen binding sites in this patient; however, hirudin did not show any inhibitory effect in this patient's spontaneous platelet aggregation (data not shown), so the mechanism of action of P-PACK in this setting is not clear.

This patient demonstrates that there can be a wide spectrum of platelet counts and variable decreases in the larger vWF multimers within one patient with type IIB vWD, depending on the physiologic conditions. The patient further suggests that the presence or absence of thrombocytopenia is related to the level of circulating endogenous larger vWF multimers. A family recently reported14 with a normal baseline vWF multimer pattern on agarose gels and increased RIPA at low concentrations of ristocetin may also represent part of this spectrum. In that family, the addition of low concentrations of ristocetin to the patients’ PRP caused binding of the largest vWF multimers, resulting in a residual type IIB multimer pattern. While the molecular defect(s) is unknown in type IIB vWd patients and in this family, it seems likely that the defect(s) leads to an increased ability of the abnormal vWF to bind to GP Ib.

Clinical evaluation of pregnant patients who present with thrombocytopenia should include a careful questioning about life-long bleeding problems and any family history of excesive bleeding; if the history is positive, evaluation of the patient’s vWF, including multimer studies, should be carried out. The clinical management of pregnancy in patients with type IIB vWD requires close clinical and laboratory supervision and conservative treatment with cryoprecipitate and platelets.

ACKNOWLEDGMENT
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REFERENCES
Thrombocytopenia associated with pregnancy in a patient with type IIB von Willebrand’s disease

ME Rick, SB Williams, RA Sacher and LP McKeown