Deficiency of Platelet Membrane Glycoprotein Ia Associated With a Decreased Platelet Adhesion to Subendothelium: A Defect in Platelet Spreading

By H. Karel Nieuwenhuis, Kjell S. Sakariassen, Wim P.M. Houdijk, Patricia F.E.M. Nievelstein, and Jan J. Sixma

A bleeding disorder with absent collagen-induced platelet aggregation and adhesion has been described in a patient whose platelets failed to express surface glycoprotein Ia. We studied the interaction of her platelets with subendothelium in an annular perfusion chamber and the interaction with purified human collagen type III in a rectangular perfusion system under flow conditions. Platelet adherence was almost completely absent both at low and high shear rates. The few platelets which adhered remained in the contact stage without subsequent spreading and aggregate formation. Addition of a monoclonal antibody, which was directed against the von Willebrand moiety of FVIII-VWF, to the blood, completely abolished platelet adherence at high shear rates and had a partial effect at low shear rates. These data indicate that von Willebrand factor plays a role in the initial attachment (contact stage) of platelets to subendothelium. We conclude that the bleeding disorder and excessively prolonged bleeding time in our patient are caused by a new specific defect of the platelet-vessel wall interaction.

MATERIALS AND METHODS

Patient and normal subjects. Patient G, a 34-year-old woman, is a previously reported patient.14 She had a history of easy bruising following minor trauma from her teens on. At the age of 21 she had an accident and a large subcutaneous hematoma developed from the groin to the ankle. Her menstrual bleedings have been heavy, but have been well-controlled with oral contraceptives and tranexamic acid (AMCA). There was no prolonged hemorrhage from small cuts; nose bleeding or spontaneous gingival bleeding did not occur so far. She underwent tonsillectomy (age 9) and appendectomy (age 15) without bleeding complications. A cesarian section (age 23) and tooth extractions (age 26 and 28) were performed without undue blood loss after cryoprecipitate infusion. A prolonged bleeding time and absent collagen-induced platelet aggregation were first discovered in 1973. Coagulation studies were normal and von Willebrand's disease was ruled out by repeated demonstrations of normal factor VIII-VWF parameters (VIII:C 140%, VWF:Ag 89%, VWF:ristocetin cofactor 94%, normal mobility of VWF in crossed immunoelectrophoresis). Her mother also showed easy bruising, but had not undergone any operative procedures or injuries. Her maternal grandfather had been admitted to the hospital several times because of severe epistaxis. Apart from this the family history was negative.

We recently reported14 that the patient's platelets were totally unresponsive to collagen. High doses of several types of collagen induced neither shape change, secretion, nor aggregation. In contrast, her platelets aggregated normally in response to ADP, arachidonic acid, thrombin, PAF-acether, ionophore A23187, epinephrine, and ristocetin. Study of platelet adherence to collagen in a static system according to Legrand17 disclosed a severe defect of adhesion. Her platelets failed to express surface glycoprotein Ia (15% to 25% of normal).

Healthy adult volunteers from the hospital staff served as normal controls. The patient and the control subjects had not ingested any drugs for at least 2 weeks prior to the study. Informed consent was obtained from all donors and experiments were performed in accordance with the Declaration of Helsinki.

Blood collection. Blood samples were collected by venepuncture into polystyrene tubes containing 0.1 vol 110 mmol/L trisodium citrate.

Platelet interaction with subendothelium. The interaction of blood platelets in flowing blood with subendothelium was studied under steady flow18 in an annular perfusion chamber according to Baumgartner.1 Segments of a human umbilical artery were exposed for five minutes to flowing citrated blood at wall shear rates of 300 sec\(^{-1}\) and 1800 sec\(^{-1}\). Before perfusion the endothelium was removed by brief exposure to air and the vessels were treated with aspirin in order to inhibit prostacyclin production.

Platelet interaction with subendothelium was quantified by a morphometric technique.2 The artery segments were fixed in 2.5%
Platelet Adhesion in GPIIa Deficiency

Glutaraldehyde after perfusion and subsequently postfixed in 2.0% osmium tetroxide. The specimens were embedded in epon, and sections of 1 μm thickness were stained at 70 °C for two minutes with basic fuchsin and methylene blue. Platelet adherence was studied at a magnification of 1,000 × with an especially constructed micrometer in the ocular of the light microscope and evaluated as contact-platelets (platelets attached but not intimately bound to subendothelium) or spread-platelets (platelets at various stages of spreading on the subendothelium, including platelets at the base of platelet aggregates).

Platelet interaction with collagen. The interaction of blood platelets in flowing blood with purified human fibroblast collagen type III was studied with a rectangular perfusion chamber as previously described. A glass microscope cover slip was coated with 30 μg/cm² of collagen type III by spraying with a retouching air brush. Collagen type III was isolated from human umbilical arteries as described. Perfusion was performed during five minutes at wall shear rates of 300 sec⁻¹ and 800 sec⁻¹. The platelet coverage was evaluated by light microscopy, with the help of a Quantimet 720 image analyzer (Imanco; Royston, UK).

Inhibition of FVIII-VWF-mediated platelet adherence with a monoclonal antibody against VWF. In some experiments perfusates were incubated and vessel segments were pretreated with CLB-RAg 35 as previously described. Per fusates were incubated for ten minutes at 37 °C with CLB-RAg 35 (10 μL ascites/mL blood) before a perfusion run. CLB-RAg 35 (kindly provided by Dr. J.A. van Mourik, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, the Netherlands) is a monoclonal antibody against von Willebrand factor that inhibits ristocetin-induced platelet aggregation and VWF binding to platelets and that blocks VWF-mediated platelet adherence to subendothelium without interfering with the binding of VWF to collagen.

Influence of an antibody against fibronectin on platelet adherence. In some experiments, vessel segments were pretreated with rabbit antihuman fibronectin F(ab')₂ fragments (Cappel Laboratories, Cochranville, Pa.), as described, and perfusion studies were performed using washed patient's platelets resuspended in fibronectin-free plasma or in normal plasma. The perfusate was reconstituted by adding washed red cells to the resuspended platelets (hematocrit 0.4; platelet count 190 x 10⁶/L).

Statistical analysis. Statistical analysis was performed by means of Student's t test, and considered significant at P < 0.05.

RESULTS

Platelet adherence to collagen type III and subendothelium. Perfusion studies in a rectangular perfusion chamber showed that platelet spreading on the subendothelium, including platelets at the base of platelet aggregates, was observed in normal blood (Table 1). Inhibition of FVIII-VWF-mediated platelet adherence with a monoclonal antibody against VWF resulted in a marked reduction of platelet adherence at high shear rate in the patient (P < 0.001) and the controls (P < 0.01). At low shear conditions, the antibody induced a decreased platelet deposition in the patient (P < 0.01) but not in the controls.

Platelet adherence to artery subendothelium when using the patient's blood was also severely decreased (Table 1). Morphometric evaluation disclosed that the resultant platelet adhesion consisted of platelets in the contact stage, but not in the spread stage (Fig 1). Platelet aggregates were absent. In contrast, when using blood of normal subjects, platelet adherence was predominantly composed of spread platelets and about 30% to 40% of the spread platelets were covered with aggregated platelets.

Addition of CLB-RAg 35 to the blood together with preincubation of the vessel wall with the antibody (in order to block VWF present in the subendothelium) completely abolished contact of the patient's platelets with the subendothelium at high shear rate (P < 0.001), but had only a slight nonsignificant effect at low shear rate. In the controls, the antibody totally inhibited platelet adhesion at high shear rate (P < 0.001) and reduced adhesion to a less but significant extent (P < 0.01) at low shear rate.

Preincubation of the vessel wall with antifibronectin and perfusion with patient's platelets resuspended in fibronectin-free plasma gave a similar low platelet deposition at low shear rate as perfusion with normal plasma without preincubation of the vessel wall.

DISCUSSION

In a previous report we described a patient with easy bruising and a prolonged bleeding time, whose platelets were totally unresponsive to collagen; other agonists, however,
under flow conditions similar to those found in vivo, and evaluated platelet adherence with a morphometric technique in order to distinguish the initial attachment (contact) and subsequent spreading.

The adherence of our patient's platelets to collagen and subendothelium was severely impaired and was characterized by a total absence of spread platelets on subendothelium. This defect differs completely from the adhesion defects in von Willebrand's disease and Bernard Soulier syndrome. In contrast to these disorders, the patient's platelets were unable to spread on exposed subendothelium both at low and high shear rates and, apparently as a result of this, aggregates were absent.

The few platelets of our patient that adhered to the subendothelium were bound to the vessel wall by a small area of the membrane. This contact could be an interaction of collagen, von Willebrand factor, and the platelet membrane receptor for VWF, or an interaction of collagen with the binding site of the collagen receptor. In order to define the role of VWF in the contact stage, we performed perfusion studies with a monoclonal antibody against VWF. VWF completely mediated contact in the patient at high wall shear rates (comparable with those in the microvasculature) and had a partial effect at low shear conditions. It is not clear as yet which factor is responsible for the remaining contact at low shear rate. In this and a previous study we could not demonstrate a role for fibronectin at low shear rate.

These data support the hypothesis that the defect in platelet adhesion in von Willebrand's disease is associated with a reduced ability of platelets to attach to subendothelium upon initial contact. The attachment of the patient's collagen-unresponsive platelets to subendothelium via VWF at high shear rate indicates that platelets can adhere via VWF without any stimulation by collagen. This agrees with studies that showed that VWF first binds to subendothelium and then mediates platelet adherence, suggesting that VWF undergoes a structural alteration on subendothelium that enables it to bind to a platelet receptor. Stimulation of platelets subsequently can expose other binding sites (like GPIIb-III) for VWF, but its physiologic significance is not clear as yet because of the inhibition of this binding by fibronectin.

The absolute failure of the patient's platelets to spread on exposed subendothelial matrix and purified collagen points to the important role of collagen in platelet activation, subsequent spreading, and aggregate formation. We conclude that the hemorrhagic diathesis and the prolonged bleeding time in our patient can be ascribed to a new, specific defect of the platelet-vessel wall interaction. The mildness of the bleeding disorder, however, points to the important role of other agonists, such as thrombin, in hemostasis.

REFERENCES

with platelet adhesion to subendothelium and with the factor VIII von Willebrand protein. J Lab Clin Med 87:586, 1976


Deficiency of platelet membrane glycoprotein Ia associated with a decreased platelet adhesion to subendothelium: a defect in platelet spreading

HK Nieuwenhuis, KS Sakariassen, WP Houdijk, PF Nievelstein and JJ Sixma