CONCISE REPORT

Presence of a Platelet Aggregating Factor in the Plasma of Patients With Thrombotic Thrombocytopenic Purpura (TTP) and its Inhibition by Normal Plasma

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Three patients with thrombotic thrombocytopenic purpura (TTP) were treated by infusion of normal plasma with dramatic responses. The plasmas collected from these patients during relapse induced in vitro aggregation of washed platelets from both normal donors and the patients during remission. The platelet aggregating factor was not dialyzable or adsorbable by Al(OH)3 and was not inactivated by disopropylfluorophosphate, hirudin, or heparin in the presence of normal amounts of antithrombin. In contrast to the platelet aggregation induced by platelet isoantibody, the platelet aggregating activity of TTP plasma diminished as a function of time when it was incubated with normal plasma at 37°C. These observations suggest that at least some instances of TTP appear to be due to deficiency of a plasma inhibitor to counteract a platelet aggregating factor demonstrated to be present in the plasma of these patients.

THROMBOTIC THROMBOCYTOPENIA PURPURA (TTP) is a disorder, usually fatal, characterized clinically by thrombocytopenia, microangiopathic hemolytic anemia, fluctuating neurologic signs, and renal abnormalities and pathologically by the presence of intravascular hyaline thrombi consisting of platelets and fibrin.1 The pathogenesis of this disease is still unknown. Observations have been reported recently from this center on the effectiveness of normal fresh or stored plasma in the management of TTP.2 These findings prompted us to investigate the effects of plasma obtained from patients with TTP upon washed platelets and its interaction with normal plasma.3

We report here the presence of a plasma platelet aggregating factor (PAF) in three patients with TTP and a time-dependent inhibition of this PAF by normal plasma in vitro.

MATERIALS AND METHODS

Patient 1 with TTP. An 18-yr-old white female, a primigravida in her 19th week of pregnancy, was admitted to Jackson Memorial Hospital with severe headache, transient numbness of hands, feet, and face, microangiopathic hemolytic anemia (hemoglobin 6.4 g/dl; hematocrit 20%; reticulocyte count 27%; with numerous schistocytes), thrombocytopenia (platelet count 6 × 10⁹/liter), fever, increased blood urea nitrogen (60 mg/dl), and proteinuria (5.5 g/24 hr). Splenectomy was performed. Histology

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of the spleen revealed scattered occlusions by a hyaline PAS-positive substance in both intravascular and subendothelial locations and proliferation of endothelial cells. The patient was successfully treated with normal fresh-frozen plasma, outdated plasma, or plasma from which cryoprecipitate had been removed as described elsewhere. After 10 mo she achieved complete remission (CR) and has remained in CR for the past 15 mo.

**Patient 2 with TTP.** A 21-yr-old white female, a primigravida in her 20th week of pregnancy, was transferred to Jackson Memorial Hospital with fever, changing neurologic symptoms (dysarthria, amnesia, numbness of the mouth, and weakness of the left arm and leg), microangiopathic hemolytic anemia (Hb 6.8 g/dl; reticulocyte count 33%; with numerous fragmented erythrocytes), thrombocytopenia (platelet count 12 × 10^9/liter), and gross hematuria. The clinical symptoms totally disappeared and the laboratory tests became nearly normal within 36 hr after she received 1 unit of whole blood and 12 units of fresh-frozen plasma. At each instance of recurrent thrombocytopenia, she responded with an increase in platelet count after infusion of either fresh or outdated plasma. She delivered spontaneously a viable infant weighing 1.6 kg (3.5 lb) 2 wk before term. Both mother and child were doing well at this writing.

**Patient 3 with TTP.** A 22-yr-old black female was admitted semicomatose to the Miami Baptist Hospital. Laboratory data shortly after admission were as follows: Hb 7 g/dl; reticulocyte count 25%; platelet count 6 × 10^9/liter. Many schistocytes were present on the peripheral blood smear. Later the patient developed gross hematuria and generalized seizures. After no response on high-dose steroids, a splenectomy was performed on the third hospital day. On day 4 there was still no improvement in consciousness, and weakness of the right upper extremity was noted. Her platelet count remained at 7 × 10^9/liter. On day 5 she developed focal seizures while receiving diphenylhydantoin and steroids. At this time she was infused with 10 units plasma over a 24-hr period, with prompt neurologic improvement and a rise in platelets to over 200 × 10^9/liter within several days. During the next several weeks 3 U of plasma were required daily to prevent thrombocytopenia. She experienced no further neurologic disorders. Gradually her plasma requirement decreased, and she was discharged in complete remission 8 wk after admission.

**Patient 4 with platelet isoantibody.** A 64-yr-old male developed platelet isoantibody stemming from multiple whole blood and platelet transfusions for the removal of colon cancer and treatment subsequently for GI bleeding and pancytopenia caused by chemotherapy. His thrombocytopenia became refractory to multiple platelet transfusions.

**Controls.** Normal healthy laboratory personnel were used as normal controls.

**Collection of plasma.** Blood was collected either into polyethylene tubes containing 3.8% sodium citrate (9 parts blood to 1 part citrate) using a two plastic syringe technique or into PA-10 Blood-Pack units containing 75 ml citrate dextrose solution USP formula A (Fenwall Laboratories). Platelet-poor plasma (PPP) was prepared by centrifuging first at 2,400 g for 20 min at 4°C, after which the supernatant was decanted and spun at 10,000 g for 10 min at 4°C. The PPP was divided into small aliquots and stored at −80°C in polyethylene tubes.

**Platelet washing technique.** Nine parts of whole blood were drawn from the antecubital vein into polyethylene tubes containing one part 3.8% sodium citrate using the double plastic syringe technique. The platelet-rich plasma (PRP) was prepared by centrifugation at 180 g for 10 min at room temperature. Platelet washing was performed according to the method of Walsh et al. with slight modification. After a 1/30 vol of 35% bovine albumin (Sigma) was introduced at the bottom of conical plastic tubes, the PRP was centrifuged at room temperature for 15 min at 1650 g. The supernatant PPP was removed with a siliconized Pasteur pipette. The platelets and the albumin were suspended in the same volume of calcium-free Tyrode's buffer pH 7.3. The platelets were washed twice with the same technique and then resuspended in the same buffer. Platelet concentrations were adjusted to about 750 × 10^9/liter for the platelet aggregation studies.

**Method to detect platelet aggregating activity and its inhibition by normal plasma.** Platelet aggregation was performed in a Chrono-log platelet aggregometer using a 609-mu red filter. After warming the cell containing 0.4 ml plasma to 37°C, 0.1 ml platelet suspension was added and the percentage decrease of optical density resulting from platelet aggregation was recorded.

**Concentration of platelet aggregating factor and platelet isoantibody.** Nine parts of plasma and one part of 1/3 diluted Al(OH)₃ (Amphojel, Wyeth) was incubated at 37°C for 10 min with frequent mixing and then centrifuged at 10,000 g for 10 min at 4°C. A 50% solution of polyethylene glycol (PEG) 6000 was added to the supernatant (final concentration 6%) followed by stirring for 30 min at 4°C. The
mixture was centrifuged at 18,000 g for 30 min at 4°C and the precipitate dissolved in 1/10 of the original plasma volume of calcium-free Tyrode's buffer.

**Concentration of platelet aggregating factor inhibitor.** Normal plasma was adsorbed with Al(OH)₃ as described above. The adsorbed plasma was brought to 30% saturation with ammonium sulfate, stirred for 30 min at 4°C, and centrifuged at 18,000 g for 30 min. The supernatant was adjusted to 50% saturation with ammonium sulfate, mixed at 4°C for 30 min, and centrifuged as above. The precipitate was dissolved in 1/10 of the original plasma volume in calcium-free Tyrode's buffer and dialyzed at 4°C for 48 hr against 3 changes of the same buffer.

**Study of the effects of diisopropylfluorophosphate (DFP), hirudin, heparin, and aluminum hydroxide adsorption.** Fifty microliters of 0.04 M diisopropyl fluorophosphate (DFP) (Sigma) was added to 950 μl TTP plasma (final concentration 2 mM). The mixture was incubated at 4°C for 4 hr and then dialyzed for 24 hr against 200 vol calcium-free Tyrode’s buffer. A control TTP plasma with buffer added instead of DFP was dialyzed in the same fashion.

Hirudin (Sigma) was added to TTP plasma to make a final concentration of 1 U/ml, and the mixture was incubated for 10 min at 37°C before testing.

Heparin (Organon, W. Orange, N.J.) was added to TTP plasma to a final concentration of 2 U/ml, and the mixture was incubated for 1 hr before testing.

Aluminum hydroxide adsorption was performed as described above. The adsorbed plasma contained less than 5% of the original activities of factors II, VII, IX, and X.

**Determination of proteinase inhibitors.** Antithrombin was assayed functionally according to the method of Biggs and immunologically by Laurell's immunoelectrophoretic technique using monospecific anti-human antithrombin serum (Atlantic Antibodies, Westbrook, Me.). C1 inhibitor, α₂-macroglobulin, and α₁-antitrypsin inhibitor were measured by Laurell’s immunoelectrophoretic technique using monospecific antisera (Atlantic Antibodies).

**RESULTS**

The TTP plasmas and plasma concentrated with 6% PEG 6000 obtained during relapse from all three patients with TTP induced the aggregation of washed platelets from normal donors (Fig. 1). This aggregation induced by TTP plasma or its 6% PEG concentrate was inhibited by prior incubation at 37°C for 1 hr with normal plasma or the 30%–50% ammonium sulfate fraction of normal plasma. In contrast, platelet aggregation induced by platelet isoantibody was not inhibited by its incubation with the 30%–50% ammonium sulfate fraction of normal plasma.

The plasmas obtained from all three TTP patients during relapse caused aggregation of washed platelets not only from normal donors but also from themselves during remission. This is in marked contrast to the behavior of the platelet isoantibody, which did not aggregate washed autologous platelets. Interestingly, the inhibition of TTP plasma--induced platelet aggregation by normal plasma was time dependent, as shown in Fig. 2.

The platelet aggregating activity of the TTP plasmas was not affected by DFP, hirudin, heparin, or aluminum hydroxide adsorption. The factor that caused aggregation was not dialyzable. Antithrombin levels determined by either functional assay or immunologic method were normal. C1 inhibitor, α₂-macroglobulin, and α₁-antitrypsin inhibitor also were normal when they were measured by immunologic methods.

**DISCUSSION**

It was reported in 1976 that as many as 60% of patients with TTP achieved remission when treated with whole blood or plasma exchange transfusions. It was speculated that the removal of immune complexes explained the success of such
The three patients described here each presented as classic clinical examples of TTP with microangiopathic hemolysis and thrombocytopenia, and the disease process in each was abruptly reversed by the infusion of normal plasma. We have demonstrated that the plasmas from these patients induced aggregation of washed platelets from normal donors as well as the patients' own platelets during remission. The platelet aggregating activity of the TTP plasmas diminished as a function of time when incubated with normal plasma prior to testing. Therefore, the behavior of PAF was distinctly different from that of platelet isoantibodies and, in addition,
the factor was equally active against the patients' own platelets. Since the PAF of TTP plasma was not inhibited by DFP or hirudin, it must not be a thrombin or other serine protease.

The source of PAF in TTP plasma is not known. It may be a normal plasma component, it may be converted from an inactive plasma precursor, or it may be released into the plasma from the tissues. If PAF is not adequately counteracted by the platelet aggregating factor inhibitor (PAFI) present in normal plasma, the PAF-induced platelet aggregates could lead to occlusion of the microcirculation and result in fluctuating neurologic signs, renal dysfunction, thrombocytopenia, and hemolytic anemia. Either an acquired2 (as appears to be the case in TTP) or a hereditary10 deficiency of PAFI would be expected to intensify the severity of the clinical symptoms.

One soluble platelet-activating factor has been described that is secreted from IgE-sensitized basophils upon interaction with specific antigen in rabbit,11 rat,12 and man.13 Whether or not the PAF in TTP plasma has the same property as that secreted from basophils requires further investigation.

The nature of PAFI is not known. We demonstrated that it is not antithrombin, α2-macroglobulin, α1-antitrypsin inhibitor, or Cl inhibitor. Further investigation of the properties of this PAFI is currently being undertaken in our laboratory.

REFERENCES

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