Case report

Post-transfusion purpura

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Post-transfusion purpura (PTP) is a rare, self-limited syndrome characterized by severe thrombocytopenia and purpura appearing approximately 1 week after a blood transfusion. Considerable evidence supports the concept that PTP results from the presence of an antibody directed against the platelet isoantigen Pla_1.[1] Pla_1 is a specific platelet antigen present in more than 97% of the population.[2] Of 20 cases thus far reported,[1–9] all except one[8] of those tested for Pla_1 antigen have had Pla_1-negative platelets and anti-Pla_1 in the plasma.

We now report the second fatal case of PTP with data identifying an IgG platelet antibody with specificity for Pla_1.

Case report

A 48-year-old white woman, gravida 2, para 2, was referred to The Cleveland Clinic Foundation on January 26, 1975, for evaluation and treatment of arteriosclerosis and severe coronary artery disease. Her medical history included two normal pregnancies, appendectomy in 1934, cholecystectomy in 1941, and hysterectomy in 1963; all surgical procedures were said to have been performed without blood transfusion. Because of coronary artery disease with angina pectoris, she was taking nitroglycerine, isosorbide dinitrate, and diphenylhydantoin. She was also

taking diazepam and furosemide. There was no history of drug sensitivity or bleeding tendency.

On physical examination vital signs were normal. Examination of the heart disclosed a grade II/VI systolic ejection murmur at the apex. Examination of the peripheral vascular system revealed bruits over both femoral arteries and the left common carotid artery.

Admission laboratory data included a blood count with a hemoglobin of 12.5 g/dl, hematocrit 38%, red cell count 4.3 × 10^6/mm^3, white cell count 7200/mm^3 with a normal differential and adequate platelets in the peripheral blood film. Electrocardiogram, serum electrolytes, urinalysis, and SMA-12/60 were normal. Prothrombin time was 11 seconds with a control of 11 seconds.

Coronary cineangiography demonstrated severe occlusions of the anterior descending and circumflex branches of the left coronary artery, and the patient had saphenous vein bypass grafts to the anterior descending and circumflex branches on January 30, 1975. During this procedure, six units of compatible group O, Rh negative blood were transfused.

The postoperative course was complicated by a temperature of 103 F and atrial fibrillation on the third postoperative day; this was treated with methicillin and digoxin. Because cultures remained negative, the antibiotic was discontinued after 2 days. She became afebrile and progressed satisfactorily until the evening of the seventh postoperative day, when clear vesicular lesions developed on the lips and buccal mucosa; they subsequently became hemorrhagic. She also noted the presence of purpuric lesions over her legs, feet, and buttocks. Shortly thereafter, she had massive gastrointestinal bleeding.

The platelet count was 2000/mm^3 on the ninth postoperative day. Prothrombin time was 13 seconds with a control of 13 seconds. Partial thromboplastin time was normal. Bleeding time was greater than 15 minutes, and clot retraction was absent. Plasma fibrinogen was 440 mg/dl. The direct Coombs' test was negative.

The diagnosis of PTP was considered, and she was given hydrocortisone sodium succinate, 100 mg intravenously every 6 hours, with fresh whole blood and concentrated platelet transfusions during the next 2 days. No systemic reaction to transfused material was noted. However, the platelet count remained unchanged.

Plasmapheresis was initiated at that time; cardiac arrhythmias developed, culminating in ventricular tachycardia and hypotension, which did not respond to therapeutic measures; she died on the 11th postoperative day.

Autopsy revealed severe coronary atherosclerosis with massive gastrointestinal bleeding. Bone marrow showed moderate erythroid hyperplasia with normal morphology and many megakaryocytes.

Materials and methods

Platelet antibodies were detected in the patient's serum by two independent methods.

Immunoglobulin binding. Platelet-binding immunoglobulins were measured by a solid-phase radioimmunoassay similar to that described by Soulier et al.[10] EDTA buffer (veronal-buffered saline pH 7.5 containing 0.01 M EDTA and 0.1% gelatin) was used for diluting and washing the platelets instead of saline. Commercially available monospecific antisera against human IgG, IgM, and IgA (Behring Diagnostics, Somerville, New Jersey) were labeled with ^{125}I by the iodine monochloride method.[11]

The patient's serum was run against platelets from two normal subjects (one blood group O− and one A+). This serum was tested against no other platelets because of insufficient quantity. Sera from six patients known to have immune

thrombocytopenia (four patients with SLE and two patients with ITP) were tested against platelets from one of the donors. Sera from seven normal individuals were tested against a panel of platelets from seven normal sources (three O+, three A+, and one O−).

Platelets were obtained from 10-ml samples of blood collected in CPD, donated by seven healthy individuals (not characterized for Pla_1). Aliquots of EDTA buffer containing 2×10^7 platelets per mm^3 were incubated at 37 C for 15 minutes with serial dilutions of test serum in EDTA buffer. After washing, sensitized platelets were mixed with labeled antiglobulin. After a second incubation and washing, the radioactivity bound to the platelets was measured. The amount of platelet antibody in each of the three immunoglobulin classes tested for was then calculated as follows: the bound radioactivity for each tube was compared with the anti-immunoglobulin radioactivity bound to known amounts of purified human immunoglobulin attached to formalinized O− human erythrocytes by the $CrCl_3$ method.[12]

Cytotoxicity. Specificity of the patient's platelet antibody for Pla_1 was determined by Dr. Richard Aster using a 51-chromium release assay as previously described.[13] Platelets bearing Pla_1 from four different donors and platelets without Pla_1 from a single donor were used.

Results

With the immunoglobulin-binding assay, the patient's serum was found to contain large amounts of IgG antibody which reacted with platelets from two donors. Considerably less IgM and IgA antibodies were found (*Table*). In all three immunoglobulin classes, the amount of antibody detected was somewhat less using platelets from the O− donor. Sera from normal individuals tested against a panel of platelets from normal sources showed no immunoglobulin binding, and sera from patients with SLE and ITP demonstrated the presence of antibody (*Table*). In the SLE patients (cases 2–5), as in the PTP

Table. Platelet-binding immunoglobulins in sera of patients and normal individuals

		Platelet antibody (μg/ml)		
Patient	Disease	IgM	IgG	IgA
1	PTP	0.524 (.337*)	7.71 (4.60*)	0.613 (0.00*)
2	SLE	0.348	1.68	0.00
3	SLE	14.4	13.9	5.96
4	SLE	0.00	0.483	0.00
5	SLE	0.00	4.00	0.00
6	ITP	1.17	0.080	0.00
7	ITP	0.00	4.54	10.4
8–14	Normal	0.00†	0.00†	0.00†

Except where indicated, platelets used as substrate were from a single A+ individual.
* Same serum tested against O-platelets.
† Seven normal sera tested against a panel of seven platelet substrates (3, O+; 3, A+; and 1, O−).
PTP = post-transfusion purpura, ITP = idiopathic thrombocytopenic purpura, SLE = systemic lupus erythematosus.

patient (case 1), IgG antibody predominated. Although both the ITP patients (cases 6 and 7) had IgG antibody, in one most of the antibody was IgM, and in the other it was IgA.

The small sample size of ITP and SLE patients in this report precludes drawing a firm conclusion about the frequency with which platelet-binding immunoglobulins are detected by this method. However, in contrast to Soulier's method which failed to identify antibody in 21 of 22 patients with ITP, our method detected antibody in both patients with ITP and all four patients with SLE.

Of the seven normal volunteers, none reacted with their own or other sources of platelets.

To determine the specificity of the PTP patient's platelet antibody, her serum was tested against a panel of platelets from different sources which had been characterized for Pla_1; these studies were performed by Dr. Richard Aster using a 51-chromium release assay. The results indicated that the patient's serum contained antibody which reacted with Pla_1 positive platelets, but not with Pla_1 negative platelets.

Discussion

In this report, we have described the second fatal case of PTP in which the platelet antibody was characterized not only as to specificity by standard methodology,[13] but also as to immunoglobulin class by a new immunoglobulin-binding method. PTP is a syndrome characterized by transient, but severe thrombocytopenia following blood transfusion.[1] Our patient, a middle-aged multiparous woman, developed hemorrhagic vesicles and purpura approximately 1 week following transfusion, the usual course of PTP.[1] The condition is usually self-limiting; however, it can be life-threatening, because of thrombocytopenia and attendant severe bleeding. Of 20 cases reported, only one terminated in death.[6] Plasmapheresis and exchange transfusion have been shown to be more effective than corticosteroids in treating PTP.[5,7]

Current concepts suggest that patients whose platelets lack a major platelet antigen, Pla_1, may be sensitized by this antigen either during pregnancy or during transfusion.[7] Following a subsequent exposure to Pla_1-bearing platelets during transfusion, the antibody causes rapid destruction of autologous (Pla_1-negative) platelets by an unknown mechanism. With one exception,[8] all reported cases have had previous exposure to homologous platelets either during pregnancy or transfusion (5 to 15 years before the transfusion that is followed by purpura). After platelets have reappeared in the circulation, the antibody can no longer be detected.[2]

Several interesting aspects of this syndrome remain unexplained: (1) the mechanism by which anti-Pla_1 can lead to the destruction of the patient's platelets which lack Pla_1 antigen; (2) the rarity of this syndrome (20 cases reported in the past 17 years); (3) the self-limited nature of the syndrome, and the disappearance of antibody from the blood after recovery.

Shulman et al[1] have suggested that Pla_1 antigen may be present in soluble form in the plasma of certain individuals. If blood from such a person is transfused to a previously sensitized Pla_1-negative recipient, the soluble antigen could stimulate anti-

body formation, and then combine with the antibody to form immune complexes having a special affinity for sites on the platelet surface. The recipient's platelets would then be coated with complexes and destroyed as "innocent bystanders" in a fashion similar to quinidine purpura. However, Abramson et al[7] were unable to demonstrate Pla_1 antigen in donor plasma. Shulman et al[1] have also shown that infusion of autologous serum-containing anti-Pla_1 antibody after recovery from an episode of PTP did not cause thrombocytopenia, whereas infusion of a smaller amount of the same serum to a Pla_1-positive individual resulted in thrombocytopenia.

A variety of techniques are available for detecting platelet antibodies. Most of the reported methods have relied on the detection of secondary results of antigen-antibody combination, such as agglutination, complement fixation, or destruction of platelets. None of these methods is entirely satisfactory since antibody populations of a given specificity (e.g., anti-Pla_1) may exhibit great differences in their ability to promote such secondary effects. Competitive-binding assays have been used to detect platelet antibody in patients with ITP[14,15] but not PTP.

The immunoglobulin-binding test described in this paper represents an advance in this area, since the technique depends only on the ability of antibody to combine with antigen. Furthermore, the test can be quantitated, and information regarding the immunoglobulin class composition of the antibody population can be obtained. As was shown by Soulier et al,[10] if suitable panels of antigenically characterized platelets are available, it can also be used to determine the exact specificity of a platelet antibody. In our results using the immunoglobulin-binding technique, it is apparent that IgG antibody is present in most patients with autoimmune thrombocytopenia as suggested by Karpatkin et al.[16] In one case of PTP,[7] the antibody detectable by platelet aggregation was absorbed completely by anti-IgG_3. In our case, the platelet antibody was also largely in the IgG class, although small amounts of IgM and IgA antibody were also found; this predominance of IgG antibody is not unexpected given the clinical characteristics of PTP which suggest a secondary immune response.

Summary

PTP is usually a rare and self-limited disease. A fatal case and description of a new method identifying platelet antibody are reported. The patient was a middle-aged multiparous woman in whom severe thrombocytopenia and purpura developed 7 days after blood transfusion. She had a platelet antibody of IgG class with specificity against Pla_1 antigen. The immunoglobulin-binding test described in this paper represents an advance in detection of platelet antibodies in PTP, since the technique relies only on the ability of antibody to combine with antigen rather than the secondary results of antigen-antibody combination. This test can be used for quantitation of immunoglobulin and determination of immunoglobulin classes as well as the specificity of platelet antibody.

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