

Isoantibody Specificity in Post-transfusion Purpura

By Jon P. Gockerman and N. Raphael Shulman

Antiplatelet antibody in the serum of a fatal case of post-transfusion purpura showed specificity for the PI^{A1} platelet isoantigen in that the antibody was adsorbed by all PI^{A1} -positive platelets tested but not by PI^{A1} -negative platelets. However, some PI^{A1} -positive platelets that fixed complement with the reference anti- PI^{A1} did not fix complement with this patient's antibody. When used with these platelets, the patient's antibody competitively blocked the complement-fixing activity of reference

anti- PI^{A1} . There was no evidence for lack of antigenic sites on the noncomplement-fixing platelets or for antibodies with more than one specificity in the patient's serum. These studies, therefore, indicated that the complement-fixing activity of different examples of anti- PI^{A1} is variable, and that this variation is probably due to distribution of PI^{A1} antigenic sites on cell surfaces rather than to differences in antigenic structure that affect antibody affinity.

IN 1961, Shulman et al. delineated a syndrome characterized by the sudden onset of thrombocytopenia approximately 1 wk following a blood transfusion.¹ They showed the thrombocytopenia was associated with development of a complement-fixing antibody against a platelet antigen that they named PI^{A1} . Two other patients in the previous literature appear to represent the same syndrome,^{2,3} and eight cases of post-transfusion purpura subsequently have been reported in each of which the same platelet antigen was involved.^{1,4-7} An additional case of post-transfusion purpura appeared in the literature after this paper was submitted.¹³

We are reporting the tenth case of post-transfusion purpura and the first reported death from it. We have further explored the serologic reactions involving the PI^{A1} antibody and antigen.

MATERIALS AND METHODS

The serum samples were stored at -20°C until use. Quantitative platelet complement fixation studies and antibody elution procedures were done as previously described by Shulman et al.^{1,4} Platelet agglutination studies were done by the method of Dausset and Malinvaud.⁸ Inhibition of clot retraction was done by the method of Lucia et al.⁹ Fractionation of serum was done using Sephadex G-200 and 0.147 M NaCl as eluting fluid. Platelet preparations were made as described before and were used at concentrations of $10^6/\text{cu mm}$ unless otherwise noted.⁴

CASE REPORT

Mrs. M.O., a 49-yr-old white married female, was in excellent health until November 1967, when she developed sensory deficits involving the left eighth and twelfth cranial

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nerves that progressed by May 1969 to include cerebellar symptoms of ataxia. By January 1970 these symptoms of a posterior fossal tumor led to admission on the neurosurgery service at Duke University Medical Center for surgery.

Her past medical history of significance included pregnancies in 1949 and 1952 with normal gestations and deliveries, and tonsillectomy, appendectomy, and hysterectomy, all performed without transfusion. There was no history of having received any blood products or of abnormal bleeding, and there was no family history of a bleeding tendency. She had taken no medications before her hospital admission in 1970.

Her preoperative hemoglobin was 15.3 g/100 ml, and her white blood cell count 5500/cu mm. No platelet count or clotting studies were done. Her blood urea nitrogen, sugar, and electrolytes were normal. On January 1, 1970, a large neurilemmoma was removed from her left posterior fossa without excessive bleeding. However, during the procedure her blood pressure dropped from 100/60 to 80/40 mm Hg, and she was given 3 U of blood without evidence of transfusion reaction. As is routine, she received postoperative medication of methylprednisolone sodium succinate 120 mg/day and glycopyrrolate 0.3 mg/day, and a single dose of urocholine was given on January 21 for urinary retention.

Her postoperative course from January 16 to the evening of January 23 was benign. However, on the evening of January 23 (seventh hospital day), the patient was found in a coma. Blood was oozing from the craniotomy site, from venipuncture sites, and around stitches of a tarsorrhaphy that had been performed that same morning without abnormal bleeding. An emergency tracheostomy was associated with excessive bleeding. Blood examination on January 17 and 20 had shown adequate platelets on smear. The peripheral blood smear on the evening of January 23 showed no platelets, and there was no clot retraction. A sternal bone marrow aspirate showed normal megakaryocytes with budding of platelets. A Lee White clotting time was 14 min, and the prothrombin time was 16 sec with control of 13.5 sec. Her initial hematocrit on January 23 was 42%, and red cell morphology was normal. There was a leukocytosis with a normal differential.

The evening of January 23, she was given 2 U of fresh blood and 16 U of platelets, but no platelets were counted in her blood after these transfusions and only a rare platelet was seen on smear. Approximately 12 hr later her platelet count was 7000/cu mm and platelets were not difficult to find on smear. Twenty-four hours after the clinical onset of her bleeding she died of a respiratory arrest.

A postmortem examination showed massive hemorrhage into the left cerebellum, medulla, and pons, with herniation of the right cerebellum over the foramen magnum. There was diffuse hemorrhage in the GI tract, bladder and in subendocardium. The bone marrow showed hyperplasia with adequate megakaryocytes. There was no gross or microscopic evidence of disseminated intravascular clotting.

RESULTS

Post-transfusion purpura was suspected, and the patient's serum (M.O.) was assayed for antiplatelet antibody by several techniques. M.O. sera did not inhibit clot retraction of normal blood when mixed 1 part serum to 10 parts blood, nor did her serum at a 1:1 dilution agglutinate platelets. However, her serum fixed complement with normal platelets as shown in Fig. 1. The quantitative complement fixation curve with a fixed concentration of platelets and varying concentrations of antibody is typical of those found in previous cases of post-transfusion purpura.⁴ When M.O. serum was fractionated by Sephadex G-200 filtration, the complement-fixing activity was found to be in the second peak that contained protein of approximately 150,000 mol wt.

The complement-fixing antibody appeared to be directed specifically against the PI^{A1} antigen. This was based initially on complement fixation reactions

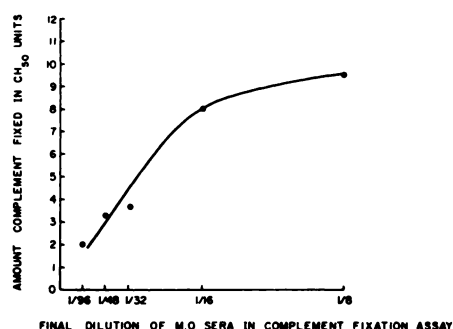


Fig. 1. Amount of complement fixed as function of antibody concentration.

with three PI^{A1} -positive platelet preparations and two PI^{A1} -negative preparations phenotyped with anti- PI^{A1} from the original case of post-transfusion purpura (P.K.).¹ However, when a subsequent larger panel of platelets of known phenotype was tested, one platelet preparation (J.L.) that reacted with the original anti- PI^{A1} (P.K.) did not fix complement with serum from patient M.O.

Because of this unexpected reaction, sera with anti- PI^{A1} specificity from ten different cases of post-transfusion purpura were tested against J.L. platelets. While nine of these gave the same complement-fixing reaction as with other usual normal PI^{A1} -positive platelets, one post-transfusion serum (E.S.) did not fix complement with J.L. platelets. Because of the failure of M.O. and E.S. sera to fix complement with an apparently PI^{A1} -positive platelet (J.L.), further studies of the antigenic specificity of M.O. and E.S. sera and J.L. platelets were undertaken.

The complement-fixing activity of anti- PI^{A1} (P.K.) with J.L. platelets was inhibited by the presence of the two sera (E.S. and M.O.) that did not fix complement with J.L. platelets (Table 1). The inhibitory reaction was similar to that of "blocking" antibodies that develop in mothers of infants with neonatal purpura due to PI^{A1} incompatibility.¹⁰

To determine whether antibodies with anti- PI^{A1} specificity in E.S. serum attached to J.L. platelets, adsorption and elution studies were performed

Table 1. Evidence for Blocking of Complement-fixing Activity on J.L. Platelets

Sera Used (ml)				Amount Complement Fixed (CH ₅₀)	Amount Complement Blocked (CH ₅₀)
P.K.	E.S.	M.O.	Mother*		
0.05	—	—	—	3.3	—
0.05	0.08	—	—	0.8	2.5
—	0.08	—	—	0	—
0.05	—	0.08	—	1.8	1.5
—	—	0.08	—	0	—
0.1	—	—	—	9.5	—
—	—	—	0.1	0	—
0.1	—	—	0.1	1.2	8.3

*Sera from a mother whose child had neonatal purpura caused by anti- PI^{A1} .

Table 2. Effects of Adsorptions and Elution Studies on Complement Fixation Activity

Procedure Used in Treating Sera	Amount Complement Fixed in C ₅₀ Units on Various Platelet Preparations	
	B.L.	J.L.
E.E. sera unadsorbed	3.1	0
E.E. sera adsorbed with J.L. platelets	0	0
Eluate of J.L. platelets adsorbed with E.S. sera	2.2	0
E.S. sera adsorbed with B.L. platelets	0	0
E.E. sera adsorbed with P.K. platelets	3.5	0
P.K. sera unadsorbed	3.4	3.4
P.K. sera adsorbed with J.L. platelets	0	0
Eluate of J.L. platelets adsorbed with P.K. sera	3.0	3.7

(Table 2). Adsorption of E.S. sera with J.L. platelets removed all of the anti-PI^{A1} antibody that fixed complement with other platelets. The same number of J.L. platelets as normal PI^{A1}-positive platelets were required for complete adsorption of the antibody. Eluates made from J.L. platelets that had been incubated in E.S. serum contained an antibody that fixed complement with PI^{A1}-positive platelets but not with PI^{A1}-negative platelets or with J.L. platelets. PI^{A1}-negative platelets obtained from patient P.K. did not adsorb antibody from E.S. serum.

DISCUSSION

Post-transfusion purpura, in every case, has been associated with development of anti-PI^{A1} and has occurred only in individuals who have been PI^{A1}-negative when platelet typing has been done after recovery.⁴ Thus, the disease is caused by sensitization to a "foreign" platelet isoantigen. All patients have a history of being previously transfused or pregnant 5-15 yr prior to the transfusion that results in purpura. Apparently a repeat exposure to a large dose of the PI^{A1} antigen stimulates an amnestic response, usually with a high titer, complement-fixing antibody within 1 wk. It is not as yet clear why severe thrombocytopenia develops in the sensitized individuals. Shulman et al. have previously shown that infusion of a patient with her own anti-PI^{A1}-positive antibody after recovery from post-transfusion purpura did not cause thrombocytopenia,⁴ whereas infusion of much less of the same serum into a PI^{A1}-positive individual resulted in thrombocytopenia. It is conceivable that attachment of antibody to PI^{A1}-negative platelets of patients who develop post-transfusion purpura may be dependent on combination of the antibody with residual circulating PI^{A1} antigen from transfused platelets. Possibly isoantigen in combination with antibody is adsorbed on platelets as a complex just as antigen-antibody complexes are adsorbed in drug purpura.¹² The resulting thrombocytopenia, as in drug purpura, is initially fulminant. In contrast to the prompt clearing of drug purpura once the drug is stopped, post-transfusion purpura may persist in mild form for a period of approximately 1 mo. Quantitative considerations of the post-transfusion purpura reaction that may account for persistence of purpura have been detailed elsewhere.¹²

Antibodies developed in post-transfusion purpura have all given complement-fixing reactions with P^I^{A1}-positive platelets. The antibody in the present case (M.O.), as well as E.S. serum, fixed complement, but not with all platelets that were P^I^{A1}-positive. However, all P^I^{A1}-positive platelets adsorbed antibody from M.O. or E.S. serum, whether or not they fixed complement with it; none of the P^I^{A1}-negative platelets adsorbed the antibody. Thus, the antibody in M.O. and E.S. sera showed P^I^{A1} specificity. Why some platelets (e.g., J.L.) bearing the P^I^{A1} antigen (as detected by complement fixation with P.K. and most other examples of anti-P^I^{A1}) do not give complement fixation with certain anti-P^I^{A1} sera is unclear. This doesn't appear simply to be a variation in the amount of antigen on the platelet, for J.L. platelets adsorbed an amount of antibody equivalent to platelets that fixed complement. A possible explanation may be that the antibody response to the foreign platelets results in more than one antibody being produced and a second non-complement-fixing antibody (possibly of different specificity) could, with certain platelets, block the complement-fixing activity of the anti-P^I^{A1} antibody. On the other hand, it is known that heterozygous P^I^{A1.2} platelets fix less complement than do homozygous P^I^{A1.1} platelets, and hence, it is conceivable that an occasional individual may have P^I^{A1}-positive platelet with an unusual distribution of antigenic sites that prevents the secondary reaction of complement-fixation on the cell surface.¹ Whatever the eventual explanation for this phenomenon, it is apparent that some P^I^{A1}-positive platelet preparations are not suitable for establishing the diagnosis of post-transfusion purpura by the complement fixation technique.

The initial thrombocytopenia in post-transfusion purpura is severe and frequently associated with bleeding. In our case this resulted in the death of the patient. The best treatment for the initial severe thrombocytopenia is not entirely clear. Massive exchange transfusions have been used with success^{1,4} presumably because the amount of antiplatelet antibody is decreased both by the exchange and by binding to the transfused platelets.

The syndrome could be prevented by cross-matching platelets, but since more than 97% of the population is P^I^{A1}-positive, the mismatch is rare, and suitable donors for a P^I^{A1}-negative patient would be difficult to find.⁴

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