Nature of Platelet Antibody in Evans Syndrome: A Case Report*

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ABSTRACT

The association of autoimmune thrombocytopenia and autoimmune hemolytic anemia (Evans Syndrome) has been recognized previously. Recently the present authors investigated a patient with this disorder. Both IgG and C3d were found on the patient's red cells and the serum contained a polyspecific IgG red cell antibody demonstrable only by the antiglobulin test. IgG antiplatelet antibody was demonstrated on circulating platelets as well as in the serum from the patient by using 125I-staphylococcal Protein A (ISPA) assay. Absorption of serum with normal platelets resulted in removal of platelet antibody, but not the red cell antibody. Furthermore, eluate prepared from platelets sensitized with patient serum contained an IgG antibody directed specifically against platelets, since it did not cross react with red cells. Our data suggest the presence of two distinct antibodies, one directed against platelets and the other against red cells in our patient with Evans Syndrome. Both these antibodies are IgG in nature and belong to subclass other than IgG3 since staphylococcal Protein A (SPA) binds to all subclasses of human IgG except IgG3.

Introduction

Evans et al1,2 initially described the association of autoimmune hemolytic anemia and thrombocytopenia. As reviewed by Pirofsky,3 many investigators subsequently confirmed the existence of this syndrome. Two autoantibodies, one directed against red cells and the other
against platelets were suspected, based on the lack of correlation between hemolysis and thrombocytopenia. Mattoth et al. demonstrated separate and distinct antibodies, but the study did not address the question of cross reactivity. Recently the present authors observed a patient with autoimmune hemolytic anemia with thrombocytopenia in whom were demonstrated two separate autoantibodies directed against red cells and platelets, respectively, and the platelet directed antibody did not crossreact with red cells.

Materials and Methods

Platelet Collection

Blood was collected into ethylene diamine tetraacetic acid (EDTA) vacutainer tubes* and centrifuged at 200 × g for 20 min to harvest platelet rich plasma (PRP). This PRP was centrifuged at 2000 × g for 10 min to obtain a platelet pellet. The pellet was washed three times with EDTA-phosphate buffered saline (PBS-EDTA: 0.027M Phosphate, 0.14M NaCl and 0.009M Na₂EDTA). All centrifugations were carried out at room temperature.

Serological Techniques

Red cell antibodies were investigated by standard techniques. Platelet antibody was identified using ¹²⁵I-staphylococcal Protein A (ISPA) as previously described by us. Briefly, in the indirect assay, 1.5 × 10⁸ washed platelets in 0.2 ml PBS-EDTA were incubated with an equal volume of serum for 30 min at 37°C. Excess unbound serum was removed by two washings of platelets with PBS-EDTA containing 1 percent bovine serum albumin (BSA). The final button was resuspended in 0.2 ml of the same buffer and the sensitized platelets were incubated with 0.2 ml of diluted ISPA (specific activity 5.1 μci per μg) for 20 min at room temperature. Unbound radioactivity was removed by two more washings of platelets and platelet bound CPM (counts per minute) of 125-I was determined in an Auto-Gamma Scintillation spectrometer.† In the direct assay, platelets obtained from the patient were incubated with ISPA following three washes, and no serum incubation was required.

In ISPA technique, a concomitant negative control serum obtained from a normal AB blood group donor and positive control consisting of serum containing polyspecific HLA antibody were included. The assays were performed in duplicates. ISPA binding to platelets with 14 normal sera was 4.1 ± 1.0 percent (mean ± S.D.). Mean + 3 S.D. ISPA binding is considered the upper value for normal sera. In the direct assay, the average ISPA binding with normal washed platelets was 0.9 percent (N = 4), with washed A Rh negative red cells 0.7 percent (N = 4), and with B Rh negative red cells 0.8 percent (N = 4) (table I).

Serum absorption and elution of antibody from platelets was performed as previously described.7

Case Report and Results

A 72-year-old white man was admitted for pericardial effusion. Eleven weeks prior to present admission, the patient was treated at another hospital for paroxysmal nocturnal dyspnea. A moderate pericardial effusion was demonstrated by echocardiography. Gross blood was aspirated on percutaneous pericardiocentesis and, during the procedure, he developed cardio-pulmonary arrest. Resuscitation measures included treatment with digoxin, quinidine, apresoline, intravenous fluids, and endotracheal intubation. Two units of packed red blood cell transfusion and 48 hour assist with an intraaortic balloon were needed. No difficulty in the crossmatching was reported. Patient was begun on anticoagulation with coumadin and discharged home five weeks prior to present admission.

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At age 52, he suffered an automobile accident with liver laceration treated with three abdominal surgeries and multiple blood transfusions. A cholecystectomy was performed at age 57 for a gangrenous gall bladder. Hypertension was diagnosed at age 68, but the patient received anti-hypertensive medicines for only one year.

Following discharge from the last hospital, he felt increasingly weak, with onset of fainting spells. On examination, he appeared thin and chronically ill but in no acute distress. Supine pulse rate was 88 per min and, upon standing, it was 90 per min. Temperature and respiration were normal. Supine blood pressure was 122/82 mm Hg; but upon standing, it decreased to 94/60 mm Hg, with on occasion as low as 65/40 mm Hg. Jugular venous pressure was elevated to 6 cm and a loud pericardial rub was heard over the entire precordium. Heart sounds were distant and examination of lungs was unremarkable. Abdomen was soft, non-distended, non-tender, and had multiple healed surgical scars. Liver was palpable 2 cm below the right costal margin and was non-tender. Spleen was not palpable. Neurological examination was normal. No occult blood was noted upon rectal examination. A small, soft, 1 cm size lymph node was present in right axilla.

A complete blood count on admission revealed hemoglobin: 9 g per dl; hematocrit: 28 percent; white blood cells: 6200 per µl; platelets: 239,000 per µl; and reticulocyte count of 2.3 percent. Red cell morphology and indices were normal. An electrocardiogram revealed sinus rhythm, left axis deviation, left bundle branch block, and non-specific ST-T wave changes. An echocardiogram revealed a large pericardial effusion, left ventricular hypertrophy, akinesis of the apex and the lateral wall, and moderate to severe hypokinesis of the anterior wall of the left ventricle. Paradoxical movement of the interventricular septum was also noted. Antinuclear antibody test showed mild fluorescence with a homogenous pattern. Rheumatoid factor and lupus erythomatosus cell preparation were negative. Liver and spleen scan and a computerized axial tomographic examination of abdomen revealed no abnormality. Bone marrow examination showed normal cellularity and normal maturation of all cellular elements. Right axillary node biopsy showed reactive hyperplasia.

Patient was treated with bed rest and was maintained on digoxin, coumadine, quinidine, and aresoline. One episode of atrial fibrillation with a rapid response was successfully treated with increase in the dose of quinidine from 600 mg per day to 800 mg per day. On the 26th hospital day, patient was begun on 100 mg of prednisone per day. Improvement in strength with resolution of orthostatic hypotension and fainting spells were noted within a week. Patient was discharged on 32nd hospital day.

Serial circulating platelet count and hematocrit values are shown in figure 1. Admission platelet count was 239,000 per µl which decreased to 41,000 per µl on 24th hospital day. Prednisone therapy (100 mg per day) was initiated on 26th hospital day. The platelet count rapidly increased to 127,000 per µl at the time of discharge. During outpatient visits over six weeks, platelet count ranged from 93,000 per µl to 134,000 per µl.

At the time when prednisone therapy was begun, the hematocrit was 29 percent. Seven days later, at the time of discharge, it was 38 percent. Follow up values on outpatient visits demonstrated stabilization of the hematocrit to 45 percent. Prednisone dos-
age was reduced stepwise to 20 mg every other day along with improvement in hematologic picture.

Serologic Studies

A blood sample obtained on admission revealed a 3+ positive direct as well as indirect Coombs test. The serum contained an IgG red cell antibody which reacted with all red cell preparations tested. This antibody reacted only in the antiglobulin phase. An eluate obtained from patient's red cells contained an antibody active in the antiglobulin phase which reacted with all red cell preparations tested. However, it appeared to have anti-c, anti-Kell, and possible anti-e specificity. Both IgG and C3d were demonstrated on the patient's red cells.

The serum obtained on admission contained a platelet antibody demonstrated by ISPA technique although the circulating platelet count was normal. Untreated patient serum resulted in 20.8 percent ISPA binding which is five fold greater than the average 4.1 percent binding with normal sera. Subsequent to one absorption of patient's serum with normal platelets, the ISPA binding decreased to 5.0 percent, which is well within the normal range. Nonetheless, absorbed serum reacted as well as unabsorbed serum against red blood cells by indirect Coombs test. An eluate prepared from the platelets sensitized with patient's serum bound 13.3 percent of ISPA which is greater than three fold average binding with normal sera. ISPA binding was 4 percent with patient's circulating platelets as compared to 0.9 percent with platelets from normal donors. This represents greater than four fold increase in platelet associated IgG on patient's circulating platelets.

Patient's red cells were also found to contain increased bound IgG with ISPA assay demonstrating 9.1 percent ISPA binding.

The serologic studies were performed on serum sample obtained on day of admission, and similar results were also noted on samples obtained on 3rd and 13th hospital day. The ISPA binding with patient's circulating red cells and platelets were performed on samples collected on, the 13th hospital day, at which time hematocrit was 28 percent and the platelet count was 91,000 per μl. These studies were not repeated following prednisone therapy.

Discussion

The association of thrombocytopenia and autoimmune hemolytic anemia (Evans Syndrome) has been described previously. Our patient presented with autoimmune hemolytic anemia with normal platelet count. Since the patient was on quinidine, drug induced immune hemolytic anemia and thrombocytopenia were suspected. During hospitalization, platelet count decreased and reached a nadir of 40,000 per μl by the 23rd hospital day.

The patient was promptly begun on corticosteroids while quinidine was continued. Hematocrit returned to normal in three weeks and improvement in platelet count was also noted. This response to therapy, even though quinidine was continued, rules out the possibility of quinidine induced cytopenias.

Platelet antibody in Evans Syndrome has previously been identified by a platelet agglutination technique. In our patient with this disorder, both the platelet and red cell antibody were found to be IgG in nature since the staphylococcal Protein A is known to bind to human IgG but not the other immunoglobulin classes. Furthermore, IgG antibody of subclass 1, 2, or 4 was involved since the staphylococcal Protein A does not bind to human IgG₃. Platelet antibody was found
to be specific since it failed to cross react with red cells. Moreover, immune thrombocytopenia was resistant to treatment despite adequate response of autoimmune hemolytic anemia since our patient continued to have mild thrombocytopenia.

References


