CONCISE REPORT

Quantitation of Platelet-Associated IgG by Radial Immunodiffusion

By Bernard S. Morse, Dennis Giuliani, and Murray Nussbaum

Platelet-associated IgG (PAIgG) was measured by a simple radial immunodiffusion technique using washed solubilized platelets and commercially available immunoplates. Subjects with normal platelet counts had PAIgG levels of 1.5-7.0 fg/platelet. Subjects with idiopathic immune thrombocytopenic purpura (ITP) had levels ranging from 5.7 to 70.5 fg/platelet. All patients with recurrent ITP and 85% of patients with acute ITP had elevated PAIgG.

CURRENT methods of measuring platelet-associated IgG (PAIgG) are complex and limited in their availability to a few research centers. We have simplified the quantitation of PAIgG by using a radial immunodiffusion technique with commercially available immunoplates and have determined the levels in a variety of disorders in which platelet antibodies were suspected. The results obtained by this technique are comparable to those of the other more complex methods.

MATERIALS AND METHODS

After obtaining informed consent, 10-30 ml of blood were collected in EDTA or ACD tubes from 20 normal subjects and 71 patients with thrombocytopenias of varying etiology. Samples were processed within 24 hr of collection. A platelet button was obtained by first centrifuging the blood specimen at 20°C and 207 g for 10 min, removing the platelet-rich plasma, and then centrifuging it at 20°C and 2000 g for 5 min. The platelet button was resuspended in 1 ml of aqueous 1% ammonium oxalate for 10 min; 0.1 ml of aqueous saturated sucrose solution was added and the remaining plasma was removed by applying the entire mixture to a 1 x 10 cm column of sepharose 2B, equilibrated with phosphate-buffered saline (PBS).

Platelets and contaminating leukocytes were found in the turbid-appearing void volume. The total number of platelets and leukocytes in the effluent was counted by electronic particle counting (Coulter Thrombocounter and D2, respectively) in an aliquot of the suspension. Specimens containing platelets of less than 25,000/μl were recounted using a 20-μl aliquot. Because contaminating leukocytes were less than 1%; a 200-μl aliquot was routinely used for the leukocyte count. Specimens containing more than 4% leukocytes were discarded. Alternatively, plasma contamination could be removed by washing the platelet button 5 times at 20°C in 16 x 95 mm polystyrene tubes with phosphate-buffered saline (PBS) containing 0.01 M EDTA. Specimens were divided into aliquots containing 10⁸ platelets. In severely thrombocytopenic patients, as few as 0.25 x 10⁸ platelets could be used.

After resuspension in PBS, the platelets were again centrifuged at 20°C and 2000 g for 5 min and the supernate discarded. The tubes were inverted and allowed to drain for several minutes. The interior of each tube was then carefully dried with cotton swabs. At this point, the platelet button could be analyzed for IgG or stored at -20°C.

Elevated PAIgG was also found in 17% of patients with recovered ITP, 40% of patients with SLE and thrombocytopenia, 57% of patients with thrombocytopenia occurring during the course of septicemia, and 100% of patients with IgG myeloma in whom the serum IgG level was clearly elevated, regardless of the platelet count. The results are similar to reports that used more complex techniques.

RESULTS

Initially, specimens were analyzed in duplicate on separate immunoplates, resulting in an insignificant mean percentage difference in ring diameters of 3.4%. No differences were detected using blood collected in EDTA or ACD if platelets were stored or analyzed within 24 hr of collection. Since the low-level IgG plates are standardized to quantitate 160-2600 ng of IgG, the lowest level of detection is 1.6 fg IgG/platelet for 10⁸ platelets, 3.2 fg for 0.5 x 10⁹ platelets, and 6.4 for 0.25 x 10⁹ platelets.

Figure 1 illustrates the values obtained from the 20 normal subjects with normal platelet counts and from the 71 patients with various illnesses. The normal subjects with normal platelet counts had PAIgG levels ranging from 1.5 to 7.0 fg/platelet (mean 3.8 ± 1.9). Twenty-seven patients with immune thrombocytopenia had PAIgG levels ranging from 5.7 to 70.5 fg/platelet.
platelet (mean 20.3 ± 15.7). PAIgG levels were elevated in 11 of 13 patients with acute ITP and all 14 patients with chronic or recurrent ITP. The means for acute ITP and recurrent or chronic ITP were 21.2 and 19.4, respectively. The platelet counts and PAIgG levels before and after treatment of 10 patients with various forms of immune thrombocytopenia are summarized in Table 1. All 10 patients, regardless of the form of therapy, showed a drop of PAIgG as platelet counts improved. Only 1 of 6 patients (17%) with recovered ITP showed an elevated value of 10.7 fg/platelet. None of these patients had received therapy for at least 6 mo. Twelve patients with nonimmune thrombocytopenia of varying etiologies showed PAIgG levels ranging from 1.6 to 16.7 fg/platelet (mean 6.2 ± 5.3). This group included 5 patients with acute leukemia, 2 patients with megaloblastic anemias (1 due to folate deficiency, the other had pernicious

Table 1. Platelet IgG Levels in Immune Thrombocytopenia: Response to Treatment

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Treatment</th>
<th>Platelet Count (cu mm)</th>
<th>Platelet IgG (fg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute ITP</td>
<td>Prednisone</td>
<td>Before 20,000</td>
<td>After 252,000</td>
</tr>
<tr>
<td>Acute ITP</td>
<td>Prednisone</td>
<td>Before 15,000</td>
<td>After 280,000</td>
</tr>
<tr>
<td>Recurrent ITP</td>
<td>Prednisone</td>
<td>Before 50,000</td>
<td>After 190,000</td>
</tr>
<tr>
<td>Recurrent ITP</td>
<td>Prednisone</td>
<td>Before 100,000</td>
<td>After 240,000</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>Prednisone</td>
<td>Before 10,000</td>
<td>After 360,000</td>
</tr>
<tr>
<td>SLE</td>
<td>Prednisone</td>
<td>Before 100,000</td>
<td>After 250,000</td>
</tr>
<tr>
<td>Acute ITP</td>
<td>Splenectomy</td>
<td>Before 39,000</td>
<td>After 345,000</td>
</tr>
<tr>
<td>Recurrent ITP</td>
<td>None</td>
<td>Before 80,000</td>
<td>After 140,000</td>
</tr>
<tr>
<td>Heparin</td>
<td>D/C heparin</td>
<td>Before 112,000</td>
<td>After 400,000</td>
</tr>
<tr>
<td>Tolazamide</td>
<td>Prednisone</td>
<td>Before 5000</td>
<td>After 50,000</td>
</tr>
<tr>
<td></td>
<td>D/C tolazamide</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
anemia), 2 patients with cirrhosis of the liver, and 3 patients with myeloproliferative disorders (1 polycythemia and 2 with myelofibrosis). Elevated PAIgG levels were found in 1 of the patients with acute leukemia and in the 2 patients with megaloblastic anemia. The PAIgG levels of the ITP patients were significantly different when compared to normals and the patients with nonimmune thrombocytopenia (p < 0.001). No significant difference was noted between PAIgG levels of normals and the levels of the nonimmune thrombocytopenia group (p = 0.4). Serum IgG levels were normal in the above 3 groups.

Two of 5 (40%) of patients with SLE and thrombocytopenia and 1 of 3 (33%) of SLE patients with normal platelet counts had elevated PAIgG. Seven myeloma patients with elevated levels of IgG had elevated PAIgG levels. Only 1 showed a low platelet count of 80,000 cu mm. Two patients with IgA myeloma and 1 patient with kappa light-chain myeloma had normal PAIgG. Four of 7 patients (57%) with thrombocytopenia, noted during the course of sepsis, had elevated PAIgG levels. Four patients with idiopathic autoimmune acquired hemolytic anemias showed normal PAIgG levels.

**DISCUSSION**

Currently available techniques for quantitating PAIgG, including Fab anti-Fab, antiglobulin consumption, radioactive labeled Coomb’s test, and 125I-labeled staph protein A binding, are complex and require rigid standardization.5 It has been suggested that a few regional centers be utilized for the investigation of patients suspected of having platelet antibodies.5 Quantitation of PAIgG by radial immunodiffusion obviates this restriction because of its relative simplicity. Immunoplates are commercially available and have a shelf life of 1 yr. The minimal requirement of 160 ng of IgG is easily met by obtaining 0.25 x 10⁶ platelets from patients with ITP even with very low platelet counts. Ten to 30 milliliters of whole blood is sufficient for most situations. Platelets can be analyzed immediately or stored frozen at -20°C.

The results of the present method and their clinical correlation are similar to the reports utilizing various other techniques. In the present study, 85% and 100% of patients with acute and recurrent ITP, respectively, had elevated PAIgG. Similarly, elevations of PAIgG were reported in 76%-100% of cases of ITP using the antiglobulin consumption test,5,6,10,12 in 91% using staph protein A binding,5 in 90% using the radiolabeled Coomb’s test,4 and in 94% using the Fab anti-Fab method.2 Elevated PAIgG levels were also found in all of our patients with IgG myeloma regardless of the platelet count, confirming the observation of McGrath et al.13 and emphasizing the necessity for measuring serum IgG levels in patients with hypergammaglobulinemia and elevated PAIgG levels. This study also found elevated PAIgG levels in patients with sepsis and thrombocytopenia (57%) and in patients with SLE and thrombocytopenia (40%), as has been reported by others.3,14

The measurement of PAIgG by radial immunodiffusion is adaptable to most clinical laboratories. Hopefully, this technique will provide wider experience with PAIgG measurement and allow further insight into its clinical significance.

**ACKNOWLEDGMENT**

The authors wish to thank the Calbiochem-Behring Corporation for the generous supply of LC-Partigen immunoplates.

**REFERENCES**

Quantitation of platelet-associated IgG by radial immunodiffusion

BS Morse, D Giuliani and M Nussbaum