The Relationship Among Platelet-Associated IgG, Platelet Lifespan, and Reticuloendothelial Cell Function


Platelet-associated IgG (PAIgG) has been reported to be elevated in nonthrombocytopenic patients who have a normal platelet lifespan. This has been interpreted as indicating that PAIgG is a nonspecific finding in these patients and not a determinant of platelet survival. It is important to recognize that the reticuloendothelial (RE) system plays an important role in the clearance of antibody-sensitized cells. In this study, we related the level of PAIgG and the platelet lifespan to the RE function in patients with: (A) idiopathic thrombocytopenic purpura (ITP), and (B) five patients with elevated levels of PAIgG yet normal or near-normal platelet counts. RE function was assessed by measuring the clearance of autologous chromium-labeled red cells sensitized with a precise amount of alloantibody (2,000-3,600 molecules of IgG/cell). Eight patients with immune thrombocytopenia had significantly shortened platelet survivals (<2-113 hr). In contrast, the five patients with elevated PAIgG, yet normal or near-normal platelet counts, all had normal autologous platelet survivals (186-222 hr). These patients also had significantly impaired clearance of IgG-sensitized red cells, with an average of 85% of the infused red cells remaining in the circulation at 60 min (normal 42% ± 14%, n = 10). In this study, every patient with elevated PAIgG and normal RE function had a shortened platelet lifespan. Those patients with elevated PAIgG and impaired RE function did not invariably have a shortened platelet lifespan. The observation that the PAIgG is elevated in some patients whose platelet survival is normal does not indicate that PAIgG is not biologically relevant. It indicates that these patients may have RE blockade and do not clear IgG-sensitized cells.

The biologic relevance of platelet-associated IgG (PAIgG) is uncertain. The high frequency of elevated PAIgG in idiopathic thrombocytopenic purpura (ITP) suggests that the PAIgG could mediate the thrombocytopenia. However, elevated PAIgG has been reported in a variety of thrombocytopenic disorders that have not been classically considered to be of immune etiology. Some investigators have investigated the biologic relevance of PAIgG using platelet survival studies. Often, an approximate inverse relationship between the level of PAIgG and the platelet survival is noted; however, two groups of investigators reported that certain thrombocytopenic subjects with raised PAIgG have a normal platelet survival. This could be interpreted as indicating that the binding of IgG to these platelets is not a determinant in their survival.

It is important to recognize that the function of the reticuloendothelial (RE) system contributes to the rate of clearance of antibody-sensitized cells. For example, patients with chronic liver and inflammatory diseases have been reported to have defective reticuloendothelial function and do not clear IgG-sensitized erythrocytes. Similarly, certain otherwise healthy individuals with the HLA-B8, DR3 alloantigens have impaired reticuloendothelial cell function. Thus, it is conceivable that patients with reticuloendothelial cell “blockade” might have increased levels of PAIgG, yet not clear these platelets because of impaired RE function. If this hypothesis were correct, then one would predict that those patients with elevated PAIgG and normal RE function would always have a shortened platelet survival, whereas the platelet survival would not necessarily be shortened in those patients with elevated PAIgG but impaired RE function. In this article, we describe a study relating the results of the PAIgG test to the platelet survival and reticuloendothelial cell function in a group of selected patients.

MATERIALS AND METHODS

Patients and Controls

Healthy controls. The clearance of IgG-sensitized autologous red cells was studied in ten healthy Rhesus-positive individuals.

Patients with immune thrombocytopenia. Eight patients with immune thrombocytopenia were studied. Six had idiopathic thrombocytopenic purpura (ITP) and two had immune thrombocytopenia in association with chronic lymphocytic leukemia.

Patients with elevated PAIgG and thrombocytopenia not caused by ITP. Five patients with normal platelet counts or mild thrombocytopenia were also studied. All of these patients had elevated levels of PAIgG.

All studies were performed after informed signed consent was obtained. The study was permitted following review by a university approved Ethics Review Committee and a Nuclear Physics Review Committee.
In Vivo Studies

Determination of Platelet Survival

An autologous platelet survival was performed if the patient had a platelet count greater than 50,000/μl. Homologous platelets (ABO compatible) were used if the patient’s platelet count was less than 50,000/μl. One unit of whole blood was collected into CPA-A and the platelets isolated by differential centrifugation. Following labelling with 150 μCi of 35sodium chromate, the platelets were washed once with platelet-poor plasma and infused into the recipient. As a check on quality control, subsamples were taken for determination of platelet labeling efficiency, nonplatelet chromium, and red cell contamination in the labeled platelet preparation. Following infusion of the radiolabeled platelets, 5 ml of whole blood was collected daily for 7 days for determination of mean platelet lifespan. This was calculated by computer-assisted analysis of the radioactive decay (gamma function analysis).13 Using this method, the platelet lifespan in healthy individuals was 144 ± 10 hr (mean ± SD).

Determination of Reticuloendothelial Cell Function

The in vivo reticuloendothelial cell function was assessed by measuring the clearance of IgG-sensitized, chromium-labeled autologous erythrocytes.9 Only Rh-positive individuals were studied. All labeling procedures were performed under sterile conditions.

Whole blood from a Rh-positive individual was collected into acid citrate dextrose (ACD) (6:1, v:v) and the hematocrit determined on a subsample. The hematocrit was used to calculate the amount of whole blood required to give 2 ml of packed red cells. This amount of whole blood was centrifuged (1,550 g for 10 min) and the plasma discarded. The erythrocytes were incubated (37°C for 10 min) with 25 μCi 51Cr at 37°C, washed once, and then incubated (37°C for 15 min) with 2 ml of a 1:100 dilution of anti-D (Win-Rho, Rh Institute, Winnipeg, Manitoba). This concentration of anti-D was used because it consistently resulted in binding ranging from 2,100 to 3,600 molecules IgG/erythrocyte (n = 10). A moderate level of sensitization of the red cells was selected, as preliminary studies demonstrated that very high levels of sensitization made RE blockade difficult to demonstrate, and very low levels of sensitization made the study technically difficult to perform because of the long time required for cell clearance.

The erythrocytes were washed twice with 0.9% sodium chloride, the supernatant discarded, and the cells resuspended to 6 ml. Subsamples were taken for determination of the total cell count, red cell specific activity, the direct antiglobulin test, and quantitation of red cell-associated IgG.

Each subject received an average of 1.74 ± 0.22 × 10^10 red cells (n = 10). Following infusion of the red cells, a 3-ml whole blood sample was collected into EDTA at 5, 10, 15, 20, 30, 60, and 90 min for measurement of red cell radioactivity. The whole blood radioactivity was plotted on semilog paper against the time following infusion of the red cells, and the percentage of radiolabeled red cells remaining in the circulation was determined at 60 min.

The red cell survival was performed at least 48 hr and, at most, 72 hr before the platelet survival. Even in those subjects with impaired RE function, all of the IgG-sensitized radiolabeled red cells had been cleared from the circulation by 24–48 hr after infusion.

In Vitro Studies

Red Cell-Associated IgG

The amount of IgG on the red cells was quantitated using an immunoradiometric assay (IRMA).13 In brief, 100 μl of 125I-anti-IgG was incubated for 1 hr at 37°C with 100 μl of a series of dilutions of 4 times washed red cells suspended in 0.15 M, pH 7.4, phosphate-buffered saline (PBS). One hundred microliters of IgG-Sephasorb (10^10 beads) was added and incubated at 37°C for 30 min. The IgG beads were prepared by covalently binding human IgG (Cohn fraction 2, Sigma Chemical Co., St. Louis, MO) to agarose beads (Sephasorb HP-Ultrapure, Pharmacia Fine Chemicals, Dorval, Quebec) using carbonyl-diimidazole. The red cells were lysed with 100 μl of 1% Triton, the beads washed twice with 1.5% bovine albumin, and the radioactivity measured in a gamma counter. A standard curve was prepared using a similar protocol, except that increasing concentrations of human IgG standard were used. The amount of IgG per cell was calculated by dividing the concentration of IgG standard that caused 50% inhibition of binding of the 125I-anti-IgG to the IgG beads by the number of red cells that also caused 50% inhibition. The results were expressed as molecules of IgG per red cell using Avogadro’s number.

Platelet-Associated IgG

PAIgG was measured using the immunoradiometric assay.14

Determination of HLA

The HLA type of the controls and patients was determined using antisera trays provided by the National Centre of the Canadian Red Cross Blood Transfusion Service. The DR alloantigens were determined on B lymphocytes separated from whole blood samples using anti-HLA-DR specific alloantisera provided by the National References Laboratory of the Canadian Red Cross Blood Transfusion Service.

RESULTS

Ten healthy nonthrombocytopenic Rh-positive individuals had reticuloendothelial cell function evaluated using IgG-sensitized red cells. All had normal platelet counts and normal levels of platelet-associated IgG (less than 5.5 fg IgG/platelet). The amount of IgG per red cell ranged from 2,100 to 3,600. The percent of the infused red cells remaining in the circulation at 60 min was 42% ± 14% (Fig. 1). None of these subjects had the HLA-B8, DR3 antigens.

Eight patients with immune thrombocytopenia were studied. None of the patients had had a previous splenectomy, and all were Rh-positive (Table 1). The platelet lifespan was measured in 7 of the patients and was reduced in all. The clearance of IgG-sensitized red cells was performed in all patients and impaired in 4 patients (Table 1). Four of the five patients with the most severe thrombocytopenia (platelet count less than 10,000/μl) had impaired reticuloendothelial cell function.

Five patients with normal platelet counts or mild thrombocytopenia were also studied (Table 2). All subjects had autologous platelet survival studies, and the results were normal in all (186–222 hr). All of these patients had significantly impaired reticuloendothelial function as assessed by the clearance of IgG-sensitized chromium-labeled autologous red cells.

A graphic presentation of the clearance IgG-sensi-
Fig. 1. The in vivo red cell clearance curves for 51Cr-labeled IgG-sensitized autologous red cells. The percentage radioactivity in the whole blood sample (ordinate) is plotted against the time following infusion (abscissa). The shaded area represents the mean ± SD clearance for 10 controls. The (△—△) curve belongs to the third patient in Table 1 (shortened platelet survival with normal RE function) and the (○—○) curve belongs to the fourth patient in Table 2, who had a normal platelet survival but elevated level of PAIgG.

Table 3 includes only those patients with elevated levels of PAIgG. In this table, the results of the platelet survival are related to the results of the RE function study. As shown, all patients with normal RE function and elevated levels of PAIgG had a significantly shortened platelet survival. More importantly, every patient with elevated PAIgG and a normal platelet survival had impaired RE function.

DISCUSSION

The biologic relevance of an elevated level of platelet-associated IgG (PAIgG) has been challenged on the basis that elevated PAIgG can occur in patients who have a normal platelet count, or more importantly, a normal platelet survival. However, none of these studies addressed the question of reticuloendothelial (RE) function. It is possible that platelets sensitized with identical amounts of IgG might have a shortened lifespan in patients with normal RE function, yet a normal lifespan if the RE function was impaired. The current study was designed to address this issue. We studied Fc-dependent reticuloendothelial function by measuring the rate of clearance of chromium-labeled autologous Rh-positive red cells sensitized with anti-D. The advantage of this technique is that it measures the in vivo clearance of autologous red cells sensitized by antibody whose spatial orientation is known, i.e., the antibody binds to the erythrocytes through its Fab terminus, making its Fc binding site available for interaction with macrophages. Because other investigators have demonstrated that the rate of clearance in healthy individuals depends on the amount of antibody sensitizing the red cells, we sensitized the red cells with a moderate amount of antibody (2,000–3,600 molecules IgG/red cell). This level of sensitization was
chosen because preliminary studies demonstrated that very high levels of sensitization made RE blockade difficult to demonstrate, and very low levels of sensitization made the study difficult to perform because of the long time required for cell clearance. As a quality control check on our technique of sensitization, we measured the amount of antibody on the infused red cells using an immunoradiometric assay. The patients studied were purposely selected because they had disorders reported by other investigators to be complicated by depressed RE function. The results of the study indicated that all patients with elevated PAIgG, but normal RE function, had shortened platelet survivals. This is consistent with the hypothesis that PAIgG is a major determinant of platelet survival. We also observed that every patient with elevated PAIgG, but a normal platelet survival, had impaired reticuloendothelial function. We hypothesize that the elevated PAIgG in those patients with normal platelet lifespan and impaired RE function might have resulted in an increased rate of platelet clearance had RE function been normal. Although crosstransfusion studies would have directly tested this hypothesis, such studies were not performed.

It is possible that the spatial orientation of the IgG bound to the platelet determines whether the platelet will be cleared by the RE system. This issue was not addressed in the current study; however, the inability of the patients with impaired RE function to clear red cells sensitized by alloantibody indirectly suggests that the spatial orientation of antibody binding to platelets may not be a critical determinant in platelet clearance.

The five patients (Table 2) with impaired RE function all had disorders other investigators have reported to be complicated with impaired RE function. Of interest, and we believe not coincidentally, every one of these patients had markedly elevated levels of serum IgG. Kurlander and coworkers have demonstrated in vitro that monomeric IgG can compete with IgG oligomers for the Fc receptors of phagocytic cells. We believe that similar events occur in vivo. This subject will be addressed in a separate report.

We observed that 3 of 8 patients with immune thrombocytopenia also had impaired reticuloendothelial cell function. All of these patients had shortened platelet survivals. Those patients with impaired RE function plus a shortened platelet lifespan tended to have the most severe thrombocytopenia, with platelet counts less than 10,000/μl. It is possible that the impaired RE function in these patients was caused by competition for macrophage receptors between the IgG-sensitized platelets and the chromium-labeled IgG-sensitized erythrocytes. The numbers of IgG-sensitized platelets would be far greater than the number of IgG-sensitized red cells; therefore, the IgG-sensitized platelets could be rapidly cleared, yet the IgG-sensitized red cells not cleared. Data consistent with this hypothesis, namely that there can be competition for macrophages by IgG-sensitized cells, has recently been reported.

The findings of this study indicate that patients whose platelets carry elevated levels of PAIgG can have a normal platelet survival if the function of the reticuloendothelial system is impaired. These studies are consistent with the hypothesis that PAIgG is a

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Percentage of Sensitized Red Cells Remaining in the Circulation at 60 min</th>
<th>Platelet Count (per μl)</th>
<th>PAIgG (μg IgG/Platelet)</th>
<th>Platelet Lifespan (hr)</th>
<th>HLA Typing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>42% ± 14%</td>
<td>&gt; 150,000</td>
<td>&lt; 5.5</td>
<td>144 ± 10</td>
<td></td>
</tr>
<tr>
<td>Cirrhosis (alcoholic)</td>
<td>87%</td>
<td>133,000</td>
<td>23.4</td>
<td>186</td>
<td>1, 2</td>
</tr>
<tr>
<td>Chronic infection (lung abscess)</td>
<td>80%</td>
<td>140,000</td>
<td>12.8</td>
<td>200</td>
<td>2, −</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>&gt;90%</td>
<td>138,000</td>
<td>24</td>
<td>215</td>
<td>2, w24(9)</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>&gt;90%</td>
<td>195,000</td>
<td>8.0</td>
<td>222</td>
<td>1, w26(10)</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>80%</td>
<td>542,000</td>
<td>6.0</td>
<td>203</td>
<td>2, w24(9)</td>
</tr>
</tbody>
</table>

ND, not done.

Table 3. The Relationship Between Platelet Survival and Reticuloendothelial Function for the 11 Subjects With Elevated Levels of PAIgG in Whom Both the Platelet Lifespan and Reticuloendothelial Cell Function was Measured

<table>
<thead>
<tr>
<th>Reticuloendothelial function</th>
<th>Normal</th>
<th>Shortened</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Impaired</td>
<td>5</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>6</td>
<td>11</td>
</tr>
</tbody>
</table>
major determinant of platelet survival, and the apparent paradox of raised PAIgG and a normal platelet survival can be explained on the basis of impairment of reticuloendothelial function. Unless normal reticuloendothelial function is demonstrated, it is not appropriate to conclude that elevated PAIgG is a nonspecific finding in a nonthrombocytopenic patient or a patient whose platelet lifespan is normal.

ACKNOWLEDGMENT

The authors would like to thank Michele Bérubé for her secretarial assistance.

REFERENCES

The relationship among platelet-associated IgG, platelet lifespan, and reticuloendothelial cell function

JG Kelton, CJ Carter, C Rodger, G Bebenek, J Gauldie, D Sheridan, YB Kassam, WF Kean, WW Buchanan and PJ Rooney