Influence of the Amount of Platelet-Bound IgG on Platelet Survival and Site of Sequestration in Autoimmune Thrombocytopenia

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Seventeen patients with idiopathic autoimmune thrombocytopenic purpura were studied in order to establish whether the amount of platelet-bound antibody influenced the rate and site at which these platelets were destroyed. Platelet-bound antibody was measured by a quantitative antiglobulin consumption technique, and platelet survival and sites of sequestration were determined by the use of 51Cr-labeled platelets and surface radioactivity measurements. A correlation significant at the 5% level was observed between the amount of platelet-bound antibody and the rate of platelet destruction. A highly significant correlation (p < 0.001) existed between antiplatelet antibody and spleen: liver surface radioactivity ratios, demonstrating that greater amounts of antibody bound to platelets result increasingly in their destruction within the liver.

The immune destruction of erythrocytes and platelets occurs as the result of their removal from the circulation by cells of the reticuloendothelial system. It has been demonstrated that, for erythrocytes, the rate at which this occurs is influenced by the amount of antibody present on the surface of the cell—greater amounts of antibody resulting in more rapid rates of destruction.1,2 It has also been demonstrated that the spleen participates more efficiently in the destructive process than does the liver. Thus, cells lightly sensitized with antibody are predominantly destroyed within the spleen and only heavily sensitized cells within the liver.3,4

Platelet antibody in autoimmune thrombocytopenic purpura is an IgG immunoglobulin,5,6 and recently described techniques have made it possible to measure the amount of this antibody on the surface of the platelet.7,8 Therefore, we have sought to determine if, as in the case of red blood cells, the amount of antibody bound to the platelet influences its rate and site of destruction.

Materials and Methods

Patient Material

Seventeen newly diagnosed patients with idiopathic autoimmune thrombocytopenic purpura (AITP) were studied. There were 15 females and 2 males, whose ages ranged between 19 and 41 yr. The diagnosis of AITP was made on the basis of (1) thrombocytopenia; (2) increased numbers of megakaryocytes in bone marrow aspirates and trephine bone biopsies; (3) an impalpable spleen; (4) the absence of preceding infection and drug ingestion; (5) negative serologic tests for the diagnosis of a collagen vascular disorder; (6) shortened survival in vivo of 51Cr-labeled platelets; and (7) elevated antiplatelet antibody. All investigations were completed before corticosteroid treatment was instituted.

Platelet-Bound IgG (Platelet-Bound Antibody)

This was measured by the quantitative antiglobulin consumption technique described by Dixon et al.7 and the results are expressed as ng IgG/10^6 platelets.

Measurement of Platelet Mean Lifespan (MLS)

Platelets were labeled with 400 μCi Na2 51CrO4 in ACD plasma according to the recommendations of the International Committee for Standardisation in Haematology.9 Autologous platelets were used in all patients except 5 whose blood platelet counts were < 20 x 10^9/liter. In these patients, ABO Rh-compatible isologous platelets from HbsAg-free donors were used. Five-milliliter whole blood samples were taken at 2.5, 5, 10, 15, 30, 45, and 60 min, 2, 3, 6, 24, and 48 hr, and daily thereafter for up to 7 days, depending on the patient's clinical status. Blood samples were counted in a gamma spectrometer to a standard deviation of 2% or less, and the blood activity curve for each patient was analyzed, using linear, exponential, and gamma functions on a Univac 1106 computer using a generalized nonlinear least-squares curve-fitting technique incorporating a chi square estimate of goodness-of-fit.10

Organ Uptake of 51Cr-Labeled Platelets

The sequestration of 51Cr-labeled platelets within the liver and spleen was measured, using appropriately collimated NaI (T1) detectors situated over the spleen (anterior and posterior probes) and liver.11 Particular care was taken in positioning the probes in order to ensure patient to patient reproducibility. Sufficient counts were accumulated to achieve a standard deviation of 2% or less, and the relative uptake of labeled platelets into these organs was expressed as the spleen: liver ratio 48 hr after injection of the dose.

Results

Platelet-Bound IgG

In 17 untreated patients with AITP, platelet-bound IgG (antibody) ranged from 21 to 213 ng/10^6 platelets compared with 1.8–14.8 for 48 normal control subjects. A significant correlation was observed between the amount of platelet-bound antibody and the presenting peripheral blood platelet count, \( r = -0.6823, p < 0.01 \) (Fig. 1B).
Platelet MLS

Platelet survival curves were well described by one or more exponential functions. The number of exponentials necessary to obtain fits to the data statistically acceptable at the \( p = 0.05 \) level were 1, 2, 3 and 4 in 1, 3, 10, and 1 patients, respectively (an acceptable fit to the data could not be achieved in two patients). All exponentials were used to obtain the best fit to the survival data, but having done so, because the magnitude of the slowly decaying third and fourth exponentials were small and the uncertainty in their parameters relatively large by comparison with the first two, platelet MLS was calculated by using the first two components only (Fig. 2). The half-lives of these first and second components ranged from 0.03 to 0.62 hr and 1.6 to 12.8 hr, respectively.

A progressive shortening of platelet MLS was observed with increasing amounts of platelet-bound antibody. The linear plot of platelet-bound antibody against the two exponential estimates of platelet MLS appeared hyperbolic (Fig. 3A), and with a log–log plot of the data (Fig. 3B), these two measurements achieved a correlation that was significant at the 5% level: \( r = -0.6002, p < 0.05 \).

Organ Uptake of \( ^{51} \text{Cr}-\text{Labeled Platelets}

Antiplatelet antibody levels plotted against spleen: liver surface radioactivity ratios assumed a hyperbolic distribution (Fig. 4A), and these measurements were highly significantly correlated with a log–log plot of the data: \( r = -0.7621, p < 0.001 \) (Fig. 4B). Thus, greater amounts of antibody bound to platelets result increasingly in their destruction within the liver.

DISCUSSION

The aim of this study has been to determine whether or not increasing amounts of platelet-bound antibody result in more rapid rates of platelet destruction and in progressively greater platelet sequestration within the liver. The significant inverse correlation between
initial platelet counts and platelet-bound antibody levels observed in this and other studies implies that the amount of antibody present is an important factor determining the severity of the disease. Consequently, it would be reasonable to anticipate that the highest levels of antibody would be associated with the most rapid rates of platelet destruction, and this is shown to be the case in the present study. However, the correlation between platelet antibody and platelet MLS was only significant at the 5% level. In autoimmune hemolytic anemia, both the IgG subclass and the additional binding of complement to the red cell membrane influence the rate of hemolysis. Recently, complement has been identified on platelets from patients with AITP, and IgG subclass differences have also been briefly reported. It is possible, therefore, that a better correlation between platelet MLS and the amount of platelet-bound antibody may emerge in future studies that take these additional variables into account. In five instances, labeled isologous platelets were administered and for the purpose of the study it has to be assumed that these platelets became rapidly sensitized in vivo with the same amount of antibody as the patients' own platelets. This assumption may well be invalid, and the use of isologous platelets may be a further reason for not having obtained a better correlation, although deleting these five patients did not improve the correlation statistic.

In theory, the exponential is the mathematical function that describes the curve form of blood cells eliminated randomly from the circulation by an extrinsic factor. Our observation that the antibody-mediated elimination of $^{51}$Cr-labeled platelets results in survival curves best described by exponentials is consistent with this theory. With a single exception, the platelet survival curves required more than one exponential for their adequate description. This may imply differing rates of sequestration of sensitized platelets within the liver and spleen or within other sites of the reticuloendothelial system. The data obtained in the present study, however, do not permit an evaluation of this possibility, and accordingly, we have not assigned a physiologic role to the component exponentials.

Early studies presented evidence that suggested that the site of platelet sequestration in AITP depended on the severity of the disease process. The infusion of platelet alloantibodies or AITP plasma caused circulating $^{51}$Cr platelets to become sequestrated within the spleen and liver, and larger infusions caused greater accumulation within the latter organ. Hepatic platelet destruction was also observed to be greatest in AITP patients with the lowest platelet counts and most rapid rates of platelet destruction. Hence, it was suggested that the degree of platelet antibody sensitization determined the site of platelet destruction, and the results of the present investigation confirm this to be the case. The technique of surface radioactivity measurements is associated with certain limitations reviewed elsewhere, and it only needs to be stated here that the technique is not quantitative. In this regard, more meaningful data may be expected to follow from the use of newly described techniques using $^{51}$In-oxine-labeled platelets that enable quantitative measurements of platelet destruction within organs to be made.

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