Platelet Kinetics in Patients With Idiopathic Thrombocytopenic Purpura and Moderate Thrombocytopenia

By David Stoll, Douglas B. Cines, Richard H. Aster, and Scott Murphy

We studied ten normal subjects and 20 patients with stable, untreated idiopathic thrombocytopenic purpura (ITP) and platelet counts in the range of 35,000 to 110,000/μL. The diagnosis was made by clinical criteria. Platelet-associated IgG was increased in all nine of the nine patients studied. Autologous platelets were labeled with chromium 51 and reinforced for measurement of mean cell life and platelet production rate. Mean cell life was calculated by two methods, weighted mean and multiple hit, with excellent agreement between the two. As expected, mean cell life was significantly reduced in ITP patients as compared to the normal subjects (2.9 days v. 8.0 days, P < .001).

However, mean platelet production rates in ITP patients and normal subjects, 3.5 and 3.8 \times 10^{10} platelets/k/d respectively, were not significantly different. Platelet production rate was above and below the normal range (2 to 5.6 \times 10^{10} platelets/k/d) in two and four patients, respectively. We conclude that the rate of platelet production is not increased in most patients with ITP who have platelet counts greater than 35,000/μL. We did find that platelet size was increased in eight of the 12 patients in whom it was measured, including two of the patients with low platelet production.

All studies reported were performed prior to splenectomy. There were no preceding or coincidental relevant medical conditions, and thrombocytopenia persisted when drugs were withdrawn. The 20 ITP patients were studied from 1969 to 1983.

The patient’s own platelets were labeled with chromium51 by methods previously described6 and reinforced. At 30 minutes, and at 1½ hours postinfusion and daily thereafter, 10-ml samples were drawn until platelet-bound radioactivity was less than 10% of the value on the day of infusion. Platelet-bound radioactivity was determined after harvesting platelets from these samples as previously described.7 Estimated blood volume (EBV) was determined from height and weight by means of standard tables.

Platelet mean cell life (MCL) was calculated by each of two methods, the weighted mean method8 and the multiple-hit method.8 A Monroe 210 table-top computer and a Perkin-Elmer 3230 computer were used for the two methods, respectively. The percentage of in vivo recovery (% recovery) immediately after infusion was calculated from the formula:

\[
\text{EBV(mL)} \times \text{“zero” time cpm/10} \times 0.85 \times \text{total cpm infused}
\]

where 0.85 is a previously determined correction factor for incomplete harvesting of platelets from the 10-ml samples.9 “Zero” time cpm was determined by two methods. With the weighted mean analysis, the cpm for the samples on the day of infusion were averaged. With the multiple-hit method, the mean cell life was extrapolated to zero time to obtain this value. Platelet production rate (PPR) was calculated from the formula:

\[
\text{platelet count/μL} \times \text{EBV (mL)} \times 1,000 \times \text{% recovery as decimal} \times \text{MCL (d)} \times \text{body wt (k)}
\]

and expressed as platelets/k/d. The correction for % recovery is based on the concept that the total body platelet mass is divided between a circulating pool and a pool in the spleen.9 Percentage recovery reflects the portion of total body platelet mass that is in the circulation.

Measurements of platelet-bound IgG and plasma IgG antiplatelet activity were performed on platelets from seven patients, using a radiolabeled antiglobulin technique.10 The amount of platelet-bound IgG is determined by measuring the binding of anti-IgG labeled with 125I and is expressed as a percentage of the total radioactivity to which the platelet had been exposed according to the formula:

\[
\text{% binding} = \frac{\text{cpm (platelet)} - \text{cpm (test tube)}}{\text{cpm (total)} - \text{cpm (test tube)}} \times 100
\]
The amount of labeled anti-lgG that binds to normal platelets is determined by the method of Luiken et al. (normal value: 2,100 ng IgG/10^9 platelets).

Platelet sizing was performed on 12 patients by two methods. Platelets from seven patients were studied by oil phase microscopy to determine the percent of cells with a diameter greater than 3 µm, normal range: 2,100 ng IgG/10^9 platelets. Platelets from five patients were studied by estimating the mean platelet volume (normal range: 6 to 9 µm^3). Because the methods of studying platelet-associated immunoglobulin and platelet size were not available during the first portion of the study period (1969 to 1983), not all patients had these measurements. Platelet counts were performed on peripheral venous blood by phase microscopy.

Standard statistical methods were used for calculation of mean values, SD, linear regression, correlation coefficient, and analysis of variance. Student's t test was estimated by a standard t table and was considered significant if P < 0.05.

Written informed consent was obtained from the normal volunteers. Studies on the patients were part of their diagnostic evaluation.

RESULTS

Table 1 contains laboratory and clinical information for each patient. Included are sex, ages, initial platelet count, extent of response to corticosteroids (response defined as a twofold rise in platelet count in less than four weeks) and the most recent platelet count. Fifteen patients responded to corticosteroid therapy. Five patients (7, 9, 12, 15, 19) underwent splenectomy. Of these five, only patient 7 had a platelet count less than 35,000/µL at the time of splenectomy. In patients 9, 12, 15, and 19, splenectomy was performed because of the presence of thrombocytopenia, not severe thrombocytopenia or clinical hemorrhage. The 15 patients who have not had splenectomy have been followed for an average of 4.1 years (1.5 to 8 years) with no therapy subsequent to the corticosteroid trial and no hemorrhagic problems. When initial platelet counts of these 15 patients were compared with the last count obtained, there was an increase in 12 of 15 patients, but this was not statistically significant (P > 0.05) by Student's paired t test.

Platelet-associated immunoglobulin and platelet sizing were performed if the patient was seen in a time interval when these studies were available. Nine of nine patients tested for platelet-associated immunoglobulin had significantly increased values (Table 2). Furthermore, six of seven patients tested had circulating antiplatelet antibody in their plasma. Eight of twelve patients had increased platelet size (Table 1).

Figure 1 shows the relationship between the percentage of recovery, MCL, and PPR for normals (Fig 1A) and for ITP patients (Fig 1B) as determined by the weighted mean and multiple-hit methods. In all cases, we found a statistically significant relationship between the two methods of analysis (P < .001). The r

Table 1. Laboratory and Clinical Information

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<th>Sex</th>
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<th>Date</th>
<th>Pit Count (x 10^3/µL)</th>
<th>Recovery (%)</th>
<th>MCL (d)</th>
<th>ppr (x 10^11/Pit/h/d)</th>
<th>Platelet Size (Normal-Increased)</th>
<th>Platelet-Associated Immunoglobulin (Normal-Increased)</th>
<th>Steroid Response</th>
<th>Splenectomy</th>
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<td>Yes</td>
<td>1983 92</td>
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</table>

Patient mean: 68.4 ± 22.7; Normal mean: 52.8 ± 14.7. These calculations were obtained by the weighted mean method. MCL, mean cell life; PPR, platelet production rate; ND, not done.
Regression lines are:

\[ y = mx + b \]

respectively. Panel B shows the relationship for ITP patients.

In this study, we have confirmed the reports of many previous investigators, which showed that the MCL in ITP was reduced when compared to that in normal subjects. In a group of 14 untreated ITP patients, Harker\(^2\) found the mean MCL to be 0.32 days, whereas from their study of 31 ITP patients, Branhog et al\(^1\) found the mean MCL to be 0.67 days. In those two studies, mean MCL in normal controls were 9.9 and 6.6 days, respectively. Our MCL of 2.9 days for the ITP group (compared to a control of 8.0 days) was much longer than in the two previous studies. The explanation for this is that different groups of patients were studied. In the two previous studies, most patients

Table 2. Platelet Antibody Measurements

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Direct Test (Platelet-IgG(^{+}))</th>
<th>Indirect Test (Plasma)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Percent Binding</td>
<td>SD</td>
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<tr>
<td>Normal Population (n = 200)</td>
<td>0.11</td>
<td>0.06</td>
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- Patients 3 and 4 had increased platelet-IgG by the method of Luiken et al\(^1\) 58.180 and 13.217 ng IgG/10\(^9\) platelets, respectively; assays for circulating plasma antiplatelet antibody were not performed in these two patients.
- These results were obtained as described in Materials and Methods.
- Binding of anti-IgG \(^{+}\) to the platelet is expressed as a percentage of the total available radioactivity.
- Within normal limits (<2 SD).

The validity of the kinetic measurements relies on the quality of the data obtained during the first few hours after infusion of the labeled platelets; that is, any early extravascular pooling would result in artifactually low early circulating radioactivity and therefore an artifactually prolonged MCL. For each patient, we divided the 1\(\frac{1}{2}\)-hour value by the 30-minute value and found the mean ratio to be 0.99 ± 0.03 (± 1 SEM). In addition, in ten of the patients, we obtained a sample at three hours after infusion and calculated the ratio of this last point to the average of the first two points. The mean was 1.05 ± 0.03 (± 1 SEM, not significantly different from 1.0). These results suggest that there was no early extravascular pooling, at least during the first three hours.

**DISCUSSION**

Figure 2 demonstrates the relationship between the platelet count and the percentage of recovery, MCL, and PPR in the control and ITP groups, using the weighted mean method of analysis. Only the relationship of platelet count vs PPR of the ITP group was statistically significant (slope = +0.04, \(P < .05\)).

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**Fig 1.** Relationship of % recovery, MCL, and PPR as determined by weighted mean and multiple-hit methods. PPR is expressed as \(\times 10^9\) platelets/k/d. Panel A shows the relationship for normal individuals. Regression lines are: \(y = 1.13x - 7.40, r = .98; y = 1.28x - 2.02, r = .91;\) and \(y = .95x + .10, r = .98,\) respectively. Panel B shows the relationship for ITP patients. Regression lines are: \(y = .71x + 16.8, r = .91; y = .59x + .74, r = .74;\) and \(y = .85x + .84, r = .90,\) respectively. The observed slope differs significantly from one only in the measurement of mean cell life in patients.

**Fig 2.** Relationship between platelet count and % recovery, MCL, and PPR for normals and ITP patients using the weighted mean method of analysis. PPR is expressed as \(\times 10^9\) platelets/k/d. When the two groups were analyzed separately, there were no statistically significant correlations between the variables except for the relationship between platelet count and PPR in ITP patients. The regression line for that relationship is: \(y = 0.91 + 0.04x, r = 0.48, P < .05.\)
had platelet counts less than 35,000/μL whereas all of our patients had platelet counts above 35,000/μL. The previous studies concluded that there was a significant positive correlation between the MCL and platelet count in ITP. We did not find such a correlation in our less severely affected patients. It is of interest that Branehog et al also found no correlation between these two variables when the analysis was limited to patients with only moderate thrombocytopenia.

The percent recoveries of labeled infused platelets in our ITP and normal groups (48.5% and 57.1%) were not statistically different (P > .05). Branchog et al found a low % recovery in ITP patients as compared with normal subjects (27% v 60%, P < .001), which was attributed to rapid clearing of platelets damaged by the labeling procedure. Harker found no such difference in his study, with recovery in ITP subjects being 60% as compared to the control value, 65%. As both investigators used autologously labeled platelets in the same proportion of patients (Harker, seven of 14, Branchog et al, 14 of 31) and studied patients with the same degree of thrombocytopenia, the discrepancy cannot be attributed to these factors. Our results support a normal recovery in ITP, but we cannot explain why our results differ from those of Branchog et al. We do feel that our study has an advantage in that only the patient's own platelets were used for kinetic studies.

Both Harker and Branehog et al found PPR to be significantly higher in ITP than in control subjects (7.6 and 2.3 times normal, respectively). Our results demonstrated no such difference as the rates of platelet production were essentially the same in the control and ITP groups (3.8 x 10⁹ platelets/k/d v 3.5 x 10⁹ platelets/k/d, respectively, P > .10). In comparing PPR with platelet count, Branchog et al found the relationship best expressed as an inverted sigmoid function, whereas Harker found a reciprocal correlation that was best expressed as a straight line function. In both studies, low platelet counts were associated with high PPR. We also found the relationship best expressed as a linear function but in contrast to previous studies, low platelet counts were associated with low rates of platelet production. Again, it is important to identify the degree of thrombocytopenia in the patient groups being investigated. As Branchog et al pointed out in their study, turnover values fell within the range of the control group in those patients with platelet counts >50,000/μL. In those patients with platelet counts <50,000/μL, turnover progressively increased. We did not find an increase in PPR in patients with lower platelet counts. We feel that this is due to our selection of patients with only moderate thrombocytopenia.

At first glance, our observation that PPR is not increased in this group of patients is surprising. One would assume that there would be a compensatory increase. Although the diagnosis of ITP was made by clinical criteria, there is little doubt that the thrombocytopenia was immunologically mediated. Fifteen of the 20 patients responded to a trial of steroids. Increased platelet-bound IgG was found in all patients studied, and six of seven patients had plasma antibody as well. It may be that the ITP autoantibody damages the megakaryocyte. Common antigens for the megakaryocyte and platelet have been demonstrated with heterologous antiplatelet serum and monoclonal antibodies. In fact, McMillan et al demonstrated that splenic lymphocytes from patients with ITP produced IgG capable of binding to normal human megakaryocytes as well as circulating platelets.

On the other hand, one can question the widely held view that the rate of platelet production is stimulated in normal physiology by moderate thrombocytopenia in the range seen in our patients. This view is derived from observations of more severely thrombocytopenic patients and patients with the "compensated thrombocytolytic state." In the latter patients, the platelet count is normal but MCL is reduced, indicating an increase in PPR. However, most of these patients have underlying pathology such as systemic lupus erythematosus, substitute heart valves, or advanced vascular disease. Their increased PPR may be a nonspecific process reactive to the underlying illness rather than a physiologic response to the reduced MCL.

In contrast to the normal PPR in our patients, we found an increase in the size of the platelets in eight of the 12 patients in whom it was measured. Recent work has indicated that at least two factors influence the proliferation of megakaryocytes. The first augments the number of megakaryocyte precursor cells (CFU-Meg), and ultimately the number of platelets produced. The second influences the maturation of maturing megakaryocytes, perhaps leading to a population of larger platelets. If marrow function is normal in ITP, it appears that the normal physiologic response to thrombocytopenia with platelet count > 35,000/μL is to elicit activity of the second factor in some cases but not of the first.

An international group has recently made recommendations concerning methods of analysis of platelet survival studies. In this study, we have used two: the multiple-hit method and the weighted mean method. The correlation between the two was excellent (Fig 1). At this time, the multiple-hit method is most commonly used; however, it requires extensive computer facilities. The weighted mean method can be carried out with a desk-top computer and yields similar results.
Several methodologic criticisms can be raised about our study. First, the choices of the multiple-hit and weighted mean models are arbitrary, although they are currently the most widely used. In the past, the exponential model has been used in analyzing platelet survival time in ITP. This now seems inappropriate since the exponential model is simply a special case of both the weighted mean and the multiple-hit models. When the disappearance of radioactivity is exponential, analysis by all three models will yield the same MCL. However, we have analyzed our 20 patients by the exponential model. Although the calculated MCL is shorter, the mean PPR is not significantly greater than the normal mean (P = .5). Second, the calculation of MCL by any model requires that the values in the first few hours be accurate. Our analysis of the first three hours showed no evidence for early extravascular pooling (Results section, last paragraph). However, because these patients were outpatients, we could not obtain points at 6, 12, and 18 hours, which would have been ideal. Third, the 20 patients were studied over a 14-year period, whereas the controls were all studied in the fifth year of that period. Undetected variations in technique could have occurred during the 14-year period. However, the results in our controls are not significantly different from the results in a similar series of controls studied two years before the start of the current study.5

Most previous reports of patients with ITP have emphasized more severely affected patients. With the increased routine use of automated platelet counting, patients such as ours are reaching medical attention more frequently. Little is known about their natural history. Of our 20 patients, five underwent splenectomy. In four of the five, the indication was the presence of moderate thrombocytopenia, not progression to more severe thrombocytopenia with hemorrhage. Two patients have died, one from a perforated viscus and the other from postoperative complications after splenectomy. Fifteen patients who did not have splenectomy have been followed for an average of four years and have received no therapy other than an initial, short course of steroids in 11 of the 15. Their last platelet counts have not changed in a statistically significant way from those when they were first evaluated, although there has been a trend towards improvement. In fact, one patient spontaneously became normal. It appears that these patients are able to tolerate such a degree of thrombocytopenia without therapy and that most do not progress to more severe disease.

REFERENCES

Platelet kinetics in patients with idiopathic thrombocytopenic purpura and moderate thrombocytopenia

D Stoll, DB Cines, RH Aster and S Murphy