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Research Article

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Mechanisms of Thrombocytopenia in Chronic Autoimmune Thrombocytopenic Purpura

Evidence of Both Impaired Platelet Production and Increased Platelet Clearance

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Abstract

Mechanisms of thrombocytopenia were studied in 38 patients with mild to moderately severe chronic autoimmune thrombocytopenia (AITP). ^{51}Cr and ^{111}In-labeled autologous platelet turnover studies and in vitro analysis of committed megakaryocyte progenitors (CFU-Meg) were used as independent measures of platelet production. Autologous ^{111}In-labeled platelet localization studies were performed to assess platelet clearance. Although there was no increase in the frequency of marrow CFU-Meg, a specific increase in the CFU-Meg [^3H]TdR suicide rate was seen which was inversely correlated with the platelet count ($P < 0.001$). Platelet turnover studies showed significant numbers of patients had inappropriate thrombopoietic responses to their reduced platelet counts. Platelet-associated antibody levels correlated inversely with platelet turnover suggesting that antiplatelet antibody impairs platelet production. The circulating platelet count was best predicted by an index relating platelet production (i.e., turnover) to the spleen-liver platelet clearance that correlated directly with platelet survival ($P < 0.001$).

In summary, both depressed platelet production and increased platelet clearance by the liver and spleen contribute to the thrombocytopenia of AITP.

Introduction

Autoimmune thrombocytopenic purpura (AITP)[1] is a disorder characterized by thrombocytopenia, increased levels of platelet-associated immunoglobulin and normal to increased numbers of marrow megakaryocytes (1–3). In studies of patients with acute AITP, a markedly shortened platelet survival and increased platelet turnover have been the accepted kinetic parameters of this condition (4, 5). Several investigators have described a close correlation between the circulating platelet count and platelet survival (6–9), suggesting thrombocytopenia results from accelerated peripheral destruction of platelets. However, autologous platelet turnover studies in 25 patients with chronic AITP showed that platelet production was decreased in a significant number (10).

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1. *Abbreviations used in this paper:* AITP, autoimmune thrombocytopenic purpura; BFU-E, erythroid progenitor; CFU-GM, granulocyte-macrophage colony-forming cell; CFU-Meg, megakaryocytic cell; [^3H] TdR, tritiated thymidine; RE, reticuloendothelial; TBC, total body count.

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The role of platelet production in the pathogenesis of AITP has been controversial. Early kinetic and morphological studies suggested that a number of patients had suppressed platelet production (11–14). In contrast, Branehog (4) and Harker (5) reported platelet turnover to be normal to markedly increased in AITP. However, more recent studies from several laboratories that examined autologous platelet kinetics (15–19) confirmed the existence of patients with impaired thrombopoiesis. Furthermore, morphological studies demonstrating binding of both polyclonal and monoclonal antiplatelet antibodies to megakaryocyte membranes (20–23) provide a possible mechanism for impaired platelet production.

This study was designed to further evaluate the pathogenesis of chronic AITP. We prospectively assessed levels of platelet-associated immunoglobulin, the numbers and cell cycle characteristics of megakaryocyte progenitor cells, autologous platelet survival and turnover, and the rate and pattern of platelet sequestration in 38 patients with chronic AITP of mild to moderate severity. In addition, we assessed how treatment modifies these measurements. Finally, for comparative purposes, we retrospectively analyzed homologous platelet survival and turnover measurements in another 13 patients.

Methods

Patients

All 38 patients studied prospectively (19 females and 19 males) initially presented with thrombocytopenia, increased platelet-associated Ig or complement and normal to increased numbers of megakaryocytes on bone marrow examination. Screening studies (CBC, chemistries, ANA, ESR, reticulocyte count, and serum protein electrophoresis) were performed to rule out other systemic illnesses. Patients with evidence of drug-mediated thrombocytopenia or an underlying disease such as systemic lupus erythematosis, malignancy, or microangiopathy were exlcuded from the study. Several patients were referred as therapy failures. Eligibility for study required a stable platelet count above 15×10^9/liters so that ^{111}In-oxine and/or ^{51}Cr autologous platelet kinetic studies and direct platelet-associated Ig measurements could be performed. The patients were analyzed in three treatment groups: 18 were initially studied on no treatment, 13 on prednisone, and 7 postsplenectomy. Of these patients, nine were later studied either on prednisone ($n = 4$) or postsplenectomy ($n = 5$). A total of 47 studies were performed.

The studies were approved by the University of Washington Human Subjects Review Committee and written informed consent was obtained from each subject.

In vitro studies

Platelet counts. Platelet counts were performed with an electronic particle counter (Series 810 Platelet Analyzer, Baker Instruments Corp., Allentown, PA) on fresh whole blood samples collected in EDTA (10%). Platelet counts less than 30×10^9/liter were confirmed by phase contrast microscopy.

Platelet antibody tests. Levels of platelet-associated IgG, IgM, or C3 were determined using a ^{125}I-radiolabeled platelet antiglobulin assay similar to that described by Cines and Schreiber (24). An $F(ab')_2$ fraction of rabbit anti-human IgG and an anti-human IgM antibody were prepared by established techniques (25, 26). Standardization of anti-human IgG was performed using an ^{131}I-labeled IgG PL^{A1} alloantibody (27). Goat anti-human C3 was obtained from Atlantic Antibodies, Scarborough, ME, and used as the complete Ig. ^{125}I-labeling of antibodies to IgG, IgM, and C3 reagents were performed by the chloramine-T method (28). Platelet-rich plasma was obtained by centrifugation of peripheral blood collected in EDTA (10%) at 200 *g* for 9 min. Unbound Ig was removed from the platelets by passage over a sepharose 2B-CL column (equilibrated in Tris-buffered saline, pH 7.0) (29). The platelet eluate was collected and counted. A calibrated amount of ^{125}I-$F(ab')_2$ rabbit anti-human IgG was added to 10^8 platelets, and the mixture was incubated at 37°C for 30 min. The plateletes were then washed three times in phosphate-buffered saline/bovine serum albumin (1.6%) buffer with centrifugation at 2,000 *g* for 10 min. The radioactivity of the platelet button was determined with a gamma spectrometer and the result for the patient's platelets was compared with simultaneously analyzed normal control platelets. When the value for the control platelets fell within our normal range (< 3.6 fg IgG/platelet), the absolute quantity of IgG bound per platelet for patient specimens was calculated. In those cases where the concurrent control results varied above our established normal range, we were able to assess the presence of increased IgG relative to the concurrent control, but an absolute quantitation was considered unreliable. Detection of platelet-associated IgM and C3 was performed in an identical manner, but absolute quantitation was not done. Normal ranges for platelet-associated IgM or C3 were established on a daily basis by concurrently run normal control samples. Abnormal results were considered to be > 2 SD from control values.

Assay of hematopoietic progenitors. Megakaryocytic and granulocyte-macrophage colony-forming cells (CFU-Meg and CFU-GM, respectively) were assayed in methylcellulose as previously described (30). Heparinized iliac crest marrow aspirates were diluted two- to threefold in Hanks' balanced salt solution (HBSS, Gibco, Grand Island, NY). Low-density mononuclear cells were obtained by centrifugation of the marrow cells at 1,500 *g* for 20 min at room temperature on Ficoll-diatrizoate (1.077 gm/ml; LSM, Litton Bionetics, Kensington, MD). The mononuclear cell fraction at the interface was washed three times in HBSS and suspended to a final concentration of 10^5 cells/ml in Iscove's Modified Dulbecco's Medium (Gibco) made semisolid with 0.9% methylcellulose and supplemented with 25% human plasma, 5% phytohemagglutinin-stimulated lymphocyte conditioned medium, 5×10^{-5} M B-mercaptoethanol, 10 mM L-glutamine, and penicillin-streptomycin. All studies were performed with plasma from the same normal donor. The cell suspension was dispensed in 1-ml volumes into 35-mm plastic tissue culture dishes (Lux, Miles Laboratories Inc., Naperville, IL). The cultures were incubated at 37°C in a high-humidity 5% CO_2-95% air mixture for 13 d. Megakaryocyte and granulocyte-macrophage colonies were enumerated by examination with an inverted microscope using previously described criteria (30). Early erythroid progenitors (BFU-E) were assayed concomitantly as previously described (31). Five replicate cultures were performed for each study.

Determination of the percentage of hematopoietic progenitors in DNA synthesis. The percentage of each progenitor type in DNA synthesis was measured by exposing 5×10^6 marrow mononuclear cells in vitro to 100 μCi of high specific activity (25 Ci/mmol) tritiated thymidine (^{3}H-TdR) (Amersham International, Amersham, UK), and the effect on colony formation was determined as previously described (32). As controls, marrow cells were incubated with TdR or with 100 μCi [^{3}H]TdR plus excess TdR (1 mg).

In vivo studies

Platelet kinetic studies. All survival studies were performed with autologous platelets at a time when the platelet count was stable. Platelet turnover was calculated by the formula: turnover = (platelet count/μl $\times$ 90%)/platelet survival (d) $\times$ % recovery. At stable platelet counts, platelet turnover = platelet production = platelet destruction (33).

In the patients with platelet counts > 20×10^9/liter, platelet survival studies were performed with ^{51}Cr and/or ^{111}In-labeled platelets. Those patients with counts < 20×10^9/liter were studied exclusively with ^{111}In-labeled platelets. Women of childbearing age were excluded from ^{111}In-labeled platelet studies. ^{51}Cr-labeled platelet survival was measured according to the method of Harker and Finch (33). ^{111}In-oxine (Mediphysics, Emeryville, CA or Chicago, IL) platelet survivals were performed using a closed bag modification of Thakur et al. (34) as previously described (35). Venous samples for determining platelet radioactivity following platelet injection were drawn at 0.5, 1, and 4 h on day 1, twice daily on days 2 and 3, and daily on days 4, 5, and 6. Platelet survival was calculated by computer-assisted analysis of the radioactive decay (gamma function analysis) (36). To establish normal values, ^{51}Cr-labeled autologous platelet survivals were performed in a control group of 16 healthy volunteers (12 males, 4 females) aged 25–60 yr (37). Similarly, normal values for ^{111}In-autologous survivals were established in a group of 10 healthy male volunteers. To ensure comparability between ^{111}In- and ^{51}Cr-labeled autologous platelet survival measurements, we performed 15 paired ^{51}Cr- and ^{111}In-autologous survivals in patients with platelet counts > 20×10^9/liter during the initial phase of this study. Survivals averaged 4.3 d±2.3 by ^{51}Cr and 4.5 d±1.2 by ^{111}In. No significant difference between survival data using the two isotopes was found by paired *t* analysis ($P > 0.10$).

Platelet localization studies. Following ^{111}In-oxine-labeled platelet injection, serial anterior and posterior whole-body imaging was performed at 2, 24, 48, 72, and 96 h in the AITP patients and at 2, 76, 96, and 216 h in normal subjects. Imaging data obtained closest to each patient's platelet survival time was analyzed for comparisons between treatment groups. A large field-of-view camera was used, collecting both the 174- and 247-keV photo peaks of ^{111}In. Total body as well as liver and spleen images were determined under identical conditions at each imaging interval using a scan speed of 12 cm/min into a 64 × 64 computer matrix. Organ activity was expressed as a percentage of the total body activity at each scanning time using the geometric mean method (38). To establish normal values for sites of platelet sequestration, 10 normal male volunteers underwent ^{111}In-platelet labeling with quantitative analysis of sites of platelet sequestration. The percentage of total body platelet counts (TBC) present in the liver and spleen at the end of the imaging study was considered a representative measure of reticuloendothelial (RE) clearance.

Statistical analysis. Data were analyzed by *t* statistics for two means, by paired *t* statistics, and by basic statistics for two variables where appropriate. Data are presented as the mean±1 SD.

Results

Platelet counts. Platelet counts for patients in each treatment group are shown in Fig. 1. Two patients, both in the "no treatment" group, had platelet counts too low (4 and 11×10^9/liter) to perform autologous platelet kinetic or antibody studies. Platelet counts averaged 78±47 $\times 10^9$/liter, 96±54 $\times 10^9$/liter, and 212±250 $\times 10^9$/liter, respectively, in the no treatment, prednisone-treated, and postsplenectomy groups.

Assays of hematopoietic progenitors. Marrow culture data were not available for 15 patients who declined a repeat bone marrow aspiration on referral to the study. Assays for CFU-Meg were performed on 29 occasions on 23 patients. BFU-E and CFU-GM were assayed concurrently to determine whether any observed changes in CFU-Meg were specific. As shown in Table I, the frequencies of CFU-Meg, BFU-E, and CFU-GM in the untreated, prednisone-treated, the splenectomized groups did not differ significantly from normal or from each other.

The [^{3}H]TdR suicide results are summarized in Table II. Among the untreated and prednisone-treated patients, there was

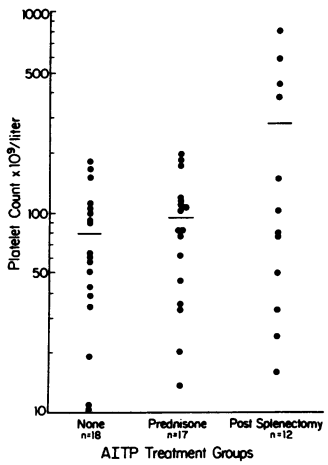

Figure 1. Bars represent the mean platelet count for each group. Normal platelet count is 250×10^9/liter ±40. *n*, number of patients studied.

Table II. Percentage of Progenitors in the DNA Synthetic Phase of the Cell Cycle

Treatment (No. patients)	CFU-MEG	BFU-E	CFU-GM
Untreated (14)	64±17* (33–90)	20±7 (9–28)	34±7 (18–40)
Steroids (10)	52±10‡ (36–62)	21±12 (5–42)	28±6 (12–38)
Postsplenectomy (6)	34±19 (7–63)	21±10 (14–32)	26±5 (21–31)
Normal (10)	21±13 (0–38)	21±6 (14–32)	27±6 (16–32)

* $P < 0.001$ or ‡$P < 0.01$ compared with normal controls.

a 2.5- to threefold increase, compared with normal, in the percentage of CFU-Meg in the DNA synthetic phase of the cell cycle. In contrast, the mean [^{3}H]TdR suicide value for CFU-Meg in the splenectomized patients was not significantly different from normal. The cycling activity of BFU-E and CFU-GM in the three treatment groups was not changed from normal.

When the CFU-Meg cell cycle kinetics was correlated with the peripheral platelet count rate for all patients (Fig. 2), a significant inverse correlation was found ($r = -0.65$; $P < 0.001$). However, for the patients with platelet counts $< 170 \times 10^9$/liter at the time of study, there was no significant relationship between these two parameters despite a mean suicide rate (62%±17; $P < 0.01$) that was significantly higher than the normal controls.

Autologous platelet survival studies. Autologous platelet survivals were all less than normal with the exception of two postsplenectomy patients. Furthermore, 36 of the 44 survivals performed were disproportionately reduced for their platelet count (37). Platelets were cleared exponentially in most patients and survival curves were never multiphasic. There was a significant correlation ($r = 0.64$; $P < 0.001$) between the platelet count and platelet survival when all patients were included (Fig. 3). However, when analyzed by treatment group, only the splenectomized patients showed a significant correlation between these two parameters ($r = 0.84$; $P < 0.001$). Furthermore, if only the 36 patients with platelet counts of $< 170 \times 10^9$/liter were analyzed, there was no significant relationship between platelet count and survival ($r = 0.36$; $P > 0.10$).

Autologous platelet turnover studies. Platelet turnover averaged $41 \pm 5 \times 10^9$ platelets/liter per d in 15 normal controls (37). Table III gives turnover results for all patients by treatment group. In the patients on no treatment, 7 of 17 (41%) had decreased platelet turnovers whereas 9 of 17 had platelet turnovers within the normal range. Thus, 94% (16 of 17) of untreated patients demonstrated an inappropriate thrombopoietic response to their low platelet counts. In contrast, 7 of 15 (47%) patients in the prednisone group demonstrated high turnover rates. Among the splenectomized patients, the four with high turnovers benefited from splenectomy (platelet counts were $> 170 \times 10^9$/liter postsplenectomy). In contrast, in the remaining eight patients with postsplenectomy platelet counts below the normal range ($15–160 \times 10^9$/liter), four had decreased platelet turnovers and four were within the normal range. There was a significant, direct correlation ($r = 0.68$; $P < 0.001$) between log platelet count and platelet turnover for all patients, and this correlation remained significant for the thrombocytopenic patients ($r = 0.63$; $P < 0.001$) (Fig. 4).

Autologous ^{111}In platelet localization studies. In both the untreated and prednisone-treated patients, the average liver-spleen uptake of autologous platelets was significantly increased compared with control values but not from each other (Table IV). This increase was due to increased splenic sequestration as

Table I. Progenitor Frequency

Treatment (No. patients)	CFU-MEG	BFU-E	CFU-GM
Untreated (14)	15±9 (5.2–38.3)	136±46 (80–214)	88±38 (43–182)
Steroids (10)	11±5 (0.4–18)	87±20 (48–103)	60±20 (14–86)
Postsplenectomy (6)	14±5 (5.0–19.3)	137±36 (99–171)	114±34 (76–159)
Normal (10)	9.7±8.8 (3.0–36)	98±44 (44–201)	86±41 (29–170)

Mean±SD/10^5 low-density marrow cells (range).

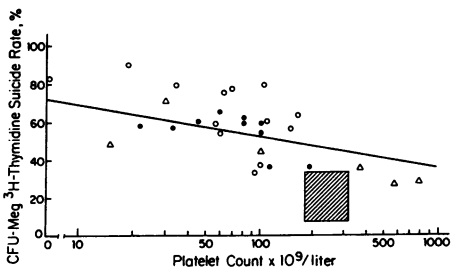

Figure 2. There is a significant inverse correlation between ^{3}H-thymidine suicide rate (%) of CFU-Meg and the log platelet count for all patients. (○) No treatment, (●) prednisone, (△) postsplenectomy, $r = -0.065$, $P < 0.001$. Hatched area represents the normal range.

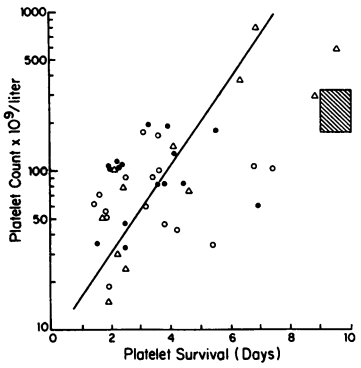

Figure 3. There was a direct correlation between log platelet count and autologous platelet survival; $r = 0.64$, $P < 0.001$. Patients on no treatment (○), prednisone (●), or postsplenectomy (△) were studied. The hatched area represents the normal range.

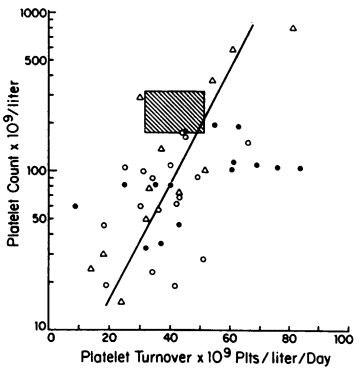

Figure 4. There was a direct relationship between log platelet count and autologous platelet turnover; $r = 0.068$, $P < 0.001$. Patients were on no treatment (○), prednisone (●), or postsplenectomy (△). Hatched area represents the normal range.

there was no significant increase in liver sequestration in untreated (13%±4) or prednisone-treated patients (11%±4) compared with controls (14%±2). In contrast, patients in the splenectomized groups had significantly increased liver activity (41%±13) compared with controls ($P < 0.001$) and untreated ($P < 0.001$) and prednisone-treated ($P < 0.005$) patients. However, there was a significant disparity in the mean clearance rates when the postsplenectomy patients were subdivided into treatment responders and nonresponders. The four patients who failed splenectomy showed significantly higher mean clearance rates ($P < 0.01$) than the total normal spleen-liver clearance, whereas those patients who responded had clearance rates significantly below normal ($P < 0.005$) (Table IV).

Production/clearance index. The contribution of both platelet production and spleen-liver platelet clearance to the platelet count was best expressed by an index relating platelet production to RE clearance: index = platelet turnover (expressed as a fraction of normal)/spleen-liver clearance (expressed as a fraction of normal). As shown in Fig. 5, there was a significant correlation between the production/clearance index and the circulating platelet count when all patients were included ($r = 0.88$; $P < 0.001$). In addition, this correlation remained significant when the thrombocytopenic patients were analyzed separately ($r = 0.78$; $P < 0.001$).

Platelet antibody data. Among 38 patients, 33 (87%) demonstrated increased platelet-associated antibody or complement at the time of study. 28 patients had elevated IgG; of these patients, three also showed elevated IgM and another six had elevated C3. Five patients had raised levels of IgM or C3 alone. There was a significant inverse correlation ($r = -0.6$; $P < 0.001$) between autologous platelet-associated IgG and the log platelet count (data not shown). Because our data suggested a direct relationship between platelet production and platelet count (Fig. 4), we examined the relationship of platelet turnover (or production) with platelet-associated IgG levels (Fig. 6). There was an inverse correlation between log platelet turnover and platelet-associated IgG levels ($r = -0.65$; $P < 0.001$). Thus, high levels of platelet-associated antibody were associated with low platelet turnover and low platelet counts. In contrast, there was no relationship between platelet-associated IgG and platelet survival ($r = -0.25$; $P > 0.10$) (data not shown).

Homologous platelet survival and turnover studies. The unexpectedly prolonged survival of radiolabeled autologous platelets observed in our prospectively studied patients led us to review a series of homologous platelet survival measurements previously performed in a group of 13 AITP patients (Table V). Some of these patients also had autologous platelet survival measurements. These patients met the same diagnostic criteria as the prospectively studied patients, and none had been previously pregnant or transfused. All of these patients had homologous or autologous platelet survivals performed using only ^{51}Cr as the isotopic label. Most of the homologous platelet survivals were performed because the patients were severely thrombocytopenic rendering ^{51}Cr autologous survivals technically unreliable. Platelet counts for the nine patients who had only homologous platelet survivals performed (patients 1–9) averaged $12\pm8 \times 10^9$ platelets/liter, platelet survivals were 0.33±0.26 d, and turnovers averaged $80\pm35 \times 10^9$ platelets/liter per d, 1.9× normal. Six patients were on no treatment, two were on steroids, and one was a splenectomy failure.

Table III. Platelet Turnover

	Turnover*	Untreated	Prednisone	Postsplenectomy	Total
	platelets/liter per d				
Decreased	$<31 \times 10^9$	7 (41%)‡	2 (13%)	4 (33%)	13 (30%)
Normal	$31–51 \times 10^9$	9 (53%)	6 (40%)	4 (33%)	19 (43%)
Increased	$>51 \times 10^9$	1 (6%)	7 (47%)	4 (33%)	12 (27%)
	Total	17	15	12	44

* Platelet turnover in 15 normal controls averaged $41\pm5 \times 10^9$ platelets/liter per d; $< 31 \times 10^9$ platelets/liter per d was < 2 SD from normal; $31–51 \times 10^9$ platelets/liter per d was within ±2 SD of normal; and $> 51 \times 10^9$ platelets/liter per d was 2 SD above normal. ‡ Data are given as No. of patients in each category (% of patients in corresponding treatment group).

Table IV. ^{111}In Platelet Localization in Liver-Spleen

	No.	Liver-spleen platelet radioactivity*
		%
Normals	10	40±4
Patient treatment groups		
None	11	55±6§
Prednisone	6	63±11§
Postsplenectomy‡	8	41±13
Nonresponders	4	53±3‖
Responders	4	29±5§

* Results are expressed as the percentage of total body ^{111}In-labeled platelet radioactivity±SD. ‡ Postsplenectomy nonresponders had platelet counts between 15 and 150 × 10^9/liter and responders had platelet counts between 290 and 790 × 10^9/liter. The average results for each test group are compared with the normal values; §$P < 0.005$, ‖$P < 0.01$.

Four patients (10–13) (Table V) had both homologous and autologous platelet survivals performed on the same therapy at relatively constant platelet counts within a 14-d period. For patient 13, the donor was an identical twin. The homologous platelet survivals were significantly shorter than the autologous (1.5 d±1.0 vs. 3.6 d±1.4, respectively, $P < 0.01$) and corresponding turnovers were greater (70±46 × 10^9 platelets/liter per d vs. 42±49 × 10^9 platelets/liter per d, respectively, $P < 0.025$).

Discussion

This study was performed to elucidate mechanisms of thrombocytopenia in AITP. We used in vitro colony-forming assays to examine the hematopoietic progenitor cell response in AITP, while autologous platelet turnover studies provided an indirect measure of platelet production.

There was no significant difference in CFU-Meg frequency between the AITP treatment groups and normal controls. This finding is in accord with animal studies in which marrow CFU-Meg numbers showed little change in response to thrombocytopenia induced by antiplatelet serum (39, 40) or exchange transfusion (41). However, this data must be interpreted with caution as the expression of progenitor cell numbers per 10^5 nucleated cells is potentially subject to artifact from peripheral blood contamination. Indeed, the number of total body progenitors is the most meaningful denominator but is not obtainable in man. However, of more significance in our study was the finding that the percentage of CFU-Meg in DNA synthesis was inversely correlated with the platelet count. This measurement is not subject to the same artifact as the assessment of colony numbers. Similar kinetic observations have been reported in thrombocytopenic animals (39, 41, 42). BFU-E and CFU-GM, assayed concurrently, were unaffected. The latter observation is important, as chronic antiplatelet antibody-induced thrombocytopenia in a murine model caused a nonspecific proliferative response involving a variety of progenitor classes (39) not seen in our studies. Although given the single designation CFU-Meg, the megakaryocytic stem cell compartment likely encompasses a spectrum of progenitors that are heterogenous in their physical characteristics and proliferative activity (32, 43–45). Whether the increased percentage of CFU-Meg in DNA synthesis in AITP reflects increased cycling activity of the entire compartment or only a subset of more mature and responsive progenitors is unclear. In addition, how this response is mediated remains to be determined.

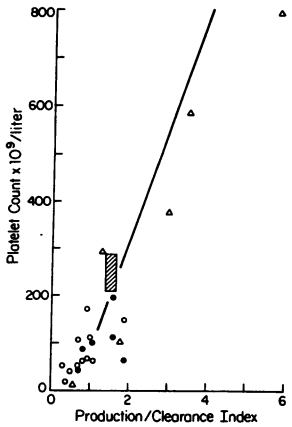

Figure 5. There was a direct correlation between platelet count and the production/clearance index for all patients; $r = 0.88$, $P < 0.001$. Patients were on no treatment (○), prednisone (●), or postsplenectomy (△). Hatched area represents the normal range.

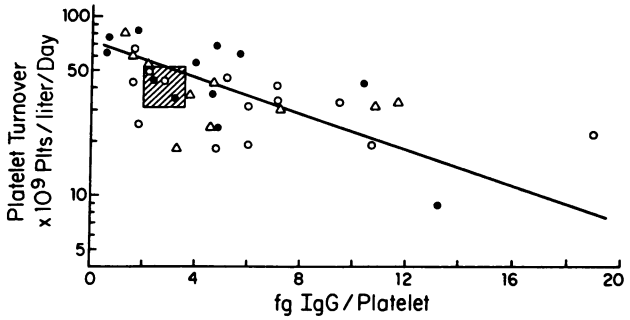

Figure 6. There is an inverse relationship between log platelet turnover and fg IgG/platelet for patients receiving no treatment (○), prednisone (●), or postsplenectomy (△); $r = -0.65$, $P < 0.001$). Hatched area represents the normal range.

In contrast to other studies, our platelet turnover calculations were based only on autologous platelet survival measurements. Overall, 30% of the AITP patients showed decreased platelet production, 43% had production rates within the normal range, and 27% had increased platelet production. Furthermore, we showed a direct correlation between platelet count and platelet turnover ($P < 0.001$). Of interest is the comparison of the turnover results between the untreated and the prednisone-treated groups. A significantly greater proportion of untreated patients exhibited inappropriately low platelet turnovers (41%) compared to the prednisone group (13%), although the mean survivals for the untreated group (3.3 d±1.7) and the prednisone groups (3.3 d±1.5) were essentially identical. Although comparisons between treatment groups must be interpreted with caution, these results suggest that although platelet survival is shortened in AITP and clearly influences the severity of the disease, a more significant factor in determining the platelet count is the rate of effective platelet production that may be improved by prednisone treatment.

In previous studies, estimated rates of platelet production based on either platelet turnover measurements or megakaryocyte quantitation (4, 5) were increased. Our reduced platelet

Table V. Homologous vs. Autologous Platelet Kinetic Measurements

Patient	Homologous platelets				Treatment	Autologous platelets			
	Platelet count	Recovery	Survival	Turnover		Platelet count	Recovery	Survival	Turnover*
	$\times 10^9$/liter	%	d	$\times 10^9$ platelets/liter per d (xN)		$\times 10^9$/liter	%	d	$\times 10^9$ platelets/liter per d (xN)
1	24	88	0.72	34 (0.8)	None				
2	22	42	0.44	107 (2.6)	None				
3	19	53	0.69	47 (1.1)	None				
4	12	83	0.14	93 (2.3)	None				
5	12	65	0.13	128 (3.1)	None				
6	10	55	0.41	40 (1.0)*	None				
	7	54	0.48	24 (0.6)*	None				
7	5	113	0.07	71 (1.7)	Prednisone				
8	4	15	0.33	73 (1.8)	Prednisone				
9	3	55	0.04	123 (3.0)	Postsplenectomy				
Average±1 SD	12±8	63±29	0.33±0.26	80±35 (1.9±0.9)					
10	9	56	0.25	93 (2.3)	None	25	43	3.9	13 (0.3)*
					None	31	47	4.3	14 (0.3)*
11	84	71	1.0	106 (2.6)	Prednisone	60	71	3.5	22 (0.5)
12	87	35	0.7	320 (7.8)	Prednisone	98	40	1.9	116 (2.8)
13	132	64	2.9	64 (1.6)‡	Prednisone	74	71	5.2	18 (0.4)
Average±1 SD	78±51	56±19	1.5±1.2§	146±118 (3.6±2.9)‖		64±31	56±17	3.6±1.4	42±49 (1.0±1.1)

(xN) is turnover value expressed as a proportion of the normal value; i.e., 41 $\times$ 10^9/liter per d. * Repeat platelet kinetic measurements from the same individual demonstrating reproducibility of the measurements. ‡ Donor was an identical twin. By paired t statistic, the average homologous platelet data was compared with autologous data; §$P < 0.01$, ‖$P < 0.025$.

turnover measurements are a direct reflection of the relatively long platelet survivals we observed. Our shortest platelet survival was 1.4 d in contrast to the survivals of minutes to hours previously reported (4, 5, 46). There are two potential explanations for the discrepancies between our survival measurements and those of others. First, the nature of this study, which required the patients to have stable platelet counts for 1–2 wk, usually prevented us from studying severely thrombocytopenic patients. Therefore, this is a study mainly of patients with chronic AITP of mild to moderate severity. It is possible that platelet kinetics in these patients are significantly different from those in the more severely affected patients. However, against this are data from two of our patients who were very thrombocytopenic at the time of study; i.e., platelet counts of < 25,000/μl. Their autologous ^{111}In platelet survivals were 1.9 and 2.5 d and corresponding platelet turnovers were 14 and 19 $\times$ 10^9 platelets/liter per d, respectively. Furthermore, when our data are combined with other In-labeled autologous platelet kinetic measurements in similarly severely thrombocytopenic patients reported in the literature, there have been 25 such patients studied (15, 17, 47). For 12 of 25 (48%), platelet survivals were > 1.0 d, and platelet turnovers were reduced by at least 2 SD from the investigators' average normal values in 17 (68%). These data indicate that disease severity may not adequately explain the disparate platelet survival results.

A second possible explanation is that previous investigators routinely used homologous platelets for survival measurements; such homologous platelets may have influenced the results. Although Branehog (4) and Abramson (48) have reported no difference between homologous and autologous platelet survivals in 10 patients with AITP, seven of the patients studied had platelet counts of less than 26 $\times$ 10^9/liter, making autologous ^{51}Cr survival results unreliable. Furthermore, recent studies by du Heyns et al. (47) found ^{51}Cr-labeled homologous platelet survivals to be significantly shorter than ^{111}In-labeled autologous survivals in patients with AITP. He attributed this survival discrepancy to antibody-induced loss of the ^{51}Cr label but provided no direct evidence for this assertion. However, when only autologous platelets were subjected to dual labeling, ^{51}Cr survivals were only minimally reduced compared to ^{111}In survivals (2.5 d±2.2 vs. 2.7 d±2.4, respectively) (17). Similar dual-labeled autologous platelet survival measurements in 15 of our AITP patients showed no difference in survival measurements using either isotope. Thus, we postulate that the markedly reduced platelet survivals (and correspondingly high turnovers) previously reported are due to a more marked effect of the patient's antibody on the clearance of homologous platelets rather than a differential effect of the antibody on the platelet label.

To evaluate the latter hypothesis, we reviewed ^{51}Cr radiolabeled homologous platelet survival measurements previously performed in a group of 13 AITP patients. In nine patients, homologous platelet survivals had been used to determine platelet kinetics because the patients were severely thrombocytopenic with platelet counts of < 25 $\times$ 10^9/liter. These studies did demonstrate the markedly reduced platelet survivals (range, 0.04–0.72 d) and normal to increased platelet turnovers (range, 34–128 $\times$ 10^9 platelets/liter per d) reported by others (4, 5). Again, this data may suggest a different disease process in severely thrombocytopenic, compared to moderately thrombocytopenic, patients. However, as further evidence for a differential effect on antibody on homologous compared to autologous platelets, data were available from four additional AITP patients who had

both homologous and autologous platelet survivals performed at similar platelet counts on the same therapy. There was a significant reduction in homologous compared to autologous platelet survivals in all four patients, including one patient whose donor was an identical twin. In addition, as all of these survival measurements were performed using ^{51}Cr as the radiolabel, the survival differences cannot be accounted for by a differential loss of chromium compared with an indium label. A differential effect of antibody on homologous platelets in AITP patients may be analogous to patients with autoimmune hemolytic anemia where homologous red cell survivals have been demonstrated to be shorter than similarly measured autologous survivals (49).

Assuming that autologous survival measurements accurately reflect steady-state kinetics in our patients, a significant number of thrombocytopenic AITP patients appear to have decreased or ineffective thrombopoiesis. As these patients have normal numbers of megakaryocyte progenitors showing increased cycling activity, the defect in thrombopoiesis likely occurs at the more terminal branches of megakaryocytopoiesis. It has been shown that platelet antibodies bind to megakaryocyte membranes in AITP patients (23) and such antibodies may interfere with megakaryocyte platelet maturation or release (50). Alternatively, some of the newly formed platelets may be subject to immediate immunologically-mediated marrow RE clearance and never enter the circulation. Both of these mechanisms are consistent with our finding that higher levels of IgG bound to autologous platelets are associated with greater reductions in platelet turnover. Thus, the platelets that actually circulate may have a relative resistance to antibody-mediated clearance. Proof of this concept would require the use of an in vivo label such as selenomethionine that is incorporated into megakaryocytes and, thus, into newly formed platelets. Patterns of platelet release in AITP patients compared with normals would substantiate whether ineffective platelet production occurs in this disorder.

The relationship of the platelet production/clearance index to the platelet count underlines the importance of both rates of platelet production and platelet removal in determining the final platelet count. Comparison of treatment groups suggests that prednisone increases the platelet count by improving platelet production with no significant effect on spleen-liver clearance. To determine whether this is a valid observation requires analysis of patients studied in a serial fashion both before and after treatment with prednisone. Such studies are ongoing. However, three patients in this study were analyzed on no treatment and then subsequently on prednisone. All three doubled their platelet turnover rates on prednisone whereas total RE clearance showed no significant change in one and actually increased in two patients (data not shown).

The effect of splenectomy appears to be more complex. Those patients who respond show significantly lower clearance rates (29%±5 TBC) and significantly higher turnover rates (57±4 $\times 10^9$ platelets/liter per d) after splenectomy than the nonresponders (53%±3 TBC; 32±3 $\times 10^9$ platelets/liter per d, respectively). This suggests that splenectomy not only reduces platelet clearance but also has the potential to enhance production, perhaps by the removal of a major site of platelet antibody synthesis (51, 52). Again, serial studies are required to validate these observations. Preliminary studies in five patients studied before and after splenectomy showed significant decreases in RE clearance in the four responders. Two of the responders also showed a significant increase in platelet turnover rates whereas two were unchanged. The one patient who failed splenectomy demonstrated no change in RE clearance or platelet production (data not shown).

In summary, megakaryocyte progenitors in AITP show increased cell cycle activity in response to peripheral thrombocytopenia. As megakaryocytes are generally increased in this disorder, the antibody-mediated decreases in platelet production we observed in many AITP patients must occur at or beyond the level of the mature megakaryocyte. We postulate that the ultimate effect of the antibody is to prevent newly formed platelets from entering the circulation. However, the circulating platelet count represents a combined response to platelet production as well as sequestration. Steroids and splenectomy variably influence the production/clearance rate to improve platelet count. Further studies are needed to clearly delineate the role of platelet production in this disorder, how the autoantibody impairs platelet production, and the specific mechanisms whereby treatments are effective in individual patients.

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