Platelet Destruction in Autoimmune Thrombocytopenic Purpura: Kinetics and Clearance of Indium-111-Labeled Autologous Platelets

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Using autologous ^{111}In-labeled platelets, platelet kinetics and the sites of platelet destruction were assessed in 16 normal subjects (13 with and three without spleens), in 17 studies of patients with primary autoimmune thrombocytopenic purpura (AITP), in six studies of patients with secondary AITP, in ten studies of patients with AITP following splenectomy, and in five thrombocytopenic patients with myelodysplastic syndromes. In normal subjects, the spleen accounted for 24 ± 4% of platelet destruction and the liver for 15 ± 2%. Untreated patients with primary AITP had increased splenic destruction (40 ± 14%, $p < 0.001$) but not hepatic destruction (13 ± 5%). Compared with untreated patients, prednisone treated patients did not have significantly different spleen and liver platelet sequestration. Patients with secondary AITP had similar platelet counts, platelet survivals, and increases in splenic destruction of platelets as did patients with primary AITP. In contrast, patients with myelodysplastic syndromes had a normal pattern of platelet destruction. In AITP patients following splenectomy, the five nonresponders all had a marked increase (>45%) in liver destruction compared to five responders (all <40%). Among all patients with primary or secondary AITP, there was an inverse relationship between the percent of platelets destroyed in the liver plus spleen and both the platelet count ($r = 0.75$, $p < 0.001$) and the platelet survival ($r = 0.86$, $p < 0.001$). In a stepwise multiple linear regression analysis, total liver plus spleen platelet destruction, the platelet survival and the platelet turnover were all significant independent predictors of the platelet count. Thus platelet destruction is shifted to the spleen in primary and secondary AITP. Failure of splenectomy is associated with a marked elevation in liver destruction. The magnitude of spleen and liver destruction appears to be of considerable importance in the severity of the disease, as reflected in the platelet survival and platelet count.

J Nucl Med 30:629–637, 1989

Until recently, the role of the reticuloendothelial cell component of the liver and spleen in platelet clearance in autoimmune thrombocytopenic purpura (AITP) has been difficult to study, and thus there is limited knowledge of the importance of destructive mechanisms in the thrombocytopenia and shortened platelet survival that characterize AITP. Earlier studies using chromium-51- (^{51}Cr) labeled platelets for determining sites of platelet destruction had critical shortcomings and led to conflicting conclusions. These shortcomings are largely related to the limitations of ^{51}Cr as an isotope. The combination of a low injected dose of ^{51}Cr-labeled platelets (10–30 μCi) and the low gamma photon yield (9%) gives a small number of imageable photons, precluding accurate quantitation of uptake in entire organs. Usually, localization studies performed using ^{51}Cr-labeled platelets obtained count rates from small nonimaging probes (usually 2 cm diameter) variably positioned over the liver, spleen or heart, and allowed

Received July 25, 1988; revision accepted Dec. 21, 1988.

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only crude approximations of organ activity (*1,2*). Typically, activity from a small portion of the spleen was expressed as a ratio relative to the heart or the liver, but none of the measures permitted the calculation of the actual percentage of platelets destroyed in the liver or spleen. The different techniques of data expression produced large variability from one patient to another, with various spleen/liver or spleen/heart ratios showing 14- to 352-fold differences between patients with AITP (*3–6*). Another limitation in most studies was the use of donor rather than autologous platelets that could cause spurious results because of alloimmunization or differences in binding characteristics of antibody to allogenic compared to autologous platelets. In addition, most prior studies made no attempt to correct for isotope attenuation. Thus, numerous methodologic limitations restrict the conclusions that can be drawn from the earlier attempts at quantitating the sites of platelet destruction in AITP.

The recent availability of indium-111 (^{111}In) as a platelet label allows the quantitative evaluation of platelet distribution, since ^{111}In has abundant gamma emissions that can be readily detected by standard gamma cameras. In addition, the high labeling efficiency of [^{111}In]oxine permits the use of autologous platelets even in patients with relatively low platelet counts (*7–9*). Furthermore, since there is minimal loss of ^{111}In following initial organ retention, measuring organ activity at a time point beyond the platelet survival can quantitatively assess sites of platelet destruction (*10*).

This study was designed to quantitatively evaluate the sites of platelet destruction in groups of AITP patients who were untreated, receiving prednisone, or were splenectomized. We also sought to determine the sites of platelet destruction in patients with immune-mediated thrombocytopenia associated with other disorders, which we have called "secondary AITP". For comparison, a group of healthy normal subjects and a group of thrombocytopenic patients with myelodysplastic syndromes were also studied. In addition, we evaluated the relationship of the amount of platelet removal in liver and spleen to both platelet survival and to platelet count.

METHODS

Patients and Normal Subjects

Sixteen normal subjects, 19 patients with primary AITP, seven with secondary AITP, and five with myelodysplastic syndromes were studied. Normal subjects and patients with myelodysplastic syndromes had only a single study, while five of the patients with primary or secondary AITP had more than one study at different stages of their disease (two subjects had baseline, prednisone, and postsplenectomy studies and three had both prednisone and postsplenectomy studies). The numbers of studies performed according to patient diagnosis and treatment are given in Table 1.

TABLE 1
Diagnosis and Treatment Categories

	Treatment groups		
	Untreated	Prednisone	Post-splenectomy
DIAGNOSIS			
Normal subject	13	0	3
Primary AITP	11	6	8
Secondary AITP	4	2	2
Myelodysplastic syndrome	5	0	0

Normal subjects were volunteers who had normal platelet counts, no known diseases, and were not receiving any medication. Three of the normal subjects had a prior splenectomy as a result of trauma. The diagnosis of primary AITP was based on the following criteria: a platelet count $< 100 \times 10^9$/l prior to treatment, a positive test for platelet associated immunoglobulin or complement, the absence of ingestion of drugs that cause thrombocytopenia, a bone marrow examination demonstrating normal to increased numbers of megakaryocytes, and the absence of other underlying diseases that may cause thrombocytopenia such as malignancy, collagen vascular disease, or microangiopathy. Platelet associated immunoglobulin and complement were assayed using radiolabeled anti-human IgG and C_3 reagents and a modification of a Coombs anti-globulin assay (*11*). A patient was considered to have secondary AITP if the same platelet count, antibody and bone marrow criteria as for primary AITP were met and an underlying disease was also present. The seven subjects with secondary AITP had a variety of associated disorders (quinidine induced thrombocytopenia -1, non-A, non-B, hepatitis -1, recurrent thrombotic thrombocytopenic purpura -1, rheumatoid arthritis -1, malignancy -2, and AIDS-related complex -1). As thrombocytopenic controls, five patients with myelodysplastic syndrome were also studied. In addition to thrombocytopenia, four of these patients had pancytopenia and one had leukopenia. Three of the five had autologous bound anti-platelet antibodies and two did not. However, the morphologic features on bone marrow examination and the in vitro growth characteristics of their hematopoietic stem cells confirmed the diagnoses of myelodysplastic syndrome.

Patients with primary or secondary AITP who were studied following splenectomy were further subdivided into those patients with a postoperative platelet count of $> 170 \times 10^9$/l (splenectomy success) and those with a postoperative platelet count of $\leq 170 \times 10^9$/l (splenectomy failure) determined at least 2 mo following splenectomy. The latter platelet count is > 2 s.d. below our average normal platelet count of $250 \pm 40 \times 10^9$/l obtained in a large group of normal subjects. One of the postsplenectomy patients received prednisone at the time of study and the remainder did not.

Subjects less than the age of 21 yr and women of childbearing potential were excluded due to the radiation involved. This study was approved by the University of Washington Human Subjects Review Committee and all subjects gave informed consent.

Platelet Labeling

Autologous platelet labeling was performed as previously described (*12,13*) using a closed blood bag modification of the

technique of Thakur et al. (*14*). In subjects with platelet counts greater than 50×10^9/l, 80 ml of whole blood were drawn into 20 ml of acid citrate dextrose (ACD); in subjects with platelet counts of less than 50×10^9/l, 160 ml of blood were drawn into 40 ml of ACD. The blood was centrifuged (350 *g* × 15 min) to obtain platelet-rich plasma. The pH was adjusted to 6.5 with 0.15*M* citric acid and a platelet pellet was formed by centrifugation of the platelet-rich plasma (1,300 *g* × 15 min). After removing the supernatant platelet-poor plasma, the pellet and bag were washed with Ringer's-citrate-dextrose (RCD), and the platelets were resuspended in 1 ml of RCD. The platelets were incubated for 20 min with ~ 1 mCi of ^{111}In oxine (Mediphysics, Emeryville, California, or Amersham, Chicago, IL). Twenty milliliters RCD plus 20 ml platelet-poor plasma were added and a platelet pellet was reformed (1,300 *g* × 15 min). The pellet and bag were again washed with RCD and the platelets were resuspended in 5 ml of autologous platelet-poor plasma. Contaminating red or white cells were removed by a slow centrifugation (200 *g* × 5 min). The volume of the labeled platelet suspension was recorded and the suspension was counted in a dose calibrator prior to injection into the patient. The mean percentage of ^{111}In activity free in the plasma prior to platelet injection was 6 ± 6% (± s.d.) in normal studies, 5 ± 5% in baseline primary AITP studies, 6 ± 4% in prednisone primary AITP studies, 6 ± 3% in post-splenectomy primary AITP studies, 8 ± 9% in secondary AITP studies, and 6 ± 6% in studies of patients with myelodysplastic syndromes. The mean injected dose was 345 ±17 μCi in normal subjects with spleens, 350 ± 10 μCi in normal subjects postsplenectomy, 273 ± 75 μCi in baseline primary AITP patients, 316 ± 59 μCi in prednisone treated primary AITP patients, 323 ± 37 μCi in primary AITP postsplenectomy patients, 309 ± 92 μCi in secondary AITP patients, and 245 ± 100 μCi in patients with myelodysplastic syndromes.

Platelet Imaging and Quantitative Analysis

Serial anterior and posterior whole-body imaging was performed using a large field-of-view gamma scintillation camera equipped with a medium-energy parallel hole collimator with collection of both the 173 and 247 keV photon peaks of ^{111}In using 20% energy windows. The first six patients were studied by acquiring individual static spot images from the head to the feet (five anterior and five posterior images for 500 sec per image). The mean total-body counts in the anterior projection were 398,310 ± 148,430 at 2 hr and 199,810 ± 216,230 at 72–96 hr. The corresponding posterior whole-body counts at 2 and 72–96 hr were 374,620 ± 122,260 and 61,770 ± 64,540, respectively.

For the subsequent 39 subjects who underwent imaging, whole-body anterior, and posterior scans were acquired at a scan speed of 12 cm/min into a 64 × 64 computer matrix, with a mean scan time of 1,148 ± 128 sec per scan. In normal subjects the mean anterior whole-body counts were 88,100 ± 24,400 at 2 hr, 41,400 ± 13,900 at 72–96 hr, and 21,700 ± 6,000 at 216 hr. The corresponding posterior counts were 90,100 ± 24,700 at 2 hr, 42,700 ± 13,100 at 72–96 hr, and 20,900 ± 3,000 at 216 hr. Among thrombocytopenic subjects, the mean anterior whole-body counts were 94,700 ± 36,400 at 2 hr and 43,500 ± 22,600 at 72–96 hr, and the corresponding posterior whole-body counts were 93,500 ± 34,300 at 2 hr and 50,300 ± 15,500 at 72–96 hr. The two imaging techniques gave comparable values for liver and splenic activities in 11 patients studied by both methods (r = 0.91). The whole-body scan technique obviates the problems inherent in realigning multiple spot images. Patients with thrombocytopenia and shortened platelet survivals were typically imaged at 2, 24, 48, and 72 or 96 hr following labeled platelet injection, while normal subjects were studied at 2, 72 or 96, and 216 hr following labeled platelet injection. Two of the normal subjects with spleens did not have serial images obtained, but did have serial blood drawn for platelet survival and turnover; thus platelet localization data over time are reported on 11 of the 13 normal subjects with spleens.

Image quantitation was performed as follows. All images on a given subject were realigned using a computer algorithm that positioned the liver and spleen in the same location in the image matrix, such that the same regions of interest (ROIs) could be applied to all serial images. For every study on each subject, spleen, liver, and whole-body activities were obtained using visually determined ROI on zoomed images for both the anterior and posterior images. The amount of ^{111}In-labeled platelet activity present in the whole body, liver, and the spleen was calculated using the geometric mean technique in which organ activity equals the square root of anterior organ counts times posterior organ counts as described by Heyns et al. (*15–17*). Liver and spleen activity were expressed as a percentage of simultaneously acquired total-body ^{111}In activity. In addition, at the final imaging time, the total liver plus spleen activity as well as a spleen/liver ratio were calculated. This method corrects for errors resulting from photon attenuation that occur if only an anterior or a posterior image is obtained and has been shown to be accurate for quantitation of liver and spleen ^{111}In activity (*16,17*). In addition, this method eliminates the effects of variations in scintillation camera efficiency since the activity of each organ is expressed relative to a simultaneously acquired whole-body image (*17*). We did not employ any arbitrary background subtraction, nor did we fit the data to a curve. To assess the intraobserver reproducibility, 18 images were blindly analyzed 3 or more months apart. There was no significant difference by paired t-testing in liver activity (19 ± 5 vs. 18 ± 4%) or spleen activity (34 ± 16 vs. 34 ± 16%) and the correlation coefficients for both were high (r = 0.996 and r = 0.920). Thus, the methods used for computer analysis of the images were highly reproducible.

Determination of Autologous Indium-111 Platelet Recovery, Survival, and Turnover

Following labeled platelet injection, 5 ml venous blood samples were typically drawn at 30 min, 2 hr, and daily thereafter (except Sundays). In normal subjects, a mean of 8 ± 1 samples were obtained and in patients with primary or secondary AITP a mean of 6 ± 1 samples. Platelet survival was calculated by the gamma function. The proportion of labeled platelets remaining in the systemic circulation after infusion (i.e., recovery) was calculated from the platelet activity per ml at zero time, multiplied by the estimated blood volume, and divided by the platelet ^{111}In activity injected. Platelet destruction, measured as platelet turnover per microliter per day, was calculated from the peripheral platelet count divided by the platelet survival time in days and corrected for recovery (*18,19*). Platelet counts were documented

TABLE 2
Platelet Kinetics in Nonsplenectomized Subjects: Normals, AITP, and Myelodysplastic Patients

	Platelet count × 10^9/l	Recovery (%)	Survival (days)	Turnover (platelets ×10^9/l/day)
Normal subjects (13)*	279 ± 39	65 ± 11	7.7 ± 1.2	52 ± 11
Primary AITP				
Untreated (11)	83 ± 56	68 ± 20	3.3 ± 2.1	37 ± 16
Prednisone (6)	109 ± 36	81 ± 20	2.7 ± 0.7	44 ± 12
Secondary AITP				
Untreated (4)	101 ± 87	44 ± 7	3.7 ± 2.0	45 ± 18
Prednisone (2)	25, 74	36, 44	2.5, 3.3	25, 46
Myelodysplastic (5)	68 ± 64	68 ± 14	5.1 ± 1.3	17 ± 15
All primary and secondary AITP				
Untreated (15)	89 ± 62	61 ± 20	3.4 ± 2.0	40 ± 17
Prednisone (8)	94 ± 43	70 ± 25	2.8 ± 0.7	42 ± 12

* Number of studies performed

to be stable based on 2–6 platelet counts obtained during each study.

Statistical Analysis

Data were analyzed by unpaired t-testing. The relationship between liver and spleen platelet clearance and other variables was determined by linear regression and stepwise multiple linear regression. All data are expressed as the mean ± s.d.

RESULTS

Platelet Kinetic and Localization Findings

Nonsplenectomized Subjects

Normal subjects. The mean platelet count, recovery, survival, and turnover in the normal subjects are shown in Table 2. At the final imaging time, normal subjects had a mean splenic sequestration of 24 ± 4% of the total-body ^{111}In activity and liver sequestration of 15 ± 2% (Table 3). The spleen/liver ratio was 1.6 ± 0.3 and the mean total liver plus spleen activity was 40 ± 4% at the final imaging time.

Primary AITP Patients

The 11 patients with untreated primary AITP had a mean platelet count of 83 ± 56 × 10^9/l ($p < 0.0001$ vs. normals). Mean platelet survival was significantly shortened ($p < 0.0001$) and mean platelet turnover was reduced compared to normal ($p < 0.02$) (Table 2). Following platelet injection, there was a progressive increase in the splenic uptake of labeled platelets from 2 hr to 72 hr. At both the 72 hr and the final imaging time, untreated patients with primary AITP had significantly greater ^{111}In platelet activity present in the spleen than did the normal subjects (both $p < 0.05$) (Table 3). In contrast, 72 hr and final liver activity were similar between untreated primary AITP subjects and normal

TABLE 3
Platelet Localization in Non-splenectomized Subjects: Normals, AITP, and Myelodysplastic Patients

	Spleen activity (% whole body)			Liver activity (% whole body)			Spleen/liver ratio	Spleen plus liver activity
	2 hr	72 hr	Final	2 hr	72 hr	Final	Final	Final
Normal subjects (11)	32 ± 2	32 ± 5	24 ± 4	11 ± 1	14 ± 2	15 ± 2	1.6 ± 0.3	40 ± 4
Primary AITP								
Untreated (11)	29 ± 5	42 ± 15	40 ± 14	14 ± 3	13 ± 6	13 ± 5	4.1 ± 2.7	54 ± 10
Prednisone (6)	28 ± 11	40 ± 15	40 ± 16	11 ± 5	17 ± 3	17 ± 3	3.7 ± 2.3	56 ± 6
Secondary AITP								
Untreated (4)	43 ± 2	43 ± 9	45 ± 10	15 ± 4	18 ± 3	17 ± 4	2.8 ± 0.8	62 ± 10
Prednisone (2)	26, 44	40, 52	40, 53	19, 11	16, 11	16, 10	2.5, 5.3	56, 63
Myelodysplastic (5)	23 ± 8	22 ± 11	24 ± 7	11 ± 3	14 ± 3	15 ± 2	1.7 ± 0.7	42 ± 9
All primary and secondary AITP								
Untreated (15)	33 ± 8	43 ± 14	42 ± 13	14 ± 3	14 ± 6	14 ± 5	3.7 ± 2.4	55 ± 10
Prednisone (8)	30 ± 12	42 ± 14	41 ± 14	12 ± 5	14 ± 9	16 ± 11	3.7 ± 2.1	57 ± 6

subjects. The total liver plus spleen platelet uptake (54 ± 10%) and the spleen/liver ratio (4.1 ± 2.7) at the final imaging time were both significantly greater than in normal subjects ($p < 0.001$).

Six patients with primary AITP were studied on prednisone treatment at a mean dose of 47 ± 17 mg/day (range 25 to 65 mg). Although prednisone treated patients did not as a group differ from the untreated AITP patients in their mean platelet counts, survivals, turnovers or splenic or liver sequestration patterns (Tables 2 and 3), the two study patients with serial untreated and prednisone treatment measurements showed increases in both platelet counts and turnovers while splenic uptakes remained abnormally elevated.

Secondary AITP Patients

Untreated and prednisone treated secondary AITP patients had mean platelet counts, survivals, and turnover measurements not significantly different from patients with primary AITP but significantly less than normal subjects (all $p < 0.05$) (Table 2). Liver and spleen uptake were also very similar to that seen in patients with primary AITP and significantly different from normal (Table 3). Thus, platelet kinetic and platelet destruction patterns in this group of patients with secondary AITP appeared similar to those in primary AITP.

Myelodysplastic Patients

The patients in this group had platelet counts, survivals, and turnovers similar to the primary and secondary AITP patients (all p = NS) (Table 2). However, in contrast to the AITP patients, spleen plus liver uptake (42 ± 9%) and the spleen/liver ratio were normal in myelodysplastic patients (Table 3).

Summary of Platelet Sequestration Patterns in Nonsplenectomized Subjects

Using the normal control data, 95% confidence limited for splenic (18–32%) and liver (8–20%) platelet sequestration were defined. Overall, in the treated and untreated patients with primary or secondary AITP, 19 of 23 (83%) demonstrated an isolated increase in splenic uptake, one (4%) demonstrated an isolated increase in liver uptake, and one (4%) had both increased spleen and liver uptake, and two (9%) had normal spleen and liver uptakes (Table 4). None of the five myelodysplastic patients had an abnormal pattern of platelet sequestration.

TABLE 4
Patterns of Platelet Destruction in Nonsplenectomized AITP Patients*

	Increased spleen only	Increased liver only	Both increased	Normal liver and spleen
Primary AITP				
Untreated (11)†	8	1	0	2
Prednisone (6)	6	0	0	0
Secondary AITP				
Untreated (4)	3	0	1	0
Prednisone (2)	2	0	0	0
All AITP	19 (83%)	1 (4%)	1 (4%)	2 (9%)

* Abnormal values were defined as exceeding the 95% confidence limits of the values in normal subjects.
† Number of studies.

Splenectomized Subjects

Three patients with primary AITP and two patients with secondary AITP were classified as splenectomy responders and five patients were nonresponders. Both postsplenectomy patients with secondary AITP were responders. The five responders had normal platelet survivals and turnovers while the five nonresponders had reduced survivals and turnovers (both $p < 0.01$) (Table 5). An even greater separation between responders and nonresponders was apparent in the amount of liver platelet sequestration. Liver uptake was

TABLE 5
Platelet Kinetic and Localization Data in Splenectomized Subjects

	Platelet count × 10^9/l	Recovery (%)	Survival (days)	Turnover (platelets × 10^9/l/day)	Liver activity (% whole body) 2 hr	72 hr	Final
Normal subjects (3)*	402 ± 143	99 ± 1	8.2 ± 1.7	47 ± 20	19 ± 5	26 ± 2	29 ± 4
Splenectomy successes†							
Primary AITP (3)	567 ± 127	100 ± 0	9.6 ± 4.3	42 ± 6	12 ± 0	26 ± 4	26 ± 4
Secondary AITP (2)‡	318, 363	67, 54	8.8, 8.4	48, 76	21, 26	30, 32	32, 38
All primary and secondary AITP (5)	476 ± 154	84 ± 22	9.1 ± 3.1	50 ± 15	18 ± 7	25 ± 6	29 ± 7
Splenectomy failures							
Primary AITP (5)	73 ± 67	77 ± 28	2.6 ± 0.9	30 ± 16	36 ± 12	51 ± 4	54 ± 4

* Number of studies
† Splenectomy successes were patients with platelet counts > 170 × 10^9/l and failures had platelet counts ≤ 170 × 10^9/l.
‡ Individual values are given for the two patients in this group.

increased (range 22–55%) above normal in all AITP patients following splenectomy. However, the five responders had relatively lower values (mean 29 ± 7%, range 22–38%) compared to the nonresponders. All nonresponders showed liver uptakes of 45% or greater (mean 54 ± 4%, range 45–55%, $p < 0.001$ vs. responders). Three postsplenectomy patients had accessory spleens detected by ^{111}In platelet scanning; one was a splenectomy success and two were failures. However, the accessory spleens did not account for more than 2% of total platelet destruction in either of the splenectomy failures.

Relationship Between Platelet Count, Kinetic and Localization Data

Among all 33 primary and secondary AITP studies, the platelet count was inversely correlated with liver and spleen platelet sequestration ($r = 0.75$, $p < 0.001$) (Fig. 1). Platelet survival was also inversely related to the liver and spleen uptake ($r = 0.69$, $p < 0.001$) (Fig. 2). The platelet count was directly related to the platelet survival ($r = 0.86$, $p < 0.001$). The direct relationship between platelet count and platelet survival was apparent in all three AITP subgroups (untreated, prednisone, postsplenectomy). In contrast, the relationship between platelet count and platelet sequestration was apparent only for the splenectomized patients. There was no significant difference in these relationships between patients with primary or secondary AITP. Neither the platelet count nor platelet survival was related to the spleen/liver ratio in the total AITP group or within subgroups. Furthermore, there was no relationship between platelet survival and platelet turnover or platelet recovery.

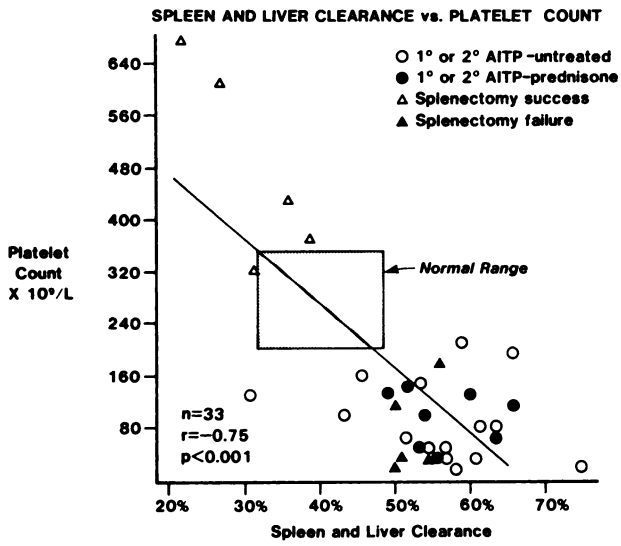

FIGURE 1
There was a significant inverse relationship between spleen plus liver platelet destruction and the platelet count ($r = 0.75$). The hatched areas represent the range seen in the normal subjects.

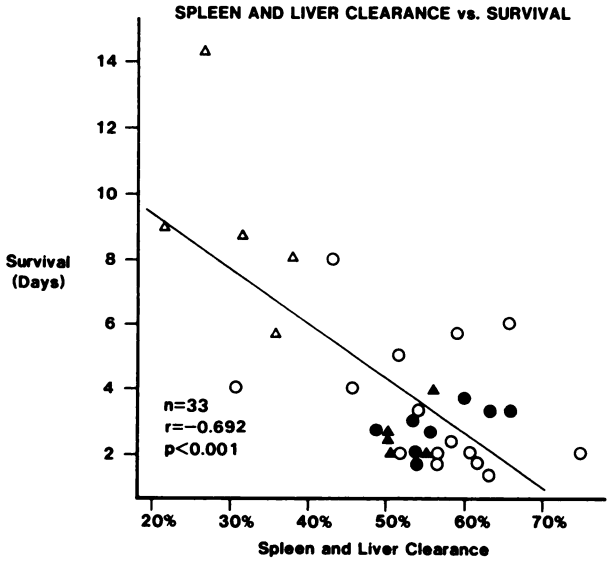

FIGURE 2
Spleen plus liver destruction also was inversely related to the platelet survival ($r = 0.69$). The symbols are the same as for Figure 1.

To better examine the relative contributions of several factors (survival, turnover, and liver plus spleen sequestration) on the platelet count, we performed stepwise multiple linear regression and partial correlations in patients with primary and secondary AITP ($n = 33$). In this analysis, total liver plus spleen sequestration, platelet survival, and platelet turnover all contributed significantly to the platelet count. The importance of the total liver and spleen sequestration was also shown by the fact that only liver plus spleen sequestration entered the model as a significant predictor for platelet survival.

DISCUSSION

Sites of Platelet Destruction in AITP

Following the injection of ^{111}In-labeled autologous platelets, serial gamma camera images were obtained to quantitate platelet uptake in the liver and spleen as a percentage of total-body platelet activity. Early activity results largely from platelets present in the circulating blood volume in the liver and spleen or in the exchangeable splenic pool (*19–23*). Patients with AITP and normal subjects had similar findings at the 2-hr imaging time, but by 72 hr AITP patients had increased splenic uptake. At later time points beyond the platelet survival time (designated as the final imaging time in this study) radioactivity measurements largely reflect platelet destruction by the spleen or liver, as virtually all viable platelets in either the circulating or exchangeable pools would be destroyed. The validity of the ^{111}In measurements of platelet destruction in the spleen and liver

depends on the fact that these organs retain the ^{111}In activity after platelet destruction. Peters et al. (*10*) documented that there was minimal elution of ^{111}In from the spleen over an 8-day period once uptake occurred; minimal change occurred in liver activity as well. Thus, it is reasonable to conclude that measurements of liver or spleen activity made at or beyond the platelet survival time closely represent the labeled platelets that were destroyed in these organs.

In normal individuals, spleen and liver uptake at the end of the normal platelet lifespan accounted for 40 ± 4% of the total platelet activity. The remaining 60% of the platelets were presumably removed in the reticuloendothelial system of the bone marrow. However, such uptake cannot be accurately quantitated with platelet imaging techniques because of the diffuseness of the bone marrow reticuloendothelial system and the resulting low gamma counting rates.

Using strictly defined normal ranges based on 95% confidence limits for spleen and liver uptake in the normal subjects, 83% of our nonsplenectomized patients with primary or secondary AITP had increased splenic uptake without increased liver uptake, one (4%) had only increased liver uptake, and two (9%) had no evidence of increased uptake in the liver or spleen suggesting predominant marrow reticuloendothelial uptake. Thus our data strongly indicate that increased splenic platelet destruction is the most frequent abnormality in either primary or secondary AITP. Both increased destruction as well as depressed platelet production contribute to the thrombocytopenia of AITP (*24*). The increased splenic destruction of platelets may explain, in part, why splenectomy usually improves the platelet count in this condition.

Using variable criteria, other smaller studies with ^{111}In-labeled platelets have similarly shown that most AITP patients have increased splenic uptake while a few patients have demonstrated increased liver and spleen uptake or diffuse reticuloendothelial destruction (*7–9,25*). In addition, Heyns et al. in a study of ten AITP patients, found half to have predominant liver sequestration, as defined by a ratio of spleen to liver activity of < 1.4 (*9*). This subgroup was characterized by lower platelet counts and shorter platelet survivals than the patients with predominantly splenic sequestration. We were unable to confirm these findings in our patient population as only two of 23 patients showed increased liver uptake. These discrepant results may relate primarily to differences in patient populations; Heyns et al. evaluated a subgroup with lower platelet counts than we studied. Our results, therefore, may not apply to patients with more severe thrombocytopenia. In addition, two of their five subjects with predominantly liver destruction had very low platelet recoveries (15% and 16%), which we did not observe in any patient.

Prednisone Effects

The pattern of platelet removal in the primary and secondary AITP patients receiving prednisone did not differ from that seen in untreated patients. Furthermore, there was no difference in the spleen/liver ratio or in the platelet survival between the treated and untreated groups. In addition, in the two patients who had serial studies prior to and while receiving prednisone, both had increases in the platelet count which were not associated with improvement in platelet survival, only an increase in turnover. Thus, our findings do not support the concept that prednisone therapy is equivalent to a "medical splenectomy" (*26*). Our data is more consistent with the hypothesis that steroids promote effective thrombopoiesis, that could be achieved if prednisone prevented marrow removal of a damaged population of platelets. Unfortunately, this potential effect of prednisone on the marrow cannot be directly evaluated by platelet imaging. Our conclusions regarding the potential effects of steroids are tempered by the small number of observations and the need for pre- and post-treatment measurements in the same patients.

Platelet Removal in Secondary AITP and Myelodysplastic Syndromes

The group designated as having secondary AITP was clinically heterogeneous. Nevertheless, all had relatively clear evidence of immune-mediated thrombocytopenia as reflected by anti-platelet antibodies in association with normal to increased bone marrow megakaryocytes. Despite the clinical heterogeneity, the pattern of platelet destruction noted was nearly identical to that seen in patients with primary AITP. These findings suggest that similar mechanisms of platelet destruction are operative in both of these immune mediated thrombocytepenias.

The small control group of thrombocytopenic patients with myelodysplastic syndromes was also clinically heterogeneous. However, common to all of these patients was evidence of underproduction of viable platelets resulting from a bone marrow defect which involved other cell lines in addition to platelets. The pattern of platelet removal in the liver and spleen in these patients was strikingly different from that seen in patients with AITP and closely resembled the pattern seen in normal subjects, even in the three patients who had increased levels of anti-platelet antibodies. Furthermore, the reduced platelet survivals in this group were consistent with the degree of thrombocytopenia (*27*). This data suggests that antibodies in such patients may not be of clinical importance to platelet removal mechanisms. The findings in the myelodysplastic patients further underscore the uniqueness of the increased splenic destruction seen in patients with anti-platelet antibodies and primary or secondary AITP.

Postsplenectomy Results

Quantitative ^{111}In platelet localization studies in patients with AITP following splenectomy have not been previously reported. We studied a relatively high proportion of splenectomy failures due to the referral sources of our patients. We noted a striking increase in the magnitude of liver platelet uptake in the five splenectomy nonresponders (54 ± 4%) compared to the responders (29 ± 7%). Liver uptake in excess of 45% was noted in all splenectomy failures, but in none of the splenectomy responders. Our results emphasize that splenectomy success is not simply because of removal of the splenic platelet "filter" as suggested by Aster (*1*) since we encountered several patients with no evidence of residual splenic tissue who were splenectomy failures.

Without pre- and postsplenectomy data in the same patients, we could not determine what factors might predict splenectomy response. Whether platelet localization studies can predict response needs to be readdressed using the superior imaging characteristics of ^{111}In. Prior studies using ^{51}Cr-labeled platelets have been conflicting, with some studies suggesting that platelet uptake measurements predict splenectomy response (*28–31*) while other studies were negative (*32–35*).

Of interest was the finding of accessory spleens in three of our subjects studied postsplenectomy. Davis et al. (*36*) also described the visual detection of accessory spleens by platelet imaging without quantitative uptake data in two patients with AITP who failed splenectomy; neither patient responded to removal of the accessory spleen. In our patients, an accessory spleen accounted for only 0.8% and 1.3% of platelet destruction in two splenectomy failures and 6% in one splenectomy success. The pathophysiologic importance of accessory spleens in AITP is uncertain. Our data suggests that increased platelet destruction in the accessory spleen cannot account for the persistent thrombocytopenia. We cannot exclude continued platelet antibody production in the accessory spleen as a factor.

All subjects studied after splenectomy exhibited liver sequestration that was greater than that seen in any normal nonsplenectomized subject. The increase in hepatic uptake in the three normal asplenic subjects (29 ± 4%) was the same as that observed in AITP splenectomy responders (29 ± 7%) while AITP splenectomy nonresponders had markedly greater hepatic uptake (54 ± 4%). These data differ from the findings of Heyns et al. (*37*) who noted no increase in hepatic destruction of platelets in normal asplenic subjects compared to normal subjects with spleens.

Determinants of the Platelet Count in AITP

Our data document a relatively close inverse relationship ($r = -0.75$) between the total liver and spleen platelet uptake and the platelet count in patients with primary or secondary AITP suggesting that the absolute quantity of platelets destroyed in these organs is an important determinant of the platelet count. Additionally, the total liver and spleen platelet uptake correlated inversely with platelet survival ($r = 0.69$), further documenting the importance of liver and spleen platelet destruction in the thrombocytopenia of patients with AITP. The relative contribution of the spleen compared to the liver in platelet destruction appeared unimportant since there was no correlation of the spleen/liver ratio to either the platelet count or platelet survival. The importance of the liver and spleen platelet destruction was further upheld in the stepwise multiple linear regression analysis. Three factors—platelet uptake in the spleen and liver, platelet survival, and platelet turnover—accounted for 90% of the variability in platelet count in AITP patients.

SUMMARY

Our data document increased splenic destruction in nearly all patients with either primary or secondary AITP. Platelet destruction patterns were remarkably similar in primary and secondary AITP and did not appear to be influenced by prednisone therapy. Among all AITP patients, the total of liver and spleen platelet destruction has a direct relationship with both platelet survival and the platelet count. Whether the increased spleen and liver destruction is due to an immune mediated shift in the normal pattern of platelet destruction or is because of other mechanisms remains to be resolved. Following splenectomy, patients with AITP have increased amounts of liver destruction of platelets, with patients who fail splenectomy demonstrating a particularly marked increase in hepatic destruction.

ACKNOWLEDGMENT

This work was supported by the Medical Research Service of the Veterans Administration and by NHLBI Grant HL31641.

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