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Detection of Accessory Spleens With Indium 111-Labeled Autologous Platelets

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In two patients with recurrent immune thrombocytopenia, accessory splenic tissue was demonstrated by radionuclide imaging following administration of indium 111-labeled autologous platelets. In one of these patients, no accessory splenic tissue was seen on images obtained with technetium 99m sulfur colloid. This new technique provides a simple means for demonstrating accessory spleens and simultaneously evaluating the life-span of autologous platelets.

Key words: accessory spleen, immune thrombocytopenia, platelet survival, radionuclide imaging

INTRODUCTION

The role of accessory spleens in the relapse of immune thrombocytopenic purpura (ITP) after splenectomy has been reviewed recently [1]. The exact prevalence and pathophysiologic significance of residual splenic tissue in patients with ITP remains uncertain. However, accessory splenectomy has resulted in remission of ITP or in a decreased requirement for steroids and immunosuppressive drugs in some patients [2–5].

The presence of accessory splenic tissue is suspected when a delayed relapse of ITP occurs following an initially satisfactory response to splenectomy. The absence of Howell-Jolly bodies in circulating erythrocytes also suggests that functioning splenic tissue is present [6, 7]. Usually, this suspicion may be confirmed and the accessory splenic tissue may be localized by radionuclide imaging with technetium 99m sulfur colloid. However, accessory spleens are not always visualized by this imaging method [8] and may not remove all Howell-Jolly bodies [9]. Therefore, neither technique can provide conclusive proof of asplenia.

We have recently studied two patients in whom ITP had relapsed following splenectomy. In both patients, accessory splenic tissue was localized by imaging with indium 111-labeled autologous platelets.

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CASE REPORTS

Case 1

A 44-year-old woman had developed Hodgkin disease in 1952 at age 18. She was treated with mantle radiotherapy and since had shown no evidence of recurrent disease.

In 1965, at age 31, she developed multiple petechiae. Her platelet count was 10,000/μl, and a bone marrow aspirate showed megakaryocytic hyperplasia. ITP was diagnosed. Because of a poor response to corticosteroid therapy, splenectomy was performed. There was transient improvement, but then her platelet count again decreased and did not respond to high doses of corticosteroids. Azathioprine therapy did lead to remission, and except for a brief relapse, which was successfully managed with azathioprine, she was well for 12 years.

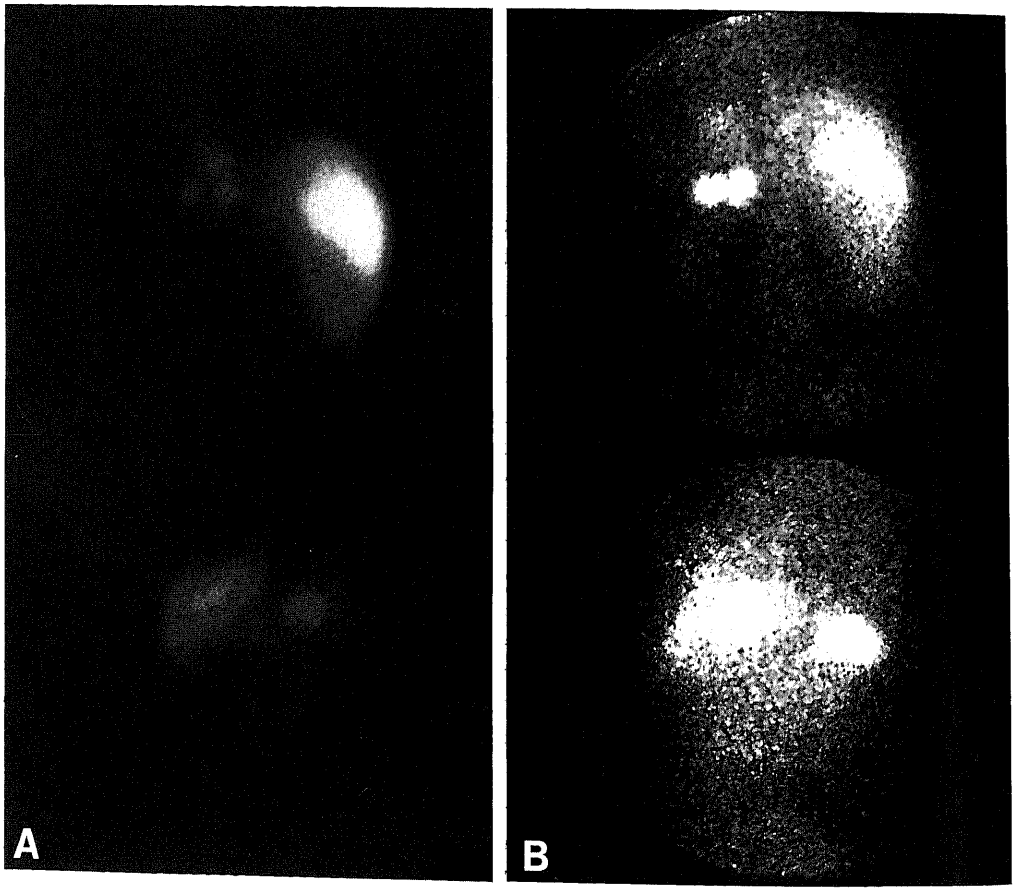

Fig. 1. Patient 1. Posterior and left lateral images of the ^{99m}Tc sulfur colloid scan (A) demonstrate two accessory spleens. These are confirmed on the corresponding images obtained with ^{111}In-labeled autologous platelets (B). The platelet imaging study was performed nine days after the liver spleen scan. The images shown were obtained 52 hours after administration of labeled platelets, but the accessory spleens also were seen on four-hour and 20-hour images.

In December 1977 thrombocytopenia recurred and was unresponsive to trials of prednisone, azathioprine, vincristine, and cyclophosphamide. Her peripheral blood smear at this time showed six Howell-Jolly bodies per 1,000 erythrocytes. Liver and spleen images obtained with ^{99m}Tc sulfur colloid suggested that two accessory spleens were present (Fig. 1A). Imaging with ^{111}In-labeled autologous platelets clearly demonstrated these to be sites of platelet localization (Fig. 1B). Two accessory spleens, weighing 9.6 and 8.5 gm, respectively, were subsequently removed. Both showed hilar arterial blood supplies and hyperplastic splenic tissue, with many foamy and non-foamy histiocytes replacing the lymphoid tissue.

She recovered from surgery uneventfully and, while on prednisone therapy (100 mg per day), her platelet count rose to a maximum of 285,000/μl on the fifth postoperative day but then fell. Cyclophosphamide was reinstituted without response and was discontinued.

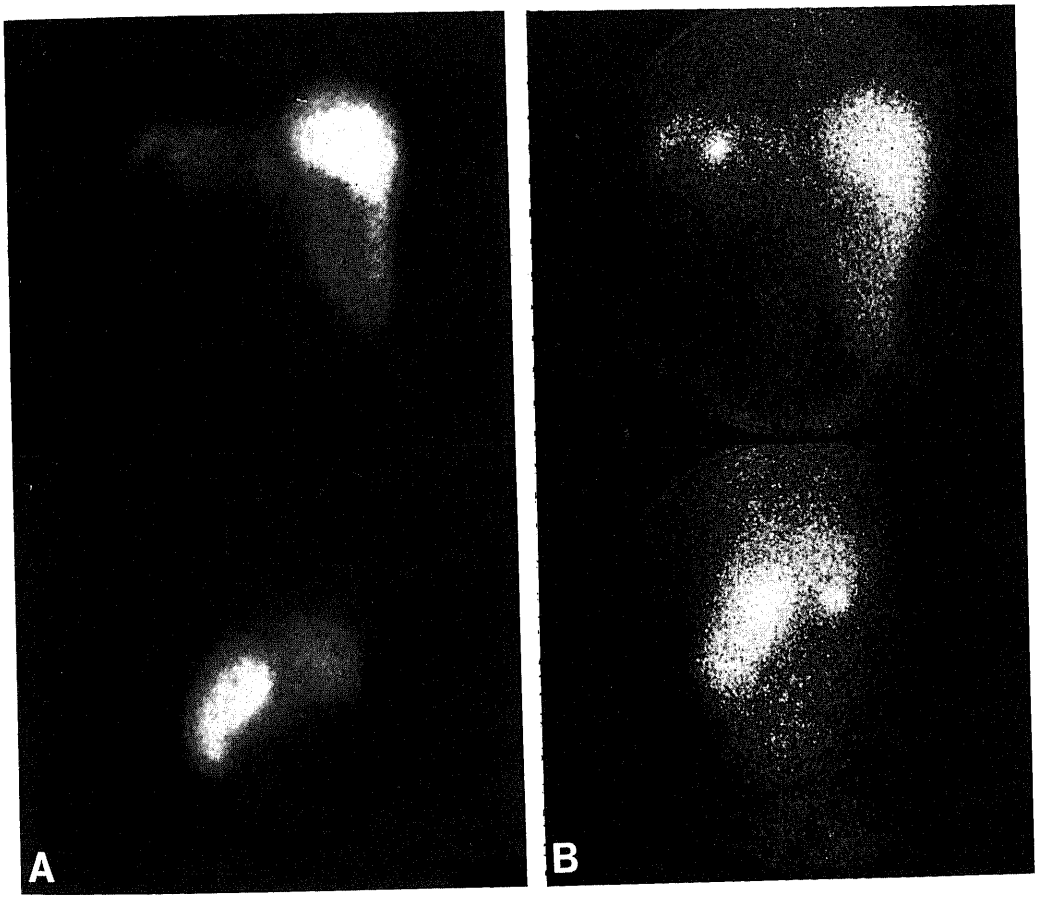

Fig. 2. Patient 2. Posterior and left lateral images of the ^{99m}Tc sulfur colloid scan (A) show no splenic tissue. The corresponding images obtained three days later with ^{111}In-labeled platelets (B) demonstrate a small accessory spleen posterior to the left lobe of the liver. The images shown were obtained 45 hours after administration of labeled platelets, but the accessory splenic tissue also was seen on two-hour, four-hour, and 20-hour images.

Prednisone was decreased to 30 mg per day, and the patient was given vinblastine-loaded platelets [10]. Subsequently, there was a progressive increase in the platelet count to the most recent level of 64,000/μl, despite discontinuation of prednisone.

Case 2

A 28-year-old woman was found to have ITP at age 18 in 1969. Her platelet count was 10,000/μl, and megakaryocytic hyperplasia was seen in her bone marrow. There was an initial response to steroids followed by an early relapse necessitating splenectomy within one month of diagnosis. The platelet count rose to 160,000/μl in ten days, but then fell again. Her thrombocytopenia was controlled after several months of therapy with prednisone and azathioprine, and she remained well for six years.

In September 1977, ITP recurred. Following a transient response to steroid therapy, azathioprine was added without benefit. In June 1978 she had severe thrombocytopenia and bleeding despite treatment with large doses of prednisone and azathioprine. Howell-Jolly bodies were absent from the peripheral smear. A ^{99m}Tc sulfur colloid scintigram did not demonstrate splenic tissue (Fig. 2A). However, images obtained with ^{111}In-labeled autologous platelets showed a small accessory spleen in the left upper quadrant (Fig. 2B). She underwent removal of a splenunculus, which measured 1.0 × 1.5 cm and weighed 1.2 gm. On histological examination, the specimen exhibited hilar arterial blood supply and hyperplastic splenic tissue, with many reactive germinal centers and abundant immunoblasts in the red pulp.

After surgery, her platelet count increased to 290,000/μl on the ninth postoperative day and remained elevated for nearly three months. However, when the steroid dose was reduced her platelet count fell suddenly to 7,000 /μl. Reinstitution of high-dose steroid therapy had no beneficial effect. Vinblastine-loaded platelets were given, and subsequently her platelet count increased (most recently to 82,000/μl while she was receiving 20 mg of prednisone every other day).

METHODS

The studies with ^{111}In-labeled platelets were performed after written, informed consent had been obtained from each patient. Autologous platelets, harvested from approximately 85 ml of each patient's blood, were labeled with ^{111}In as we have recently described [11]. The fractional labeling efficiency was determined by assaying the activity of the final labeled platelet suspension and the wash solutions in an ionization chamber.

After the labeled platelets were injected into the patients, serial 2 ml blood samples were obtained at five-minute intervals for 15 minutes, 15-minute intervals for two hours, at four, 24, 28, 44, and 52 hours, and then daily for up to five days. One milliliter aliquots of each sample, together with a standard prepared from the original injectate, were counted in a NaI crystal, well-type, scintillation detector. The remainder of each sample was centrifuged in an Eppendorf mini-centrifuge at 18,000g for two minutes. Plasma and cell fractions were separated and counted to determine the cell-associated proportion of whole blood activity. The recovery of labeled platelets in the circulating blood was calculated from the estimated blood volume and the activity/ml of each sample. Mean platelet life-span and initial recovery were calculated from the weighted mean of the linear and semilogarithmic least-squares estimates [12].

Several additional blood samples were obtained from the second patient at varying times for separation of erythrocytes, leukocytes, and platelets on density gradients. The individual cell fractions were counted to assess relative activity.

Images of the chest and abdomen were obtained with a large-field-of-view scintillation camera fitted with a medium-energy collimator. The camera spectrometer was set to detect both photopeaks of ^{111}In. Both patients also underwent conventional liver-spleen scintigraphy with ^{99m}Tc sulfur colloid.

RESULTS

Conventional ^{99m}Tc sulfur colloid images demonstrated two accessory spleens in the first patient but did not demonstrate splenic tissue in the second patient (figs. 1A and 2A). The images obtained with ^{111}In-labeled autologous platelets clearly showed the accessory spleens in both subjects (Figs. 1B and 2B).

The first patient's platelet count was 31,000/μl on the day of the study; 24% of these platelets were recovered in the platelet-rich plasma obtained from 86 ml of blood. The labeled platelet suspension contained 148 μCi of ^{111}In associated with 6.3×10^8 platelets and 1.0×10^7 contaminating leukocytes. The labeling efficiency was 9.3%. The estimated initial recovery of labeled platelets in the circulation was 85%, and the platelet life-span was 6.0 days. Seventy hours after beginning the study, the two accessory spleens were removed and counted. They contained 8.5% of the total injected activity; the ratio of splenic activity to that in blood at the same time was 33:1.

The platelet count of the second patient on the day of the study was 29,000/μl. The injectate contained 390 μCi of ^{111}In associated with 1.9×10^9 platelets (representing 76% of the original platelets) and 8.3×10^6 contaminating leukocytes. Less than 3% of the injected activity was erythrocyte associated. Labeling efficiency was 45%. The initial platelet recovery was 56%, and the life-span was 4.3 days. The erythrocyte-associated activity did not exceed 2% in any sample. The accessory spleen contained 0.4% of the injected dose; the spleen/blood activity ratio was 82:1.

DISCUSSION

Relapse of ITP following an initial response to splenectomy is occasionally associated with hypertrophied accessory splenic tissue. Removal of an accessory spleen has been reported to result in remission of ITP or in amelioration of its severity [1–4]. The presence of Howell-Jolly bodies on the peripheral smear has, with occasional exceptions [9], been considered proof of asplenia. However, in our first patient, this index of the "pitting" function of the spleen was misleading and, although many Howell-Jolly bodies were seen, two accessory spleens were detected by both ^{99m}Tc sulfur colloid and ^{111}In platelet imaging.

The ^{99m}Tc sulfur colloid scan is usually considered an adequate method to detect accessory splenic tissue. However, in our second patient, the ^{111}In platelet images detected a small accessory spleen that was not visualized by ^{99m}Tc sulfur colloid scintigraphy, most likely because of the overlapping activity in the left lobe of the liver.

In comparison to ^{99m}Tc sulfur colloid, ^{111}In-labeled platelets are theoretically superior for localizing small islands of functioning splenic tissue. In dogs, the spleen/liver activity ratio achieved with ^{111}In platelets averaged 2.3:1 [13], whereas with ^{99m}Tc sulfur colloid, a much lower ratio (0.86:1) is obtained [14]. Active platelet sequestration by the spleen

in patients with ITP would be expected to further increase the spleen/liver activity ratio and thus might further improve the ability to visualize small accessory spleens.

The use of ^{111}In-labeled platelets also permits the evaluation of autologous platelet kinetics concurrently with imaging studies. In both of our patients, with life-span of autologous platelets was shortened compared to that previously observed in normal subjects [11]. However, the survival of autologous platelets was surprisingly long when compared to results obtained by others in patients with ITP using ^{51}Cr-labeled homologous platelets [15–17]. Our results support the suggestion of Baldini [18] that circulating platelets in patients with severe ITP may be more resistant to injury by antibody than are homologous transfused cells.

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