Shortened Platelet Survival as a Cause of Thrombocytopenia in Mice with L1210 Leukemia

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ABSTRACT

Thrombocytopenia is a frequent complication of acute leukemias of humans and animals. To define the possible causes of this decrease in platelets, we have studied platelet kinetics in mice after transplantation of 10^6 ascites cells from mice bearing L1210 leukemia. The circulating half-time of 51Cr-labeled platelets was reduced to approximately one-half that of controls when studied 1 or 3 days posttransplantation. Recovery of transfused 51Cr-labeled platelets was reduced to approximately one-half that in controls when studied 3 days after introduction of L1210 cells. Megakaryocyte concentration showed no change during the 5-day survival after i.v. infusion of leukemic cells but was increased on Day 5 after i.p. inoculation with an average host survival of 7 days. Megakaryocyte diameter distributions were significantly shifted toward larger sizes beginning on Day 2 after i.v. inoculation and on Day 3 after i.p. inoculation. Twenty-four-hr [3H]thymidine labeling indices of megakaryocytes were significantly increased beginning on Day 3 after i.v. inoculation but were significantly decreased on Days 5 and 6 after i.p. introduction of L1210 cells. We conclude that the decrease in platelets in mice transplanted with L1210 leukemia results primarily from shortened platelet survival and organ pooling. Megakaryocytes remain normal in concentration but increase in size, a usual response to decreases in platelet count.

INTRODUCTION

Decreases in blood cells are frequently associated with acute leukemias of humans and animals. Law et al. (11) described a decrease in platelet counts and hemoglobin concentration of DBA mice inoculated with L1210 leukemia. We confirmed the finding of thrombocytopenia after L1210 inoculation and, in addition, found platelet depression with L1210 cells placed in diffusion chambers in the peritoneal cavity of mice (8). However, the changes in platelet kinetics which result in this thrombocytopenia had not been elucidated. In this paper, we describe studies of megakaryocytopoiesis and platelet survival in mice inoculated with L1210 leukemia.

MATERIALS AND METHODS

Female C57BL6 X DBA/2 (hereafter called BD2F1) mice weighing 14 to 22 g were obtained from The Jackson Laboratories, Bar Harbor, Maine. Platelets and WBC were counted by phase microscopy. Packed cell volumes were determined by a micromethod.

Tumor Cells. L1210 ascites from DBA/2 mice were used for inocula. The L1210 cells were maintained by weekly passage in DBA/2 mice by Dr. Thomas Avery of this institution. Cells were diluted to the desired concentration with Earle's basal salt solution, and 10^6 cells were injected either i.v. via a tail vein or i.p.

Collection of Blood Samples. Blood was collected from the retroorbital plexus for determination of blood cell concentrations. Blood for platelet and WBC determination was collected using the Unopette system (Becton-Dickinson Immunodiagnostics).

Determination of Platelet Survival. Platelet survival was estimated using 51Cr. Platelets were obtained by differential centrifugation from whole blood (19 or 20 ml) collected from female BD2F1 mice into acid citrate via cardiac puncture. In each experiment, the platelets were resuspended in Hanks' balanced salt solution without calcium and magnesium, and incubated with 500 or 250 μCi of Na251CrO4 at room temperature for 45 min. The platelets were then pelleted and washed twice with mouse plasma diluted approximately 1:1 with the Hanks' balanced salt solution described above and resuspended in the plasma-Hanks' solution. Each recipient mouse received 0.1 ml of 51Cr platelet suspension via a tail vein. 51Cr platelets were transfused on Day 1 (1.357 x 10^5 cpm) or Day 3 (5.975 x 10^5 cpm) after i.v. inoculation of 10^6 L1210 cells. Blood samples (10 μl) for radioactivity determination were obtained by puncturing a tail vein with a 27-gauge needle at 1 or 2 hr and 1, 2, 3, and 4 days after transfusion. The mice were warmed under an examination lamp to cause vasodilation approximately 10 min before each sampling. The blood was collected using 10-μl Unopette pipets and diluted in a Unopette reservoir containing 0.99 ml of 1% ammonium oxalate. The Unopette reservoir was assayed for radioactivity in a Packard gamma scintillation spectrometer. 51Cr bound to contaminating erythrocytes accounted for 3.5% of the radioactivity in the labeled platelet suspension in both studies. To construct platelet survival curves, the circulating radioactivity was expressed as a percentage of the initial count (1 to 2 hr). The proportion of the platelet radioactivity injected which was circulating at 1 to 2 hr, referred to as platelet recovery, was also calculated. A blood volume of 6% of body weight was used to calculate the total circulating radioactivity.

Bone Marrow Megakaryocyte Concentration. The average number of megakaryocytes per high-power field (x500) was determined on 5-μm sections of sternal marrow that had been fixed in Bouin's fixative, decalcified, and stained with hematoxylin and eosin. One entire longitudinal section was evaluated for each mouse. Since bone marrow megakaryocyte concentration determined in this manner is a reflection of megakaryo-
cyte size, as well as absolute number (9), megakaryocyte diameters were measured on the same marrow sections. The megakaryocyte concentrations of the L1210-inoculated mice were corrected for changes in average megakaryocyte diameter induced by the leukemia. The concentrations of megakaryocytes from L1210 mice were divided by the quotient of average megakaryocyte diameter of L1210-bearing mice divided by the average megakaryocyte diameter of control mice.

Megakaryocyte Diameter. Megakaryocyte diameters were measured using an eyepiece micrometer at a magnification of ×1250. The diameter was expressed as the square root of the product of 2 measurements made at right angles. Fifty megakaryocytes in each of the marrow sections described above were measured. Frequency distributions of the diameters for each group were constructed and compared using a 2-sample rank test.

Tritiated Thymidine Labeling Indices of Bone Marrow Megakaryocytes. Mice were given [3H]thymidine (1 μCi/g body weight; specific activity, 1.9 Ci/mmol) (Becton-Dickinson Immunodiagnostics) i.p. 24 hr prior to sacrifice. Autoradiograms were made on a longitudinal sternal marrow section prepared as above by dipping the sections in Kodak NTB-2 emulsion, exposing them in light-tight boxes at 4°C for 40 days, developing them in Kodak D-19 developer, and staining them with hematoxylin and eosin. The numbers of labeled and unlabeled megakaryocytes in one section from each mouse were determined, and the labeled cells were expressed as a percentage of the total megakaryocytes.

Statistical Analysis. Means were analyzed using Student's t test.

RESULTS

Inoculation of 10⁶ ascites cells from L1210-bearing mice i.v. or i.p. produced a marked drop in blood platelet concentration first noted on Day 2. The nadir, platelet count approximately 10% of control, occurred on Day 4 (Chart 1). The platelet counts of the i.v.-inoculated mice remained low on Day 5. The average survival with the i.v. route of inoculation was 5 days. The platelets of the i.p. inoculated group showed a slight recovery between Days 4 and 7. Seven days was the average survival with the i.p. route of inoculation.

Platelet Recovery. Mice inoculated with L1210 1 day previously demonstrated 13% less circulating 51Cr-labeled platelet radioactivity at 2 hr after platelet transfusion compared to controls (p < 0.02) (Chart 2). The initial (1-hr) circulating radioactivity level in mice inoculated 3 days previously with L1210 averaged 43% less than controls.

Platelet Survival. The disappearance rate of the radioactivity circulating at 1 to 2 hr was twice as fast in the L1210-bearing mice with the circulating half-time being 22 and 20 hr, respectively, in the mice inoculated 1 and 3 days previously with L1210 cells, compared to 40 and 44 hr in the controls (Chart 2).

Megakaryocyte Concentration. Megakaryocyte concentration was significantly increased on Day 5 (p < 0.01) in the i.p. inoculated group but had returned to control levels by Day 6 (Chart 3). Although some mice in the i.v.-inoculated group showed increased megakaryocytes, the means of the i.v.-inoculated mice and controls did not differ.

Megakaryocyte Diameter. The megakaryocyte diameter distributions are shown in Chart 4. The diameter distributions were shifted toward larger sizes from Day 2 (p < 0.02) and Day 3 (p < 0.01), respectively, in the i.v.- and i.p.-inoculated groups. The largest increase in average diameter (21%) occurred on Day 5 in the i.p.-inoculated group. The average diameter was increased 10 to 12% on Days 3 to 5 in the i.v.-inoculated group.

Tritiated Thymidine Labeling Indices of Megakaryocytes. Tritiated thymidine labeling indices of bone marrow megakaryocytes were significantly increased from Day 3 in the i.v.-inoculated group (Table 1). Labeling indices of i.p.-inoculated mice remained within the control range, except for Days 5 and
Chart 3. Megakaryocyte concentration after inoculation of 10⁶ L1210 ascites cells. ■, no treatment controls (17 mice); □, L1210 inoculated i.v. (4 mice/interval except 3 on Day 3); ●, L1210 inoculated i.p. (3 mice/interval). Bars, S.E.

DISCUSSION

Inoculation of mice with L1210 leukemia produced marked thrombocytopenia within 4 days, as reported in earlier studies (8, 11). Although the nadir was lower after i.v. inoculation, mice inoculated by either i.p. or i.v. routes showed similar declines in platelet concentration.

The initial recovery of transfused ⁵¹Cr-labeled platelet radioactivity in leukemic mice differed significantly compared to controls but also with time after inoculation of leukemic cells. Organ pooling is the most likely explanation for decreased platelet recovery, especially in the Day 3 leukemic mice since: (a) splenomegaly and hepatomegaly are apparent by this time; and (b) the disappearance rate of ⁵¹Cr-labeled platelets in these mice was the same as that in Day 1 leukemic mice.

The circulation of ⁵¹Cr-labeled platelets in leukemic mice was reduced to 0.5 that of controls when ⁵¹Cr-labeled platelets were decreased to one-half and four-fifths of control, respectively.

Chart 4. Megakaryocyte diameter distributions after inoculation of 10⁶ L1210 ascites cells. ■, L1210 inoculated i.p.; □, L1210 inoculated i.v.; ●, no treatment controls. Each curve represents diameters of 100 to 200 megakaryocytes from 2 to 4 mice (50 megakaryocytes/mouse).
were given either 1 or 3 days after Li 2i 0 inoculation. This same disappearance rate suggests that: (a) the factors responsible for producing the shortened platelet survival are present in the same concentrations on both Days 1 and 3; (b) only a certain platelet population is affected; or (c) the removal of damaged platelets from the circulation is less efficient as the leukemia progresses. These findings of shortened platelet survival and reduced platelet recovery resemble those observed by Cowan (3) in patients with acute nonlymphocytic leukemia, in which platelet recovery was reduced and platelet survival was decreased to about one-half of control.

The mechanism responsible for shortened platelet survival in this murine leukemia is unknown. Gasic et al. reported that Li 210 cells did not cause platelet aggregation in vitro (7), although other tumor cells do (6, 7, 15). Earlier studies using mice implanted with diffusion chambers containing L1210 cells suggest that the factor(s) producing shortened platelet survival were diffusible (8).

The lack of a decrease in megakaryocyte concentration as the disease progresses indicates that differentiation of precursors into megakaryocytes is not affected. On the other hand, erythropoiesis is inhibited after inoculation of L1210 cells (2) while granulocytes are increased (11). The increase in megakaryocytes on Day 5 in the i.p.-inoculated group may result from increased precursor differentiation into megakaryocytes in response to the thrombopoietic stimuli of thrombocytopenia (10, 12), or from arrest of megakaryocyte maturation.

The shift in megakaryocyte diameter distributions toward larger sizes in the leukemic mice is probably the response to decreased platelet count since a shift toward larger megakaryocytes (5, 10, 16) with a higher level of polyplody (14, 16) occurs after induction of experimental thrombocytopenia. Similarly, the increased [3H]thymidine labeling indices of megakaryocytes beginning on Day 3 in the i.v.-inoculated group are probably also a response to thrombocytopenia and may result from more rapid transit of megakaryocytes through the maturation phase, as well as more time spent in polyplody formation (4, 12, 13). In contrast, the labeling indices of megakaryocytes in the i.p.-inoculated mice showed a marked decrease on Day 5 which may be associated with the route of [3H]thymidine injection (i.p.) and the presence of a large volume of ascites in these mice. These lower labeling indices could also reflect a block in postpolyplody development of megakaryocytes.

A difficulty with the L1210 model for studying the mechanism of decreased platelet circulation time is the short duration of survival of animals, which precludes steady-state investigation of platelet kinetics and raises the issue of the influence of morbidity on results. Although morbidity during the last day or so of survival may complicate studies, the changes in platelet recovery and survival described here occur before the leukemic process has progressed to this point.

In conclusion, transplantation of L1210 ascites cells into mice produces thrombocytopenia with shortened platelet survival and decreased platelet recovery but no reduction in megakaryocytes. Thus, shortened platelet survival and increased organ pooling constitute the basis for the thrombocytopenia of L1210 leukemia.

REFERENCES
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