Platelet Production After Administration of Antiplatelet Serum and 5-Fluorouracil

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Although 5-fluorouracil induces relatively minimal thrombocytopenia in mice compared with antiplatelet serum, the ensuing rebound thrombocytosis is much greater. It also occurs several days later. Administration of 5-fluorouracil 24 hr after antiplatelet serum suppresses the increase in platelets produced by antiplatelet serum, while the subsequent thrombocytosis is indistinguishable from that produced by 5-fluorouracil alone. It is concluded that the thrombocytosis that occurs after 5-fluorouracil originates from a class of primitive stem cells, or early megakaryocyte precursors, which are not killed by the drug. Thrombocytosis occurring after antiplatelet serum is derived from more mature precursor cells, which are sensitive to 5-fluorouracil.

There is considerable evidence that the production of platelets by megakaryocytes normally reflects the level or mass of circulating platelets. Thus, thrombocytosis produced by hypertransfusion of platelets is followed by rebound thrombocytopenia, while thrombocytopenia induced by exchange transfusion or the administration of antiplatelet serum is followed by an increased production of platelets. Changes in the replicative activity of megakaryocytes are undetectable until 18–25 hr after application of the stimulus. This time lag is thought to represent either a delay in the production of humoral substance (thrombopoietin) responsible for altered thrombopoiesis, or the necessary changes that must occur in unrecognizable megakaryocyte precursor cells, or both.

Recently, it has been found that mice recovering from the cytotoxic effects of 5-fluorouracil (150 mg/kg body weight) exhibit a marked increase in megakaryocytopoiesis in bone marrow. The appearance of increased numbers of megakaryocytes is followed by a high level of platelets in the peripheral blood 11 days after 5-fluorouracil. The degree of rebound thrombocytosis exceeds that reported after antiplatelet serum, although the latter produces a much greater depression in the circulating platelet level. This suggests a more complex system of control of platelet levels than considered hitherto.

The dose of 5-fluorouracil used in the above experiments kills proliferating hemopoietic cells for up to 2 days. If, normally, either stem cells or committed megakaryocyte precursors remain out of cycle for long periods, they might escape the lethal effects of 5-fluorouracil and thus be responsible for the enhanced megakaryocytopoiesis. We sought to distinguish which of these two cell types is responsible by using antiplatelet serum to promote replicative activity among committed megakaryocyte precursors and then administering 5-fluorouracil 24 hr later.

**MATERIALS AND METHODS**

Female mice of the C57BL/6J strain, aged about 18 wk, were used for the experiments. Fifty mice were injected intravenously, via the tail vein, with 50 µl antiplatelet serum and were then divided into 2 groups: one, comprising 28 mice, received no further injection; the other was injected intravenously with 5-fluorouracil (150 mg/kg body weight) 24 hr later. A third group of 22 mice was injected with 5-fluorouracil at the same time. A fourth group comprised uninjected controls. At times (Fig. 1), mice were bled for platelet counts, each mouse being used once only. Control mice also were bled at each time point.

Antiplatelet serum was obtained from rabbits that had been immunized by multiple serial subcutaneous and intramuscular injections of washed frozen-thawed platelets, suspended in Freund’s adjuvant, from C57BL/6J mice. Preliminary experiments indicated that 0.05 ml of antiplatelet serum was the lowest dose that produced reproducible, severe, acute thrombocytopenia (platelet counts approximately 5% of normal).

Platelet counts were carried out using phase microscopy on 20 µl blood sampled from the retroorbital plexus and diluted 1:50 in 1% ammonium oxalate.

**RESULTS AND DISCUSSION**

Thirty minutes after the administration of antiplatelet serum, the circulating platelet count was reduced to 7% of normal (Fig. 1). Rapid recovery occurred between days 2 and 5, when a maximum plateau was reached at 1.6 times normal. The platelet...
THROMBOCYTOSIS AFTER 5-FLUOROURACIL

Fig. 1. Mean peripheral blood platelet counts (±SEM) after antiplatelet serum, 5-fluorouracil, and antiplatelet serum plus 5-fluorouracil (4 mice per point except day 15 when there were 2; controls are the mean of 16 mice). Times of injection of antiplatelet serum (APS) and 5-fluorouracil (SFU) are shown.

Count returned to normal by day 12. This response is similar to those reported by others.3,5

Five-fluorouracil given 24 hr after the antiplatelet serum effectively prevented the rise in platelet counts seen with the antiplatelet serum alone, a result consistent with 5-fluorouracil killing megakaryocyte precursors that had been stimulated to proliferate by antiplatelet serum. The small elevation in the platelet count seen 4 days after 5-fluorouracil can be accounted for by platelet release from maturing megakaryocytes, which are beyond their replication phase at the time of 5-fluorouracil injection. The subsequent fall and rise in the platelet count was indistinguishable from that after 5-fluorouracil alone.

We have concluded that the later and more marked thrombocytosis, which begins about 9 days after 5-fluorouracil, does not originate from megakaryocyte precursors normally present in the bone marrow and responsive to thrombocytopenia induced by antiplatelet serum. Rather, it would appear to originate from proliferation of a class of "primitive" (pre-CFU-S) stem cells in the bone marrow that survive 5-fluorouracil and are efficient at regenerating the megakaryocyte population of a lethally irradiated host.14 The possibility also exists that the increased thrombopoiesis may derive from early committed megakaryocyte precursor cells with high proliferative potential that are generally noncycling, if this type of cell is present in the megakaryocyte pathway of differentiation. There is evidence for such a cell in the granulocytic-macrophage series.15 It remains to be established whether or not the megakaryocyte progenitors that can be grown in vitro (CFU-M16) are involved in the post-5-fluorouracil rebound thrombocytosis.

Once the cell type responsible is stimulated to proliferate and produce megakaryocytes, the normal mechanisms controlling platelet production appear to be relatively ineffective, and a marked rebound thrombocytosis occurs. Further study of the effects of 5-fluorouracil on mouse bone marrow may help to extend knowledge concerning stem cell commitment and the control of megakaryocyte precursors.

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