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The Mitotic Index of Megakaryocytes of Mice after Acute Thrombocytopenia[1] (39762)

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Megakaryocytopoiesis in mice has received relatively little attention (1), although other aspects of hematopoiesis have been extensively studied in mice. However, mice have been used by several investigators in tests of presumptive thrombocytopoietic agents. For example, it has been shown that plasma or plasma extracts from platelet-poor donors and thrombocytopoietin-rich kidney cell culture medium increase the amount of label in newly formed platelets of mice injected with radioactive tracers (2–5). Presumably thrombocytopoietic agents increase platelet production by stimulating megakaryocytopoiesis, although direct evidence is lacking. To our knowledge studies of effects on megakaryocytopoiesis of mice of treatments that stimulate or inhibit platelet production have not been made.

It has been observed in rats that one of the more marked responses of the megakaryocyte system to a demand for platelets induced by acute thrombocytopenia is an increase in the mitotic index (6). It seemed likely that mice would respond similarly and that changes in the mitotic index of megakaryocytes of mice might, therefore, be used to provide direct evidence of stimulation of platelet production through stimulation of megakaryocytopoiesis. Consequently, we examined the effects of acute thrombocytopenia on the mitotic index of mouse megakaryocytes during a period of 6 days after a single injection of antiplatelet serum. The response was similar to that observed in rats, with a peak mitotic index occurring about 32 hr after induction of thrombocytopenia.

Methods. Male C3H mice (approximately 11–12 weeks of age and weighing about 28 g) were used in these experiments. The mice were injected intraperitoneally with a single dose of antiplatelet serum (APS), which had been made in rabbits against mouse platelets and absorbed with mouse erythrocytes (7). Mice were killed at intervals, femurs were collected, and longitudinal sections were cut and stained with hematoxylin and eosin. The mitotic index (percentage of the megakaryocyte population in endomitosis) and numbers of megakaryocytes per high-power field were determined on these sections. A minimum of two longitudinal sections per mouse was examined. The photomicroscopy field is rectangular in shape and has dimensions of 236 × 152 μm. Megakaryocytes were readily distinguished from other marrow cells primarily by the sizes of both their nuclei and whole cells. The earliest cells of the megakaryocytic series, including diploid and tetraploid cells, were not included in the counted samples because they cannot be distinguished from blast cells of other hematopoietic cell lines by usual hematologic staining methods. Neither were pieces of cytoplasm that were devoid of any nuclear material included.

Platelet counts were taken before, and at intervals after, treatment. Serial platelet counts were made on additional mice similarly injected with antiserum; mice were observed for the duration of the experiments to check the response of the peripheral platelet count to the dose of antiserum used. Platelet counts were made on blood obtained from the retro-orbital sinus and were counted by the phase microscope method.

Results. The average platelet count of 219 untreated C3H male mice was 1.6×10^6 platelets/mm^3 of blood. Injection of 0.05 cm^3 of APS reduced the platelet count to approximately 5% of the pretreatment count (Figs. 1A and B). Early platelet return was slow, but the peripheral count increased rapidly after about 36 hr and reached a maximum at about 5 days. Platelet values of serially counted mice are shown in Fig. 1A and values of mice killed at inter-

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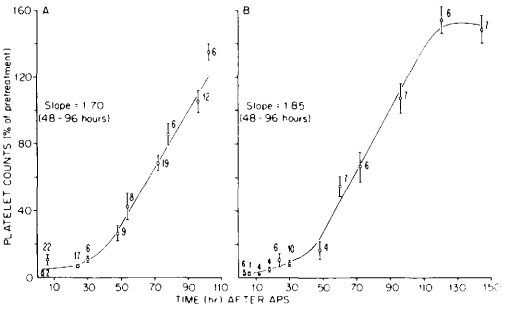

FIG. 1. (A) Serial platelet counts of mice injected ip with 0.05 cm^3 of antiplatelet serum, plotted as percentages of the pretreatment platelet count. The numbers by the symbols indicate the number of mice counted. Vertical bars indicate ± 1 SE. (B) Platelet counts of mice killed for marrow samples at intervals after ip injection of 0.05 cm^3 of antiserum.

vals for marrow samples are shown in Fig. 1B. In both figures the line between 48 and 95 hr represents a linear regression. Note the similarities in values and slopes of the two sets of data.

The mitotic indices at intervals after APS are shown in Fig. 2. The mitotic index rose to a peak around 32 hr (actual peak may occur a few hours later) after induction of thrombocytopenia and later showed a transient oscillation below normal. When compared with controls by Student's *t* test, the probability values were less than 0.001 at 24 and 32 hr, less than 0.005 at 48 and 96 hr, and less than 0.01 at 60 hr.

The number of megakaryocytes per high-power field was greater in experimentals than in controls by Student's *t* test at 32, 48, 60, and 72 hr (Table I).

Discussion. The responses of the platelet counts and of mitotic indices of megakaryocytes of C3H mice to thrombocytopenia induced with antiplatelet serum were similar to those previously described for rats (8, 9). After reduction of the platelet count to a few percent of normal, marked thrombocytopenia persisted for about 1 1/2 days followed by a period of rapid entry of platelets into the circulation. Thrombocytopenia persists while the regulatory mechanism is carrying out its function of stimulating the megakaryocyte system to produce new platelets (6, 10). New platelet production at an increased rate begins around 36 hr after antiserum injection and continues for about an additional 3 1/2 days, at which time the platelet count has exceeded the normal level. It was shown in rats that one of the steps in this response to thrombocytopenia is an increase in the endomitotic activity of megakaryocytes, which undergo additional replication of DNA with the result that the average ploidy level of the megakaryocyte population increases by about one doubling. The results presented here indicate that the same sequence of events takes place in mice and that the time relations are very similar to those observed in rats, the apogee of the endomitotic index occurring around 32–38 hr after induction of thrombocytopenia. This parameter can be used, therefore, as an indicator of stimulated megakaryocytopoiesis. The next step is to determine whether this method is sufficiently sensitive to be used as an assay for presumptive thrombocytopoietic agents.

An increase in the number of megakaryo-

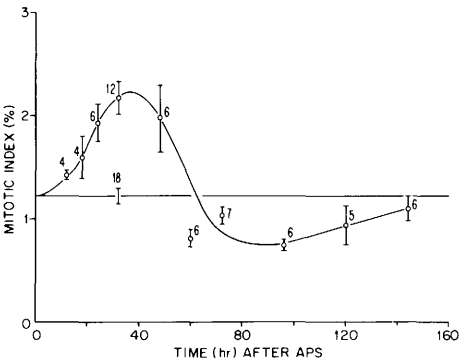

FIG. 2. Mitotic index of megakaryocytes of mice at intervals after ip injection of 0.05 cm^3 of antiplatelet serum.

TABLE I. MEGAKARYOCYTES (MKC) PER HIGH-POWER FIELD (HPF) AT INTERVALS AFTER INDUCTION OF THROMBOCYTOPENIA BY ANTIPLATELET SERUM.

Time after treatment (hr)	Number of mice	MKC/HPF (average)	SE	*P*
Control	9	3.1	0.13	–
24	3	3.1	0.07	>0.5
32	9	3.6	0.15	<0.05
48	6	3.6	0.15	<0.05
60	3	4.1	0.25	<0.005
72	4	4.0	0.22	<0.005
96	3	3.5	0.15	<0.2
120	2	3.2	0.15	>0.5

cytes was also observed. This increase may in part be only apparent and related to increases in megakaryocyte size. In addition to changes in mitosis and other parameters after stimulation of megakaryocytopoiesis, megakaryocytes also get larger (11, 12). Larger cells will appear in more sections, thereby increasing the apparent number (13). Changes both in size and in number probably contribute to the measured increase in number of megakaryocytes. No matter what the cause, there is a measurable change. However, the change in endomitotic index is more pronounced, occurs earlier, and is easier to measure, and therefore is a more satisfactory index of stimulated megakaryocytopoiesis. It is suggested that determination of the mitotic index of megakaryocytes of mice may constitute a suitable method for assay of stimulation of megakaryocytopoiesis by presumptive thrombocytopoietic substances.

Summary. C3H mice were made severely thrombocytopenic by injection of antiplatelet serum. The peripheral platelet counts and the endomitotic index and number of marrow megakaryocytes were determined at intervals during the 6 days following treatment. Both the endomitotic index and the number of megakaryocytes increased and returned to pretreatment levels during that time, indicating a transitory stimulation of megakaryocytopoiesis in response to thrombocytopenia.

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