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Stimulation of Mouse Megakaryocyte Endomitosis by Plasma from Thrombocytopenic Rats[1,2] (40431)

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In 1961 it was shown that injection of plasma or serum from platelet-poor donor rabbits (1) or rats (2) produced an increase in the peripheral platelet count of recipient animals. Subsequently, it has been demonstrated that such plasma also increases the labeling index of the circulating platelet population in rabbits (3) or rats (4, 5) injected with [^{75}Se] selenomethionine or $Na_2{}^{35}SO_4$, respectively. Other refinements and modifications of these experiments have supported the concept of a humoral agent that regulates thrombocytopoiesis (for a review see ref. 6).

All of these investigations suggest that thrombocytopenia induces an increased production and release of a thrombocytopoietic agent (thrombocytopoiesis-stimulating factor, TSF) into the blood, whose function is to stimulate megakaryocytopoiesis. Indeed, it was reported that injection of plasma from thrombocytopenic donors increased the number of megakaryocytes in the marrow of rats (7), and more recently it was found that in humans reinfusion of plasma from thrombocytopheresed donors after their recovery from thrombocytopenia resulted in an increase in immature megakaryocytes (8). In addition, treatment of mouse marrow cultures with plasma from thrombocytopenic donors stimulated maturation of megakaryocyte precursors (9). Beyond that there is little information about the direct action of TSF on megakaryocytopoiesis.

It was suggested (5), based on the effects of stimulation and suppression on megakaryocytopoiesis (10–12) and on stimulation of platelet production by plasma of platelet-poor donors (5), that TSF operates by stimulating megakaryocyte endomitosis. Consistent with this hypothesis was the finding that stimulation of megakaryocytopoiesis by induced thrombocytopenia initiates an increase in the endomitotic index of rats (12, 13) and mice (14). The endomitotic index of megakaryocytes was determined in mice injected with plasma obtained from thrombocytopenic donor rats. Endomitosis was stimulated by this plasma; this result provides additional evidence for the presence of a humoral thrombocytopoietic agent in such plasma and for the ability of the megakaryocyte cell line to respond to it.

Materials and methods. Platelet-poor plasma was obtained by making CD rats (Charles River, 3 to 4 months old, average body weight 563 g) thrombocytopenic by iv injection of 0.25 ml of rabbit anti-rat platelet antiserum. The rabbit antiserum was prepared as previously described (2), including absorption with rat red blood cells, and was kept frozen until used. Platelet counts were taken 3½ hr after antiserum injection, and only those rats whose counts were markedly reduced (counts averaged 11% of pretreatment counts) were used as donors of TSF plasma. Plasma was collected approximately 4 hr after the injection of the platelet antiserum. Both normal and thrombocytopenic rats were bled from the dorsal aorta into heparinized syringes (heparin drawn into the syringe and then flushed back out). The blood was centrifuged for 15 min at 1200*g* in 12-ml plastic tubes. The plasma was removed, pooled into 50-ml plastic tubes, and centrifuged again for 30 min at 1200*g* to remove any remaining platelets.

The plasma from donor rats was injected sc into C3H mice (10–12 weeks old, weight ~

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23 g), the total dose being divided into two equal parts given at 0 and 6 hr. Mice were exsanguinated 32 hr after the initial injection by having their cervical blood vessels severed. Their femurs were collected and fixed in Zenkerformol. Longitudinal sections about 5 μm thick were cut and stained with hematoxylin and eosin. The total number of megakaryocytes and the number of megakaryocytes in endomitosis were scored on 4 sections from each mouse, and the percentage of megakaryocytes in endomitosis was calculated (14). The number of megakaryocytes counted for each mouse ranged between 488 and 1879. Two replicates of the experiment were done, with three mice per group in one experiment, and five per group in the second. Results were comparable within identical treatment groups, therefore the data were combined.

Results. The effects of the various treatments on the endomitotic index of marrow megakaryocytes of mice are presented in Table I. It is apparent that plasma from normal rats in the doses used had no effect on the index; the average values of groups 1, 2, and 3 were almost identical. However, in mice injected with plasma from platelet-poor rats the index was significantly increased ($P < 0.001$) at both dose levels over that of any of the control groups. Moreover, there appeared to be a dose-response relation in the groups treated with TSF, indicated by a significantly higher value ($P < 0.05$) in the group receiving a dose of 2 ml/mouse.

TABLE I. ENDOMITOTIC INDEX OF MOUSE MEGAKARYOCYTES 32 HR AFTER TREATMENT.

Group	Treatment	Dose (ml/mouse)	No. of mice	Mitotic index ± SE	Groups compared, *t* test
1	Saline	1	8	0.87 ±0.06	
2	NRP[a]	1	8	0.91 ±0.07	1 & 2, $P > 0.5$
3	NRP	2	8	0.87 ±0.03	1 & 3, $P > 0.5$
4	TSF[b]	1	8	1.44 ±0.09	2 & 4, $P < 0.001$
5	TSF	2	8	2.08 ±0.30	3 & 5, $P < 0.001$
					4 & 5, $P < 0.05$

[a] NRP = plasma from normal rats.
[b] TSF = plasma from thrombocytopenic rats.

Discussion. The results presented here demonstrate the presence in plasma of thrombocytopenic donors of an agent that either directly or indirectly stimulates endomitosis of megakaryocytes. Although the simplest interpretation is that of a direct stimulation of megakaryocyte endomitosis by TSF, other mechanisms seem equally possible with our present state of knowledge. For example, TSF might initiate a change in megakaryocytes that are in late stages of differentiation which in turn might stimulate endoreduplication of less differentiated megakaryocytes by way of a feedback mechanism.

Recipient mice responded to this plasma agent (TSF) in much the same way that mice respond to severe thrombocytopenia induced by injection of antiserum against platelets, in which an increase in the endomitotic index of megakaryocytes is observed (14). Therefore, this finding provides a hematopoietic basis for previous reports of an increase in the number of circulating platelets and an increased uptake of platelet radioactive labels in mice, rats, and rabbits treated with plasma from platelet-poor donors (6). The results are also consistent with *in vitro* studies in which putative TSF's promoted the appearance and maturation of megakaryocytes in cultures of marrow (9).

Our observations suggest a dose-response relationship between the amount of TSF administered and the degree of response in the megakaryocyte population. In addition, these data support earlier indications that the stimulatory agent is not species specific.

Summary. Injection of plasma from thrombocytopenic donor rats resulted in an increase in the endomitotic index of megakaryocytes of recipient mice 32 hr after the initial treatment with plasma. The results suggested a dose-response relationship between the amount of plasma administered and the degree of stimulation of megakaryocytopoiesis. These findings demonstrate that an agent capable of stimulating megakaryocytopoiesis is released in response to thrombocytopenia and that this factor can be successfully transferred between species. They also substantiate the assumption that the increase in peripheral platelet numbers and in platelet labeling after administration of presumptive TSF occurs via stimulation of megakaryocytopoiesis.

1. Spector, B., Proc. Soc. Exp. Biol. Med. **108,** 146 (1961).
2. Odell, T. T., McDonald, T. P., and Detwiler, T. C., Proc. Soc. Exp. Biol. Med. **108,** 428 (1961).
3. Evatt, B. L., and Levin, J., J. Clin. Invest. **48,** 1615 (1969).
4. Cooper, G. W., Cooper, B., and Chang, C., Proc. Soc. Exp. Biol. Med. **134,** 1123 (1970).
5. Harker, L. A., Amer. J. Physiol. **218,** 1376 (1970).
6. McDonald, T. P., Scand. J. Haematol. **18,** 5 (1977).
7. Odell, T. T., McDonald, T. P., and Howsden, F. L., J. Lab. Clin. Med. **64,** 418 (1964).
8. Adams, W. H., Liu, Y. K., and Sullivan, L. W., J. Lab. Clin. Med. **91,** 41 (1978).
9. Nakeff, A., Dicke, K. A., and van Noord, M. J., Ser. Haematol. **8,** 4 (1975).
10. Ebbe, S., Stohlman, F., Jr., Overcash, J., Donovan, J., and Howard, D., Blood **32,** 383 (1968).
11. Harker, L. A., J. Clin. Invest. **47,** 458 (1968).
12. Odell, T. T., Jackson, C. W., Friday, T. J., and Charsha, D. E., Brit. J. Haematol. **17,** 91 (1969).
13. Odell, T. T., Murphy, J. R., and Jackson, C. W., Blood **48,** 765 (1976).
14. Odell, T. T., and Boran, D. A., Proc. Soc. Exp. Biol. Med. **155,** 149 (1977).

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