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USE OF [^{75}Se] -METHIONINE AS A TRACER OF THROMBOCYTOPOIESIS
III- Evidence for two different in vivo thrombocytopoiesis-stimulating factors

E. DASSIN, J. BOUREBIA and Y. NAJEAN

Formation de recherche associée n°4,
I.N.S.E.R.M., Hôpital Saint-Louis, 2
pl. du Dr A. Fournier, 75010 Paris,
France

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SUMMARY: Thrombocytopenia was induced by injecting hydroxy-urea into normal rats. When serum from these rats was injected into normal recipients, platelet production was stimulated. As expected, a dose-dependent effect was obtained in the peripheral platelet number as well as in [^{75}Se] -Methionine incorporation into platelet proteins. Serum from non-thrombocytopenic animals which were bone-marrow depleted stimulated platelet production. The response was dose dependent. Moreover, the data suggest that bone-marrow depletion can induce secretion of a thrombocytopoiesis-stimulating factor (TSF_M) which could act upon a different target cell than the thrombocytopenia-induced thrombocytopoiesis-stimulating factor (TSF_T).

INTRODUCTION

The regulation of the production of the myeloid cell lines has been extensively studied, but, for technical reasons, less is known in the case of platelets than for erythrocytes and granulocytes. It is generally accepted that any depletion of mature cells induces the secretion of a specific, circulating, factor able to favour differentiation and proliferation of the precursor cells of this cell line (1, 2). A short-range regulation could also be dependent on the bone-marrow cell density (3).

The use of [^{75}Se] -Methionine as a tracer for the megakaryocyte line enables platelet production to be quantified (4, 5). The thrombocytopoietic activity of serum collected from hydroxy-urea treated rats was tested with this method. Serum from thrombocytopenic rats and from bone-marrow depleted animals without thrombocytopenia were collected and tested. The existence of at least two different thrombocytopoiesis-stimulating factors is suggested.

MATERIAL AND METHODS

Normal Wistar rats, weighing between 250 and 300 g and fed on standard pellet food were used. Hydroxy-urea (HU) was dissolved in 0.15 M NaCl and then injected intraperitoneally as follows: 0.2×10^{-2} moles/kg on days 0 and 1, and then 0.13×10^{-2} moles/kg on days 2 and 4. The control group received 0.15 M NaCl alone. 24 hours (second group) or

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7 days (first group) after the first HU injection, blood samples were taken by cardiac puncture under ether anaesthesia (Fig 1). Serum samples were preparated by incubating blood samples for 1 hour at 37°C and then centrifuging at 1500g for 15 min.

Different volumes of serum from each group of rats were injected intraperitoneally into normal recipients. We also injected HU at 10% of the level given on day 0 into five normal rats, because it is well known that 24 hours after its injection, 10% of the HU remains in the blood circulation of the serum donors (6). No effect upon platelet production was found at this concentration.

Platelet production is given comparing platelet number on day 4 (the maximum) with those obtained on day 0. [^{75}Se] -Methionine was injected intravenously at different times after the serum injection. We have previously shown that its incorporation into specific platelet proteins was an index of cellular production (7). To calculate the true production, it was necessary to take away the non-specific incorporation of the tracer, which was probably due to adsorption of labelled plasma proteins (principally albumin and fibrinogen) onto platelet plasma membranes. Moreover, the specific incorporation of the tracer into proteins (principally

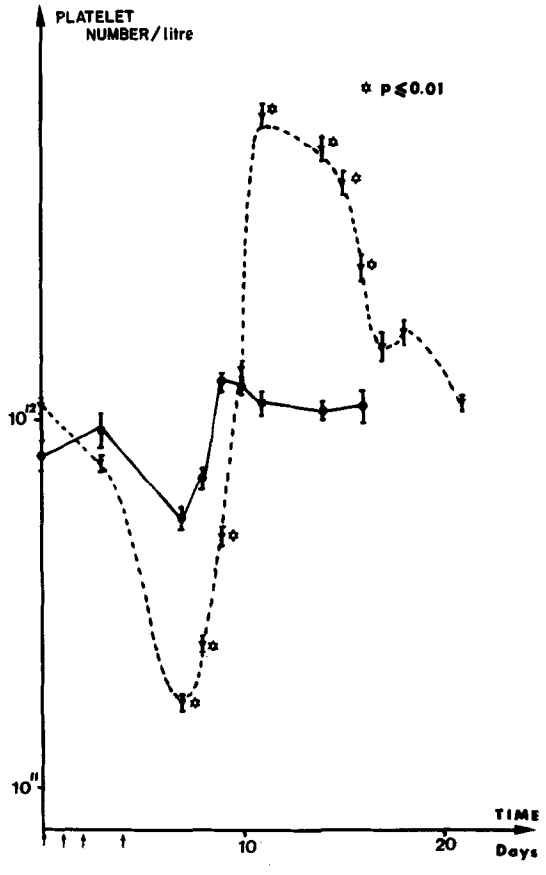

Fig 1. Curve of platelet count after hydroxy-urea infusion in rats (x - x) or saline infusion (• - •). The vertical lines indicate the standard errors. Serum samples were prepared on days 1 and 7 for assays of thrombopoietic activity.

acto-myosin) synthetized by megakaryocytes, which occurs 2 to 3 days before the liberation of platelets into the blood circulation, may be possible for only a very short time (a quarter of an hour after the tracer injection, no free [^{75}Se] -Methionine was found in plasma samples).

Results were compared using the statistical Wilcoxon test.

RESULTS

1. Serum from thrombocytopenic rats (obtained on the 7th day after the first HU injection) was infused into normal rats where it induced thrombocytosis and an increase of [^{75}Se] -Methionine incorporation into platelet proteins. Such an effect was dose-dependent (Fig 2b). The maximum [^{75}Se] -Methionine incorporation was obtained when the tracer was injected 24 hours after the thrombocytopoiesis-stimulating factor (TSF_T) (Fig 3b).

2. Serum from non-thrombocytopenic rats with depleted bone-marrow (obtained 24 hours after the first HU injection) also demonstrated a dose-dependent thrombocytopoiesis-stimulating effect (Fig 2c). The highest [^{75}Se] -Methionine incorporation was obtained when the tracer was injected 36 hours after thrombocytopoiesis-stimulating factor from bone-marrow depleted rats (TSF_M) (Fig 3c). Differences in the level of incorporation between curves 3b and 3c were significant ($\alpha < 0.01$), at the 24th and at the 36th hour.

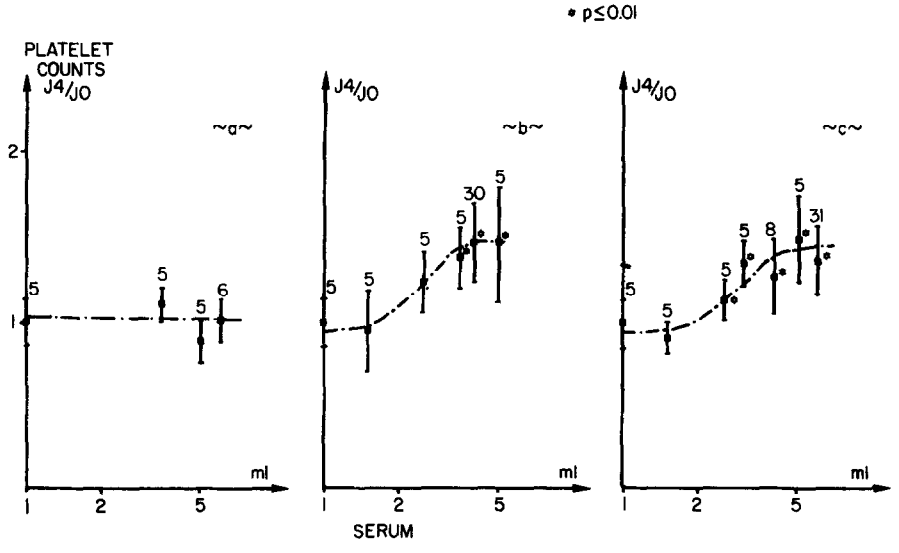

Fig 2. Platelet count 4 days after infusion of different doses of normal rat serum (a), serum of thrombocytopenic rats (day 7) (b), serum of marrow-depleted but non-thrombocytopenic rats (day 1) (c). The vertical lines indicate the standard errors and the numbers represent the number of rats at each treatment.

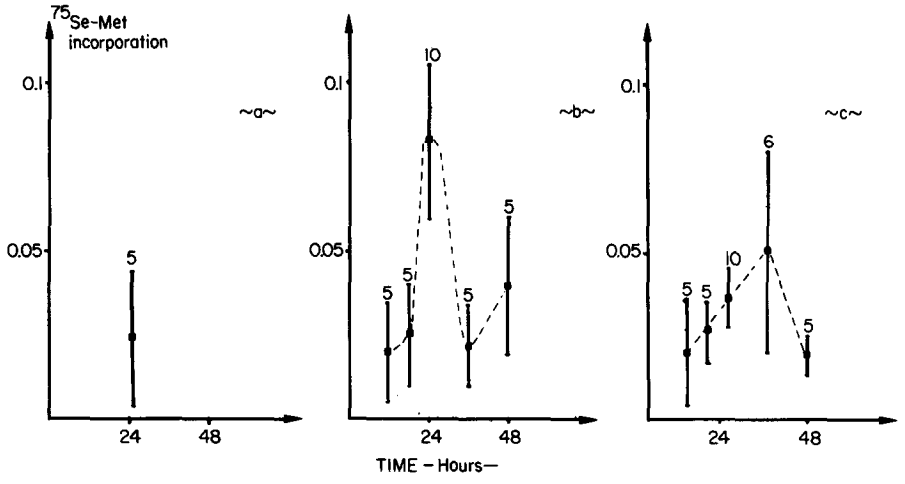

Fig 3. The variation of the incorporation of $[^{75}Se]$ -Methionine into the specific platelet proteins on the 7th day, with the delay between infusion of thrombocytopoietic material and of the tracer. (a): infusion of normal rat serum; (b): infusion of thrombocytopenic rat serum; (c): infusion of serum of marrow-depleted non-thrombocytopenic rats. The vertical lines indicate the standard errors and the numbers represent the number of rats at each treatment.

DISCUSSION

A thrombocytopoiesis-stimulating factor (TSF_T) whose secretion increases when animals are thrombocytopenic has often been observed. As in previous experiments, such an effect was dose-dependent, and a function of the severity of thrombocytopenia (1, 2). This paper reports new observations on the timing of thrombopoietic activity. $[^{75}Se]$ -Methionine was incorporated into specific platelet proteins at the end of the replication phase. If an appropriate correction was made to subtract non-specific binding of radioactive plasma proteins to the circulating platelets (7), the delay between infusion of the thrombocytopoiesis-stimulating factor (TSF_T) (secreted by thrombocytopenic animals) and the maximum $[^{75}Se]$ -Methionine incorporation does measure the time needed for the appearance of a cohort of $[^{75}Se]$ -Methionine-incorporating megakaryocytes, in the bone marrow. The observed delay (24 hours) suggests that the TSF_T acts on a 2 N committed cell: the cell generation cycle in the rat is indeed about 9 hours (8) and most of the maturing ($[^{75}Se]$ -Methionine incorporating) cells are 16 N (1).

Another new observation was of the presence of a thrombocytopoiesis-stimulating factor (TSF_M) which increased when animals were bone-marrow depleted, but still not thrombocytopenic. It is generally assumed that bone-marrow depletion can induce stem cell proliferation,

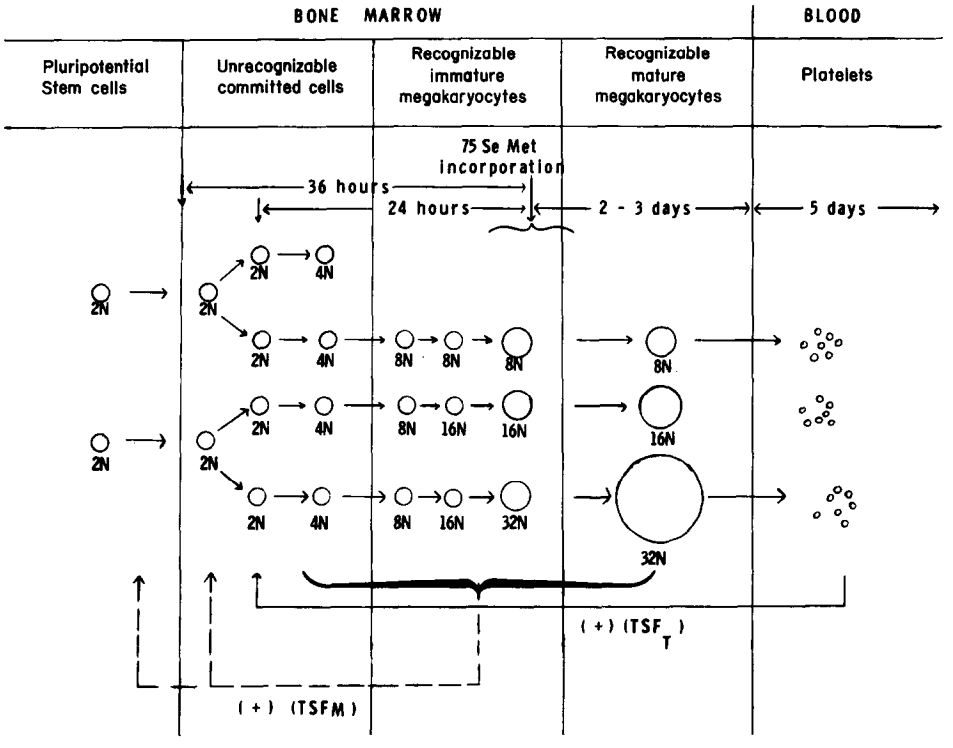

Fig 4. Suggested model of thrombocytopoiesis (adapted from Ebbe (1)) and possible sites of action of TSF_T (thrombocytopoiesis-stimulating factor from thrombocytopenic animals) and TSF_M (thrombocytopoiesis-stimulating factor from bone-marrow depleted non-thrombocytopenic rats).

by short-range interaction (3). Our experiment seemed to demonstrate that such a depletion also induces stimulation through a circulating activator. Its physiological target cell is apparently different from that of the thrombocytopoietic activity from thrombocytopenic rats, since the cohort of $[^{75}Se]$-Methionine-incorporating megakaryocytes appeared at the 36^{th} hour only when active material was infused; this means that the target cell would be a less differentiated cell than that responsive to the TSF_T (Fig 4).

As for other cell lines, thrombopoietic activation and probably inhibition depends on the number of circulating platelets. Such factors would modulate late events of cell division and maturation (late-committed stem cell and early megakaryocytes). Our experiments suggest no increase in the release of platelets, in agreement with other published curves (9, 10, 11, 12, 13), which do not show any early thrombocytosis in normal receptors. On the other hand, megakaryocytopoiesis could be regulated by the cycle-cell status of the CFU_M compartment (14), by a

feedback mechanism involving the marrow population of maturing megakaryocytes. By analogy with other myeloid cell lines, it has been suggested that such a control would be locally mediated (14), but our experiments suggest that such an activation could be mediated by a circulating factor. Ebbe et al., use parabiosis between Sl/Sl^{d} and normal mice, to show abnormal macro-megakaryocytopoiesis in the normal parabionts, which suggests the generation of a thrombopoietic substance by Sl/Sl^{d} mice; such a factor could be generated to compensate for a reduced number of circulating platelets (15).

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