EFFECT OF STIMULATION OF THROMBOCYTOPOIESIS IN HEALTHY AND IRRADIATED DONOR MICE ON THE FORMATION OF EXOGENOUS COLONIES IN THE SPLEEN OF RECIPIENTS

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The number of precursor cells forming colonies when transplanted into the spleen of recipient mice irradiated in a dose of 850 R was increased in the bone marrow of intact CBA mice irradiated in a dose of 150 R, after receiving an injection of plasma with increased thrombocytopoietic activity. These results indicate that the sensitivity of the hematopoietic organs to thrombocytopoietic stimulation is preserved in the irradiated animal.

It is now accepted, in principle, that it is possible to stimulate megakaryocytopoiesis and thrombocytopoiesis considerably and to increase the number of platelets circulating in the blood stream in irradiated animals [1-3, 5]. It is therefore particularly important to study the state of hematopoietic precursor cells in the irradiated organism and their ability to differentiate into the megakaryocyte series.

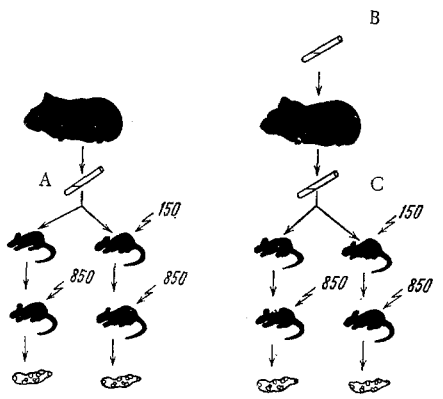

Fig. 1. Scheme of the study of the effect of irradiation and thrombocytopoietic plasma on the composition of exogenous hematopoietic colonies in irradiated recipient mice. A) "intact" plasma; B) antithrombocytic serum; C) "thrombocytopoietic" plasma; Numbers denote doses of irradiation (in R).

The object of the present investigation was to study the effect of stimulation of thrombocytopoiesis on the ability of the bone marrow of healthy and irradiated mice to form exogenous hematopoietic (megakaryocytic) colonies in the spleen.

EXPERIMENTAL METHOD

CBA mice aged 3 months were used. The scheme of the experiments is shown in Fig. 1. The bone marrow donors were unirradiated mice and mice irradiated in a dose of 150 R. From 2 to 3 hours after irradiation the donors received a single injection of "intact" or "thrombocytopoietic" guinea pig plasma in a dose of 0.3 ml. The plasma was obtained from the blood of guinea pigs after receiving a preliminary injection of antithrombocytic serum obtained by a modified method [4]. The thrombocytopoietic activity of the plasma was judged from its ability to induce thrombocytosis in intact recipient mice on the 3rd-5th day after receiving an intraperitoneal injection of the test plasma. The donors were decapitated 24 h after receiving the injection of plasma and their tibial bone marrow was extracted, mixed in medium 199, and suspended by passing it through needles of decreasing diameter. The suspension of bone marrow cells was injected intravenously into recipient mice which had been irradiated 24 h previously in a dose of 850 R. Cells from unirradiated bone marrow were

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TABLE 1. Composition of Exogenous Hematopoietic Colonies (in %) in Recipient Mice After Stimulation of Thrombocytopoiesis in Healthy and Irradiated Donor Mice (M ± m)

Index	Injection of intact plasma		Injection of thrombocytopoietic plasma		Injection of physiological saline
		irradiation		irradiation	
No. of animals in group	14	16	23	18	10
No. of cells injected	4×10^4	8×10^4	4×10^4	8×10^4	4×10^4
Mean number of colonies per section through spleen	7,0±0,7	3,0±0,3	8,3±0,8	3,3±0,6	6,5±1,2
Comp. of colonies (in %):					
erythroid	48,0±5,9	58,4±11,8	47,4±3,6	60,3±11,8	53,8±9,4
granulocytic	18,4±3,5	12,5±4,7	7,8±1,2	3,5±4,0	15,8±4,7
megakarytocytic	7,1±4,7	4,2±2,4	21,3±3,0	20,7±4,0	6,0±1,6
undifferentiated	16,3±3,5	18,7±4,7	16,1±3,6	13,8±4,0	21,6±6,3
mixed	10,2±3,5	6,2±2,4	7,3±2,4	1,7±2,0	3,1±1,6

injected in a dose of 4×10^4, and cells from irradiated bone marrow in a dose of 8×10^4. The marrow was irradiated with Co^{60} γ-rays (dose rate 50 R/sec). In each group bone marrow from 5 donors animals was pooled. The recipient mice received an aqueous solution of antibiotics (polymyxin and neomycin, 1000/ml of each) to drink for 7 days after irradiation. The recipient mice were decapitated on the 9th day after irradiation and the spleen was extracted, fixed in Carnoy's fluid, and embedded in paraffin wax. Three longitudinal sections 8μ in thickness were cut from each spleen, at different levels so that the same colony did not occur twice in a section. The sections were stained with hematoxylin-eosin and hematopoietic colonies were identified [8].

EXPERIMENTAL RESULTS

Histological examination of the spleens of the recipient mice revealed 5 types of hematopoietic colonies. Erythroid colonies (Fig. 2a), consisting of erythroblasts and pronormoblasts, were the largest and they accounted for about half the total number of all colonies. Granulocytic colonies (Fig. 2b) consisted mainly of cells of the myelocyte type, although more mature cells could also be seen in them. Megakaryocytic colonies (Fig. 2c) usually contained up to 10-15 cells of the megakaryocyte and promegakaryocyte type. Undifferentiated colonies contained young (blast) cells with a large nucleus and small quantity of cytoplasm. Mixed colonies consisted of cells of 2 or, rarely, 3 types (Fig. 2d).

The experimental results are summarized in Table 1.

Injection of plasma of intact guinea pigs into the mice donating the bone marrow had no significant effect on the composition of the exogenous colonies. Just as in the control group (injection of physiological saline), about half of the colonies were erythroid, 16-21% were undifferentiated, and 15-18% of the total number of colonies were granulocytic. The number of megakaryocytic colonies was small, not more than 6-7%. Irradiation of the donor mice in a dose of 150 R led to a substantial decrease in the total number of colonies, but did not cause any marked changes in their cell composition. For instance, the number of granulocytic, megakaryocytic, and mixed colonies was reduced while the number of erythroid colonies was increased.

Injection of thrombocytopoietic plasma into unirradiated donor mice was followed by a slight increase in the total number of colonies and by considerable changes in their cell composition. For instance, the number of megakaryocytic colonies was trebled, while the number of granulocytic colonies was reduced by 60%. The number of mixed colonies was slightly reduced. Injection of thrombocytopoietic plasma into irradiated donor mice had a similar effect on the composition of the exogenous colonies. Compared with the group of donor mice irradiated and subsequently receiving an injection of intact plasma, after injection of thrombocytopoietic plasma the number of megakaryocytic colonies was increased by 5 times, while the number of granulocytic, mixed, and undifferentiated colonies were reduced by 3.6, 3.6, and 1.3 times respectively.

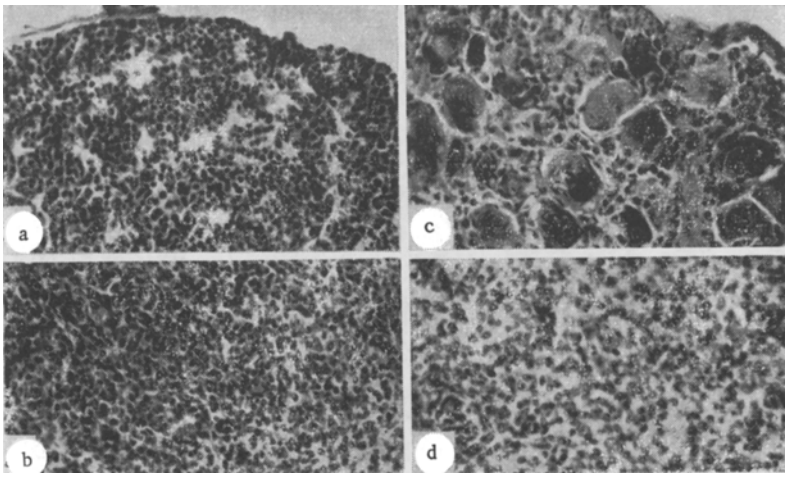

Fig. 2. Exogenous hematopoietic colonies differing in their cell composition: a) erythroid; b) granulocytic; c) megakaryocytic; d) mixed, 200 ×.

Thrombocytopoietic plasma thus affected the bone marrow both of intact and of irradiated animals, stimulating an increase in the number of cells forming megakaryocytic colonies in the spleen of the recipient mice. These results suggest that precursor cells of megakaryocytes remain sensitive after irradiation to the stimulus inducing their differentiation into the megakaryocytic series. Results obtained with mice irradiated in doses of 200-400 R, which received an injection of antithrombocytic serum immediately before or after irradiation [3, 6], are interesting from this point of view. In these animals, despite the sharp decrease in the number of circulating platelets during the first 24 h after injection of the antithrombocytic serum, the platelet level subsequently was restored faster than in animals irradiated only. Although the workers cited did not study megakaryocytopoiesis in these animals, it can be assumed that restoration of the platelet level was "true" in character and was due to stimulation of thrombocytopoiesis. Injection of normal or thrombocytopoietic human serum into irradiated mice also had a favorable effect on restoration of the platelet level [5, 7]. It can accordingly be concluded that hematopoietic precursor cells surviving after irradiation remain sensitive to the stimulus inducing their differentiation into the megakaryocytic series.

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