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EFFECT OF SIZE-FRACTIONATED THYMOCYTES ON NUMBER OF HEMATOPOIETIC STEM CELLS IN BONE MARROW OF SUBLETHALLY IRRADIATED MICE

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T lymphocytes have an influence on the functional properties of hematopoietic stem cells (CFU-S) [1, 3, 4]. In particular, thymocytes have been shown to stimulate CFU-S proliferation [8, 9]. The stimulating effect of thymus cells has been noted mainly in a nonsyngeneic system, whereas the results of studies of interaction between thymocytes and CFU-S under conditions of a syngeneic donor–recipient combination are contradictory [2, 10, 11]. Lymphocyte subpopulations may exhibit various functional properties, and methods of their fractionation have now been developed, whereby the cells can be separated without disturbance of their function [7, 12]. Repeated injections of small doses of glucocorticoids increase the number of CFU-S in the bone marrow of intact mice [5], possibly on account of changes in the cell composition of the thymus as a result of the effect of the hormone on the cortisone-sensitive subpopulation of T cells.

The object of this investigation was to study the effect of thymus cells, fractionated according to size, and obtained from intact mice and mice treated with dexamethasone, on the number of CFU-S in the bone marrow after sublethal irradiation.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57BL)F_1 hybrid mice weighing 20-24 g. A thymocyte suspension was prepared in medium No. 199 from the thymus glands of intact mice or of mice receiving an intraperitoneal injection of dexamethasone-^{21}Na-phosphate (DM) in a dose of 0.001 mg per mouse daily for 7 days. The thymocytes were fractionated by size by

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TABLE 1. Effect of Thymocytes Fractionated by Size on Number of CFU-S in Mouse Bone Marrow on 10th Day after Irradiation

No. of expt.	Irradiation	Thymocytes of intact mice			Thymocytes from mice after injection of DM		
		unfractionated	fraction I	fraction II	unfractionated	fraction I	fraction II
1	10,7±1,3 (10)	13,1±1,3 (10)	6,0±0,8*(11)	20,5±1,4* (9)	—	—	—
	7,7±0,5 (7)	—	—	—	18,5±4,3* (2)	17,4±2,3* (7)	14,6±1,9* (5)
2	5,2±0,9 (10)	9,9±1,2 (9)*	5,4±1,0 (9)	8,2±1,3 (4)	—	—	—
	13,1±0,9 (10)	—	—	—	17,0±1,1* (9)	11,8±0,4 (12)	20,7±1,2*(10)
3	7,8±1,2 (4)	7,0±1,7 (6)	3,4±1,1*(11)	20,3±1,3*(12)	—	—	—
	10,0±0,8 (6)	—	—	—	14,6±1,3* (9)	21,4±3,4* (5)	13,3±1,4 (5)
Total	9,8±0,9 (47)	10,0±1,4 (25)	4,9±1,0*(31)	16,3±1,3*(25)	16,7±2,2*(20)	16,3±2,0*(24)	16,2±1,5*(20)

Legend. *$P < 0.05$ compared with irradiation control. Number of animals given in parentheses.

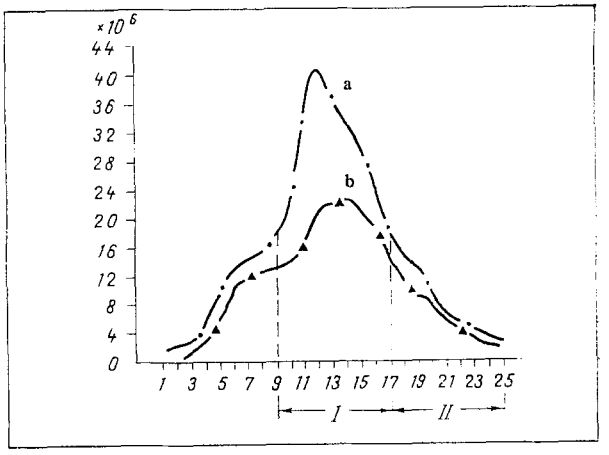

Fig. 1. Averaged distribution profile of thymocytes among fractions after sedimentation in BSA density gradient. a) Thymocytes of intact mice; b) thymocytes of mice after daily injection of DM for 7 days. Abscissa, Nos. of thymocyte fractions; ordinate, number of cells in fraction.

sedimentation at 1 g in a bovine serum albumin (BSA) density gradient on an apparatus of "Staput" type, similar to that described in [12, 13], for 3 h at 4°C, after which the separated suspension was fractionated by displacing the gradient from the chamber by solution of higher density. The number of fractions was 25. Since the first eight fractions consisted chiefly of erythrocytes, with only very few thymocytes, fractions from the 9th through the 17th and from the 18th through the 25th were pooled for biological testing; they will subsequently be described as fraction I and fraction II. The viability of the cells was estimated by staining with trypan blue. Fractionated thymocytes or unfractionated cells taken from intact and DM-treated mice, in a dose of 5×10^6 cells per mouse, were injected intravenously into mice 2-3 days after γ-ray irradiation (^{137}Cs) in a dose of 6.27 Gy, with a dose rate of 2.05 Gy/min. On the 10th day after irradiation the number of CFU-S in the bone marrow was determined by the exogenous cloning method in a lethally irradiated recipient [14]. Bone marrow cells were injected in a dose of 2×10^5 cells per mouse.

EXPERIMENTAL RESULTS

During fractionation of the thymus cells they suffered practically no damage and the proportion of dead cells after fractionation was 3-5%. Averaged distribution profiles of thymocytes among fractions are shown in Fig. 1. Pooled fraction I consisted of cells 5.9-6.7 μ in diameter, those of fraction II of cells 6.7-7 μ in diameter. Comparison of the

distribution profiles of thymocytes of intact animals and those treated with DM among the fractions revealed a relative decrease in the number of fraction I cells after injection of the hormone.

Injection of unfractionated thymocytes taken from intact animals did not significantly change the number of CFU-S in the bone marrow of the mice on the 10th day after sublethal irradiation (Table 1). After injection of the thymocytes of fraction I the CFU-S level was approximately halved, but injection of fraction II of thymus cells led to an increase in the number of CFU-S.

When unfractionated thymus cells obtained from animals treated with DM were used the number of CFU-S in the bone marrow of the irradiated mice increased. The increase in the number of CFU-S in the bone marrow was observed equally in animals receiving thymus cells of fraction I and fraction II, obtained from mice after injection of the hormone.

The thymocyte population of intact mice is thus heterogeneous in its action on hematopoietic stem cells in bone marrow after sublethal irradiation and is made up of a subpopulation of thymocytes which inhibits, and another which stimulates CFU-S formation in the period of recovery after irradiation. It can be tentatively suggested that the isolated subpopulations of thymocytes are identical with suppressor cells and helper cells of the immune response, for after fractionation of thymocytes of month old mice by sedimentation at 1g in [7] it was shown that T suppressors sediment more slowly than T helpers.

During fractionation of thymocytes obtained from mice after injection of a small dose of DM, at the level of physiological doses of corticosteroids [6], the subpopulation of cells with an inhibitory action of CFU-S was not discovered. The fraction of thymus cells which inhibit growth of CFU-S is evidently cortisone-sensitive. Repeated injections of DM in a small dose modifies the composition of the thymocyte population and reduces the number of cells with a suppressive effect on hematopoietic stem cells or on their more differentiated precursors.

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