EFFECT OF THYMIC FACTORS ON DIFFERENTIATION OF CIRCULATING AND MEDULLARY HEMATOPOIETIC STEM CELLS

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The principal functional properties of the hematopoietic stem cell (HSC), namely proliferation, differentiation, and circulation, are interdependent [1, 10]. According to the hypothesis of Rosendaal et al. [14], HSC in the bone marrow acquire mobility and the ability to receive differential stimuli after repeated passage through the cell cycle. HSC circulating in the peripheral blood differ significantly from medullary HSC in their proliferative activity, capacity for self-maintenance, settling in the spleen of irradiated animals, and expression of antigen bound with the brain [13]. The differential potential of HSC in the peripheral blood under normal conditions and during various procedures has been inadequately studied, although according to the few investigations described in the literature [2, 7], differences in differentiation of HSC in the bone marrow and peripheral blood of normal mice are not significant, but after thymectomy, differentiation of medullary and circulating HSC changes in the same direction: cloning of the cells reveals a standard decrease in the intensity of granulocytopoiesis [5].

The aim of this investigation was to study the characteristics of differentiation of HSC in the bone marrow and peripheral blood of adult animals and the effect of thymic factors on it.

EXPERIMENTAL METHOD

Experiments were carried out on 240 male CBA mice weighing 20-22 g: thymectomy was performed on 20 mice, a mock operation on another 20 mice, and 200 intact mice also were used; the animals were obtained from "Rappolovo" Nursery, Academy of Medical Sciences of the USSR. Thymectomy was performed on mice aged 4-6 weeks by the method in [12], and they were used in experiments in vivo and in vitro 11-13 weeks later.

In the experiments in vivo physiological saline or thymaline in 0.5 ml of physiological saline in a dose of 20 μg was injected subcutaneously into the mice after thymectomy and the mock operation, daily for 10 days. Thymalin is a preparation of calf thymus obtained by acetic acid extraction [4]; it consists of complexes of fractions of polypeptide nature with mol. wt. < 10,000 daltons. Two days after the end of the course of injections the mice were decapitated, and the femoral marrow and peripheral blood from four or five mice, with added heparin, were pooled to prepare cell suspensions. The cells were then washed three times in 10 volumes of medium 199. The number of HSC in the resulting suspensions was counted by the exogenous colonies method [15], by transplanting $5 \cdot 10^5$ nucleated blood cells or 10^5 bone marrow cells into a syngeneic recipient, totally irradiated with x rays in a dose of 8.5 Gy. On the 8th day after transplantation of the cells the animals were killed and their spleens fixed in Bouin's solution and used to prepare histological specimens. Differentiation and the number of HSC (taken to be equal to the number of splenic colony-forming units – CFU_S) were determined by counting hematopoietic colonies of different types in three paraffin sections through the spleen (two subcapsular and one central), stained with hematoxylin and eosin [8]. The method used, which is widely adopted nowadays to assess differentiation of HSC [3, 9], was previously described in detail [6].

In the experiments in vitro peripheral blood from normal or thymectomized animals was cultured for 1.5 h at 37°C with thymalin in a concentration of 10 μg/ml or physiological saline. Subsequently the technique was identical with that used in the experiments in vivo.

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TABLE 1. Histological Analysis of CFU_S Formed by Exogenous Cloning of Bone Marrow and Peripheral Blood Cells from Mice Stimulated with Thymalin in Experiments in vivo or in vitro

Method of stimulation with thymalin	Source of CFU_S	Conditions	No. of colonies counted under microscope	Type of heamtopoietic colonies					E/Gr
				E	Gr	Mg	Mx	U	
In vivo	Peripheral blood	Mock operation + physiological saline	10,7±0,9	5,9±0,9	1,2±0,3	1,1±0,3	0,1±0,0	2,4±1,0	4,9
		Mock operation + thymalin	6,6±1,5*	2,7±0,6*	2,3±0,8	0,8±0,4	0,0±0,0	0,8±0,3	1,2
		Thymectomy + physiological saline	8,3±1,8	4,8±1,4	0,9±0,3	1,2±0,3	0,1±0,0	1,4±0,5	5,3
		Thymectomy + thymalin	7,1±1,3*	3,1±0,7*	1,9±0,7	0,9±0,3	0,0±0,0	1,1±0,3	1,6
In vivo	Bone marrow	Mock operation + physiological saline	26,7±2,8	14,6±1,9	5,0±0,7	1,6±0,4	0,1±0,1	5,4±0,8	2,9
		Thymectomy + physiological saline	20,9±4,1	13,4±2,6	2,9±0,8*	0,8±0,3	0,3±0,2	3,6±0,8	4,6
		Thymectomy + thymalin	24,0±3,3	12,6±1,1	4,6±0,9	1,9±1,5	0,2±0,2	4,7±0,8	2,7
In vitro	Peripheral blood	Mock operation + physiological saline	8,9±1,8	6,8±1,3	1,2±0,5	0,6±0,2	0,0±0,0	0,5±0,2	5,7
		Mock operation + thymalin	10,4±1,9	7,4±1,4	1,5±0,6	0,6±0,2	0,0±0,0	1,1±0,5	5,0
		Thymectomy + physiological saline	7,6±1,1	5,5±0,9	0,9±0,3	0,7±0,4	0,0±0,0	0,7±0,4	5,9
		Thymectomy + thymalin	7,8±1,1	5,4±0,7	1,0±0,3	0,4±0,3	0,0±0,0	0,9±0,5	5,4

Legend. E, Gr, Mg, Mx, and U denote different types of hematopoietic colonies: erythroid, granulocytic, megakaryocytic, mixed, and undifferentiated respectively. *$P < 0.05$ compared with animals undergoing mock operation and receiving physiological saline. Number of medullary CFU_S given per 10^5 transplanted nucleated cells, number of blood CFU_S – per 10^6 cells.

EXPERIMENTAL RESULTS

The total number of CFU_S in blood from animals undergoing the mock operation and thymectomy, and treated with physiological saline, did not differ significantly in the experiments in vivo and in vitro, and varied from 7.6 to 10.7; the number of colonies of different types likewise did not differ significantly, and the ratio of the number of erythroid colonies to the number of granulocytic colonies (E/Gr) varied from 4.9 to 5.9 (Table 1).

Injection of thymalin into the animals undergoing the mock operation and thymectomy led to qualitatively similar changes in the colony-forming properties of the HSC from their blood: the number of erythroid colonies formed was significantly reduced, the number of granulocytic colonies was increased, and the E/Gr ratio was 1.2 and 1.6 respectively. Changes in the colony-forming properties of the blood HSC under the influence of thymalin were associated with a decrease in the number of CFU_S in circulation: In mice undergoing the mock operation this parameter fell from 10.7±0.9 to 6.6±1.5 ($P < 0.05$), and in the thymectomized mice it fell from 8.3±1.8 to 7.1±1.3.

By contrast with the experiments in vivo, treatment of blood cells from mice undergoing the mock operation and thymectomy with thymalin in the experiments in vitro was not accompanied by any change in the total number of CFU_S or in the number of hematopoietic colonies of the various types.

Blood HSC from mice undergoing the mock operation differed significantly from the blood cells of the same animals in exhibiting increased granulocytic differentiation: their E/Gr ratio was 2.9. After thymectomy, the colony-forming properties of the medullary HSC changed: The number of granulocytic colonies decreased and the E/Gr ratio was 4.6, which did not differ quantitatively from the E/Gr ratio for autologous blood cells. Thymalin in the experiments in vivo normalized the colony-forming properties of the medullary HSC of the thymectomized mice.

The results of this investigation are evidence that the colony-forming properties of medullary and peripheral blood HSC of normal animals differ significantly. Evidently under ordinary conditions cells with enhanced erythropoietic differentiation are expelled into the circulation. A change in the colony-forming prop-

erties of the circulating cells could be achieved in the experiments in vivo, but not in vitro, by injecting thymalin into normal or thymectomized mice, which led to a decrease in the number of circulating HSC. The colony-forming properties of the blood HSC of animals undergoing thymectomy and the mock operation, when stimulated by thymalin in experiments in vivo, were similar to those of the medullary HSC of normal mice.

This combined change in the number of circulating HSC and in their colony-forming properties under the influence of thymalin can be explained on the grounds that this substance inhibits erythroid differentiation of medullary HSC, and so prevents the expulsion of cells with enhanced erythroid differentiation from the bone marrow into the blood.

The effect of thymalin on HSC may perhaps be mediated through a component of the microenvironment that is present in bone marrow but not in peripheral blood, since this substance modifies the colony-forming properties of HSC in experiments in vivo but does not affect them in experiments in vitro. Differences in the results of the action of thymalin in experiments in vivo and in vitro may also depend on the absence of HSC in the peripheral blood at an earlier ancestral stage than those detectable by counting 8-10-day CFU_S. Indirect evidence of the existence of such HSC in bone marrow was published recently [11].

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