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ACTIVATION OF PRECURSORS OF T KILLERS OF HEMATOPOIETIC STEM CELLS BY THYMOSINE

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Lymph node cells from normal CBA mice, from CBA → CBA syngeneic radiation chimeras, and B mice were incubated *in vitro* with fraction 5 of thymosine, and transplanted into sublethally irradiated (CBA × C57BL)F_1 recipients, and the number of endogenous colonies in the recipients' spleen was determined. Thymosine was shown to potentiate the killer activity of lymph node cells of normal CBA mice and of CBA → CBA syngeneic radiation chimeras, but not of B mice. It is suggested that the target for the action of thymosine is the subpopulation of T_1 lymphocytes.

KEY WORDS: *thymosine; T cell; stem cell.*

It has been firmly established that lymphocytes carrying markers of T cells differentiate from bone marrow precursor cells *in vitro* under the influence of thymosine, a humoral factor of the thymus [3, 5, 7]. Expression of the markers of the T lymphocytes during incubation with thymosine has been shown to take place very quickly (in the course of 1-2 h), and to require transcription of DNA and translation of RNA, but not replication of DNA [8].

The object of this investigation was to study the effect of thymosine on precursor cells of T lymphocytes, exhibiting killer activity against allogeneic hematopoietic stem cells.

EXPERIMENTAL METHODS

CBA and (CBA × C57BL)F_1 mice aged 8-10 weeks were used. To obtain T-deficient animals, normal or thymectomized mice were irradiated in a dose of 750 R and protected with syngeneic bone marrow (10 cells).

Thymosine (fraction 5) was obtained by Goldstein's method with certain modifications [4]. Calf thymus was freed from capsule and homogenized at 12,000 rpm in 0.15 M NaCl (weight:volume = 1:3) in a type 302 Mechanika Precyzyna (Poland) homogenizer. The homogenate was incubated for 16-18 h at 4°C. The insoluble part of the homogenate was removed by centrifugation at 13,000 rpm (Beckman J-21B centrifuge, JA-14 rotor). The resulting supernatant was heated to 30°C for 15 min and the residue of thermolabile components was removed by centrifugation at 13,000 rpm (Beckman centrifuge). The supernatant was treated with 5 volumes of 90% acetone cooled to −20°C. The residue insoluble in acetone was collected by centrifugation at 2500 rpm (Mistral Type 6L centrifuge, J-344 rotor) and dissolved in 10 mM Na-phosphate buffer, pH 7.0 for treatment with ammonium sulfate. The active material was precipitated by ammonium sulfate in saturations from 25 to 50%. The preparation was kept at 4°C in ammonium sulfate (50% saturation, pH 7.0). For the biological tests the preparation was dialyzed against physiological saline. Activity of the thymosine was verified in the test of restoring the sensitivity of rosette-forming cells of thymectomized mice to the inhibitory action of azathioprine [6].

Killer activity was estimated by the ability of T lymphocytes to inactivate endogenous hematopoietic stem cells during the graft versus host reaction [1]. Lymph node cells from CBA mice were transplanted into sublethally irradiated (600 R) (CBA × C57Bl)F_1 mice, and the

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number of endogenous colonies in the recipient's spleen was determined 9 days after irradiation.

Lymph node cells of normal and T-deficient mice were incubated with different concentrations of thymosine in medium 199 at 37°C for different time intervals, thoroughly washed with medium, and transplanted into sublethally irradiated recipients.

The numerical results were subjected to statistical analysis with calculation of the standard error (m), the arithmetic mean (M), and the confidence interval (I) by the ordinary method.

EXPERIMENTAL RESULTS

The effect of incubation of lymph node cells from normal CBA mice or irradiated CBA mice receiving injections of syngeneic bone marrow with thymosine in different concentrations on their activity toward endogenous stem colony-forming units (CFU) of sublethally irradiated (CBA × C57BL)F_1 hybrids is shown in Fig. 1. The cells were incubated *in vitro* for 90 min. As Fig. 1 shows, thymosine in concentrations of 50, 100, and 250 μg/ml caused a marked increase in the ability of lymph node cells of normal CBA mice to inhibit endogenous colonies formed in the spleen of the sublethally irradiated recipients. However, when lymph node cells were used from irradiated CBA mice protected with syngeneic bone marrow (syngeneic radiation chimeras), an increase in the killer activity of their lymphocytes was observed only in the presence of thymosine in concentrations of 100 and 250 μg/ml.

The results of a study of the effect of the incubation time of lymph node cells with thymosine on the killer activity of the lymphocytes are shown in Fig. 2. Incubation of lymph node cells from normal CBA mice or from CBA → CBA radiation chimeras with thymosine for 30-90 min led to a considerable increase in the killer activity of the lymphocytes (Fig. 2). Treatment of the lymphocytes with thymosine for 5 min did not affect their activity.

In the next experiment an attempt was made to determine which cells of the killer series were the targets for the action of thymosine. For this purpose the effect of thymosine was studied on killer activity of lymph node cells from CBA → CBA radiation chimeras and or previously thymectomized CBA → CBA radiation chimeras (B mice). The results are given in Table 1. Transplantation of 0.4×10^6 lymph node cells from CBA mice into sublethally irradiated recipients was accompanied by 50% inactivation of endogenous CFU, whereas transplantation of 0.2×10^6 cells inactivated about 30% of the CFU. After incubation of lymph node cells with thymosine, transplantation of these doses of cells inhibited 90-100% of endogenous CFU, respectively. A similar effect of potentiation of the killer activity of the lymphocytes was obtained in experiments with lymph node cells of irradiated mice receiving injections of syngeneic bone marrow and with their thymus intact (syngeneic radiation chimeras). Two weeks after irradiation lymph node cells of the CBA → CBA radiation chimeras had virtually no effect on endogenous CFU during transplantation into sublethally irradiated T_1 recipients, i.e., killer activity was virtually absent. Treatment of lymphocytes of syngeneic chimeras with thymosine

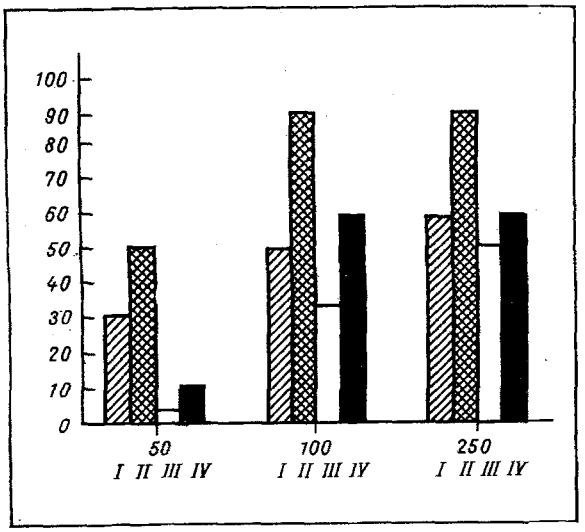

Fig. 1. Effect of thymosine concentration *in vitro* on killer effect of lymph node cells. Incubation time 90 min. Here and in Fig. 2: abscissa) thymosine concentration (in μg/ml); ordinate) inhibition of CFU (in %). I) Lymph node cells from intact mice. Dose of cells 0.2×10^6; II) lymph node cells of intact mice. Dose of cells 0.4×10^6; III) lymph node cells of radiation chimeras. Dose of cells 1×10^6; IV) lymph node cells from radiation chimeras. Dose of cells 3×10^6.

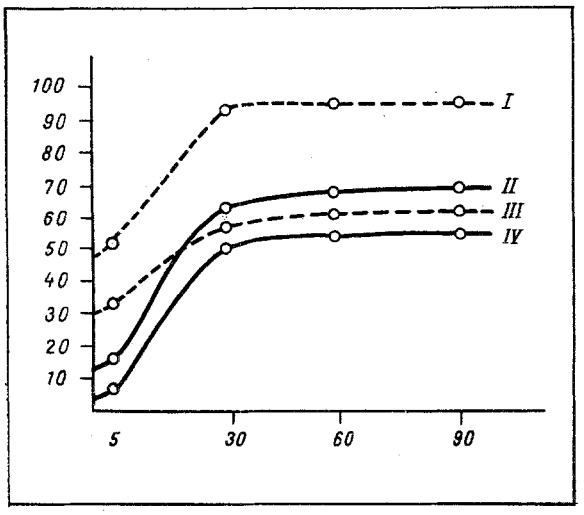

Fig. 2. Effect of incubation time with thymosine *in vitro* on killer effect of lymph node cells. Thymosine concentration 250 μg/ml. I, III) Lymph node cells of intact mice. Dose of cells: I) 0.4×10^6; III) 0.2×10^6; II, IV) lymph node cells from radiation chimeras. Dose of cells: II) 3×10^6; IV) 1×10^6.

TABLE 1. Action of Thymosine on Killer Activity of Lymph Node Cells

Donors of lymph node cells	Number of transplanted cells	Number of mice	Number of colonies per spleen	Inhibition of CFU, %	I_p
Intact CBA mice:	—	30	11,9±0,08	—	11,8–12,0
control	$0{,}4\times10^6$	25	5,4±0,07	55	5,26–5,54
	$0{,}2\times10^6$	24	7,6±0,12	36	7,36–7,84
incubation with thymosine	$0{,}4\times10^6$	10	1,0±0,06	92	0,87–1,13
	$0{,}2\times10^6$	12	3,3±0,15	73	3,0–3,6
Radiation chimeras					
control	3×10^6	10	9,1±0,3	18	8,43–9,77
	1×10^6	14	13,2±0,31	0	12,6–13,8
incubation with thymosine	3×10^6	11	4,0±0,02	67	3,6–4,4
	1×10^6	13	5,3±0,23	56	4,8–5,8
B mice					
control	3×10^6	16	7,8±0,14	35	7,5–8,1
incubation with thymosine	3×10^6	17	8,4±0,24	30	7,9–8,9

restored the killer activity of the cells on the endogenous CFU of T_1 recipients. However, incubation of lymph node cells from B mice with thymosine was not accompanied by any increase in killer activity in the population of these lymphocytes. Table 1 shows that lymph node cells of B mice did not inhibit endogenous CFU in either the control or the experimental group after treatment with thymosine.

Treatment of lymphocytes of normal mice and syngeneic chimeras, but not of B mice, thus increases their killer activity against foreign stem cells. The humoral factor of the thymus is known to induce differentiation of prethymic precursors (prothymocytes) of B cells with the antigenic and functional (response to concanavaline A) characteristics of thymus lymphocytes *in vitro*, but not to affect differentiation of B cells [2]. It has also been shown that differentiation of prothymocytes into T cells possessing helper properties takes place *in vitro* under the influence of thymosine [7]. The present investigation showed that thymosine can also induce differentiation of T killers. T killers are induced only during incubation of lymph node cells from normal mice of syngeneic radiation chimeras with thymosine, but this procedure does not affect the killer activity of lymph node cells from B mice. Hence it follows that thymosine affects cells of the killer line only in the postthymic stage of development, evidently the immediate precursors of the T killers. In other words, thymosine cannot induce differentiation of T killers unless their precursors have passed through the thymocyte stage in the thymus. The spleen of radiation chimeras 14 days after irradiation is known to be already repopulated by a subpopulation of T_1 cells which has migrated from the thymus, but does not yet contain T_2 cells [9]. The spleen of B mice contains practically no T_2 or T_1 cells. Consequently, *in vitro* T_1 cells differentiate into T killers under the influence of thymosine. In other words, in this case the target cell for the action of thymosine is the T_1 cell.

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SOME ASPECTS OF THE PATHOLOGY OF MITOSIS IN A CHINESE HAMSTER CULTURE DURING DISTURBANCES OF THE PROTEIN-SYNTHESIZING SYSTEM

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After disturbance of protein synthesis by puromycin and disturbance of transcription of chromosomal and ribosomal RNA by actinomycin D considerable changes were observed in the normal course of mitosis. An increase in the number of colchicine-like mitoses (c mitoses) sometimes accompanied, in particular, by segmentation of their cytoplasm with the formation of racemose structures, were observed. It is suggested that the development of c mitoses is associated not only with disturbances in the system of formation of the mitotic apparatus, but also with blocking of the synthesis of one of the chromosomal proteins that stabilize the spiralization of DNA strands. The other disturbance of division arising as a result of depression of metabolism, namely hollow metaphase, is associated not only with disturbances of the formation of the division spindle, but also with chromosomal changes. Selective depression of transcription of ribosomal RNA led to definite delay of anaphase and to coupling of the telomeric regions of the chromosomes, evidently on account of disturbance of the "protective membrane" of the chromosomes formed by RNA of the disintegrating nucleoli and RNA of the perichromatin granules.

KEY WORDS: *pathology of mitosis; RNA transcription; protein synthesis; cell culture.*

Pathological mitoses (PM), the mechanism of their origin, and also the applied importance of their investigation have repeatedly been examined in different types of cells [1]. In the study of the mitotic regime when protein synthesis is blocked and transcription of chromosomal and ribosomal RNA inhibited in culture, the writers discovered certain distinguishing features of the disturbance of mitosis. Knowing the character of the cytochemical changes causing them, it is possible to imagine the possible mechanisms with which these disturbances of normal cell division are connected.

EXPERIMENTAL METHODS

Observations were made on a synchronized culture of Chinese hamster fibroblast-like cells (line Bl1dii FaF-28 clone 237), in which different metabolic processes were inhibited during different periods of interphase. Protein synthesis was inhibited by puromycin (Serva, 10 μg/ml), transcription of total chromosomal RNA (cRNA) by actinomycin D (Reanal, 1 μg/ml), and synthesis of ribosomal RNA (rRNA) selectively by actinomycin D in a dose of 0.1 μg/ml. The incidence and character of PM were investigated during the 1st and 2nd waves of mitosis after synchronization of the cells by mitotic selection after preliminary treatment with colcemid.

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