INTERACTION BETWEEN THYMALIN AND LYMPHOCYTES FROM DIFFERENT SOURCES IN EXPERIMENTS *IN VIVO* AND *IN VITRO*

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Treatment of immature mouse bone marrow cells with thymus polypeptide preparations such as thymosin and thymalin *in vitro* has been shown to promote maturation of T precursors into T lymphocytes [5, 7]. Thymectomy in adult mice leads to elimination of T cells from the spleen, and the preparations mentioned above restore their number [4, 8]. The origin of the T lymphocytes which appear in the spleen of thymectomized animals under the influence of the thymus factors is not known.

The aim of the present investigation was to study the content of T lymphocytes in the bone marrow and spleen of thymectomized mice and mice undergoing a mock operation, treated with thymalin *in vivo* and *in vitro*.

EXPERIMENTAL METHOD

Thymalin, prepared by acetic acid extraction of calf thymus glands [6], is a complex of polypeptide fractions with molecular weight of under 10,000. Male CBA mice weighing 16-18 g underwent thymectomy or a mock operation, and 3-4 months later thymalin was injected subcutaneously in a dose of 2.5 μg/g on alternate days for 10 days into the animals, or intact spleen and bone marrow cells of these animals were treated with thymalin *in vitro* in a dose of 10 μg/ml at a temperature of 37°C, with constant shaking, for 1.5 h. Thymectomy [3] was performed under ether anesthesia on animals aged 3-4 weeks. After injection of thymalin into the animals or after treatment of lymphoid cells with thymalin *in vitro*, the cell suspensions in both cases were washed at least five times, for 10 min each time, at 1200 rpm with cold medium 199, after which the number of T lymphocytes in them was determined.

The T lymphocytes were detected in the complement-dependent cytotoxic test [1, 2] by means of antibrain serum. The anteserum was prepared by repeated subcutaneous immunization of a rabbit with brain homogenate from CBA mice without Freund's adjuvant [1]. The rabbit was exsanguinated 1 week after immunization, its serum was heated to 56°C for 30 min, absorbed with a homogenate of mouse liver and with mouse and sheep's red blood cells [1, 2], and used in the cytotoxicity test with lymphocytes from various sources in a dilution of 1:80. In this dilution, in the presence of fresh guinea pig complement (1:3) which, in this concentration, has no toxic action on lymphocytes, the antibrain serum caused death of 91.8 ± 1.6% of thymocytes and 1.2 ± 0.5% of bone marrow cells. The number of T lymphocytes was determined individually in each animal. In each test at least 100 cells were counted and their viability estimated with a 0.2% aqueous solution of trypan blue. The experiment was repeated at least 4-5 times.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that injection of thymalin into thymectomized mice completely restored to normal the number of T cells in the spleen, which had been sharply reduced after the operation, and increased the number of T cells in the bone marrow from 1.2 ± 0.5 to 23.9 ± 2.7%. Injection of thymalin into animals undergoing the mock operation, like its injection into thymectomized mice, increased the number of T lymphocytes to 17.1 ± 2.4% compared with 3.2 ± 1.2% after injection of physiological saline, but reduced the number of T cells in the spleen to 22.8 ± 2% compared with 33.2 ± 2.2% in the control.

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TABLE 1. Effect of Thymalin on Number of T Cells in Bone Marrow and Spleen of Mice Undergoing Mock Operation and Thymectomized Mice *in Vivo* (M ± m)

Group of animals	Source of cells	Number of T cells – cytotoxicity index of antibrain serum (in %) after treatment of mice with	
		thymalin	physiological saline
Undergoing mock operation	Bone marrow	17,1±2,4*	3,2±1,2
	Spleen	22,8±2,0*	33,2±2,2
Thymectomized	Bone marrow	23,8±2,7*	1,2±0,5
	Spleen	31,5±1,9*	2,2±0,7

Legend. Here and in Table 2 each number denotes results of 4-5 experiments (counting at least 600-800 cells). Viability of cells with normal serum was 80-90%. Asterisk indicates significant difference compared with corresponding values in control (injection of physiological saline or treatment of cells with medium 199 respectively) at the $P < 0.01$ level.

TABLE 2. Effect of Thymalin on Number of T Cells in Bone Marrow and Spleen of Mice Undergoing Mock Operation and of Thymectomized Mice *in Vitro* (M ± m)

Group of animals	Source of cells	Number of T cells – cytotoxicity index of antibrain serum (in %) after treatment of cells with	
		thymalin	medium 199
Undergoing mock operation	Bone marrow	17,5±2,1*	10,7±2,2
	Spleen	21,1±2,0*	30,0±2,0
Thymectomized	Bone marrow	28,8±3,2*	9,6±2,7
	Spleen	14,0±1,9	9,4±1,8

After treatment of spleen cells of thymectomized mice with thymalin *in vitro* (Table 2) only a tendency was observed for the number of T cells to increase. Treatment of splenocytes of mice undergoing a mock operation with thymalin, just as in the experiments *in vivo*, reduced the number of T lymphocytes. After treatment with thymalin *in vitro* the number of T lymphocytes in the bone marrow cell population of the thymectomized mice and mice undergoing the mock operation increased in the same way as was observed after the action of thymalin *in vivo*.

These results showed that not only in experiments *in vitro*, but also after injection of thymalin into animals an increase in the number of T lymphocytes is observed in the bone marrow cell population. Since it has been shown [5, 6] that treatment of bone marrow cells *in vitro* causes more rapid maturation of T precursors into T lymphocytes, it can be tentatively suggested that it has the same action *in vivo* also. At the same time it must be pointed out that the effect of thymalin on splenocytes of thymectomized mice *in vitro* was not accompanied by any significant increase in the number of T cells, whereas in experiments *in vivo* the number of T lymphocytes in the spleen was completely restored to normal under the influence of thymalin. These differences are evidence that precursors of T cells sensitive to the action of thymalin are not present in the spleen. Very probably the appearance of T cells in the spleen of thymectomized mice in experiments *in vivo* is due to their migration from the bone marrow.

The acquisition of some resistance by splenocytes of mice undergoing mock operations to antibrain serum after the action of thymalin may perhaps be evidence of screening of the T cells by this preparation. Further experiments are needed to test this hypothesis.

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