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EFFECT OF A HIGHLY PURIFIED FACTOR FROM THE THYMUS ON CELLULAR AND HUMORAL INDICES OF IMMUNITY IN THYMECTOMIZED MICE

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In experiments on thymectomized adult CBA mice the effect of a homogeneous factor of polypeptide nature from the thymus, with mol. wt. about 5000 (thymarin-III) on the cellular and humoral indices of immunity was studied in animals. Thymectomy in animals was shown to sharply reduce the number of T-cells in the spleen. Correspondingly, the ability of the mice to produce both IgM- and IgG-antibody-forming cells and humoral antibodies against a thymus-dependent antigen (sheep's red blood cells) was sharply inhibited in the mice. Subcutaneous injection of thymarin-III in a dose of 1 μg/kg into the animals daily for 7 days completely restored the T-cell population of the spleen and restored the normal immunologic reactivity of the animals.

KEY WORDS: thymus factor; thymectomy; T-lymphocytes; antibody-formation.

It has now been shown that certain factors isolated from the thymus can completely or partly restore the immunologic indices in neonatally thymectomized animals [7, 10]. Thymectomy in adult animals is known to lead chiefly to a reduction in the T_1-population of short-living lymphocytes [5] and to simulate to a considerable degree certain acquired immunodeficiency states. In this connection the possibility of restoring immunologic reactivity in thymectomized adult animals is interesting. The writers previously demonstrated the stimulating action of a highly purified factor from the thymus on the immune response to thymus-dependent antigen in intact animals [1].

The object of the present investigation was to study the effect of thymus factor on the cellular and humoral indices of immunity in adult thymectomized mice.

EXPERIMENTAL METHOD

Experiments were carried out on 105 male CBA mice weighing 16-18 g. The thymus was removed from 75 animals, under superficial ether anesthesia, by a modified method of Galkin and Drobkin [3]. A mock operation was performed on 30 mice, i.e., all stages of the operation except actual removal of the thymus.

Between 1.5 and 2 months after the operation thymus factor was injected subcutaneously into 40 thymectomized animals in a dose of 1 μg/g in 0.2 ml of physiological saline daily for 7 days. The thymus preparation

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TABLE 1. Immunologic Indices in Thymectomized Mice after Injection of Thymus Factor (M ± m)

Index	Undergoing mock operation	Thymectomized	
	physiological saline		thymarin-III
Number of T-cells (index of cytotoxicity of antibrain serum), %			
in spleen	37,6±3,0	8,8±1,3*	35,6±2,8†
in lymph nodes	65,0±2,9	59,0±2,9	77,0±2,4†
Direct AFC (IgM), per 10^6 spleen cells	170,0±33,0	47,6±7,7*	158,0±22,6†
Indirect AFC (IgG) per 10^6 spleen cells	482.0±71,3	171,4±23,8*	498,0±88.8†
Reciprocals of hemagglutinin titers	568,8±35,9	240,0±40,1*	571,4±75,3†

Legend. *) Difference significant compared with analogous indices for animals undergoing mock operation and receiving physiological saline ($P < 0.001$); †) difference significant compared with analogous indices in thymectomized mice receiving physiological saline ($P < 0.001$).

was a polypeptide with mol. wt. about 5000 (thymarin-III), isolated from fresh calf thymus glands by ion-exchange chromatography [4]. The remaining thymectomized animals and control mice undergoing the mock operation received injections of physiological saline in accordance with the same scheme. The animals of one group, consisting of both thymectomized mice and mice undergoing the mock operation, were then immunized by intravenous injection of sheep's red blood cells, carefully washed with physiological saline, in a dose of 1×10^7. Animals of the other group (without immunization) were used for determination of the number of T-cells in the spleen and lymph nodes.

The number of T-lymphocytes was determined in the complement-dependent cytotoxic test [11] with serum against CBA mouse brain in a dilution of 1:10 – a concentration which caused the death of 96 ± 1.8% of thymocytes and 5 ± 2.1% of bone marrow cells. The serum was prepared by repeated subcutaneous immunization of a rabbit with brain homogenate from CBA mice, without Freund's adjuvant [8], after which it was absorbed at room temperature with mouse liver homogenate and mouse and sheep red blood cells [2]. Fresh guinea pig serum (1:3), absorbed in the same way by mouse liver and spleen homogenates and by mouse and sheep red blood cells, was used as the complement.

To determine the number of T-cells in the lymph nodes, pools of cells from three animals were used; the spleen was tested individually in each mouse. In each test no fewer than 100 cells were counted and their viability estimated with the aid of a 0.2% aqueous solution of trypan blue. Altogether nine mice from each group were used to determine the number of T-cells in the lymph nodes and spleen.

The animals were decapitated 5 days after immunization. The sera were tested in the hemagglutination test. The number of direct and indirect antibody-forming cells (AFC) in the spleen was determined. Direct AFC were detected by the method of Jerne and Nordin [9], indirect by the method of Dresser and Wortis [6]. To detect indirect AFC, rabbit serum (1:80) against mouse IgG, isolated with the aid of caprylic acid [12], was used. The number of direct and indirect AFC was expressed per 10^6 nucleated cells. Antibodies and AFC were tested individually in each mouse. Each group studied contained 25-30 animals.

EXPERIMENTAL RESULTS

As Table 1 shows, thymectomy on adult animals led to a sharp decrease in the number of T-cells in the spleen but had no significant effect on their number in the lymph nodes. The ability to form antibodies was depressed in thymectomized mice, as shown by the weak increase in their titers and by the reduction of 67-75% in the number of AFC compared with their number in control animals undergoing the mock operation.

Injection of thymarin-III into the thymectomized animals led not only to complete recovery of the T-cell population in the spleen of the thymectomized mice, but also to some increase in their number in the lymph nodes, while the immunologic reactivity of the animals was completely restored to normal (Table 1).

The results are evidence that thymarin-III affects the thymus-dependent lymphocyte population in adult animals. The complete recovery of the T-cell population in the spleen of the thymectomized mice and the weak effect on the number of T-cells in the lymph nodes suggest that thymarin-III acts on the T_1-population of lymphocytes, with the consequent restoration of the cooperative immune response to thymus-dependent antigen.

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