# EFFECT OF POLYPEPTIDES ISOLATED FROM THE THYMUS, CEREBRAL CORTEX, AND WHITE MATTER ON INDICES OF CELLULAR AND HUMORAL IMMUNITY IN THYMECTOMIZED MICE

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UDC 612.017.1−06:[612.82+612.438].015.21

The effect of low-molecular-weight polypeptides (mol. wt. under 10,000), isolated by acetic acid extraction from the bovine thymus and cerebral cortex and white matter, on indices of cellular and humoral immunity was investigated in experiments on adult thymectomized CBA mice. Thymectomy sharply reduced the number of T cells in the spleen. The ability of these mice to produce both IgM and IgG antibody-forming cells and humoral antibodies to sheep's red blood cells, a thymus-dependent antigen, was correspondingly reduced considerably. Subcutaneous injection of polypeptides from the thymus or cerebral cortex into animals in a dose of 1 μg/g over a period of 8 days not only completely restored the T cell population of the spleen and the immunologic reactivity of the thymectomized mice, but actually raised these indices by 50-100% compared with animals undergoing a mock operation and receiving physiological saline. Extract from the white matter of the brain had no biological activity.

KEY WORDS: thymectomy; cross-reacting antigens; thymus; brain; stimulation of the immune response.

The θ antigen characteristic of the thymus-dependent lymphocyte population in various species in animals is also present in the brain [1, 7, 11]. This antigen is associated mainly with the gray matter (cortex) and is virtually absent in the white matter of the brain [1, 6].

Components isolated from the thymus have been shown to stimulate the immune response in animals, especially after thymectomy, when they replace the receptors of the T cells functionally [2, 10, 13]. The question arises whether substances contained in the cerebral cortex also possess this effect.

The object of this investigation was to compare the effect of polypeptides isolated from the thymus and the cerebral cortex and white matter on indices of cellular and humoral immunity in thymectomized mice.

## EXPERIMENTAL METHOD

Extracts from the bovine thymus and cerebral cortex and white matter were obtained by acetic acid extraction followed by precipitation of the extracted material with acetone [4] and were provided for the investigation by V. G. Morozov and V. Kh. Khavinson to whom the writer is grateful. These extracts consisted of various fractions of polypeptide material with a molecular weight of under 10,000.

The polypeptide from the thymus has been named thymarin [2]. The preparation from the cerebral cortex was provisionally named cortexin.

Experiments were carried out on 120 male CBA mice weighing 16-18 g. Thymectomy was performed on 96 animals under superficial ether anesthesia by a modified method of Galkin and Dobkin [3]. A mock operation was performed on 24 mice, i.e., all stages other than removal of the thymus were carried out.

Polypeptides isolated from the thymus or the cerebral cortex or white matter were injected in a dose of 1 μg/g body weight, in 0.5 ml physiological saline, into the experimental thymectomized animals 2.5-3 months after the operation subcutaneously for 5 days before and 3 days after immunization. Physiological saline was

Laboratory of General Immunology, Department of Microbiology and Immunology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR V. I. Ioffe.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 6, pp. 572-574, June, 1979. Original article submitted May 4, 1978.

0007-4888/79/8706-0601$07.50  

TABLE 1. Effect of Low-Molecular-Weight Polypeptides from Thymus and Cerebral Cortex and White Matter on Immunologic Indices in Thymectomized Mice (M ± m; n = 12)

| Index | Thymectomized mice receiving various substances | | | | Mice undergoing mock operation and receiving physiological saline |
|---|---|---|---|---|---|
| | physiological saline | polypeptide from white matter of brain | polypeptide from gray matter of brain | polypeptide from thymus (thymarin) | |
| Number of T cells in lymph nodes (index of cytotoxicity of antibrain serum), % | 58,5±6,1 | 57,4±6,1 | 59.6±6,1 | 60,0±5,8 | 59,3±4.7 |
| Number of T cells in spleen (index of cytotoxicity of antibrain serum), % | 4,0±0,3 | 4,6±0,4 | 40,4±1,9* | 35,8±2,1† | 28,9±2,0 |
| Number of direct (IgM) AFC in $10^6$ splenic karyocytes | 17,3±1,2 | 18,5±2,0 | 67,0±2,3* | 62,5±2,0* | 42,0±2,3 |
| Number of indirect (IgG) AFC in $10^6$ splenic karyocytes | 13,8±1,9 | 16,3±3,5 | 62,8±5,9‡ | 59,4±5,4† | 39,3±5,0 |
| Reciprocals of serum hemagglutinin titer | 253,3±14,8 | 333,3±44,5 | 1120,0±59,3* | 1066,0±59,3* | 533,3±29.6 |

*, †, ‡ Denote indices differing significantly from corresponding indices for animals undergoing mock operation and receiving physiological saline at the levels of $P < 0.001$, 0.05, and 0.01 respectively.

injected in accordance with a similar scheme into the control thymectomized animals and mice undergoing the mock operation. Of the animals undergoing thymectomy and the mock operation, some were immunized intravenously with sheep's red blood cells (SRBC), thoroughly washed with physiological saline, in a dose of $1 \cdot 10^7$ cells, and others (not immunized) were used to determine the number of T cells in the spleen and in the lymph nodes.

The number of T lymphocytes was determined in the complement-dependent cytotoxic test [14] with serum against CBA mouse brain in a dilution of 1:10, a concentration causing death of 96.0 ± 1.8% of thymocytes and 5.0 ± 2.1% of bone marrow cells. The serum was prepared by repeated subcutaneous immunzation of a rabbit with CBA mouse brain homogenate without Freund's adjuvant [11], after which it was absorbed at room temperature with mouse liver homogenate and with mouse red blood cells (MRBC) and SRBC [1]. Fresh guinea pig serum (1:3), absorbed with mouse liver and spleen homogenate, MRBC, and SRBC in a manner similar to absorption of complement with agarose [8], 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 spleens were tested individually from each mouse. In each sample no fewer than 100 cells whose viability had been estimated with 0.2% aqueous solution of trypan blue were counted. Altogether 12 mice in each group were used for determination of the number of T cells in the lymph nodes and in the spleen.

The animals were decapitated on the 4th day after immunization. Their sera were studied in the hemagglutination test. The number of direct and indirect antibody-forming cells (AFC) was determined in the spleens. Direct AFC were revealed by Jerne and Nordin's method [12], indirect by the method of Dresser and Wortis [9]. To find indirect AFC, rabbit serum (1:400) against mouse IgG, isolated with the aid of caprylic acid [15], was used. The number of direct and indirect AFC was counted in $10^6$ splenic karyocytes. Antibodies and AFC were investigated individually in each mouse. Each group studied contained 12 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 effect on their number in the lymph nodes. Ability to form antibodies was depressed in the thymectomized mice, as shown both by the small increase in their titer and by the decrease of 60-67% in the number of IgM and IgG AFC compared with control animals undergoing the mock operation.

Injection of the preparation from the thymus (thymarin) or the polypeptide from the cerebral cortex (cortexin) into thymectomized animals not only led to complete restoration of the T cell population in the spleen of the thymectomized mice, but gave a considerable increase in the number of T cells compared with animals undergoing the mock operation and receiving physiological saline ($P < 0.05$ and 0.001 respectively). The number of T lymphocytes in the lymph nodes was unchanged under these circumstances.

Restoration of the T cell population of the spleen under the influence of cortexin or thymarin in the thymectomized mice was followed not only by recovery, but also by considerable stimulation of the immune re-

sponse compared with animals undergoing the mock operation. The number of direct and indirect AFC increased by 50% ($P < 0.01$) and the hemagglutinin titer by 100% ($P < 0.001$).

The extract from the white matter of the brain did not restore the T cell population in the spleen of the thymectomized mice. It likewise had no effect on the reduced immunologic reactivity of the thymectomized animals.

The results suggest that a constituent of the cerebral cortex possessing common features with the $\theta$ antigen of the T lymphocytes can be isolated as a polypeptide fraction able to restore the T cell population of the spleen in thymectomized mice and, correspondingly, to stimulate the immune response to thymus-dependent antigen.

The full recovery of the T cell population in the spleen of the thymectomized mice, coupled with the complete absence of any effect on the number of T cells in the lymph nodes, suggest that thymarin and cortexin act on the $T_1$ population of lymphocytes, which is sensitive to thymectomy [5] and located predominantly in the spleen of animals.

It must be emphasized that cortexin was isolated by the same method [4] as the reference substance extracted from the thymus in this investigation, namely thymarin. However, the polypeptide extracted from the white matter of the brain by a similar method was inactive.

It is now generally accepted that the biologically active factor of the thymus, i.e., thymosin (thymarin in the present investigation), can confer immunocompetence on undifferentiated lymphocytes [10, 13]. We have no evidence from which it can be concluded that the brain extract (cortexin) plays any direct part in the activity of the immune system. The existence of common antigenic and biological properties in the substances isolated from the cerebral cortex and thymus may point to certain common mechanisms in the specific function of the nervous and immune systems.

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