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# ANTIGENIC KINSHIP OF POLYPEPTIDE STIMULATORS OF IMMUNOGENESIS FROM THE THYMUS AND CEREBRAL CORTEX

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The writer showed previously [4] that injection of a low-molecular-weight polypeptide from the cerebral cortex into mice has a marked stimulating action on immunogenesis, similar to the effect of a polypeptide prepared from the thymus by the same method [3]. The low-molecular-weight polypeptide isolated in this way from the white matter of the brain had no such effect [4]. It was therefore interesting to study whether the stimulators of immunogenesis from the thymus and cerebral cortex are antigenically related, or whether the cortex, where the θ-antigen is located [2, 10], contains an active substance which resembles the polypeptide from the thymus only functionally.

The object of this investigation was to make a serologic analysis of low-molecular-weight polypeptides from the thymus, cerebral cortex, and white matter of the brain in two tests: the complement fixation test and the complement-dependent cytotoxic test.

## EXPERIMENTAL METHOD

Preparations were obtained from the thymus glands and gray and white matter of the brain of calves by acetic acid extraction as described previously [7]. The lyophilized preparations had a molecular weight of under 10,000. It was shown by methods of ascending paper chromatography, ion-exchange chromatography, and electrophoresis on paper and in polyacrylamide gel that the isolated preparations were complexes of polypeptide fractions. The complex of polypeptide fractions from the thymus has been called "thymarin" [3], and the polypeptide from the cerebral cortex was given the working name of "cortexin." A preparation of thymosin with a molecular weight of 12,500, prepared from calf thymus glands by Goldstein's original method [12] in the Department of Virology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, and generously sent to the present writer for investigation, also was studied.

To determine the degree of antigenic kinship between thymarin, cortexin, and polypeptide from brain white matter, antisera were prepared against them and these were cross-absorbed

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TABLE 1. Cytotoxic Activity of Antisera against Polypeptides from Thymus, Cerebral Cortex, and White Matter of the Brain against Lymphocytes of Different Origin, before and after Their Crossed Absorption by Corresponding Polypeptides

| Source of cells | Preparation for absorption | Dilution of antisera | Cytoxicity index (in %) of antisera against | | | |
|---|---|---|---|---|---|---|
| | | | thymosin | thymarin | cortexin | polypeptide from white matter of brain |
| Thymus | — | 1:5 | 52,0±3,6 | 77,0±3,4 | 41,0±4,0 | 7,0±1,8 |
| | — | 1:10 | 45,0±3,6 | 81,6±3,2 | 49,3±4,0 | 8,0±1,9 |
| | — | 1:50 | 0 | 40,8±3,9 | 48,2±4,0 | 0 |
| | — | 1:100 | 0 | 15,5±2,6 | 21,4±2,9 | 0 |
| | Thymosin | 1:10 | 0* | 0* | 0* | N |
| | Thymarin | 1:10 | 0* | 0* | 12,0±2,3* | N |
| | Cortexin | 1:10 | 15,0±2,2* | 45,0±3,6* | 0* | N |
| | Polypeptide from white matter of brain | 1:10 | 50,0±3,2 | 76,6±3,0 | 41,0±3,6 | N |
| Bone marrow | — | 1:10 | 0 | 0 | 0 | 0 |

Legend. *) Difference significant compared with cytoxicity index of corresponding antiserum before absorption ($P < 0.001$); —) serum not absorbed; N) experiment was not done.

with the corresponding preparations. To detect the presence of common antigens in the above-mentioned polypeptides and thymosine, the antisera prepared as described above were absorbed with thymosin.

Antisera were prepared by immunization of rabbits weighing 2-2.5 kg in the footpads of the hind limbs with polypeptides in a dose of 20 mg in Freund's complete adjuvant, containing 5 mg of dried *Mycobacterium tuberculosis* cells in 1 ml. On the 18th day after the first immunization the animals were reimmunized with the same dose of the preparation but without adjuvant, directly into the popliteal lymph nodes. Ten days later the rabbits were exsanguinated and the sera were heated to 56°C for 30 min. The antisera and the sera against thymosin thus prepared were tested with the homonymous preparations in the complement fixation test (CFT), and also in the complement-dependent cytotoxic test with thymus and bone marrow cells from CBA mice. Rabbit sera against thymosin were provided by Dr. Mandi, from the Medical University, Debreczen, Hungary, to whom the author is grateful.

For the CFT polypeptides were taken in a dose of 600 mg/ml; during testing of the cytotoxic activity of the antisera no fewer than 200 cells whose viability was assessed by means of a 0.2% aqueous solution of trypan blue were counted.

The antisera were absorbed at 37°C for 1 h and for 18 h at 4°C in the ratio of 10 mg polypeptide to 1 ml of the test antiserum. Altogether 11 antisera were tested: four antisera each against polypeptide from the thymus and cerebral cortex, two antisera against polypeptide from the white matter of the brain, and one antiserum against thymosin.

## EXPERIMENTAL RESULTS

Sera against thymosin, thymarin, cortexin, and polypeptide from the white matter of the brain reacted in the CFT with homonymous antigens in dilutions expressed in the following reciprocal titers: 24 ± 3.2, 80 ± 17.7, 112 ± 10.6, and 48 ± 8.8, respectively. Antisera against the polypeptide from the white matter of the brain reacted neither with thymosin nor with thymarin nor with cortexin. Antiserum against thymosin reacted in the CFT in equal titers with thymosin, thymarin, and cortexin: 24 ± 3.2. Sera against thymarin, after absorption with cortexin, lost their ability to react with cortexin but reacted with thymarin in a titer of 12 ± 2.3. Sera against cortexin, after absorption with thymarin, lost their ability to react with thymarin, but continued to react with cortexin in a titer of 24 ± 3.2. All antisera were completely exhausted by the homologous antigens. Sera against thymarin and cortexin lost no activity whatsoever after absorption by polypeptides from the white matter of the brain.

In the complement-dependent cytotoxic test antiserum against thymosin in dilutions of 1:5 and 1:10 led to death of 52 ± 3.6 and 45 ± 3.6% of thymus cells, respectively. In a dilution of 1:50, they no longer reacted with thymocytes. Antisera against thymarin and cortexin had a higher titer: They reacted with thymocytes in dilutions of up to 1:100 and caused death of 15.5 ± 2.6 and 21.5 ± 2.9% of thymus cells, respectively. Sera against the polypeptide from white matter of the brain, on the other hand, gave practically no cytotoxic action on thymocytes

(the indices of cytotoxicity of these antisera in dilutions of 1:5 and 1:10 were 7 ± 1.8 and 8 ± 1.9% respectively). No antiserum reacted with bone marrow cells (Table 1).

It is important to note than antisera against cortexin, both in high (1:10) and lower (1:50) concentration, exhibited practically equal cytotoxic activity (49.3 ± 4 and 2 ± 4% respectively). By contrast, antisera against thymarin, which actually had a somewhat lower titer against homologous antigen than sera against cortexin, caused death of 81.6 ± 3.2% of thymus cells in the cytotoxic test in a high concentration (1:10), and to death of 40.8 ± 3.9% of thymus cells in a dilution of 1:50. If the thymocytes treated with antiserum against cortexin (1:10) were washed with cold medium No. 199 (5 min at 1200 rpm) and were additionally treated with antiserum against thymarin (1:10) — either native or after preliminary absorption with cortexin, the percentage of cells which died increased from 49.3 ± 4 to 100. These results were confirmed by crossed absorption of antisera against thymarin and cortexin with the corresponding preparations. Antisera against thymarin (1:10), after absorption with cortexin, only lowered the cytotoxicity index from 81.6 ± 3.2 to 45 ± 3.6%. Absorption of these sera with thymarin, however, completely prevented them from reacting with thymocytes. Antisera against cortexin, after absorption with thymarin, led to death of only 12 ± 2.3% of thymus cells, and they did not react at all with thymocytes after absorption with the homologous antigen. Antiserum against thymosin (1:10) was completely exhausted by thymarin and caused death of 15 ± 2.2% of thymocytes after absorption with cortexin, compared with 45 ± 3.6% before absorption. Antisera against thymarin and cortexin, after absorption with thymosin, were completely deprived of their cytotoxic activity against thymus cells. Meanwhile, antisera against thymosin, thymarin, and cortexin showed no decrease whatever in their cytotoxic activity against thymocytes after absorption with polypeptide from the white matter of the brain (Table 1).

The results are evidence that low-molecular-weight polypeptides from thymus and cerebral cortex are antigenically related and differ in their antigenic structure from the polypeptide from the white matter of the brain.

The biological activity of thymosin, thymarin, and cortexin is directed toward T-cells, for antisera against these preparations react in the cytotoxic test with thymocytes, but do not react at all with bone marrow cells. The test preparations affect the $T_1$-cell population, for in adult thymectomized mice they restore the $T_1$-cells of the spleen [1, 5].

The results of crossed absorption showed that the sera against the above-mentioned polypeptides of the thymus and cerebral cortex contained antibodies both against common antigens and against antigens characteristic of that preparation only. The antigenic constitution of thymarin corresponds more closely to antigens of the thymocytes than that of cortexin. It can be tentatively suggested that there are two varieties of θ-antigen: a θ-antigen characteristic of the thymus only, and a θ-antigen common with that of the cerebral cortex, as has already been described [9].

Since thymosin, thymarin, and cortexin are antigenically similar, and since some properties (molecular weight, amino acid composition) of the θ-antigen of the thymus and cerebral cortex are indistinguishable [8, 14, 15], and are similar to the analogous indices of thymosin and thymarin [6, 12, 13], this suggests that the stimulator of immunogenesis isolated from the thymus (thymarin) is identical with θ-antigen. The experiments of Dardenne and Bach [11], in which anti-θ-serum could not be exhausted by fraction 6a of thymosin does not contradict this hypothesis, for experiments with crossed absorption of antisera against θ-antigen and thymosin with the corresponding preparations but not absorption of anti-θ-serum by one of the fractions of thymosin, can serve as proof of identify or difference between θ-antigen and thymosin.

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# DEPENDENCE OF STRAIN DIFFERENCES IN ANTIBODY RESPONSE TO SHEEP RED BLOOD CELLS AND RESPONSIVENESS OF $F_1$ HYBRIDS ON DOSE AND METHOD OF ADMINISTRATION OF THE ANTIGEN

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KEY WORDS: immune response; sheep's red cells; strain differences.

Investigations into genetic control of the immune response have revealed strains of mice which give opposite responses to sheep's red blood cells and inheritance of a strong type of response has been shown to be dominant in character [2, 3]. In all these investigations high doses of antigen were given and were injected either intraperitoneally or intravenously. It was shown that genetic control over the immune response is effected at the level of immunocompetent cells [3, 4, 7]. It is a familiar fact, however, that besides immunocompetent cells, mononuclear phagocytes also play an essential role in immunogenesis. This suggests that under certain conditions the level of the immune response may be limited by macrophages. Such conditions are perhaps created by immunization with small doses of antigen and by the use of methods of immunization (such as intramuscular injection of antigen) dependent on access of antigen to the lymphoid organs, for which macrophages are evidently responsible.

The object of this investigation was to study dependence of strain differences in the height of the antibody response to sheep's red blood cells in strains of mice responding in opposite directions to this antigen, and also to examine how the character of inheritance of responsiveness depends on the immunizing dose and mode of administration of the antigen.

## EXPERIMENTAL METHOD

Experiments were carried out on inbred CBA, C57BL/6, and BALB/c mice and on (CBA × BALB/c)$F_1$ and (CBA × C57BL/6)$F_1$ hybrids, aged 3-4 months. All animals were obtained from the animal house of the Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR. The mice were immunized by a single intravenous or intramuscular injection of sheep's red blood cells (SRBC) in doses of $5 \times 10^5$ to $1 \times 10^9$ cells. The immunoreactivity of the animals was assessed by the number of antibody-forming cells (AFC) in the spleen by Cunningham's method of local immune hemolysis in the liquid phase [6], in the modification of Kaledin et al. [1]. The number of AFC was counted at the peak of the immune response, namely on the 4th day after intravenous and on the 6th day after intramuscular injection of FRBC.

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