COMPARATIVE ACTION OF LOW-MOLECULAR-WEIGHT FRACTIONS FROM POLYPEPTIDES OF THYMUS AND CEREBRAL CORTEX ON THE IMMUNE RESPONSE IN MICE

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The writers showed previously [3, 5] that low-molecular-weight polypeptides from the thymus and cerebral cortex stimulate the primary immune response to sheep's red blood cells (SRBC) in mice. A similar, but much weaker action on the immunologic reactivity of animals is exerted by the low-molecular-weight fraction from thymus polypeptide [4].

The object of this investigation was to compare the effects of low-molecular-weight fractions of polypeptide nature from the thymus and cerebral cortex on the primary immune response to SRBC in mice.

EXPERIMENTAL METHOD

Freeze-dried preparations of polypeptide nature with mol. wt. of under 10,000 were obtained from the thymus glands and gray and white matter of the brains of calves by acetic acid extraction [7]. The polypeptide from the thymus has been named thymarin [4], and the preparation from the cerebral cortex was given the working name of "cortexin."

By ion-exchange chromatography on the carboxylic cation-exchange resin "Biocarb" [6], under similar conditions individual fractions with a molecular weight of not more than 5000 were isolated from polypeptides from the thymus and from the cortex and white matter of the brain [8]. On investigation by the gel-filtration method all fractions were found to be homogeneous substances.

To determine the degree of antigenic kinship of the polypeptides, sera were prepared against them and cross-absorption tests carried out. The antisera were prepared by immunization of rabbits twice with the polypeptides in doses of 20 mg each time. The first time the material was injected in Freund's complete adjuvant, the second time (18 days after the first immunization) without the adjuvant, directly into the popliteal lymph nodes. The antisera thus prepared were tested with homonymous preparations in the complement fixation test (CFT) and also in the complement-dependent cytotoxic test with thymus and bone marrow cells of CBA mice. When the cytotoxic activity of the antisera was tested, at least 200 cells were counted and their viability was assessed with the aid of a 0.2% aqueous solution of trypan blue.

Absorption of the antisera was carried out at 37°C for 1 h and at 4°C for 18 h in the ratio of 10 mg polypeptide to 1 ml test antiserum. Altogether 10 antisera were tested.

Experiments were carried out on 270 male CBA mice weighing 16-18 g. The test substances were injected subcutaneously in 0.5 ml of physiological saline daily for 5 days before immunization of the animals and for 3 days thereafter. The mice were immunized by a single intravenous injection of 5×10^6 freshly obtained SRBC, washed with physiological saline. On the 4th day after immunization the hemagglutinin titer of the blood sera was determined, and the number of direct (IgM) and indirect (IgG) antibody-forming cells (AFC) was determined in the spleen cells. Direct AFC were estimated by Jerne and Nordin's method [12], indirectly by Dresser and Wortis' method [10], using rabbit serum (1:400) against mouse IgG, isolated with the aid of caprylic acid. The number of direct and indirect AFC was expressed per 10^6 karyocytes. Antibodies were determined in each mouse individually, AFC in pools of three spleens from the animals.

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TABLE 1. Effect of Low-Molecular-Weight Fractions from Brain and Thymus Polypeptides on Level of Immune Response in Mice (M ± m)

Preparation	Dose, μg/g body weight/day	Number of animals	Reciprocal of hemagglutinin titer in serum	Number of AFC per 10^6 splenic karyocytes	
				direct (IgM)	indirect (IgG)
Physiological saline	—	51	125,3±11,0	16,5±1,6	16,2±3,4
Fraction from polypeptide of white matter of brain	1,0	18	155,5±16,0	18,1±2,6	18,1±3,0
	0,25	36	137,7±18,8	17,5±0,6	16,3±0,9
Fraction from polypeptide of gray matter (cerebral cortex) (cortexin)	1,0	18	960,0±42,9*	82,5±1,2*	78,5±1,4*
	0,25	18	533,3±21,3*	64,5±1,1*	61,9±1,6*
	0,05	33	155,0±15,1	44,4±1,7*	43,0±1,5*
	0,01	18	138,6±6,2	26,5±0,9*	25,8±1,2*
Fraction from polypeptide of thymus (thymarin)	1,0	18	946,6±42,6*	72,6±2,4*	71,3±2,1*
	0,25	18	501,1±21,3*	49,1±1,0*	42,8±0,8*
	0,05	18	142,2±16,0	38,2±1,7*	37,4±1,3*
	0,01	18	138,0±21,6	17,0±0,4	16,5±1,3

*Difference statistically significant compared with corresponding index in animals receiving physiological saline ($P < 0.001$).

EXPERIMENTAL RESULTS

Characteristic properties of the polypeptides isolated from thymus, cerebral cortex, and white matter were as follows. Sera against thymarin, cortexin, and polypeptide from the white matter of the brain interacted in the CFT with homonymous preparations in dilutions of 1:64, 1:128, and 1:32, respectively. Sera against thymarin, exhausted with cortexin, lost their ability to react with cortexin, but interacted with thymarin in dilutions of 1:8-1:16. Sera against cortexin, after absorption with thymarin, lost their ability to react with thymarin, but continued to react with cortexin in dilutions of 1:16-1:32. Both groups of antisera were completely exhausted by the homonymous antigens, but they did not lose their activity after absorption with polypeptide from the white matter of the brain. Correspondingly, sera against polypeptide from the white matter of the brain reacted with neither thymarin nor cortexin.

In the complement-dependent cytotoxic test, sera (1:10) against polypeptides from thymus and cerebral cortex led to death of 81.6 ± 3.2 and 49.3 ± 4% thymus cells respectively. Sera against polypeptide from the white matter of the brain, on the other hand, had practically no effect on the thymocytes: The index of their cytotoxicity was only 8 ± 1.9%. None of the antisera reacted in the cytotoxic test with bone marrow cells.

It will be clear from Table 1 that injection of fractions from thymarin or cortexin into mice in doses of 1 and 0.25 μg/g led to a considerable increase both in the level of circulating antibodies and in the numbers of direct and indirect AFC. The preparation from the cortex in a dose of 0.25 μg/g stimulated AFC formation by a significantly higher degree than the same dose of the fraction from thymarin ($P < 0.001$). Injection of fractions from thymarin or cortexin into mice in a dose of 0.05 μg/g stimulated the production of direct and indirect AFC but did not affect the hemagglutinin level. In a dose of 0.01 μg/g only the fraction from cortexin increased the number of AFC. Injections of the preparation from thymarin in this dose had no effect in general on the immune response of the animals. Injection of the fraction from polypeptide isolated from the white matter of the brain did not affect the immunologic indices, even in cases when the preparation was injected in large doses (Table 1).

The writers showed previously that a homogenate of cerebral cortex [1] and the low-molecular-weight polypeptide cortexin, isolated from it [5], have a stimulating action on immunogenesis. The results of the present investigation confirm these observations and indicate that a biologically active low-molecular-weight homogeneous fraction, with which the stimulating effect of cortical tissue on immunogenesis is connected, can be isolated from cortexin.

Stimulation of the immune response to SRBC by fractions from thymarin and cortexin is connected with the effect of the fractions on T-cells, for sera against thymarin and cortexin reacted in the complement-dependent cytotoxic test with thymocytes but did not react with bone marrow cells.

It must be emphasized that polypeptides from the thymus and cortex and their fractions were prepared by the same methods using the same isolation techniques. They were antigenically related and had a similar molecular weight: That of the polypeptides was under 10,000, and of their fractions about 5000 and 3500, respectively.

Cross-reacting θ-antigen of the thymus and brain is known [2, 9, 11, 13, 14] to be present in the cortex but absent in the white matter of the brain. According to the results now obtained, a factor stimulating immuno-

genesis also is present in the cortex only, and not present in the white matter of the brain. It is antigenically similar to polypeptide from the thymus and differs from polypeptide from the white matter of the brain. This suggests that the cerebral cortex contains polypeptide of thymarin type, or that biologically active substances of the thymus and brain are similar to or identical with θ-antigen. The proof of this hypothesis, as well as the solution to the problem of direct participation of the active brain substance in immunogenesis, require a more penetrating and detailed investigation.

LITERATURE CITED

1. G. A. Belokrylov, Byull. Éksp. Biol. Med., No. 9, 327 (1978).
2. G. A. Belokrylov and Yu. L. Zhitnukhin, Zh. Mikrobiol., No. 9, 66 (1976).
3. G. A. Belokrylov, V. G. Morozov, V. Kh. Kavinzon, et al., Byull. Éksp. Biol. Med., No. 7, 56 (1977).
4. G. A. Belokrylov, V. G. Morozov, and V. Kh. Khavinzon, Byull. Éksp. Biol. Med., No. 12, 703 (1978).
5. G. A. Belokrylov, V. G. Morozov, and V. Kh. Khavinzon, Byull. Éksp. Biol. Med., No. 7, 51 (1978).
6. V. G. Morozov, "A factor from the thymus and its effect on the immunologic reactivity of the body," Candidate's Dissertation, Leningrad (1979).
7. V. G. Morozov and V. Kh. Khavinzon, Eksp. Khir., No. 2, 49 (1974).
8. R. T. Acton, J. Addis, G. F. Carl, et al., Proc. Natl. Acad. Sci. USA, 75, 3283 (1978).
9. R. S. Basch and J. L. Kadish, J. Exp. Med., 145, 405 (1977).
10. G. Birnbaum, Brain Res., 84, 111 (1975).
11. M. Dardenne and J. F. Bach, Immunology, 25, 343 (1973).
12. A. L. Goldstein, A. Guha, M. M. Zatz, et al., Proc. Natl. Acad. Sci. USA, 69, 1800 (1972).
13. A. L. Goldstein, T. L. K. Low, M. McAdoo, et al., Proc. Natl. Acad. Sci. USA, 74, 725 (1977).
14. L. D. McClain, M. Tomana, and R. T. Acton, Proc. Natl. Acad. Sci. USA, 159, 161 (1978).

A STUDY OF Fc-RECEPTORS OF HEART VALVE FIBROBLASTS AND THE SEARCH FOR SIMILAR RECEPTORS IN OTHER TISSUES

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All normal human and rabbit sera and also the IgG isolated from them have been shown to react with human and bovine heart valve fibroblasts. These reactions are evidently due to the presence of Fc-receptors on the membranes of heart valve fibroblasts [1]. According to observations by other workers [9], Fc-receptors appeared in cultures of human skin fibroblasts only after infection with cytomegalovirus. The problem of the presence of Fc-receptors on fibroblasts of the interstitial connective tissue (ICT) of the heart and tissues of the joints has not been studied. By now Fc-receptors have been found on the surface of various cells (on lymphocytes, macrophages, and liver, kidney, and placental cells [5, 7, 10, 11]). These receptors have been shown to differ: Some react with immunoglobulins in the form of a complex with antigen, some with monomeric immunoglobulins, some with different classes of immunoglobulins [5, 8, 13, 14, 15].

The object of this investigation was to study some special features of Fc-receptors found on heart valve fibroblasts and also to search for similar receptors on fibroblasts of the ICT of the myocardium and joints and in cultures of mouse fibroblasts (L-cells).

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