

EFFECT OF LOW-MOLECULAR-WEIGHT EXTRACTS  
OF HETEROLOGOUS THYMUS AND PINEAL GLANDS  
AND HYPOTHALAMUS ON THE IMMUNE RESPONSE  
IN MICE

G. A. Belokrylov, V. G. Morozov,  
V. Kh. Khavinson, and B. N. Sofronov

UDC 612.017.1-06: [612.438+612.826  
.33+612.826.4] .015.21

The effect of acetic acid extracts of the bovine thymus and pineal glands and hypothalamus, purified by gel filtration, on the content of circulating antibodies and the number of antibody-forming cells was investigated in experiments on mice. Extracts of the thymus and pineal glands, injected for 10 days, stimulated immunogenesis induced by injection of sheep's red cells, whereas the hypothalamic extract had an inhibitory action.

KEY WORDS: immunogenesis; thymus; pineal gland; hypothalamus.

Extracts of homologous and heterologous thymus obtained by various methods can stimulate immunogenesis. This has led to the hypothesis that a thymic hormone regulates maturation of the lymphoid tissue and the immunologic function of the organism [1, 3, 6, 8, 9, 11]. Injury to the hypothalamus [2, 10] or pinealectomy [7] are known to depress the immune response. This explains the attempts to isolate humoral factors from these organs with direct or indirect action on the immune response.

The object of this investigation was to test the action of low-molecular-weight extracts of heterologous thymus, hypothalamus, and pineal gland and also of the spleen on the ability of animals to form antibodies.

#### EXPERIMENTAL METHOD

Acetic acid extracts were prepared from bovine tissues and then purified by gel filtration as described previously [4, 5].

Female CBA mice weighing 16-18 g were used. Sheep's red cells washed three times with 0.85% NaCl solution to remove serum were used as the antigen. The substances for testing were injected subcutaneously in doses of 1 and 0.1 mg per mouse daily for 7 days before injection of the antigen and for 3 days after its injection. The control animals received physiological saline. Each group in the experimental and control series consisted of not less than 12 mice.

The immune response was determined on the fourth day after a single intravenous immunization of mice with  $1 \cdot 10^7$  red cells by the hemolysis test and by determining the number of antibody-forming cells (AFC) in the spleen by Jerne's direct method. The sera or cells were used in the form of a mixture from 3 or 4 animals. The values obtained by the investigation of 3 to 16 pools of cells or sera were subjected to statistical analysis.

#### EXPERIMENTAL RESULTS

As Table 1 shows, in four corresponding experiments in which extracts from the thymus were used

Division of Microbiology and Immunology, Research 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. 81, No. 2, pp. 202-204, February, 1976. Original article submitted November 20, 1974.

©1976 Plenum Publishing Corporation, 227 West 17th Street, New York, N.Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for \$15.00.

TABLE 1. Immune Response in Mice to Injection of Thymic Extract ( $M \pm m$ )

Index of immune response	Experiment No.	Injection of extract of thymus (per animal per day)				Injection of physiological saline
		1 mg	P	0,1 mg	P	
Reciprocal of serum hemolyzing titer	1	160±0	<0,01	160±0	<0,01	93±13,3
	2	80±0	<0,01	40±0	0,4	33±6,6
	3	83±18,6	0,3	133±16,9	<0,01	53±13,6
Number of AFC per 10 <sup>6</sup> spleen cells	1	1267±228	<0,01	637±50	0,03	477±23,5
	2	487±15,3	<0,01	386±4,7	<0,01	91±5,1
	3	258±29,7	<0,01	71±21	0,05	23±1,6
	4	37±2,4	<0,01	12,7±0,5	<0,01	2,5±0,7

Note. Each figure is the mean result of investigation of 3-6 mixtures of sera or cells, each of which was obtained from three to four animals.

TABLE 2. Number of AFC in Spleen of Immunized Mice after Injection of Extracts from Various Organs ( $M \pm m$ )

Organ from which extract was prepared	Number of AFC after injection of extract (per animal per day)				Injection of physiological saline
	1 mg	P	0,1 mg	P	
Hypothalamus	14±4,94	<0,01	63±5,0	0,05	46,5±3,49
Pineal gland	180±25,5	<0,01	140±20	<0,01	55,8±3,78
Spleen	563±114	0,4	335±20	<0,01	460±22,1

Note. Each figure is the result of investigation of 9-13 mixtures of spleen cells, each of which was obtained from 3 mice

immunogenesis was stimulated and the effect was particularly clear if based on counting the number of AFC. The higher dose of the substance had a greater stimulating effect. The highest degree of stimulation (by 11-15 times) was observed when the response in the control groups was weakest.

Unlike the thymic extract, extracts from tissue from the hypothalamic region of the brain, injected in a dose of 1 mg per animal, greatly reduced the immune response (Table 2). The preparation from the pineal gland appreciably stimulated the indices of immunogenesis: The number of AFC in the spleen reached 250-320% of the control.

Splenic extract prepared by the same method as the other extracts had a weaker effect on these indices of immunogenesis: Deviations from the control were 22-27%.

The results confirm the fact that a low-molecular-weight (10,000) substrate, stimulating immunogenesis in the heterologous organism, can be isolated from the thymus. The discovery of a substance with a similar action in the pineal gland supports the observations of Csaba et al. [7], who found that the ability of pinealectomized animals to give a primary immune response is depressed.

Since destruction of the hypothalamic nuclei, according to some observations, has an immunodepressant action, it might be expected that an extract from that region would stimulate immunogenesis. However, it was found to have an inhibitory action. Presumably destruction of areas of the hypothalamus stimulates the production of factors inhibiting immunogenesis in them.

It must be emphasized that the extracts tested were not themselves immunogenic. Their further study in relation to different forms of immune response, and also in relation to the mechanism of their action, thus appears to be highly promising.

#### LITERATURE CITED

1. V. M. Kavsan and I. S. Nikol'skii, in: Immunology [in Russian], No. 6, Kiev (1973), pp. 16-18.
2. E. A. Korneva and L. M. Khai, Fiziol. Zh. SSSR, No. 1, 42 (1963).
3. D. A. Kostadinov, Vestn. Akad. Med. Nauk SSSR, No. 10, 93 (1971).
4. V. G. Morozov and V. Kh. Khavinson, Éksper. Khir., No. 1, 19 (1973).
5. V. G. Morozov and V. Kh. Khavinson, Éksper. Khir., No. 1, 34 (1974).
6. J. Bach and M. Dardenne, Transplant Proc., 4, 345 (1972).

7. G. Csaba, M. Bodoky, J. Fischer, et al., *Experientia*, 22, 168 (1966).
8. M. Dardenne and J. F. Bach, *Immunology*, 25, 343 (1973).
9. A. L. Goldstein, A. Guho, M. M. Zatz, et al., *Proc. Nat. Acad. Sci. (Washington)*, 69, 1800 (1972).
10. T. J. Lupurello, M. Steing, and C. D. Park, *Am. J. Physiol.*, 207, 911 (1966).
11. D. Metcalf, *Cold Spring Harbor Symp. Quant. Biol.*, 32, 583 (1967).