A LOW-MOLECULAR-WEIGHT HOMOGENEOUS FRACTION OF THE THYMUS STIMULATING IMMUNOGENESIS

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The effect of a purified acetic acid extract of thymus ("thymarin") and of three of its fractions obtained by ion-exchange chromatography, and consisting of individual substances of polypeptide nature, on the immune response was studied in mice immunized with sheep's erythrocytes. Thymarin and one of its fractions with a molecular weight of about 5000 were shown to have a marked stimulating action on the thymus-dependent immune response (the formation of cells producing IgM and IgG antibodies and the circulating antibody level).

KEY WORDS: *thymus; fractions of thymus; immunoglobulins.*

It has now been established that the thymus contains substances of hormone type which promote maturation of the T lymphocyte population and participate in the regulation of immunological reactivity [2, 5, 6, 9, 12]. However, no general agreement has been reached regarding the nature of these substances and the character of their action. A polypeptide ("thymosine"), isolated from the thymus, which stimulates immunogenesis substantially both in vivo and in vitro, has attracted the most attention [8, 10, 14]. Nevertheless, the search for and isolation and identification of the hormone of the thymus still remain an urgent problem.

The writers previously isolated from calf thymus a group of low-molecular-weight polypeptides capable of stimulating antibody formation in mice. The preparation obtained by the same method from the spleen was evidently ineffective [1].

The object of this investigation was to study the effect of individual substances isolated from the thymus on immunogenesis.

EXPERIMENTAL METHOD

As original material, a preparation obtained from fresh calf thymus by acetic acid extraction from tissue dehydrated with acetone and subsequent precipitation from the supernatants by a method described earlier [4] was used. The lyophilized preparation had a molecular weight of under 10,000 and contained substances of polypeptide nature, giving three peaks on gel filtration. The preparation was given the working name of "thymarin." Three fractions, I, II, and III, accounting on average for 90%, 7%, and 3%, respectively, of the total weight of the original preparation, were isolated from thymarin by ion-exchange chromatography in a gradient of eluting solution, at pH 3.2-10.5, on the porous carboxyl cation-exchange resin Biocarb-T [3]. When these fractions were tested by gel filtration they behaved as homogeneous substances.

Experiments were carried out on 275 male CBA mice weighing 16-18 g. Each group studied consisted of at least 10 animals.

The preparations for testing were injected in different doses, in equal volumes of physiological saline, subcutaneously daily for seven days. The injections began four days

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TABLE 1. Effect of Thymus Preparations on Mouse Serum Hemagglutinin Level

Daily dose of preparation, μg per mouse	Level of hemagglutinins				
	on 3rd day	on 5th day			
	thymarin	thymarin	fraction I	fraction II	fraction III
1000	510,8±44,3*	1120,0±285,5*	400,0±59,8	1024,0±137,6*	672,0±240,7
100	190,0±74,5	800,0±179,7	480,0±89,8	280,0±44,9	960,0±179,6*
20	—	720,0±269,5	480,0±89,8	560,0±89,8	1624,6±388,1*
5	—	800,0±179,7	480,0±89,8	320,0±134,8	768,0±206,4
Control (physiological saline)	170,0±9,8	451,8±33,4			

*Here and in Table 2 differences from control are significant ($P < 0.01$).

TABLE 2. Effect of Thymus Preparations on Number of AFC in Mouse Spleen (M ± m)

Daily dose of preparation, μg per mouse	Direct AFC (IgM)		Indirect AFC (IgG)	
	per 10^6 spleen cells	per spleen	per 10^6 spleen cells	per spleen
Control (physiological saline)	24,5±1,9	4 821±302	98,9±3,3	19 483±1270
Thymarin				
100.0	47,0±2,6*	9 475±485*	157,0±5,8*	31 651±1024*
Fraction III				
1000	27,6±4,3	4 860±387	120,4±13,8	21 600±864
20,0	78,4±3,0*	11 700±525*	404,0±15,0*	60 600±1785*
1,0	42,4±3,0*	6 300±312*	221,6±13,3*	33 150±1240*

before injection of the antigen. The mice were immunized by a single intravenous injection of $1 \cdot 10^7$ fresh washed sheep's erythrocytes. The animals were decapitated 3-5 days after immunization. The hemagglutinin level was determined in the individually collected blood sera; spleen cells 5 days after immunization of the mice were tested for their content of direct (IgM) and indirect (IgG) antibody-forming cells (AFC). Direct AFC were determined by the method of Jerne and Nordin [11], indirect by the method of Dresser and Wortis [7] using rabbit serum (1:80) against mouse γ_2-globulin, obtained with the aid of caprylic acid [13].

EXPERIMENTAL RESULTS

As Table 1 shows, injection of thymarin in a dose of 1000 μg into mice led to a significant increase in the antibody titer detectable on the third and fifth days after immunization. Smaller doses of the preparation (100 μg or less) did not significantly stimulate the immune response. Of the three fractions isolated from thymarin, the most active was fraction III which, as stated above, amounts to 3% of the original preparation. When given in a dose of 20 μg, it more then trebled the quantity of hemagglutinin formed in the animals. The fact is worth noting that this fraction, in a dose of 1000 μg (50 times above the optimal dose) had no stimulating action. The same rule applies to the action of many biologically active substances. Fraction I was inactive, and fraction II stimulated antibody formation only in a dose of 1000 μg, 15 times greater than in its content in thymarin.

The action of the biologically active fraction III on immunogenesis was tested at the cellular level by determining the number of cells forming IgM and IgG antibodies in the animals' spleen. As Table 2 shows, fraction III in a dose of 20 μg stimulated the immune response most actively, as reflected by the number of both direct (IgM) and indirect (IgG) AFC. Fraction III in a dose of 1 μg also had a significant stimulating action on AFC formation. This dose of the preparation, it will be noted, caused no significant increase in the titer of circulating antibodies when tested at the same time.

The results show that a highly active substance of polypeptide nature (fraction III), with a molecular weight of about 5000, is contained in and can be isolated from the thymus.

The biological activity of this fraction is 50-100 times greater than that of the original preparation, thymarin. This substance, in a dose of 20 μg per mouse, stimulates the immune response intensively to injection of a thymus-dependent antigen, namely sheep's erythrocytes, and in a dose of 1 μg stimulates the responses of the host connected with IgM and IgG formation, but does not increase the circulating antibody level.

It will be noted that these experiments were performed on immunologically normal animals; possibly under conditions of immunological deficiency, due in particular to thymus insufficiency, this substance would have a stronger action.

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LIMITS OF PHAGOCYTIC POWER OF MACROPHAGES

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The ability of peritoneal macrophages to take up different doses of antigen (sheep's erythrocytes) and of antigens differing in physicochemical properties (sheep's erythrocytes, rat erythrocytes, and typhoid vaccine) was studied. An increase in the dose of sheep's erythrocytes injected many times over had no effect on the quantity of antigen ingested by the macrophages within a definite time interval. In macrophages taken at short periods after injection of erythrocytes of the different species of animals into the mice, ability to take up these erythrocytes in vitro was sharply inhibited. Preincubation of macrophages (in vivo or in vitro) with all the antigens tested sharply increased their ability to phagocytose typhoid vaccine.

KEY WORDS: *macrophages; phagocytosis; antigen.*

It has long been established that blockade of cells of the monocyte phagocytic systems (MPS) by inorganic particles or by injection of certain agents causes depression of antibody

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