BIOLOGICAL ACTIVITY OF THYMUS PREPARATIONS FROM SHEEP OF DIFFERENT AGES

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Immunologically active substances of the thymus are of great interest to immunobiologists, for in recent years they have not only begun to be used as a tool with which to study the molecular mechanisms of development and formation of the immune system [1, 2], but they have also found widespread clinical application. Thymus preparations are now used to restore the disturbed component of the immune system in various diseases accompanied by disturbances of thymus function [5-7]. The thymus of mammals, including man, begins its physiological involution at puberty [4]. Throughout the individual's lifetime the thymus synthesizes biologically active substances. It has been shown that the blood level of thymic serum factor falls parallel with involution of the thymus [8]. The thymus has been shown to synthesize a wide range of immunologically active peptides [3]. It is not yet clear whether the spectrum of thymic peptides and their biological activity change in the course of involution of the gland.

We have carried out a comparative analysis of biologically active thymus preparations obtained from sheep at different stages of ontogeny: fetuses (135 days of development), neonates, and adults (aged 4-6 years).

EXPERIMENTAL METHOD

Biologically active fractions from the thymus of sheep fetuses and newborn and adult sheep were obtained by the standard method [1], except that ultrafiltration in the stage of obtaining active fraction 5 (ATF-5) was carried out on filters produced by the Institute of Nuclear Physics Academy of Sciences of the Uzbek SSR.

Experiments were carried out on BALB/c mice obtained from the Stolbovaya Pure-Line Animals Nursery, Academy of Medical Sciences of the USSR. Mice aged 2-3 months and weighing 20-22 g were used in the experiments.

A model of an immunodeficiency state was obtained in mice by whole-body irradiation in a sublethal dose of 6 Gy. Five days later the animals were immunized intraperitoneally with sheep red blood cells (SRBC) in a dose of $2 \cdot 10^8$; thymus preparations were injected intraperitonally at the same time in a dose of 20 μg per mouse, and a further 4 days later, the number of antibody-forming cells (AFC) was determined in the spleen by the local hemolysis in agarose method [9]. Besides AFC, the number of immune rosette-forming cells (iRFC) was determined in the thymus, spleen, and lymph nodes of the mice by the standard method [10].

The experiments results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Sublethal irradiation sharply depressed the immune response of the mice to thymus-dependent antigen (SRBC) (Table 1). A single injection of ATF-5, isolated from the fetal thymus, into the immunodeficient animals doubled the intensity of the immune response to SRBC. Specimens obtained from new born and adult sheep had a stronger action: the intensity

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TABLE 1. Effect of ATF-5 on Immune Response to SRBC in Immunodeficient Mice (M ± m)

Intact animals	Source of ATF-5	Number of karyocytes in spleen (× 10^6)	Number of AFC	
			per spleen	per 10^5 cells
Intact (n = 10)	—	79±13,3	1941±218	39,7±7,8
Immunodeficient (n = 10)	—	44,8±11,9	78,5±10,3	3,6±1,0
Immunodeficient (n = 10)	Fetal lamb	85,0±9,9	159,0±14,6	3,9±1,0
Immunodeficient (n = 9)	Newborn lamb	62,2±11,1	326,0±81,0	10,0±2,2
Immunodeficient (n = 6)	Adult sheep	62,5±16,6	532,0±156,0	21,5±5,3

Legend. Here and in Table 2, n denotes number of animals.

TABLE 2. Effect of ATF-5 on iRFC Population of Immunodeficient Mice (M ± m)

Intact animals	Source of ATF-5	Source of mouse lymphocytes	Number of iRFC per 100 lymphocytes
Intact (n = 9)	–		17.0±1.4
Immunodeficient (n = 9)	–		13.2±1.0
Immunodeficient (n = 10)	Fetal lamb	Thymus	29.9±1.2
Immunodeficient (n = 10)	Newborn lamb		27.1±3.2
Immunodeficient (n = 5)	Adult sheep		17.4±2.8
Intact (n = 9)	–		9.8±2.1
Immunodeficient (n = 8)	–		10.4±2.1
Immunodeficient (n = 7)	Fetal lamb	Lymph node	32±47.3
Immunodeficient (n = 8)	Newborn lamb		29.5±3.6
Immunodeficient (n = 5)	Adult sheep		17.4-1.9
Intact (n = 9)	–		13.8±1.6
Immunodeficient (n = 10)	–		12.3±1.4
Immunodeficient (n = 9)	Fetal lamb	Spleen	32.2±2.5
Immunodeficient (n = 8)	Newborn lamb		35.6±3.0
Immunodeficient (n = 5)	Adult sheep		19.2±1.0

of the response was increased by 4.1 and 6.8 times, respectively. Table 1 also shows that ATF-5 preparations stimulated the proliferative properties of the karyocytes in the spleen of immunodeficient mice.

ATF-5 preparations isolated from the thymus of lambs in the early stages of development (fetus, newborn lambs), like adult sheep ATF-5, thus possess quite strong immunocorrecting activity under conditions of immunodeficiency.

An important criterion of evaluation of the immunologic status of an animal is determination of the level of antigen-lymphocytes. We studied the effect of the thymus preparations on a pool of rosette-forming cells in response to immunization with SRBC. The number of immune rosette-forming cells was determined in the thymus, lymph nodes, and spleen (Table 2).

ATF-5 obtained from fetal and newborn lambs was found to increase the number of iRFC in the lymph nodes by 2.3 times. A similar stimulating effect of preparations was recorded when the iRFC level was studied in the spleen of sublethally irradiated animals. ATF-5 from the adult sheep thymus had a very weak stimulating action on the iRFC population. Injection of thymus preparations into intact animals caused virtually no change in the immunologic parameters studied.

Thus ATF-5 obtained from sheep at different stages of ontogeny exert a well-marked immunocorrective effect. These preparations, incidentally, differ in their action on the AFC and iRFC populations depending on the source from which they were obtained. For instance, ATF-5 obtained from the fetal thymus had a weaker stimulating action on AFC, but caused maximal stimulation of iRFC. Conversely, the stimulating effect of ATF-5 obtained from the adult sheep thymus was very strong on antibody formation but minimal on iRFC. The reason may probably be the qualitative heterogeneity of the biochemical composition of the ATF-5 preparations: qualitative differences in ATF-5 preparations from different sources were found by electrophoresis, and ultimately this reflects the change in composition of the thymus peptides during ontogeny.

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