EFFECT OF T-ACTIVIN ON INVOLUTION OF THE THYMUS IN MICE

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The thymus, the central organ of the immune system, begins to undergo involution immediately after puberty in man and mice [6, 7]. Involution of the thymus, reflecting aging of the immune system, is characterized by definite morphological and immunologic changes [5-7, 10], primarily affecting the T-cell component of the immune system [5, 7, 9-11]. Parallel with this process, production of thymic factors is reduced [3, 4, 8]. With age, immediately after involution of the thymus the frequency of onset of certain infectious diseases, autoimmune diseases, and tumors increases [6, 7, 10]. Involution of the thymus is accelerated in diseases of the immune system itself. Accordingly, the question of immunocorrection to activate particular components of the immune system is an increasingly urgent problem. It has been shown that depressed immunologic functions in old mice can be restored or maintained at an adequate level by combined transplantation of bone marrow and thymus from newborn mice [6]. The appearance of new immunomodulators of thymic nature and their successful use in clinical practice in certain immunodeficiency diseases [2] provides a new approach to the problem of aging of the immune system. The key process in immunologic aging is evidently that which regulates involution and atrophy of the thymus [9].

It was accordingly decided that the study of the effect of T-activin [1] on the structure of the thymus in the course of its involution would make a useful contribution.

EXPERIMENTAL METHOD

Experiments were carried out on mature male (CBA × C57Bl)F_1 mice weighing 24.0 ± 2.0 g, divided into three groups, with five to eight animals in each group. Group 1 consisted of intact young animals (original normal), group 2 of animals receiving 20 injections each of 0.2 ml of physiological saline intraperitoneally, and killed 6 months later (control of physiological age involution), and group 3 consisted of animals receiving 20 injections each of 1.0 μg of T-activin in 0.2 ml physiological saline intraperitoneally, daily, and killed after 6 months (experiment). The animals were weighed and decapitated, after which the thymus was removed and weighed, and fixed in Bouin's fluid. Paraffin sections (4 μm) were stained with azure II and eosin, and morphometric analysis was carried out by means of Avtandilov's grid. The relative areas of the cortex and medulla and of nonparenchymatous tissue (connective tissue, adipose tissue, blood vessels, and cysts) were calculated, using a 20× objective and 10× ocular. The density of the thymocytes (Tc) in the subcortical zone and in the remaining zones of the cortex and medulla, the density of transformed Tc (tTc), figures of mitosis and pycnosis, and reticular cells in these same zones was determined with a 90× objective and 10× ocular in 20 small squares of the grid. The changes discovered were confirmed by conversion of the parameters to values per square millimeter of section and for the organ as a whole. For statistical analysis of the data the nonparametric U test of Wilcoxon, Mann, and Whitney was used.

EXPERIMENTAL RESULTS

Six months after the beginning of the experiment, when the body weight was increased a little there was a marked decrease in weight of the thymus in both experimental and control animals compared with the initial norm (Fig. 1, I). However, in experimental animals receiving T-activin this decrease was much less ($P = 0.005$) than in the control. On morphological investigation, in three of five cases marked involution of the thymus was

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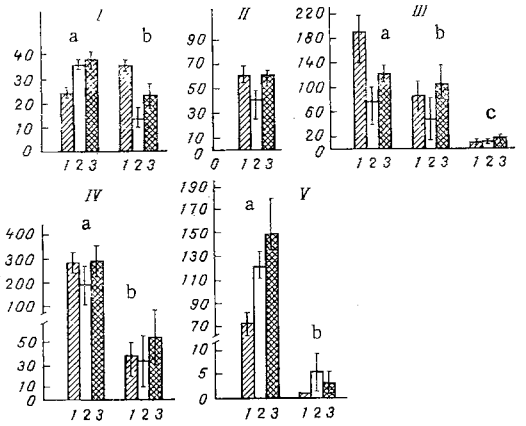

Fig. 1. Changes in body weight and weight and morphometric parameters of the thymus during its involution under normal conditions and under the influence of T-activin. Abscissa: 1) initial group (normal), 2) control group (physiological saline), 3) experimental group (T-activin); ordinate: I: a) body weight (in g), b) weight of thymus (in mg), II) area of cortex (in %), III) subcortical zone of thymus: a) number of thymocytes (in conventional units), b) number of transformed thymocytes, c) number of mitotically dividing cells (in conventional units), IV) cortical zone: a) number of Tc, b) number of tTc, V) medulla: a) number of Tc, b) number of tTc (in conventional units). Vertical lines denote limits of individual values.

observed in the control: The area of the cortex was sharply reduced and the area of the medulla increased (inversion), the boundary between cortex and medulla was less clear because of reduced density of Tc in the cortex and increased density of Tc in the medulla, the stroma in the cortex was exposed the number of diffusely distributed macrophages and mast and plasma cells was increased, and gland-like structures (cysts) appeared. In the experimental animals virtually no such changes were observed and the usual structure of the thymus was preserved.

Morphometric analysis revealed a significant ($P = 0.005$) decrease in area of the cortex and an increase in area of the medulla in the control compared with the corresponding values under normal and experimental conditions (Fig. 1, II), evidence that the development of involution of the thymus was inhibited in the experimental mice. In the subcortical zone of the thymus, the principal zone of Tc generation, a significant ($P = 0.05$) decrease was observed in the number (both in conventional units and calculated per square millimeter and in the organ as a whole) of Tc and tTc was observed in mice of the control group (age involution) compared with the original norm (Fig. 1, III, a, b).

The number of mitotically dividing cells (Fig. 1, III, c) and cells exhibiting pycnosis was unchanged whereas the number of reticular cells and macrophages was somewhat increased. A different situation was found in the experimental group in animals receiving T-activin or the 6 months before sacrifice: The number of Tc was less than normal ($P = 0.005$) but significantly greater ($P = 0.05$) than in the control (Fig. 1, III, a); the absolute and relative numbers of tTc were somewhat greater than normal and were significantly higher ($P = 0.005$) than the control values (Fig. 1, III, b). The number of mitotically dividing cells was greater ($P = 0.05$) and the corresponding parameter both in normal animals and in the control (Fig. 1, III, c). In the remaining zones of the cortex (Fig. 1, IV) all parameters studied, except reticular cells, were reduced in the control compared with normal, whereas in the experimental group they were higher than corresponding parameters in the control, and reached the initial normal values.

In the medulla of the thymus (Fig. 1, V) a significant increase was observed in the number of Tc in the control and experimental mice compared with the original norm ($P = 0.001$), but in the experimental group this increase was greater than in the control ($P = 0.001$). The other parameters studied showed no significant changes with the exception of tTc, the number of which was significantly increased in the experimental and, in particular, in the control animals.

Injection of T-activin in a daily dose of 1.0 μg for 20 days into sexually mature young male mice thus inhibits the development of involution of the thymus, as shown by a slower decrease in weight of the thymus compared with the control and by preservation of the characteristic morphology of the thymus as found in younger animals, by contrast with changes in its morphology in control mice, typical of involution. The morphometric data also indicate that T-activin delays the development of inversion of the layers of the thymus, inhibits depopulation of all zones of the cortex on account of Tc, and increases the number of tTc and of mitotically dividing cells in them, and leads to a small increase in the number of Tc in the medulla compared with the control. Analysis of the experimental results shows that T-activin, i.e., products of synthesis in the thymus, can hardly be classed as hormones, for unlike known hormone such as insulin, glycocorticoids, etc., injection of which into an animal is followed by the development of atrophy of the corresponding endocrine organs, T-activin not only does not induce more rapid involution of the thymus, but actually delays it. Against this background, under the influence of T-activin functional activity of the thymus may perhaps even be intensified.

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