EFFECT OF T-ACTIVIN ON THYMOCYTE REPRODUCTION AND MIGRATION

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T-activin exhibits immunomodulating properties both experimentally and clinically and has a therapeutic action in various pathological states [3, 6-8]. Immunologically active peptides, which include T-activin, are essential for generation of T cells [2, 9, 12, 13], the intrathymic stage of which includes reproduction, maturation, and migration of thymocytes [14].

The most active zone of proliferation is the subcapsular zone of the cortex of the thymus, from which maturing thymocytes migrate toward the medulla. Medullary thymocytes are the most mature cells of the thymus and they have the phenotype of helper cells [10].

Two different views are held on regeneration of T cells. According to the first it follows a direct line from cortical through medullary thymocytes to peripheral T cells. According to the second view migration of thymocytes from the thymus takes place at the boundary between cortex and medulla, and the medullary thymocytes are an independent population of mature cells. They persist in the thymus and secrete lymphokines, which bring about maturation of the cortical thymocytes [11, 14].

Regardless of which view is the true one, the level of intrathymic generation of T cells can be judged from a combination of morphological features and weight parameters of the thymus. These features include: the number of transformed and untransformed thymocytes and also of mitotic figures in the different zones of the cortex; the number of thymocytes per unit area in individual zones of the cortex and medulla, and also of the thymus as a whole; the relative areas of the cortex, medulla, and interlobular connective tissue.

The aim of the present investigation was to assess the effect of T-activin on reproduction and migration of thymocytes in intact mice by means of the morphometric parameters listed above, and also to compare the results of a single and fractional injection of the same dose of the preparation.

EXPERIMENTAL METHOD

Altogether 47 female (CBA × C57BL)F_1 mice weighing 27.8 g (22.1-35.0)* were used. Seven animals served as the control. T-activin in a dose of 0.5 μg was injected subcutaneously into the dorsal region. Twenty animals received this dose in one injection, another twenty animals received it as five daily injections of 0.1 μg each. The mice were decapitated on the 1st, 5th, 10th, and 15th days (five animals at each time). When fractional injections were used, the time was counted from the last injection. The thymus was weighed and fixed in Bouin's fluid. Paraffin sections were stained with azure II and eosin. The relative areas of parts of the organs were determined with a 20× objective and 7× ocular, using an Avtandilov grid [1]. The same grid, but without dots, was used to demarcate an area in the field of vision of the microscope when analyzing the other parameters. The number of transformed thymyoctyes (tT — the diameter equal to or greater than the diameter of an average thymocyte, basophilic cytoplasm), of untransformed thymocytes (utT — small and medium-sized thymocytes, basophilia of cytoplasm absent), and of mitotic figures was counted under a 90× objective and 7× ocular in twenty small squares of the grid, in the following zones: subcapsular, middle,

*Here and subsequently the limits of individual values are given in parentheses.

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TABLE 1. Changes in Weight, and in Relative Areas of Cortex, Medulla, and Interlobular Connective Tissue of the Thymus after Injection of T-Activin

Time of investigations, days	Weight of thymus, mg	Area, percent		
		cortex	medulla	connective tissue
Control	46,0 (40—67)	73,4 (65,3—83,7)	25,4 (15,7—33,3)	1,2 (0,5—2,2)
1 st	47,0 (41—56)	71,9 (66,2—76,7)	25,1 (18,6—32,0)	2,2 (0,9—3,2)
5 th	47,2 (38—53)	70,6 (66,4—71,3)	27,6 (20,1—32,7)	2,0 (0,5—3,2)
10 th	53,2 (35—64)	67,8 (62,1—71,3)	29,5 (23,0—36,0)	2,1 (1,1—3,7)
15 th	50,7 (36—61)	68,1 (52,3—77,7)	27,6 (20,6—34,6)	2,5 (1,2—4,1)

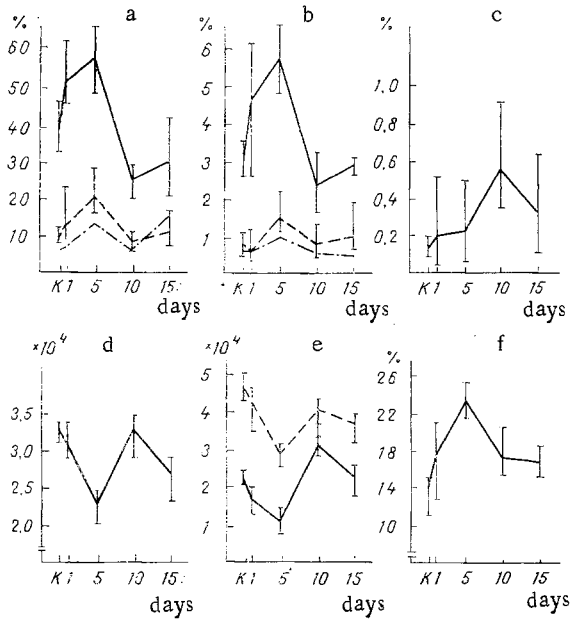

Fig. 1. Changes in structural features of reproduction and migration of thymocytes under the influence of T-activin. a) Percentage of transformed thymocytes; b) percentage of mitoses; c) percentage of plasma cells in inner zone of cortex; d) number of untransformed thymocytes per 1 mm² of thymus as a whole; e) number of untransformed thymocytes per 1 mm² of subcapsular and middle zones of cortex; f) number of medullary thymocytes as a fraction of untransformed thymocytes in the spleen as a whole. Continuous line in a, b, e represents subcapsular zone, broken line — middle zone of cortex, line of dots and dashes — inner zone of cortex. Vertical lines show limits of individual values (for inner zone of cortex these are given in the text). Abscissa, time (in days).

and inner zones of the cortex and medulla. After determination of the area of a small square, the number of tT and utT was calculated in 1 mm² of each zone of the cortex and medulla, and also in the organ as a whole, the fraction of medullary thymocytes in the total number of utT, and the percentage of tT and of mitoses were calculated for each zone of the cortex.

Plasma cells, constantly observed in the inner zone of the cortex, could not be estimated quantitatively by the method described above because of their infrequent distribution. A separate calculation of these cells was accordingly undertaken in 200 large squares of the Avtandilov grid (90× objective, 7× ocular) and their percentage of the total number of cells in the inner zone of the cortex was calculated. The data were subjected to statistical analysis by the nonparametric Wilcoxon—Mann—Whitney U test [5].

EXPERIMENTAL RESULTS

Single and fractional injection of T-activin caused similar changes, and accordingly only the results of a single injection are shown in the graphs and in Table 1.

T-activin increased the number of tT and mitoses in the cortex of the thymus (Fig. 1a, b). Curves showing changes in these parameters in the subcapsular zone coincided, but were not

identical on the first day. The increase in number of tT in this case was significant ($P < 0.005$) but the increase in the number of mitoses was not significant. The increase in the mean took place on account of a marked increase in individual variability. After a peak of the two parameters on the 5th day ($P < 0.005$) the number of tT and mitoses fell below the control values ($P < 0.005$ and $P < 0.01$, respectively), returning to normal by the 15th day. The number of mitoses also returned toward normal as regards individual variability, but the number of tT showed greater individual variability than in the control, i.e., the second parameter was stabilized to a lesser degree than the first.

Curves showing the change in number of tT and mitoses in the middle zone of the cortex differed only during the first day when the number of mitoses was equal to the control, but the number of tT was characterized by increased individual variability although the increase in the mean value was not significant. Otherwise these curves were identical: They had one peak on the 5th day ($P < 0.005$ and tT and $P < 0.01$ for mitoses), whereas on the 10th day both parameters became stablized as regards both mean values and individual variability.

In the inner zone of the cortex the number of tT, after an increase in individual variability on the first day (3.1-15.6% compared with 5.1-11.3% in the control), was characterized by an increase in mean values on the 5th day: 14.1% (9.7-16.6) compared with 7.0% (5.1-11.3) in the control ($P < 0.01$). On the 10th day the number of tT returned to the control values, but on the 15th day it was again increased to 15.7% (10.2-26.7) ($P < 0.05$). The number of mitoses remained within the limits of the control values at all times.

Plasma cells were constantly present in the thymus of the control animals (Fig. 1c), but their number was small (0.13%, 0.09-0.19). They were scattered and distributed mainly in the inner zone, less frequently in the middle zone of the cortex. Under the influence of T-activin the individual variability of this parameter was greatly increased at all times, but on the 10th day the degree of plasmatization was considerable ($P < 0.005$). After fractional administration of T-activin, the character of the curve remained as before, but the increase in the number of plasma cells was significant at all times. Even in the case of intensive plasmatization, the cells did not form groups.

T-activin had no effect on the weight of the thymus. The relative areas of cortex, medulla, and interlobar connective tissue did not change significantly, but there was a slight tendency for the fraction both of the parenchyma as a whole and of the cortex to decrease, and for the fraction of the medulla to increase.

T-activin caused changes both in the total number of thymocytes and in the number of utT per 1 mm^2. The curves showing changes in the number of utT both in the thymus as a whole (Fig. 1d) and for the zones of the cortex (Fig. 1e) had a similar wavelike pattern with a considerable drop on the 5th day ($P < 0.005$ for all curves), a rise on the 10th day, and a second drop in the 15th day. Despite the general similarity in the character of these curves, they had considerable differences. The curve representing the number of utT per 1 mm^2 of the subcapsular zone (Fig. 1e) differed from the rest by a considerable rise on the 10th day ($P < 0.005$), with a return to normal on the 15th day. Changes in the number of utT per 1 mm^2 of the middle and inner zones were identical, and accordingly only the curve for the middle zone is shown. Here the number of utT per 1 mm^2 remained below its initial level on the 10th and 15th days, although a tendency toward normalization was observed. The number of utT per 1 mm^2 of the thymus as a whole (Fig. 1d), although rising to the control level on the 10th day, fell again below normal on the 15th day ($P < 0.005$).

The number of medullary thymocytes as a percentage of all utT of the thymus (Fig. 1f) increased steadily on the 5th, 10th, and 15th days ($P < 0.005$), with a peak on the 5th day. When this parameter was calculated, transformed thymocytes in the medulla were disregarded, for their number was small and was unchanged by T-activin (0.2%). Not only the fraction of medullary thymocytes, but also the density of their distribution, was increased. For instance, on the 1st, 5th, and 10th days their number per 1 mm^2 was significantly higher than in the control, 21.7×10^3 (18.6-24.9) compared with 17.6×10^3 (13.8-19.5) ($P < 0.01$).

These results indicate that the first reaction of T-activin is intensification of the thymocyte transformation in the subcapsular zone — the mitotic index is destabilized. On the 5th day this leads to maximal intensification of both transforming and mitotic activity and their dissemination over the cortex. The result of this activity is acceleration of maturation of the thymocytes and their migration within the gland, reflected in a decrease in the number of cells in the middle and inner zones of the cortex and an increase both in the frac-

tion of medullary thymocytes and in their number per 1 mm^2. The decrease in the total number of cells on the 5th and 15th days is indirect evidence of migration of thymocytes from the thymus. Further indirect confirmation of migration is given by the tendency for the fraction of parenchyma to decrease and the appearance of perivascular lymphatic spaces filled with thymocytes on the boundary between cortex and medulla. The method of evaluation used in the investigation cannot be used to evaluate the latter phenomenon quantitatively.

Toward the end of the investigation mitotic activity was completely stabilized and back to normal, transformation was restored to a lesser degree, and structural features of migration were not stabilized, although a tendency toward stabilization was evident.

The constant presence of plasma cells in the parenchyma of the thymus of the control animals and the significant increase in their number under the influence of T-activin suggest that this phenomenon is random in character, as other workers also have described [4]. The extremely small number and the solitary arrangement of the plasma cells suggest that the immunoglobulins produced by them are intended by intrathymic use. An explanation of this phenomenon requires other methods of investigation.

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