EFFECT OF T-ACTIVIN ON PERIPHERAL ORGANS OF IMMUNITY IN INTACT AND THYMECTOMIZED MICE

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The thymus produces immunologically active factors which have been characterized to a varied degree both physicochemically and with respect to their biological activity [6, 8, 10]. The preparation T-activin has been studied intensively. Its high clinical efficacy and its ability to restore T-cell immunity in various pathological states and also in experimental immunodeficiencies have been demonstrated [2, 3, 7]. Knowledge of the fine mechanisms of action of thymic factors under normal conditions and when thymic regulation is disturbed is essential in the search for methods of correcting defects of immunity [6, 7]. Quantitative structural analysis of the organs of immunity can provide important help in the study of the mechanisms of action of these substances.

This paper describes the study of the effect of T-activin on structural features of cellular and humoral immunity in bone marrow, peripheral blood, the spleen, and lymph nodes of intact and thymectomized mice.

EXPERIMENTAL METHOD

Experiments were carried out on 94 male (CBA × C57BL)F_1 mice weighing 22-35 g. Thymectomy was performed on 45 mice at the age of 8 weeks. Intact (healthy) and thymectomized animals, 8 weeks after the operation, received a subcutaneous injection of 0.5 μg of T-activin. The substance was injected in a single dose into mice of one group, fractionally (0.1 μg daily for 5 days) into mice of the other group. Control groups consisted of intact and thymectomized animals not receiving T-activin. The mice were decapitated 1, 5, 10, and 15 days after the end of injection of the preparation. The weight of the spleen, number of nucleated cells in the bone marrow (per femur), and the number of leukocytes in 1 μl of peripheral blood were determined. The spleen and axillary lymph nodes were fixed in Bouin's fluid. Paraffin sections of the spleen and lymph nodes and films of bone marrow and peripheral blood were stained with azure II and eosin. The relative areas of different functional zones were determined in the sections by means of an Avtandilov's grid [1], with objective 20 and ocular 7 of the microscope. The number of lymphocytes, immunoblasts, plasma cells, macrophages, granulocytes, and also of mitoses and pycnotic cells was counted in thymus-dependent and thymus-independent zones of the spleen and lymph nodes per square millimeter of section under objective 90 and ocular 7 of the microscope. The results thus obtained and also data of the marrow and blood cell counts were subjected to statistical analysis by the nonparametric Wilcoxon–Mann–Whitney U test [4].

EXPERIMENTAL RESULTS

Single and fractional injection of T-activin caused similar changes in the organs of immunity. The results of the series of experiments in which the animals received a single dose of 0.5 μg of the preparation will therefore be described below.

Injection of T-activin into intact mice caused various structural changes in the organs of their immune system. Lymphocytopenia was observed in the bone marrow on the 1st, 5th, and 10th days, but by the 15th day an increase in the number of lymphocytes was found (Fig.1).

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Lymphocytosis was recorded in the peripheral blood on the 1st and 15th days, whereas in the spleen the white pulp (including the thymus-dependent zone also) was denuded of lymphocytes on the first day (Figs. 1 and 2). The number of blast cells in the T-zone of the spleen was reduced on the 5th, 10th, and 15th days, but the level of mitosis, except on the 10th day, remained within normal limits (Fig. 2). The relative area of the germinative centers on the 5th-15th days and the number of blast cells in them on the 5th day were reduced (Fig. 3). As early as on the 1st day an increase in the number of plasma cells in the cords of red pulp was observed. This parameter remained at a high level throughout the period of observation (Fig. 3). In the thymus-dependent zone of the lymph nodes a tendency was observed for the number of lymphocytes to increase on the 1st day, but then to fall on the 5th and 10th days. In the medullary cords an increase was observed in the number of plasma cells on the 1st day ($6.81 \cdot 10^3/mm^2$ compared with $3.65 \cdot 10^3$ in the control; $P < 0.05$) followed by a decrease to the control level. The number of degenerating cells in the organs studied was the same in control and experiment.

T-activin thus brought about changes in lymphocyte migration in intact animals, with a marked and early increase in the degree of differentiation of plasma cells. The different dynamics of plasmatization and of the response of the germinative centers compared with antigenic stimulation [13] suggests that T-activin is not immunogenic. The early and rapid exhaustion of the germinative centers and intensification of the plasma-cell response are evidence that the preparation stimulates pre-existing immune reactions, evidently by stimulating helper activity of the T cells [5]. The constant number of degenerating cells in the immunity system is evidence that T-activin is nontoxic.

Removal of the thymus in adult animals leads to considerable functional changes in immunity. Migration of hematopoietic stem cells from bone marrow is inhibited [9], the circulating factor of the thymus disappears from the peripheral blood, and the T-suppressor pool is reduced [14]. Blocking the central component of the T system is evidently combined with definite preservation of the peripheral component [11], and this is confirmed by the present results. For instance, the number of nucleated cells and also of lymphocytes in the bone marrow and peripheral blood of thymectomized mice remained at the same level as in intact animals. The weight of the spleen increased by 18% ($P < 0.05$) and, together with the unchanged area of the white pulp, this indicates some increase in the weight of the pulp. A tendency toward delymphization of the white pulp was found. The number of plasma cells also increased ($5.0 \cdot 10^3/mm^2$ compared with $3.0 \cdot 10^3$ in intact animals; $P < 0.05$), possibly on account of a decrease in the number of T-suppressors [12, 14]. Moderate reduction of the thymus-dependent zone (from 65.4 to 48.1%; $P < 0.05$) and a decrease in the number of blast cells in it (from $6.81 \cdot 10^2$ to $1.31 \cdot 10^2/mm^2$; $P < 0.05$) were observed in the lymph nodes.

The reaction of the immune system of thymectomized mice to T-activin had certain distinguishing features. The number of lymphocytes in the bone marrow remained within limits of the control values at all times (Fig. 1). Lymphocytosis was observed in the peripheral blood only on the 15th day; its final level was equal to that in intact animals (Fig. 1). Delymphatization of the white pulp and of the thymus-dependent zone in the spleen was recorded on the 15th day (Figs. 1 and 2). In the T-zone the number of blast cells was reduced on the 5th and 15th days but the level of mitoses was high at all times (Fig. 2). Just as in intact animals, in the thymectomized mice there was a considerable decrease in the relative area of the germinative centers from the 1st to the 15th days, and the decrease in the number of blast cells in them took place earlier and reached a lower level (Fig. 3). The plasma-cell reaction also began on the 1st day and maintained high values at all times (Fig. 3). Restoration of the relative area of the T zone from the 5th to the 15th days was observed in the lymph nodes (61.3, 63.9, and 63.4% compared with 48.1% in the control; $P < 0.05$), with intensification of blast cell formation in it ($3.62 \cdot 10^2$, $2.42 \cdot 10^2$, and $3.08 \cdot 10^2/mm^2$ compared with $1.31 \cdot 10^2$ in the control; $P < 0.05$) on the 5th-15th days, and an increase in the number of mitoses on the 1st day ($126.9/mm^2$ compared with 15.4 in the control; $P < 0.05$). The number of plasma cells remained at the control level. Absence of cell migration from the bone marrow and later delymphization of the white pulp of the spleen caused a delayed increase in the number of lymphocytes in the peripheral blood, although the final level of lymphocytosis (15th day) was equal to that in the intact animals.

The following distinguishing features of the response of thymectomized mice to T-activin were noted. Signs of lymphocyte migration from bone marrow were absent. An early and stable increase in reproductive activity of the T-zone and a smaller increase in the intensity of plasmatization, as shown by the fact that the level of plasma cells was the same as in intact

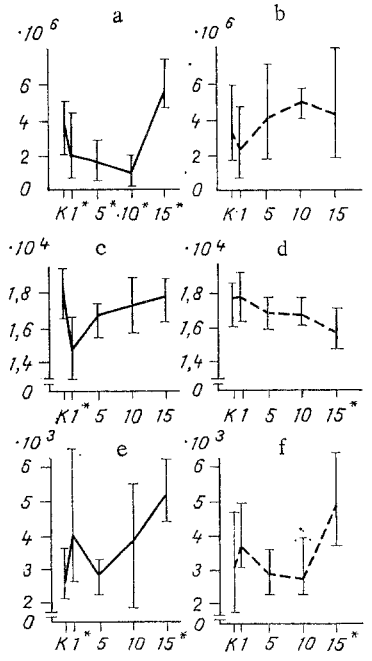

Fig. 1

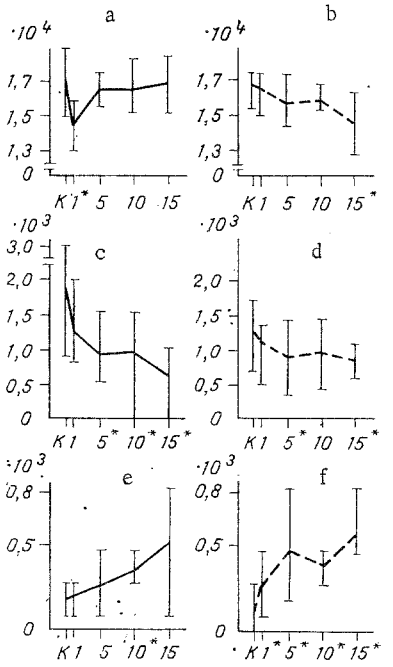

Fig. 2

Fig. 1. Changes in number of lymphocytes in peripheral organs of immunity under the influence of T-activin. a, b) Number of lymphocytes per femur; c, d) number of lymphocytes per square millimeter of white pulp of spleen; e, f) number of lymphocytes in 1 μl of peripheral blood. a, c, e) Intact animals, b, d, f) thymectomized animals. Abscissa, time in days after injection of T-activin; ordinate, number of lymphocytes; K) initial data. Vertical lines show limits of individual variations. Asterisks: significance of differences from initial values.

Fig. 2. Thymus-dependent zone of white pulp of spleen. a, b) Number of lymphocytes in 1 mm^2; c, d) number of immunoblasts in 1 mm^2; e, f) number of mitoses in 1 mm^2. Ordinate, values of parameters. Remainder of legend as to Fig. 1.

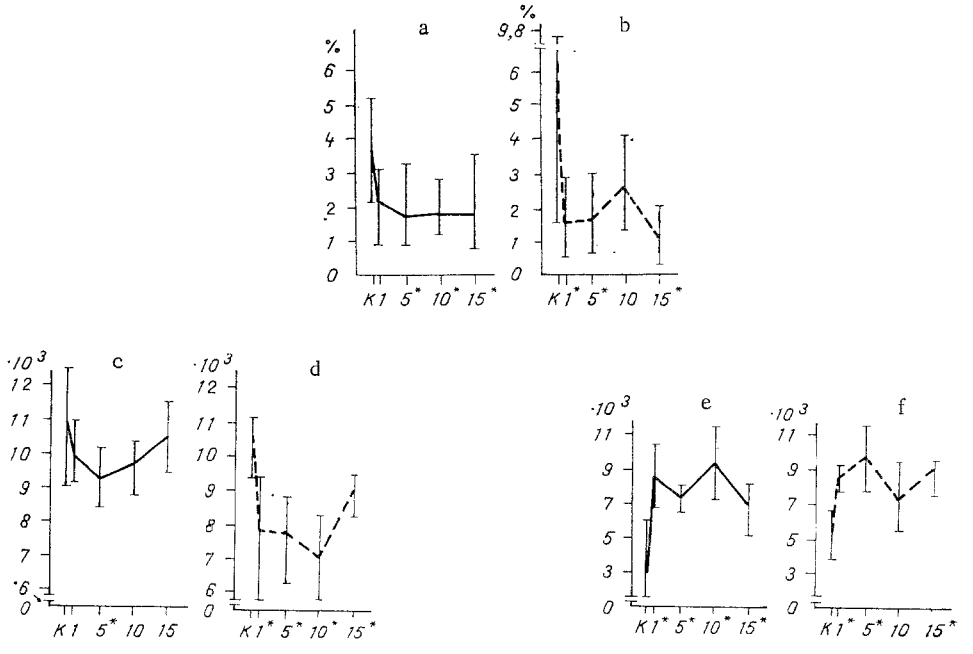

Fig. 3. B-zone of spleen. a, b) Relative area of germinative centers; c, d) number of immunoblasts in germinative centers; e, f) number of plasma cells in 1 mm^2. Ordinate, values of parameters. Remainder of legend as to Fig. 1.

mice, whereas higher initial values were observed in the thymectomized mice, was recorded in the spleen. All zones of the white pulp took part in the plasma-cell reaction. In the lymph nodes, despite restoration of the area of the T-zone and a transient increase in its reproductive activity, differentiation of plasma cells was not intensified.

After injection of T-activin into thymectomized mice, it was thus the spleen that was principally activated. T_1 cells, with affinity for the spleen and accumulating in it [15], are perhaps not completely eliminated 2 months after thymectomy and become targets for T-activin. The identical character of reaction of the germinative centers and of plasmatization in the thymectomized and intact animals is evidence that in the immunodeficiency studied the degree of preservation of the organs of immunity was sufficient for exhibition of the stimulating effect of T-activin on T helpers. Weaker stimulation of plasma cell differentiation is evidence in support of restoration of T suppressor activity under the influence of T-activin.

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