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ACTION OF THE IMMUNOMODULATOR T-ACTIVIN ON ELECTRICAL PROPERTIES OF THE THYMUS PLASMA CELL MEMBRANE STUDIED BY FLUORESCENT PROBES

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Thymic hormones, synthesized by the epithelial cells of the thymus, are known to have a definite influence on the formation of many thymus-dependent functions of T cells [1, 2, 10, 14]. An important role in the activation, differentiation, and maturation of lymphocytes is played by their membrane [7]. However, the direct action of thymic hormonal factors on the structure of the lymphocyte membrane has received only little study.

It was accordingly decided to study the action of T-activin on the electrical properties of membranes of a suspension of thymus cells. For this purpose we used the method of membrane fluorescent probes [14], which has been used with success for these purposes [5].

EXPERIMENTAL METHOD

Thymus cells were obtained from (CBA × C57Bl) mice aged 2 months in colorless Hanks' solution. Refraction of intact cells (as shown by the test of their permeability to 0.2% trypan blue) amounted to 94% of the total.

The thymocytes were stained with the following fluorescent probes: 1) aniline-naphthalene-8-sulfonate (ANS), 2-(p-dimethylaminostyryl)-4-methylpyridinium (DSM), 4-dimethylaminochalcone (DMC), and 3-methoxybenzanthrone (MBA). To 0.2 ml of a thymocyte suspension (2×10^7 cells/ml) were added 1 mM solutions of ANS or DSM in water and of DMC or MBA in dimethylformamide to a final concentration of 10 μM. The intensity of fluorescence was measured on an ML-4 microfluorometer (LOMO, USSR).

The wavelengths of exciting light for probes MBA, DMC, and DSM were 405-436 nm and for ANS 365 nm; the wavelength of emission of MBA and LMC was 520 nm, of ANS 450 nm, and DSM 550 nm, distinguished with the aid of interference filters. Measurements were made on single cells in a field of the microscope 10 μ in diameter. Apochromatic 40 × objective and 7 × ocular were used. In each preparation the intensity of fluorescence of 100 individual cells was measured. To evaluate the action of T-activin, a solution of T-activin was added to a suspension of thymocytes in Hanks' solution up to a final concentration of between 1 and 10 μg per $1 \cdot 10^6$ cells, and the suspension was incubated at 37°C for 1 h with periodic shaking. After the end of incubation the thymocytes were washed with Hanks' solution with centrifugation at 200 g for 10 min. The residue was resuspended in Hanks' solution, and the number of cells in the suspension thus obtained was counted and their viability determined.

The thymocytes were incubated with neuraminidase as follows: to a suspension of thymic lymphocytes in Hanks' solution a solution of neuraminidase was added up to a final concen-

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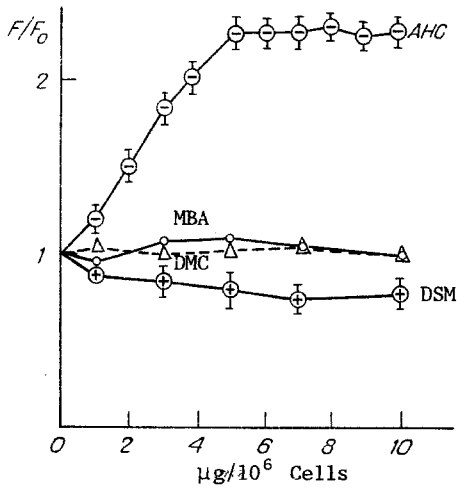

Fig. 1. Effect of T-activin on intensity of fluorescence of ANS, BMA, DMC, and DSM probes in single thymocytes. Abscissa, concentration of T-activin. F_0 and F) Intensities of fluorescence of ANS (450 nm), MBA, DMC (520 nm), and DSM probes (550 nm) in the absence and presence of T-activin. Concentration of probes 10 μM. Concentration of thymocytes in suspension $2 \cdot 10^7$/ml.

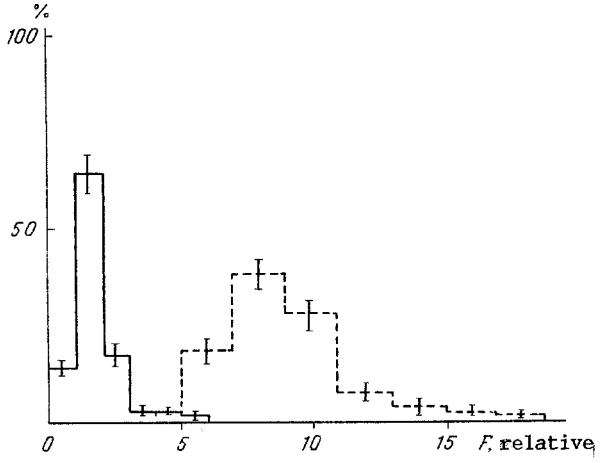

Fig. 2. Histograms of distribution of mouse thymocutes by intensity of fluorescence (F) of ANS probe in them. Fluorescence of each cell was excited at 365 nm and recorded at 450 nm. Continuous lines indicate intact cells, broken lines – cells after treatment with T-activin (5 μg/10^6 cells). Ordinate, fraction of cells (in %) giving this level of fluorescence.

tration of 50 units/ml, and the sample was incubated at 37°C for 30 min with periodic mixing. After the end of incubation the thymocytes were washed twice as described above. The thymic preparation T-activin was generously provided by V. Ya. Arion (Research Institute of Physicochemical Medicine, Ministry of Health of the RSFSR).

EXPERIMENTAL RESULTS

The results of microfluorometric determination of the thymocytes treated with T-activin and stained with the fluorescent probes MBA, DMC, ANS, and DSM, are given in Fig. 1. Clearly the intensity of fluorescence of the MBA and DMC probes did not in fact change compared with the control.

These probes are known to be located in the polar layer from the level of phosphate groups of membrane lipids to the region of location of carbonyl groups [4]. DMC likewise is sensitive to the concentration and mobility of water molecules penetration int the region of the membrane carbonyl groups [11].

Thus T-activin evidently does not induce changes in the structure of the polar layer of the thymocyte membrane at the site of these probes and does not affect the state of the water in it.

The intensity of fluorescence of oppositely charged fluorescent probes ANS and DSM changed after treatment of the thymocyte suspension from the thymus with T-activin. It was shown previously that these probes are very sensitive to a change in the charge on the membrane surface, and also a change of transmembrane potential [5]. It can also be seen in Fig. 1 that a decrease in the intensity of fluorescence of the positively charged DMS probe by about 20% and an increase in F/F_0 of the negative ANS probe by 2.3 times took place. These changes in the intensity of fluorescence can be explained, first, by an increase in the positive charge of the surface layer of the thymocyte membrane, second by a decrease in the negative charge, and third, by depolarization of the transmembrane potential on the plasma membrane.

The increase in the positive charge could be the result of binding of positively charged polypeptides of T-activin on the membrane surface. It was shown previously that an increase in the positive charge on the membranes of liposomes (of egg phosphatidylcholine) and erythrocyte ghosts, induced by means of protamine, led to similar changes in fluorescence of ANS and DSM [8]. However, for identical effects of protamine to be achieved, 20 times more of it was needed than of T-activin. Treatment of phosphatidylcholine liposomes with T-activin likewise did not lead to changes in fluorescence of ANS.

Thus no increase in the positive charge of the membrane evidently takes place on account of its binding with T-activin.

We know that a large quantity of carbohydrates is present on the surface of thymic thymocytes, including certain neuraminic (sialic) acids, which have a negative charge. It is possible that under the influence of T-activin on the thymocyte membrane processes are triggered as a result of which sialic acid residues are removed from the membrane surface. This leads to reduction of the negative charge on the membrane surface and to corresponding changes in fluorescence of ANS and DSM. The action of T-activin can be compared with the action of neuraminidase, but it is weaker. The intensity of fluorescence of ANS increases in both cases. However, after treatment of the thymocytes with neuraminidase F/F_0 was almost 3 times greater than after treatment with T-activin.

It was shown previously, moreover, that fluorescence of ANS in thymocytes can also increase because of depolarization of the plasma membrane and subsequent entry of the probe into the cell. This is reflected in histograms of distribution of thymocytes (intact and after depolarization of the plasmalemma) of the thymus, based on intensity of fluorescence of the probe in them [6]. It was this change in the histogram that was found after treatment of the thymocyte suspension with T-activin (Fig. 2). T-activin evidently leads to depolarization and lowering of the transmembrane potential on the platelet plasma membrane. As a result of this ANS enters the cell and binds with intracellular membrane structures, and this leads to an increase in its fluorescence.

The following changes of fluorescence of the ANS probe took place in a thymocyte suspension treated with neuraminidase and T-activin (5 μg to $1 \cdot 10^6$ cells): intact lymphocytes 1.00, lymphocytes treated with T-activin 2.30 ± 0.13, and lymphocytes treated with neuraminidase 6.85 ± 0.10.

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LYMPHOCYTE KINETICS IN MICE WITH ALLOXAN DIABETES

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Among the many different hematologic disturbances developing in diabetes a special place is occupied by the marked inhibition of cellular immunity [3, 4, 8]. Insulin is known to possess the properties of a general paleohematopoietim in mammals and man: proliferation of the lymphoid cells of the thymus and bone marrow and also the function of cytotoxic T lymphocytes are known to depend to the greatest degree on it, whereas the formation and function of phylogenetically younger B lymphocytes are not disturbed as a general rule if this hormone is deficient, and indeed, they may even be enhanced.

The aim of this investigation was to study the kinetics of the lymphoid cells of the thymus, lymph nodes, and blood in mice with alloxan diabetes.

EXPERIMENTAL METHOD

Experiments were carried out on 100 male BALB/c mice (from the "Rassvet" Nursery, Tomsk) weighing 18-20 g, in some of which alloxan diabetes was induced by the method described previously [3]. The blood sugar level of the diabetic animals was not lower than 14 mM. The lifespan of the peripheral blood lymphocytes was determined autoradiographically, with the use of ^{3}H-thymidine [6]. 5-methyl-^{3}H-thymidine ("Izotop" Production Combine, specific radioactivity 925 GBq/mmole) was injected intraperitoneally once daily in a dose of 40 MBq/kg for 14 days, after which all the animals were given unlabeled thymidine ("Fluka," Swizerland) from the 15th through the 30th day of the experiment in order to block reutilization of the radionuclide (the unlabeled thymidine was added to the drinking water in a concentration of 100 mg/liter). To study the dynamics of the appearance and disappearance of labeled lymphocytes, blood films were obtained throughout the experiment at intervals of 1-2 days, and subsequently used for autoradiograhic investigation [3]. Growth fractions and temporal parameters of the mitotic cycle of the lymphoblasts and prolymphocytes in the thymus and ileocecal lymph nodes were determined by the method of saturation with ^{3}H-thymidine [2, 5]. The radionuclide was injected intraperitoneally in a dose of 20 MBq/kg seven times in the course of 24 h at intervals of 4 h, or twice in the same dose at intervals of 1 and 2 h, or

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