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SHEDDING OF MOUSE THYMOCYTE RECEPTORS FOR AUTOLOGOUS ERYTHROCYTES UNDER THE INFLUENCE OF THYMIC OLIGOPEPTIDE FACTOR

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A small percentage of mouse thymocytes can interact with autologous and syngeneic erythrocytes to form rosettes [7, 8, 11]. Thymocytes forming rosettes with autologous or syngeneic erythrocytes (ARFC) are probably in the early stages of maturation [8, 9].

A hormone of the thymus detectable in serum (serum thymic factor), which controls T-cell differentiation, reduces the percentage of ARFC among thymocytes and their cytotoxicity against autologous erythrocytes [4]. A similar action on thymocytes has been observed after incubation for a short time with the low-molecular-weight oligopeptide factor isolated from a dialyzable thymus extract by adsorption on immobilized bovine serum euglobulins [2, 3]. The mechanism of reduction of affinity of the thymocytes for autologous erythrocytes in the presence of this factor is not clear.

The aim of this investigation was to study the properties of the supernatant obtained after incubation of thymocytes with thymic oligopeptide factor (TOF).

EXPERIMENTAL METHOD

Thymus glands from CBA mice aged 2 months were used. Thymocytes were isolated by a coarsely ground glass homogenizer in an excess of Hanks' solution. Autologous rosette formation was studied by the method in [8].

TOF was isolated from a saline extract of the thymus by chromatography on euglobulins, bound to sepharose 4B, as described previously [2, 3]. Activity of the preparation was tested by determining the decrease in the ability of mouse thymocytes (10^7 cells in 1 ml) to form

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TABLE 1. Shedding of Thymocyte Receptors for Autologous Erythrocytes under the Influence of TOF

Expt. No.	Intact thymocytes	Thymocytes heated to 45°C	Heated thymocytes incubated with supernatant of intact cells	Heated thymocytes incubated with supernatant of activated cells
1	18	—	10	32
2	10	6	6	9
3	21	11	11	28
4	22	5	25	27
5	10	9	9	14
6	30	12	21	29
7	—	19	20	28
8	20	19	15.	24
9	30	21	24	26
10	18	5	5	7
11	27	12	11	18

Legend. Number of autorosette-forming heated thymocytes after restoration by supernatant of syngeneic thymocytes activated by TOF (in %). For comparison of intact and heated thymocytes, and also of thymocytes incubated with supernatant of intact and activated cells, $p<0.01$.

autologous rosettes after incubation for 90 min with different concentrations of the preparation at 37°C. Activity was due to a peptide with mol. wt. of 1000 daltons [1], and was lost after treatment of the preparation with pronase. For N-terminal analysis the TOF preparation was obtained with the aid of chromatography on SE-Sephadex and again on DEAE-Sephacel, and by chromatography on immobilized euglobins. Biological activity was determined at each stage of purification. The N-terminal analysis was carried out by Yu. L. Radavskii (Institute of Bio-organic Chemistry, Academy of Sciences of the Ukrainian SSR) by a modified Gray's method [10]. Two N-ends (serine and glutamic acid), and the NH_2-group of lysine and OH-group of tyrosine inside the chain were found.

To study the mechanism of the decreased ability of the thymocytes to form autorosettes under the influence of TOF the following experiment was undertaken. 0.4 ml of a suspension of thymocytes (15×10^6 cells/ml), heated for 1 h at 45°C and washed with Hanks' solution, was incubated with 3 ml of supernatant and with syngeneic thymocytes (10^7 cells/ml) treated with TOF (10 μg/ml). The supernatant of the same suspension of thymocytes incubated without TOF served as the control. The test supernatants were incubated with heated and washed thymocytes for 1 h at 4°C. The formation of ARFC by the thymocytes was studied after washing with Hanks' solution.

The results were subjected to statistical analysis by the method of direct differences [5].

EXPERIMENTAL RESULTS

As was shown previously [6], on heating to 45°C the ability of the thymocytes to interact with autologous erythrocytes was reduced. The receptor structures responsible for autologous rosette formation passed into the medium under these circumstances. The ability of heated and washed thymocytes to form ARFC can be restored after their incubation with the supernatant obtained by heating syngeneic thymocytes.

The study of the properties of the supernatant of thymocytes incubated with TOF under physiological conditions (at 37°C) showed that after incubation with TOF receptors for autologous erythrocytes also passed into the medium, so that the supernatant obtained from TOF-treated thymocytes restored the percentage of ARFC of heated and washed syngeneic thymocytes (Table 1). The supernatant of control (not treated with TOF) thymocytes did not possess this property.

On this basis we suggest that the decrease in autoreactivity of the thymocytes under the influence of TOF takes place as a result of shedding of receptor structures responsible for autologous rosette formation from the surface of the thymocytes. Shedding of receptors for

autologous erythrocytes under the influence of TOF may perhaps reflect the formation of mature T lymphocytes with low autoreactivity.

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