DETECTION OF IMMUNOMODULATING POLYPEPTIDES IN HUMAN THYMUS CELLS BY AN IMMUNOFLUORESCENCE METHOD

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The successful study of the functional morphology of the thymus in various physiological and pathological processes in which it participates directly or indirectly largely depends on a solution to the problem of the morphological equivalence of thymus function [1, 6, 8]. This is impossible without discovery of the localization of the factors of the thymus directly in its tissue. We know that the lymphopoietic, immunoregulatory, and endocrine functions of the thymus are linked mainly with the production of a heterogeneous group of humoral factors of polypeptide nature by its cells [5, 12]. The localization of polypeptides, thymosin, the serum factor of the thymus, and thymopoietins has now been studied and their thymic origin proved [9, 10, 13]. Isolated studies of the human thymus have shown the age dynamics of thymosin α_1 and thymosins β_3 and β_4 [13]. An increase in the content of the serum factor of the thymus has been found in the thymus tissue in myasthenia and certain lymphoepithelial thymomas [15] and a decrease in its content in the case of a combination of thymoma with systemic lupus erythematosus [11]. This is in agreement with the results of quantitative determination of immunomodulating polypeptides in the human thymus in myasthenia and thymoma [3]. Thymalin is a preparation containing a group of polypeptide factors of the thymus [2, 3]. It is used as an immunomodulator in the treatment of various immunodeficiency diseases [7].

The aim of the present investigation was to determine the localization of the polypeptides of thymalin in human thymus tissue and to study their age dynamics.

EXPERIMENTAL METHOD

The thymus of eight children and of a number of adults was studied: biopsy material obtained from the thymus during operations on children with congenital heart defects aged 2 and 2.5 years was investigated; the autopsy material came from patients dying from acute respiratory virus infection, meningococcal infection, bronchial asthma, chronic lung abscess, and ischemic heart disease accompanied by atherosclerosis after coronary by-pass operations at the ages of 5 months and 1.5, 11, 28, 44, and 46 years. Histological investigation of the thymus in all cases showed that its state corresponded to the subject's age and the primary disease. To detect thymalin polypeptides in cells of the human thymus a specific antiserum to a preparation obtained at the Leningrad Research Institute of Vaccines and Sera, Ministry of Health of the USSR, by the method of Moskvicheva et al. [4] was used. Thymus polypeptides (thymalin) were determined by an indirect immunofluorescent method. Sections through lymph nodes, spleen, and skin, treated in the same manner, served as the control. Sections through the thymus, not treated by specific antiserum to thymalin, also were studied.

Sections 4-5 μ thick, cut on a cryostat from unfixed material, were washed in buffered (0.1 M phoshate buffer, pH 7.2) physiological saline and incubated consecutively with rabbit antiserum containing antibodies to thymalin (titer in the hemagglutination test 1:3200; dilution 2-4 times) and with donkey serum against rabbit γ-globulin, labeled with FITC (from the N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Sciences

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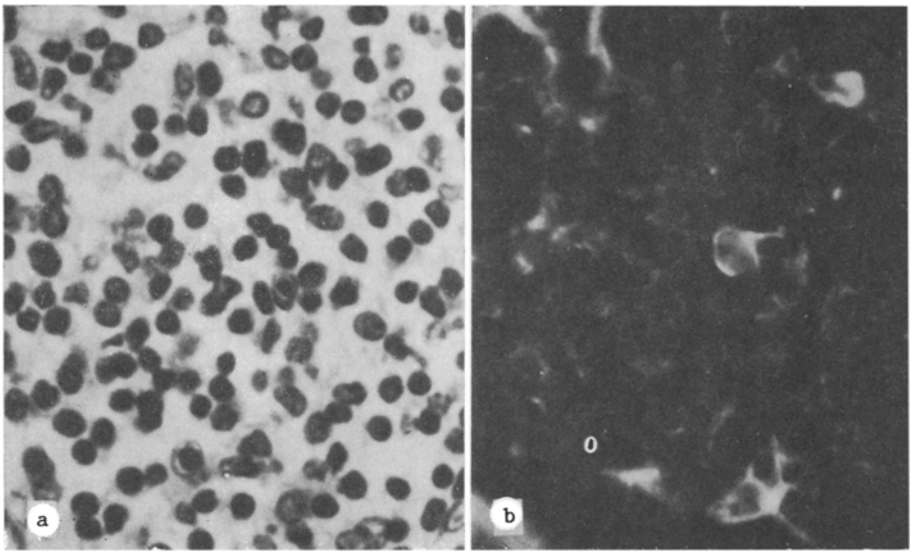

Fig. 1. Thymus. Subcapsular zone of cortex (surgical biopsy, 2 years). a) Stained with hematoxylin and eosin; b) thymalin polypeptides in bodies of epithelial cells and in their processes, forming Clark's reticulum. Small quantity of thymalin polypeptides, in the form of granules, on thymocyte membranes inside. Here and in Figs. 2 and 3b — indirect immunofluorescence method with antibodies to thymalin polypeptides. 600×,

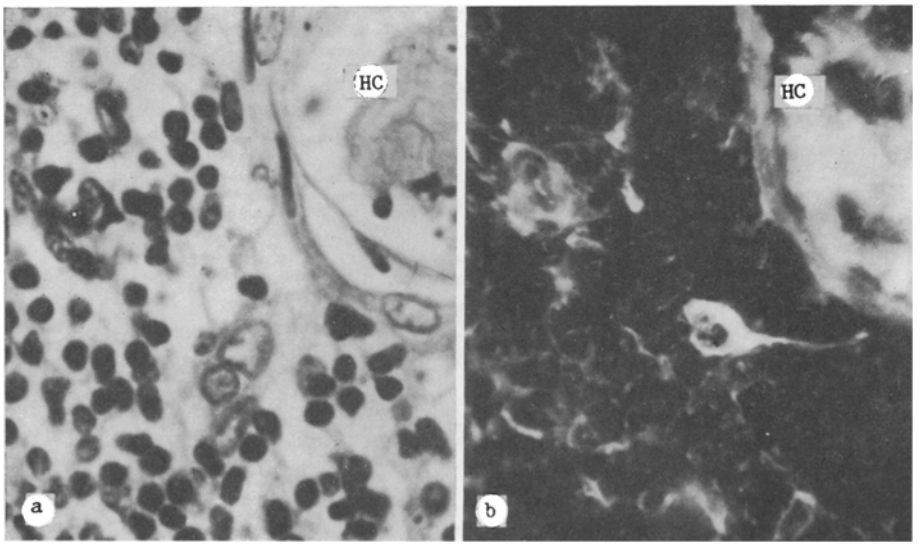

Fig. 2. Thymus. Medulla (autopsy, 44 years). a) Stained with hematoxylin and eosin; b) thymalin polypeptides in bodies and processes of epithelial cells, including cells located at periphery of Hassall's corpuscles. Granules of thymalin polypeptides on thymocyte membranes. HC) Hassall's corpuscles.

of the USSR, titer 1:64, dilution 4-8 times) for 20 min at 22°C, with rinsing at each stage with buffered physiological saline. The preparations obtained, together with the control, were studied with the LYuMAM-IZ microscope.

EXPERIMENTAL RESULTS

Cells containing thymalin polypeptides were found only in thymus tissue. These data are in agreement with results obtained in a study of the localization of thymosin α_1 [13] and of thymus serum factor [14, 15]. Binding of antibodies to thymalin by macrophages of the thymus and spleen could not be found, whereas antibodies to thymopoietin and to fractions 5 and

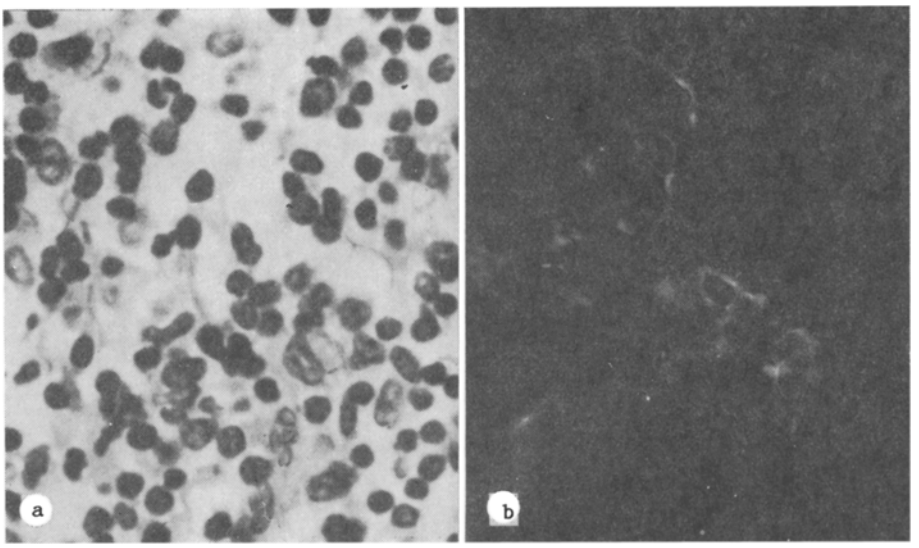

Fig. 3. Thymus. Autopsy, 46 years. a) Stained with hematoxylin and eosin. b) Thymalin polypeptides in bodies and processes of epithelial cells, forming groups of 2-5 cells.

6 of thymosin bound with these cells [9, 13]. Thymalin polypeptides were found in the form of granular and diffuse material in the bodies and processes of stellate epithelial cells, mainly in the subcapsular zone of the cortex (Fig. 1b) and in analogous cells of the medulla. Outgrowths of the epithelial cells, visible because of the thymalin polypeptides present in them, formed a network of Clark's reticulum. This distribution is also characteristic of other thymus polypeptide factors, as has been shown by immunofluorescence and immunoperoxidase methods, using polyclonal and monoclonal antibodies [9, 13-15]. It has been shown by the use of electron-microscopic immunohistochemistry that thymus polypeptide factors are present in vacuoles of the Ia-antigen-negative, keratin-containing population of eipthelial cells of the cortex and medulla of the thymus [10, 14, 15]. Thymalin also was contained by individual peripheral cells of Hassall's corpuscles (Fig. 2b), but adsorption of antibodies by debris of thymic corpuscles could be nonspecific [14].

In man between the ages of 5 months and 11 years the number of cells containing thymalin polypeptides is quite large in both the cortex and the medulla, but at the age of 28-46 years their number decreases and they are infrequently grouped into clusters of two to five cells (Fig. 3b). A similar age dynamics has been shown for thymosin α_1 [13], and this correlates with data showing a fall in the blood level of thymus factor with age [12].

It has been shown that by no means all the epithelial cells contain thymalin polypeptides. This is characteristic also of other preparations from the thymus. For instance, only about 1% of medullary epithelial cells of the thymus contain thymus serum factor [14]. With an increase in the concentration of antibodies to thymalin polypeptides the number of positively reacting epithelial cells increased only very slightly, but small amounts of polypeptides appeared under these circumstances in the form of granules on the membrane of thymocytes located in the Clark's reticulum (Figs. 1 and 2b). It can be tentatively suggested that in such cases polypeptide factors bound with thymocyte receptors were demonstrated [9].

The results of this investigation thus showed that thymalin polypeptides are contained in the epithelial cells of the thymus and that their distribution corresponds to that described previously for other polypeptide factors of the thymus and, in particular, thymus serum factor and thymosin α_1. Data on a decrease in the number of thymus cells containing immunomodulating polypeptides with age also were confirmed. The use of the immunofluorescence method of locating thymalin polypeptides opens up wide prospects for the study of thymus function under normal and pathological conditions.

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EFFECT OF IMMUNOSTIMULATORS ON MOUSE PERITONEAL EXUDATE MACROPHAGES ANALYZED BY MATHEMATICAL MODELING AND PLANNING METHODS

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Biologically active substances of varied chemical nature, isolated from plants, animals, and bacteria, and also artificially synthesized, possess immunostimulating properties [2]. Immunostimulators (IS) induce profound and lasting transformations in the plasma membrane of cells of the mononuclear phagocytic system. This is shown, in particular, by changes in the phospholipid layer of the membrane [6], and also in the activity of 5'-nucleotidase (EC 3.1.3.5) one of the principal enzymes of purine catabolism, which is a marker of the cytoplasmic membrane [4]. It is accordingly interesting to study IS by assessing their effect on 5'-nucleotidase activity.

The aim of this investigation was to analyze changes in 5'-nucleotidase activity in mouse peritoneal exudate macrophages (PEM) after intraperitoneal injection of IS, using methods of mathematical modeling and planning.

EXPERIMENTAL METHOD

In the experimental part of the work 5'-nucleotidase activity was measured in PEM of CBA mice aged 3 months, at selected time intervals after intraperitoneal injection of IS. The following IS were used: salmosan (a polysaccharide of bacterial origin), tuftsin and rigin (tetrapeptides), and a polysaccharide of animal origin (PAO).

The preparations were injected in a single dose of 100 μg per mouse. Enzyme activity was determined by a modified method of Dixon and Purdom [4]. PEM were obtained by the method in [1]. The experimental data were subjected to statistical analysis by methods of regression analysis [3].

EXPERIMENTAL RESULTS

It will be clear from Table 1 that all the IS used induced a long-term decrease in activity of this enzyme. Regression analysis of the data showed that the change in 5'-

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