DYNAMICS OF THYMALIN LOCALIZATION IN HUMAN EMBRYONIC THYMUS TISSUES

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KEY WORDS: thymus; human embryo; thymalin.

Differentiation of T lymphocytes from their precursor T-cells, reaching the thymus initially from the liver and later from the bone marrow, takes place in the thymus. This process depends on the production of hormone-like substances secreted by the thymic epithelial cells [9]. These substances include thymosin [6], thymic humoral factor [12], thymopoietin [7], serum thymus factor [5], and T-activin [1]. Serum thymus factor [4], α-thymosin [8], and thymopoietin [10] have now been identified by immunohistochemical methods

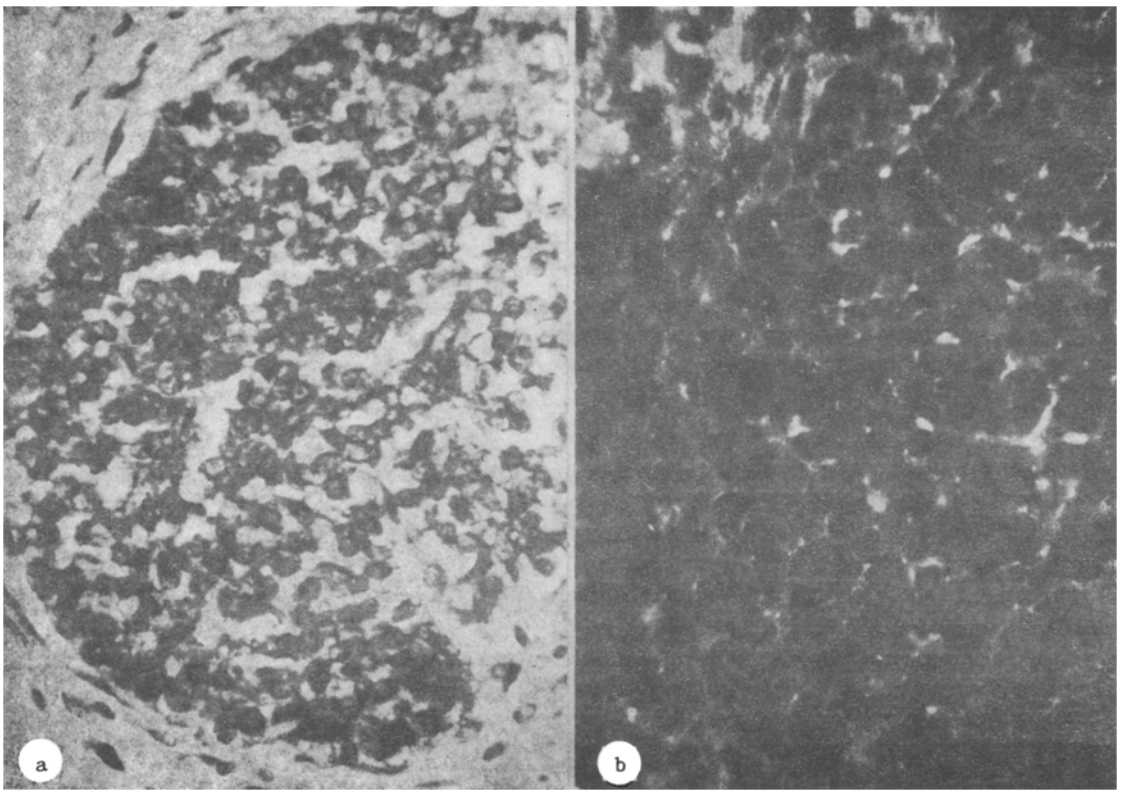

Fig. 1. Thymus of 6-week human embryo. a) Network of reticuloepithelial cells without lymphocytes. Fixation with formalin. Stained with hematoxylin and eosin. 250 ×. b) Frozen section. Indirect immunofluorescence method with antibodies to thymalin polypeptides. Diffuse arrangement of thymalin-positive cells. 800 ×.

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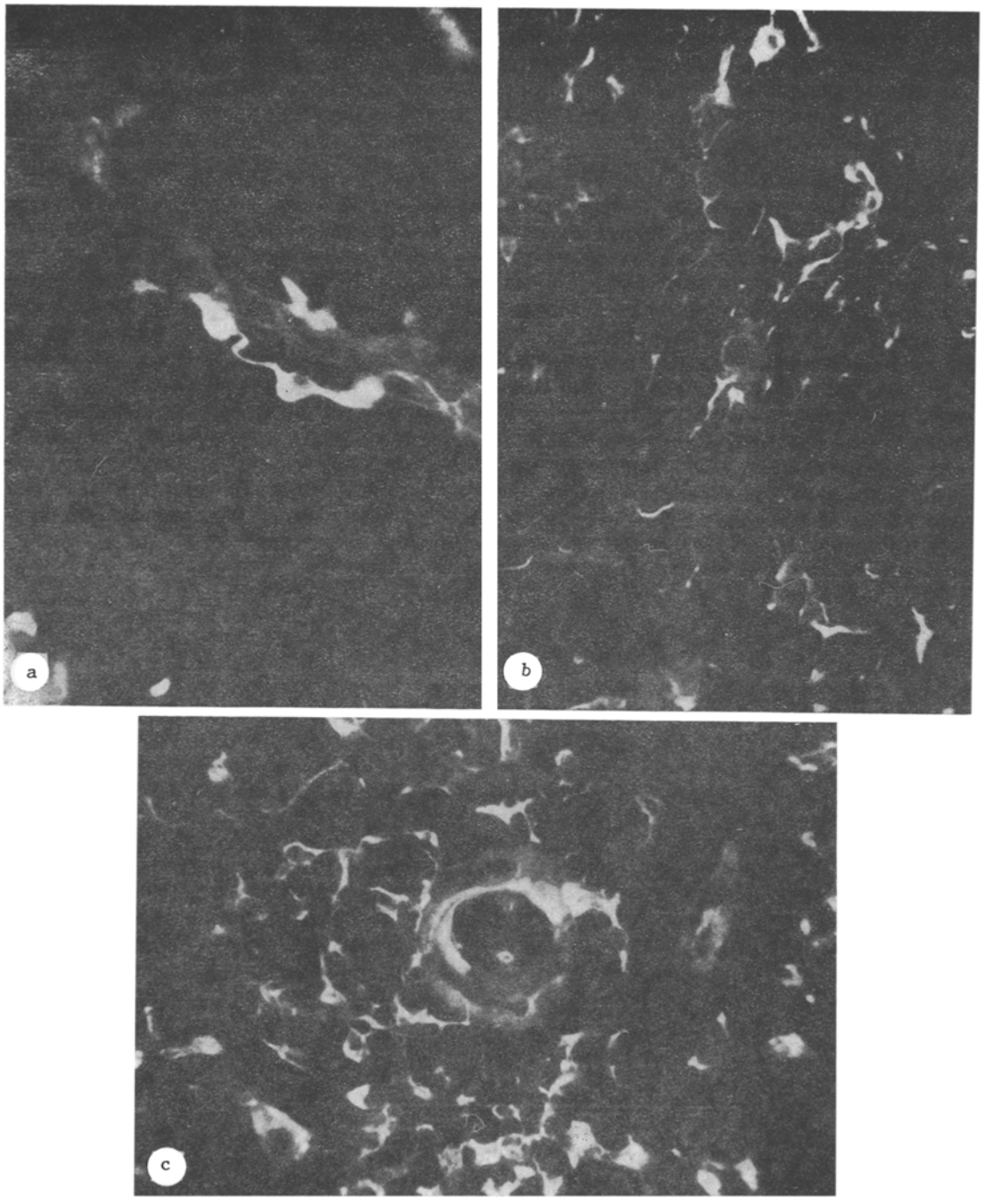

Fig. 2. Frozen sections through thymus. Indirect immunofluorescence test with antibodies to thymalin polypeptides. a) 23-week fetus. Thymalin-positive cells in cortex of thymus. 800 ×. b) 23-week fetus. Thymalin-positive cells in medulla of thymus. 800 ×. c) Child aged 3 years. Thymalin-positive cells in medulla of thymus. Hassall's corpuscle in center. 800 ×.

in the epithelial cells of the thymus. The localization of the serum factor [11] in cytoplasmic vacuoles of the epithelial cells has been demonstrated electron-microscopically. Thymalin, which is currently being used as an immunomodulator for the treatment of various immunodeficiency states [2], is a factor discovered in the USSR, which induces differentiation and functional activity of T cells.

The aim of this investigation was to determine the time when thymalin-positive cells can be found in the thymus and their distribution in the human thymus during embryogenesis.

EXPERIMENTAL METHOD

The thymus from 16 human embryos and fetuses aged from 6 to 23 weeks of intrauterine development, obtained during abortions on medical indications, and the thymus of two children (a child aged 3 years with a congenital heart defect and one aged 2 years, dying as a result of falling from a height) were studied.

Specific antiserum to thymalin was obtained from Candidate of Medical Sciences G. A. Ryzhak, at the Leningrad Research Institute of Vaccines and Sera, Ministry of the Medical and Biological Industry of the USSR, to whom the authors are grateful. Thymalin was determined by the indirect immunofluorescence method. Sections through the thymus, not treated with specific antiserum, and also sections through the spleen, liver, and intestine, treated with specific antiserum, served as the controls. Frozen sections were fixed for 5-10 min at 4°C in 96° ethanol, acidified with acetic acid (99:1), washed with buffered physiological saline, and incubated in a humid chamber consecutively with rabbit antiserum containing antibodies to thymalin (titer in the indirect agglutination test 1:3200, dilution 1:4), and with donkey serum to rabbit globulin, labeled with FITC, produced by the N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR (titer 1:64, dilution 1:4). The preparations were studied under the LYUMAM-R3 microscope.

EXPERIMENTAL RESULTS

In the early stage of embryogenesis (4-6 weeks) the thymus is an epithelial organ containing young epithelial cells, which have already started on their path of differentiation into a recticuloepithelium (Fig. 1a). Cells containing thymalin are found in the thymus of the human embryo at 6 weeks of development, before the organ has been colonized by lymphoid cells. Thymalin is found in the bodies and processes of the epithelial cells, which are stellate in shape. Meanwhile thymalin-positive cells are diffusely distributed (Fig. 1b) all over the organ, which does not yet have a cortex and medulla.

By the 12th week of development thymalin-positive cells form groups of two or three cells, distributed throughout the organ. No difference could be found in the arrangement of thymalin-positive cells in the cortex and medulla, which at this time are already separated in the thymus.

By the 23rd week of development thymalin-positive cells are arranged singly in the subcapsular zone and concentrated in the medulla, where they form a network (Fig. 2a, b). Peripheral cells of Hassall's corpuscles also can be identified as thymalin-positive cells. A similar pattern of distribution of thymalin-positive cells persisted in the thymus of children aged 2-3 years (Fig. 2c). No thymalin-positive cells were presented in control sections of the thymus, spleen, and intestine, treated by the methods described above, confirming the specificity of thymalin secretion by the epithelial cells of the thymus.

The results of the immunofluorescence study of the thymus from human embryos and fetuses and from children in the first years of life, using antithymalin serum, thus lead to the conclusion that thymalin production is specific for the epithelial cells of the human thymus, and that thymalin-positive cells can be identified in the thymus of the 6-week embryo, i.e., before colonization of the epithelial anlage of the thymus by lymphoid cells. The dynamics of distribution of the thymalin-positive cells was noted. In the 6-7-week embryo these cells are distributed diffusely over the organ, but by the 23rd week and later they are concentrated in the medulla and form only a thin layer of thymalin-positive cells in the subcapsular zone of the thymus.

LITERATURE CITED

1. V. Ya. Arion, Progress in Science and Technology. Series: Immunology [in Russian], Vol. 10, Moscow (1982), p. 45.
2. V. G. Morozov and V. Kh. Khavinson, Dokl. Akad. Nauk SSSR, 240, 1004 (1978).
3. Z. S. Khlystova, Formation of the Immunogenetic System of the Human Fetus [in Russian], Moscow (1987).
4. C. Auger, J. Monier, M. Dardenne, et al., Immunol. Lett., 5, 213 (1982).
5. M. Dardenne, M. Papiernik, J. Bach, et al., Immunology, 27, 299 (1973).
6. A. Goldstein, G. Thurman, G. Cohen, et al., Biological Activity of Thymic Hormones, ed. by D. W. van Bekkum, Rotterdam (1974).
7. G. Goldstein. Ann. N. Y. Acad. Sci., 249, 177 (1975).

8. H. Kirokawa, J. McClure and A. Goldstein, Thymus, 4, 19 (1982).
9. A. Kruisbeek, Thymus, 1, 163 (1979).
10. W. Savino and M. Dardenne, Eur. J. Immunol., 14, 987 (1984).
11. D. Schmitt, J. Monier, M. Dardenne, et al., "Cytoplasmic localization of FTS in thymus epithelial cells," Thymus, 2, 177 (1980).
12. N. Trainin, M. Small, D. Zipori, et al., "Characteristics of THF, a thymic hormone," in: D. E. van Bekkum (ed.), The Biological Activity of Thymic Hormones, Rotterdam (1974).

VARIABILITY OF PLOIDY OF HUMAN CARDIOMYOCYTES

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Publications dealing with the study of ploidy of human cardiomyocytes give data on the DNA content in single nuclei [1, 5, 9, 10, 13]. It has been shown that the ventricles of the human heart contain many tetraploid nuclei, and indeed octaploid nuclei or nuclei of even higher ploidy have been found. However, such an interpretation is incomplete. Conclusions regarding polyploidy must always include an assessment of the number of nuclei in the cell: the binuclear cell with two diploid nuclei does not differ in the properties studied from a mononuclear tetraploid cell, one with two tetraploid nuclei from a single octaploid [8]. In mice and rats 80% or more of ventricular myocytes are binuclear 2c × 2 cells (where c denotes the haploid DNA content), which are tetraploid relative to the combined genome. The ventricles of the human heart also contain many binuclear cells [10, 12]; it has recently been shown that the modal class here is 4c × 2 [3]. Now for the first time we are comparing the composition of myocytes in different layers of the myocardium. Preliminary data on ploidy of mononuclear and binuclear myocytes in the hypertrophied heart are given below.

EXPERIMENTAL METHOD

Cardiomyocytes from four persons were studied: one died after burns (46 years), another died from knife wounds of the abdominal wall (age 15 years); in two cases the hearts of persons who died were hypertrophied due to coarctation of the aorta (31 years) and general atherosclerosis (61 years). Pieces of the inner, middle, and outer layers of the heart muscle were fixed with 10% formalin in phosphate buffer (pH 7.0) and dissociated into single cells with 50% KOH [2]. We previously improved this technique and demonstrated that DNA and proteins are preserved in the cells. After carrying out the Feulgen reaction in cardiomyocytes stained with naphthol yellow, we measured the DNA content on a Vickers M-86 integrating microdensitometer [6, 7].

EXPERIMENTAL RESULTS

In the two hearts with no marked pathological changes the classes of myocytes were similar in the inner, middle, and outer layers of the heart muscle (Fig. 1). In both cases 4c × 2 myocytes predominated. In one histogram there were many tetraploid mononuclear (4c) and binuclear (2c × 2) cells; 8c × 2 cells constituted a conspicuous group, but there were only single 16c and 16c × 2 cells. In another histogram (Fig. 1b) there were almost as

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