# Development of Endocrine and Lymphocytopoietic Functions of the Thymus in Human Embryogenesis

Z. S. Khlystova, I. I. Kalinina, S. P. Shmeleva, and O. P. Ryabchikov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 130, No. 10, pp. 453-456, October, 2000
Original article submitted March 23, 2000

The endocrine function of the thymus develops earlier than lymphocytopoietic. Thymalin is produced by epithelial cells in the thymus primordium. It is released into the blood and regulates differentiation of T lymphocytes in the liver, the initial hemopoietic organ. The hormonal and lymphopoietic functions of human thymus are united on weeks 7.5-8 of embryonic life.

**Key Words:** *thymus; liver; human fetus; thymaline*

Immunogenesis in human fetus was extensively studied in Russia [1,8,9] and abroad [15,16], but it is still unknown when the two functions, endocrine and lymphocytopoietic, are united in the thymus.

Thymus was referred to the endocrine system until 1960s. When noninfectious immunology became an independent branch, the thymus was considered as the central organ regulating the immune processes in the organism. Its dual function, endocrine and immune, was finally discovered; this became an important step in the understanding of immune system functioning and attracted great attention to histophysiology of this organ, particularly, in the fetus living under specific conditions.

The fetus in the uterus develops under sterile, but not antigen-free conditions. Fetal immune system reacts to maternal factors. The development of immunogenesis system in the fetus is regulated by the laws of philoontogenetic development, but the «quality» of developing organs is determined by local conditions of fetal organism and its relationships with maternal organism. The concepts on the leading role of maternal organism supplying IgG and other components to the fetus in the maintenance of the mother-fetus equilibrium have now proved to be erroneous. The immune system of the fetus is now known to participate in this process, but the earliest stages in the development of this system deserve special studies.

We studied the development of the endocrine and immune functions of the thymus in human fetus during early embryogenesis.

## MATERIALS AND METHODS

The liver and thymus from 24 human embryos and fetuses (3-12 weeks) obtained from healthy women at maternity hospitals of Moscow were examined. The age of the fetus was evaluated by the last menses and fetal body length using special tables. Fragments of organs for morphological analysis were fixed in Carnoy fluid and embedded in paraffin. The sections were stained with hematoxylin-eosin and for ribonucleoproteins as described elsewhere [7] and examined under light, electron, and fluorescent microscope. T cell population was evaluated by immunological methods (E-RFC) [14]. B cells were evaluated by the presence of surface IgG [4]. T and B cells were counted in Goryaev's chamber per 100 lymphocytes. Specificity of rosette formation was verified by rosette canceling test with antiserum to T lymphocytes. We evaluated only two total populations of T and B lymphocytes and therefore did not decipher the phenotypical parameters of these groups, as we have described them previously [8,9]. Thymaline, a hormone presented by a complex of polypeptides [5], was measured in the thymus. Cryostat sections (4-5 μ) prepared from the fragments

Laboratory of Embryonal Histogenesis, Institute of Human Morphology, Moscow

0007-4888/00/0010-1001$25.00 

of organs frozen in liquid nitrogen [3] were dried in air, fixed in cold (5°C) acetone, and washed in cold phosphate buffered saline (PBS, pH 7.4) for 15 min. Then the sections were incubated with rabbit antithymaline antiserum (Institute of Vaccines and Sera, St. Petersburg) for 40 min, after which the sections were washed 3 times in cold PBS (3×15 min) and incubated with FITC-labeled donkey serum to rabbit globulin for 20 min. Maximum titer of antithymaline serum in indirect agglutination test was 1:3200, of antirabbit antiserum 1:64. In order to reduce nonspecific protein adsorption, FITC conjugates were treated with human liver powder before incubation with sections. Control sections were treated with intact serum or PBS. The sections were incubated in a humid chamber at 18-20°C and examined under a LUMAM-P3 fluorescent microscope.

## RESULTS

During mammalian ontogeny stem hemopoietic cells migrate into organs with the beginning of hemopoiesis. In a 5-week human embryo they migrate from the yolk sac into developing liver [16], which at that period is the only hemopoietic organ.

It was shown previously that T lymphocytes (E-RFC), though not many, are present in fetal liver [11, 12]. There is no thymus yet at this period, only two primordial cords of epithelial cells containing no lymphocytes. In adults, the thymus, as the central organ of the immune system, produces hormonal factors regulating differentiation of T lymphocytes responsible for cell immunity [6]. It can be assumed that at the earliest stages the liver is closely related to epithelial thymus primordium, which creates conditions for extrathymic differentiation of T lymphocytes in the liver.

Embryonic liver at the third week of gestation is characterized by spongy structure and contains no hemopoietic islets. The organ is presented by hepatocyte cords separated by wide sinusoids. During the 5th week, numerous hemopoietic islets appear between hepatocytes and thin sinusoidal wall (Fig. 1, *a*), occupying about 60% liver parenchyma, and 0.6% T lymphocytes (E-RFC) and 2.6% IgG-positive cells appear there (Table 1). Lymphocytes cannot enter the liver from the thymus, because there is no thymus yet. It was believed that the vicinity of the liver and blood entering from the placenta create conditions for penetration of maternal immunocompetent cells into fetal liver [11], but D. Stites [16] examined male fetal karyotypes for Y chromosome and revealed that lymphocytes in fetal liver were not maternal. He considers that lymphocytes reacting to mitogens and allogenic lymphocytes appear early in human fetal liver, T and B cells being present there. By week 12 of fetal development the liver contains 1.7% T lymphocytes (E-RFC) and 1.6% B cells. S. Clark [13] described the fine structure of thymic reticuloepithelium and secretory function of these cells. Secretory vacuoles predominate and are the most complex in epithelial cells of the thymus medulla, which indicates higher differentiation of cells in this zone. F. Bernet [2] described the function of the thymus as the central organ of the immune system producing various T cell clones regulating immune reactions in the organism. These two authors demonstrated a close relationship between epithelial and lymphoid components of the thymus performing the same function and united the function of this organ in the endocrine and immune systems. Later it was formulated that lymphocytes passing through the thymus acquired immunological competence, the obligatory condition for the development of lymphoid tissue and maturation of lymphoid cells [6]. We showed that thymus primordium appeared at the 4th week of embryonal development and looks as two cords of multilamellar epithelium in the cervical zone originating from surface epithelium of the branchial pouches. During weeks 5-6, the cord cells are extended (Fig. 1, *b*), form processes, and are gradually transformed into reticuloepithelium. The organ has no lymphocytes at this stage (Table 1). If differentiation of T lymphocytes occurs only in the thymus, embryonal organism cannot contain them at this period. However, we detected few T cells (E-RFC) in the liver of a 5-week embryo.

Immunological studies with antiserum to thymalin [10] showed that epithelial cells of a 6-week thymus primordium contained thymalin [11] but no lymphocytes. Thymalin-positive cells diffusely spread in the thymus primordium at this period. Thymalin is present in the cytoplasm, cell processes, and extracellular spaces with growing small capillaries. Electron microscopy (Fig. 1, *c*) showed that epithelial cells contain secretory vacuoles of different size. The studies showed that the epithelium forming the thymus was not yet populated with lymphocytes, but functioned as incretory tissue, releasing thymalin into the blood,

**TABLE 1.** Content of T and B Lymphocytes (in %) in the Liver and Thymus of Human Fetus

| Fetal age, weeks | | Liver ($n$=10) | Thymus ($n$=14) |
|---|---|---|---|
| 5-6 | T lymphocytes | 0.6 | 0 |
| | B lymphocytes | 2.6 | 0 |
| 7-8 | T lymphocytes | 1.7 | 5.2 |
| | B lymphocytes | 1.6 | 0 |
| 11-12 | T lymphocytes | 1.7 | 82.6 |
| | B lymphocytes | 1.6 | 1.3 |

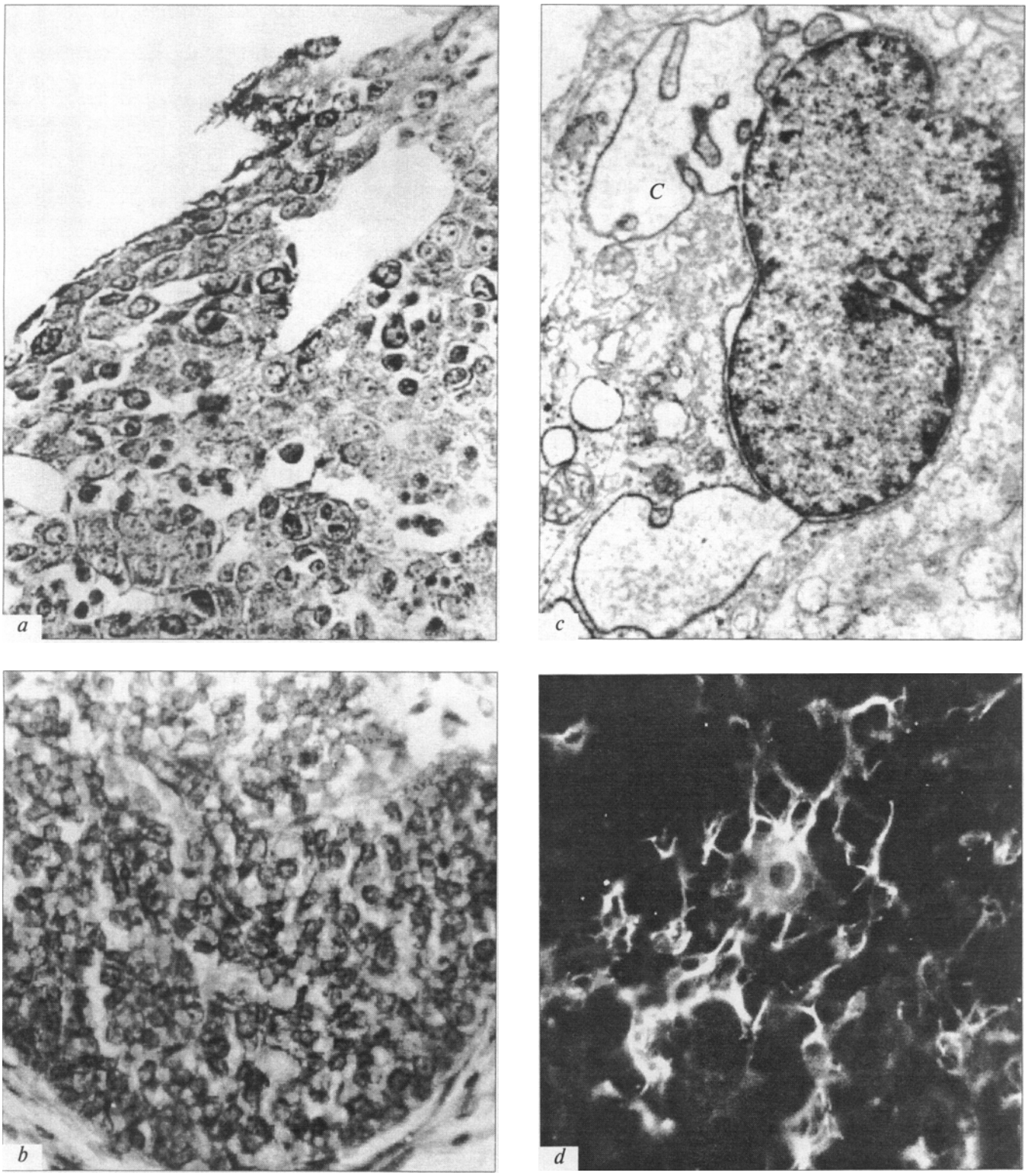


**Fig. 1.** Liver and thymus of human embryo. *a*) hemopoietic foci in embryonal liver; Brache's staining, 8500; *b*) 6-week epithelial primordium of the thymus free from lymphocytes, epithelial reticulation; periodic acid-Schiff reaction, *250; *c*) 7-week embryonal thymus, secretory vacuoles and cysts (*C*) in the cytoplasm of a reticuloepithelial cell, *12,000; *d*) 20-week fetal thymus, thymalin-positive cells in thymus medulla, *800.

thus inducing differentiation of some blood polypotent cells in the liver into T lymphocytes. The first lymphocytes in human thymus primordium appear later at 7.5-8 weeks of gestation. And during all this period they contain 5.2% T lymphocytes. The reticuloepithelium undergoes characteristic restructuring and by weeks 11-12 the cortex and medulla are detected in the organ. T cells at this period constitute up to

82.6% of lymphocyte population, and this parameter does not change to the end of embryogenesis. B lymphocytes constitute about 1.3% (Table 1). Stem or committed cells entering the organ acquire the characteristics of T lymphocytes. *In vivo* colony-formation test with mouse embryo thymocytes showed that hemopoietic colony-forming cells (splenic CFU) enter the thymus only during the earliest periods of embryogenesis.

Hence, we conclude that the endocrine function of the thymus appears earlier than lymphocytopoietic. Thymalin is produced by epithelial cells in the thymus primordium. It is released into the blood and exerts a distant effect on differentiation of T lymphocytes in the liver, the initial hemopoietic organ. The hormonal and lymphopoietic functions are united in human thymus at weeks 7.5-8 of embryogenesis.

## REFERENCES

1. N. K. Ageev, *Arkh. Patol.*, No. 12, 3-11 (1976).
2. F. Bernet, *Cellular Immunology* [in Russian], Moscow (1976).
3. I. I. Kalinina, *Byull. Eksp. Biol. Med.*, **96,** No. 12, 76-79 (1983).
4. E. R. Kudryavtseva, *Immunologiya,* No. 6, 71-73 (1983).
5. V. G. Morozov and V. Kh. Khavinson, *Immunology of Thymic Hormones* [in Russian], Moscow (1989).
6. R. V. Petrov, *Immunology and Immunogenetics* [in Russian], Moscow (1976).
7. E. Pears, *Histochemistry* [in Russian], Moscow (1962).
8. O. P. Ryabchikov, Z. S. Khlystova, *Uspekhi Sovrem. Biol.*, **101,** No. 1, 85-99 (1986).
9. Z. S. Khlystova, *Development of Human Fetal Immunogenesis System* [in Russian], Moscow (1987).
10. Z. S. Khlystova, I. I. Kalinina, and V. Kh. Khavinson, *Byull. Eksp. Biol. Med.*, **96,** No. 12, 627-630 (1983).
11. S. P. Shmeleva, Z. F. Khlystova, and O. P. Ryabchikov, *Immunologiya,* No. 5, 39-41 (1989).
12. G. Asma, R. Van den Bergh, and J. Vossen, *Clin. Exp. Immunol.*, **53,** No. 2, 429-436 (1983).
13. S. Clark, *Am. J. Anat.*, **112,** 1-33 (1963).
14. M. Jondal, G. Holm, and B. Nussenzweig, *J. Exp. Med.*, **136,** No. 2, 207-215 (1972).
15. D. Stites, M. Carr, and H. Fudenberg, *Cell. Immunol.*, **11,** 257 (1974).
16. S. Stites and Ch. Pavia, *Pediatrics,* No. 5, Pt. 2, Suppl., 795-802 (1979).