(cirrhosis of the liver) activity of bone-marrow B suppressors is depressed. It is valid hypothesis that the marked increase in the intensity of spontaneous proliferation of bone-marrow cells from patients with cirrhosis of the liver in vitro is the result of a decrease in number or depression of the function of B suppressors. The study of the causes of depression of activity of bone-marrow B suppressors will be a task for future research.

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STATE OF THE NATURAL KILLER CELL SYSTEM IN THE HUMAN FETUS

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Natural killer cells (NKC) are components of the hematopoietic system which play an important role in the maintenance of internal homeostasis and of immunologic surveillance in the body [4, 11, 13]. Phylogenetically the NKC system is the oldest component of the immune system, but its ontogenetic aspect has not been adequately studied [10, 12, 15]. Considering the role of NKC it can be postulated that the appearance of these cells and the beginning of their functioning coincide in ontogeny with the times of formation of hematopoiesis. No such investigations have been undertaken in the USSR, and the few papers published abroad do not give a complete picture of the problem.

The aim of this investigation was to study the state of the NKC system in human prenatal ontogeny, with consideration for the morphological structure of hematopoietic and lymphopoietic organs at certain stages of embryogenesis.

EXPERIMENTAL METHOD

The investigation was conducted on eight human fetuses from the 14th through the 26th weeks of prenatal development. Cells were isolated from bone marrow, spleen, liver, appendix, and tonsils on a Ficoll–Hypaque gradient (d = 1.077 g/ml) and used as effectors of natural cytotoxicity. Cells of human erythromyoleukemic strain K-562 were used as targets. Medium RPMI-1640, containing 10% bovine fetal serum, 2 mM glutamine, and monomycin (100 U/ml) were used for cell culture and the cytotoxic test.

The conditions for labeling the target cells and conducting the cytotoxic test were described by the writers previously [1, 2]. Cytotoxic activity of NKC was determined with effectors and targets in different ratios. Cells were fractionated (2×10^6/ml in a volume of 2 ml) on plastic Petri dishes in medium with 20% bovine fetal serum for 1 h at 37°C in a CO_2 incubator. Cells not adherent to the plastic were used in the cytotoxic test.

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TABLE 1. Activity of NKC in Hematopoietic and Lymphocytopoietic Organs of Human Fetuses (cytotoxicity, %)

Organ	No. of fetus	Age of fetus, weeks	Ratio of effector to target cells			
			100:1	50:1	25:1	12.5:1
Liver	1	14—15	n.d.	n.d.	0	0
	2	20—21	n.d.	0,1	0,7	0
	3	22	13,4	10,7	9,5	n.d.
	4	22	6,0	6,1	2,7	n.d.
	5	22—23	n.d.	n.d.	0	0
	6	23—24	6,1	5,6	2,5	n.d.
	7	25—26	26,8	12,1	7,8	5,3
	8	25—26	43,0	28,6	26,1	n.d.
Spleen (without adherent cells)	1	14—15	n.d.	n.d.	n.d.	n.d.
	2	20—21	0	32,6	17,2	0
	3	22	41,1	33,4	18,9	n.d.
	4	22	40,5	28,1	18,0	The same
	5	22—23	n.d.	n.d.	n.d.	» »
	6	23—24	22,1	9,6	1,5	» »
	6	23—24	6,3	3,3	1,5	» »
	7	25—26	54,2	31,9	19,2	11,4
	8	25—26	n.d.	51,2	32,1	20,1
Thymus	1	14—15	2,2	0	2,5	4,9
	2	20—21	0	0	0	0
	3	22	6,2	5,2	n.d.	n.d.
	4	22	7,7	3,6	n.d.	n.d.
	5	22—23	3,1	1,7	2,0	2,0
	6	23—24	0	0	0	0
	7	25—26	n.d.	n.d.	n.d.	n.d.
	8	25—26	3,9	n.d.	n.d.	n.d.
Tonsils	2	20—21	n.d.	0	0	n.d.
	3	22	n.d.	n.d.	n.d.	0,1
	6	23—24	0	0	0,2	n.d.
	7	25—26	n.d.	n.d.	n.d.	0
Appendix	3	22	n.d.	n.d.	2,6	1,6
	6	23—24	0	0	0	n.d.
	7	25—26	n.d.	n.d.	n.d.	0
	8	25—26	n.d.	n.d.	n.d.	1,2
Bone marrow	1	14—15	» »	» »	0,9	0,5
	2	20—21	0	0	0	0
	5	22—23	n.d.	3,4	2,9	n.d.

Legend. n.d. – Not determined.

EXPERIMENTAL RESULTS

Some NKC activity was found in 14-15-week fetuses in bone-marrow and thymus cell populations (Table 1). At these times of development the bone marrow functions as an organ of both hematopoiesis and lymphopoiesis. Among its lymphocytes there were 0.8% of T lymphocytes (E-RFC) and more than 20% of immunoglobulin-positive (Ig^+) cells, whereas the predominant cells (up to 90%) were early precursors of lympho- and myelopoiesis [8]. All the definitive component parts of the thymus are formed in the 14-week fetus (Fig. 1a): cortex, medulla, Hassall's corpuscles. Up to 80-40% of E-RFC and 1.3% of Ig^+-cells are found in the thymus [3, 5].

High NKC activity was found in the liver and spleen of fetuses aged 22-23 weeks or more. The level of cytolytic activity in these organs was close to that recorded in the spleen and peripheral blood of healthy adults [9]. It will be noted that the effectiveness of cytolysis was clearly dependent on the ratio of effectors to targets. At these stages of embryogenesis the organs studied have the following morphological picture. In the fetal spleen lymphoid follicles in which definitive T- and B-lymphocyte zones can be clearly identified, are already present (Fig. 1b). From 12 to 15% of E-RFC and up to 28% of Ig^+-cells have been found in it at this time [6, 7]. In the liver, hematopoiesis begins in the 5th week of development and can be traced until the stage under study. Up to 2.4% of E-RFC and up to 12% of Ig^+-cells are found in it. Glycogen is synthesized in the hepatocytes (Fig. 1c).

The relatively high level of NKC in the thymus of the two 22-week fetuses is interesting. Here also the level of cytolysis was strictly dependent on the ratio of effectors to targets. NKC activity was appreciably lower in a younger and in three older fetuses. Although the investigation was conducted on comparatively few fetuses, it can be tentatively suggested that the transient burst of NKC activity at the 22nd week of prenatal development is connected with

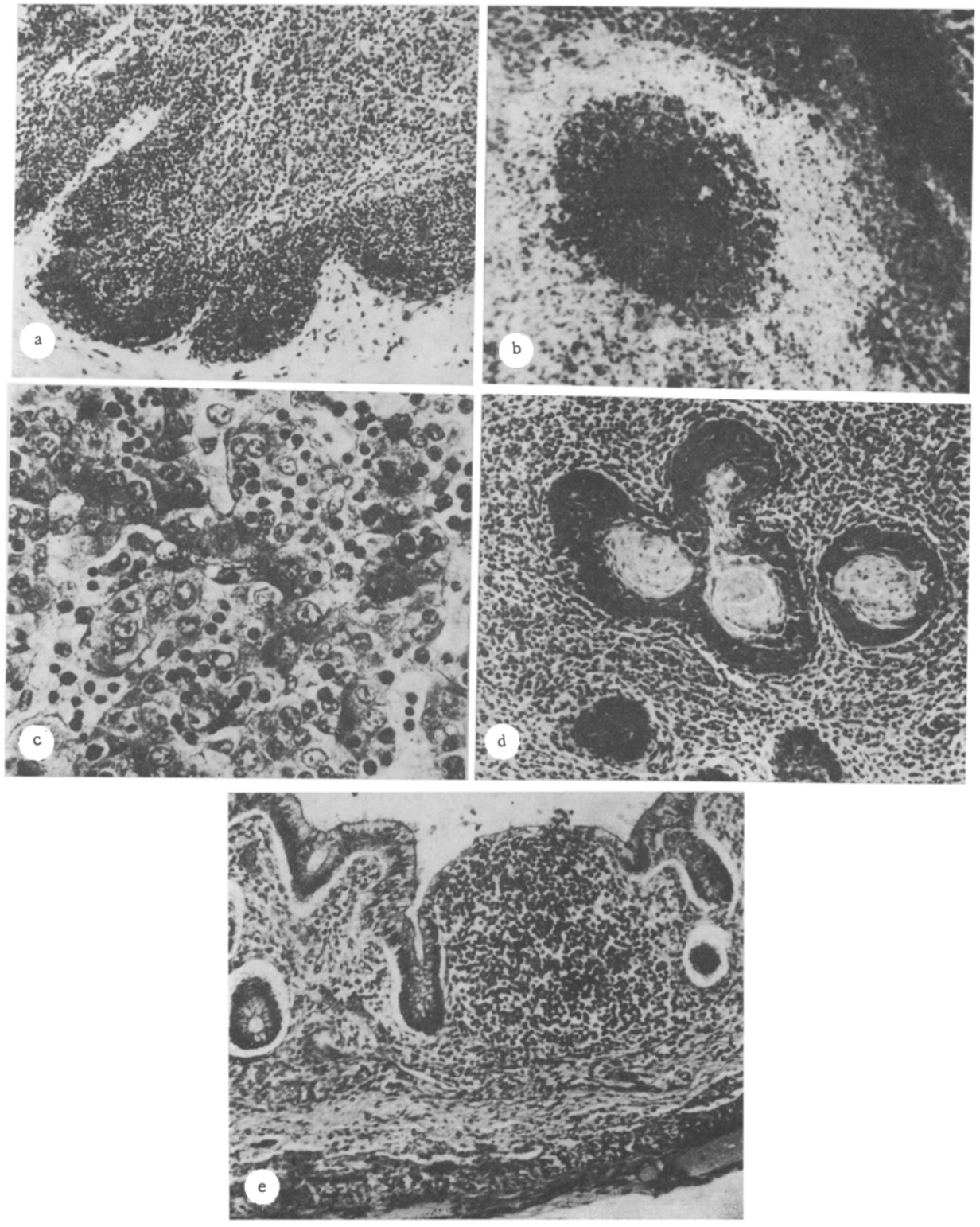

Fig. 1. Morphology of lymphopoietic and hematopoietic organs of fetuses at different stages of development: a) thymus of 12-week human fetus. Carnoy's fixative, Schiff's reagent, 120×; b) spleen of 25-week fetus. Frozen section. Luminescence. zone of B-dependent cells. Treatment with antiserum against human Ig^+-cells. Objective 10, homan 3; c) liver of 14-week fetus. Carnoy's fixative. Schiff's reagent. 500x; d) tonsil of 17-week fetus; lymphoid tissue and developing crypts; Carnoy's fixative. Stained by Romeis' method. 120×; e) appendix of 24-25-week fetus, lymphoid follicle and migration of lymphocytes to surface of mucosa. Carnoy's fixative. Hematoxylin and eosin. 160×.

intensification of migration of precursor cells at this stage from the bone marrow into the thymus and proliferation of the lymphocyte pool [12].

Exceptionally low NKC activity or its total abscence (Table 1) was observed in the appendix and tonsils at all times of testing, despite the large quantity of lymphoid tissue present in the tonsil and the large lymphoid follicles in the appendix, with migration of lymphocytes from them to the surface of the mucosa (Fig. 1d, e).

The nature of cells mediating spontaneous cytotoxicity in the fetus is particularly interesting. On removal of cells adherent to the plastic from the splenocyte suspnesion, NKC activity of the residual population fell sharply (from 22.1 to 6.3). No such decrease was observed by investigators who analyzed the population of effectors of natural cutotoxicity in adults [9] and in laboratory animals [14]. In man, effectors of spontaneous cytotoxicity of monocytic nature have been found. However, they constitute a very samll fraction of the total effector population. The effect we observed is evidence that cytotoxicity in the fetus may be mediated to a much greater extent by adhesive cells, so that a more detailed analysis of the nature of cytotoxic effectors of the human fetus is required.

Thus the NKC system functions effectively in the human fetus and the level of cytolytic activity by the 25th-26th weeks of prenatal development is close to parameters of response recorded in adults. The distribution of cytotoxic effects among the organs corresponds to the morphological picture, i.e., NKC activity is high in foci of intensive extramedullary hematopoiesis and lymphocytopoiesis. The thymus, the central organ of lymphocytopoiesis, in which the appearance of spontaneous cytotoxicity is not accompanied by a high level of lymphocytopoiesis, is an exception. This does not necessarily mean that fetal thymocytes are resistant to the regulatory action of NKC, and does not rule out elimination of these cells by NKC during anomalous migration of immature cells outside the thymus. The burst of NKC activity in the thymus of 22-week fetuses can be attributed to the high level of migrants from the liver and bone marrow. Indirect confirmation of this view may be given by data on the high sensitivity of thymocytes of 16-19-week fetuses to cytolysis by NKC [12]. Unlike the adult tonsils, fetal tonsils do not contain them. The fetal appendix likewise does not contain effectors of spontaneous cytotoxicity.

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