CLONOGENIC FIBROBLASTS OF HEMATOPOIETIC ORGANS OF THE HUMAN EMBRYO AND FETUS AT DIFFERENT TIMES OF GESTATION

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A characteristic feature of fetal hematopoiesis is a change in the type of hematopoiesis and a shift of its territory among the organs during the first 6 months of the gestation period. Since the appearance of intraembryonic intramural hematopoiesis at the 5th-6th week of the gestation period, migration of stem cells among organs and colonization of zones with an appropriate microenvironment become the basic principle of development of fetal hematopoiesis [5]. One possible component of the "inductive hematopoietic microenvironment" complex consists of fibroblasts, precursors of mechanocytes [2, 3].

The main task of this investigation was the study of clonogenic fibroblasts of the hematopoietic organs of the fetus as a factor determining the readiness of the stroma to maintain active and stable hematopoiesis.

EXPERIMENTAL METHOD

Hematopoietic organs were studied in 72 embryos and fetuses between the 5th and 27th weeks of gestation, obtained as a result of therapeutic and spontaneous abortions and miscarriages in women not under treatment with drugs.

The hematopoietic organs were investigated at the following times of fetal development: liver, 5-9 weeks — 22 investigations, 10-11 weeks — 18, 12-15 weeks — eight, 16-19 weeks — three, 20-27 weeks — seven (total 58 investigations); spleen: 10-11 weeks — three, 12-15 weeks — 16, 16-19 weeks — two, 20-27 weeks — eight (total 29 investigations); bone marrow: 10-11 weeks — eight, 12-15 weeks — 10, 16-19 weeks — four, 20-27 weeks — five (total 27 investigations).

Morphologic assessment of hematopoietic activity was undertaken on squash preparations from hematopoietic organs. To evaluate the functional activity of clonogenic fibroblasts the method of cloning of stromal precursors of hematopoietic organs, with the use of a xenogeneic feeder [1], was used.

Embryonic bone marrow was obtained from two femora, fetal marrow from one femur, flushed out with 1 ml of medium 199. Liver and spleen cells were obtained by the resuspension tech-

TABLE 1. Cell Content (number of cells x 10^6/ml) and ECF_f of Precursors of Hematopoietic Organs of Human Embryos and Fetuses at Different Times of the Gestation Period (M ± m)

Organ	Period of gestation, weeks									
	5—6		9—10		11—12		15—18		23—26	
	cell content	ECF_f	cell content	ECF_f	cell content	ECF_f	cell content	ECF_f	cell content	ECF_f
Bone marrow (n=27)	—	—	0,56±0,09	3,3±1,5	0,53±0,1	29,9±5,9	4,1±1,56	33,2±7,1	11,0±1,0	9,2±2,8
Spleen (n=29)	—	—	0,6±0,26	4,9±2,2	0,84±0,06	8,52±2,96	4,0±1,54	10,6±2,23	9,5±0,14	8,62±1,41
Liver (n=58)	0,42±0,07	4,78±1,37	33,2±11,4	3,7±1,0	36,0±6,7	4,6±1,7	19,0±8,5	0,5±0,32	22,0±0,65	0,35±0,15

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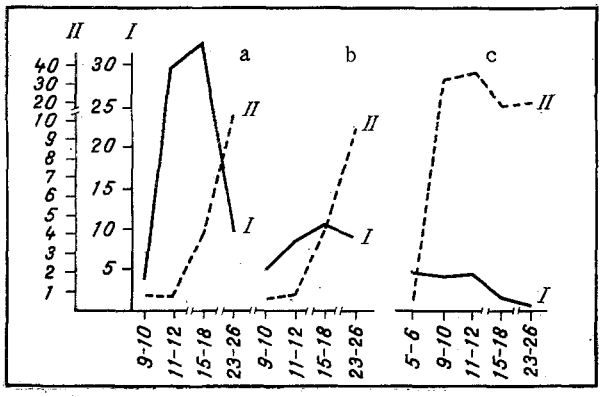

Fig. 1. Dynamics of values of ECF_f and cell content of hematopoietic organs of human embryos and fetuses at different times of the gestation period (M ± m). Abscissa, period of gestation (in weeks). I) $ECF_f/10^5$, II) cell content (number of cells $\times 10^6$/ml): a) bone marrow, b) spleen, c) liver.

nique; at all ages of gestation a constant volume of liver and spleen tissue was used. The cell suspension was filtered through a nylon filter, washed once, and the concentration of hemokaryocytes determined ain 1 ml of suspension. The cells were cultured in glass flasks whose bottom had an area of 25 and 69 cm^2 (East Germany) and in plastic flasks whose bottom had an area of 25 and 80 cm^2 (from Nunclon, Denmark), in medium 199 containing 20% of human serum of any blood group, with the addition of antibiotic (1000 U to 1 ml of medium). Bone marrow cells from noninbred rats, obtained from the femora and irradiated on a cesium source of LMB-1 type, with a dose rate of 1600 rads/min and in a dose of 6000 rads, were used as the feeder. The cultures were fixed on the 11th–12th day of cultures and stained with azure-eosin by the Romanovsky–Giemsa method, the number of colonies was counted, and the efficiency of formation of colonies of fibroblast precursors (ECF_f) per 10^5 cells ($ECF_f/10^5$) was determined.

EXPERIMENTAL RESULTS

Investigation of the femoral marrow of the embryos and fetuses showed (Fig. 1) that at different periods of gestation different values of ECF_f of bone marrow were obtained. The low ECF_f in embryos aged 9–10 weeks was followed by a sharp increase in activity of colony-forming precursors of mechanocytes at the 11th–12th week of gestation ($2.3/10^5$ and $29.9/10^5$ respectively, $P < 0.001$). No significant change was found in the cell content of the marrow and, according to the morphological data, no signs of active hematopoiesis were present. Active hematopoiesis in the femora was discovered after 14–18 weeks of gestation (Table 1). ECF_f for bone marrow at this period remained high. By the 23rd–26th weeks of gestation ECF_f of the femoral marrow was reduced to $9.2/10^5$ myelokaryocytes. The cell content of the bone marrow continued to increase under these circumstances and reached its maximal values. It will be clear from Fig. 1 that stable hematopoiesis was established in the fetal marrow 2–3 weeks after maximal activity of clonogenic stromal fibroblasts was recorded.

ECF_f of the spleen also changed depending on the age of the embryos and fetuses, but by a less marked degree than ECF_f of bone marrow (Fig. 1b). At the 9th–10th week of gestation ECF_f of the spleen was $4.9/10^5$ myelokaryocytes, and at the 12th week it rose to $8.5/10^5$ ($P > 0.05$). The maximal concentration of clonogenic fibroblasts in the spleen was considerably lower than in the bone marrow, namely $10.6/10^5$. The cell content of the spleen was increased 2 weeks after the increase in activity of clonogenic fibroblasts.

Unlike in the bone marrow and spleen, according to morphological data active hematopoiesis of erythronormoblastic character was obsered as early as the 5th–6th week of gestation in the liver. ECF_f, determined at this stage (5–6 weeks), reached its highest value for liver tissue (Fig. 1c). The cell content of the hepatic suspension rose from the 5th–6th week up to a maximum at the 11th–12th week, and then remained virtually unchanged until the 26th week of gestation. ECF_f in the liver began to fall at the 23rd–26th week. A particular feature of hepatic hematopoiesis *in vitro* was a prolonged macrophagal-histiocytic phase, which was observed until the 14th day of culture and thereafter, and which accompanied the period of fibroblast colony formation.

A study of the morphology of hematopoiesis showed that the formation of a stromal matrix is an essential condition for hematopoiesis to begin in hematopoietic organs. The lag period between formation of the stromal matrix and the appearance of the first hematopoietic islets in this case averages 2 weeks for all hematopoietic organs [5]. For instance, in the majority of bones the formation of the stromal matrix ended by the 7th-8th week, and hematopoiesis began between the 11th and 12th weeks. It was still not clear what functional changes take place in bone marrow in the period from formation of the stroma until establishment of active hematopoiesis.

The results for the efficiency of colony formation of fibroblast precursors of mechanocytes in the fetal femora showed that at the 11th-12th week there was a considerable increase in the concentration of clonogenic bone marrow fibroblasts. The increase in ECF_f was not accompanied by any direct increase in the cellular content of the bone marrow, i.e., it was evidently not connected with proliferation of differentiated medullary cells. The high concentration of clonogenic fibroblasts persisted until the 15th-18th week, i.e., until the time of formation of active hematopoiesis.

Comparison of the results for stromal bone marrow fibroblasts with information in the literature on the development of pluripotent and committed hematopoietic precursors in human fetal bones [4] showed that the highest concentration of pluripotent hematopoietic cells was observed in the fetal bone marrow at the 15th-16th week and their relative number did not change at subsequent times of gestation [4]. Conversely, the relatively low concentration of committed granulocytic precursors in the bone marrow at the 5th-18th week rose sharply until the 22nd-23rd week of gestation, and this was accompanied by an increase in the number of myelokaryocytes.

The lag period between formation of the stromal matrix (7th-10th weeks) and establishment of active hematopoiesis in the bones (15th-18th weeks) is thus characterized by a high concentration of clonogenic stromal fibroblasts and a high concentration of hematopoietic pluripotent precursor cells. Since an increase in activity of clonogenic fibroblasts in the fetal bone marrow was recorded earlier (11th-12th weeks) it can be tentatively suggested that proliferation of stromal mechanocytes precedes the appearance of hematopoietic stem cells, and may perhaps facilitate their migration on bone marrow territory. Later the increase in cell content of the bone marrow was due to an increase in the number of committed hematopoietic precursors. At this period (after 20 weeks of gestation) the concentration of clonogenic fibroblasts fell and was maintained at a level close to ECF_f of adult human bone marrow [1]. A similar rule was observed during the investigation of hematopoiesis in the fetal spleen.

The dynamics of ECF_f in the liver and its correlation with the cell content and activity of committed granulocytic precursors confirm data showing that active hematopoiesis is already taking place at the 5th-6th week of embryonic development in this organ, and is maintained at a constant level until the 24th-25th week. It must be noted that the decline of hematopoiesis in the liver is accompanied by a fall initially of ECF_f, and later in the number of colony-forming units of granulo-monocytic precursors. Differentiated hematopoietic cells continue to be detected in the liver until the 26th-27th weeks of the gestation period.

The results described above are evidence that the formation of hematopoiesis in the fetal hematopoietic organs is preceded by high activity of stromal clonogenic fibroblasts. This suggests their possible role in the creation of the necessary microenvironment for hematopoietic cells.

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