COLONY-FORMING CELLS IN ORGAN CULTURES OF EMBRYONIC LIVER

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Differentiation of hematopoietic cells persists for long periods in organ cultures of the liver of 17-20 day mouse embryos on millipore filters, and the line of colony-forming cells, i.e., presumably stem cells, is maintained. The erythroid and myeloid branches of hematopoiesis continue for the first 10 days of cultivation but after the 15th day myeloid hematopoiesis is maintained (including megakaryocytes).

Cell from 7-23 day cultures, after injection into irradiated mice, led to the formation of typical large hematopoietic colonies in the spleen, predominantly erythroid in nature.

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Cultivation of hematopoietic tissue with maintenance of its differentiation in vitro and preservation of its stem cells, capable of carrying out hematopoiesis after transplantation in vivo, meets with serious difficulties. Rapid loss of differentiation of hematopoietic cells takes place in monolayer cultures in liquid nutrient medium and in plasma cultures [3, 4, 7, 8], and populations of fibroblast-like cells appear. By the use of agar cultures and embryonic liver extract injected into the medium, Salvatorelli [10, 11] was able to maintain hematopoiesis for more than two weeks in chick embryonic bone marrow. Metcalf [5, 6] used agar cultures of mouse bone marrow with the addition of leukemic or embryonic serum, or with the provision of kidney cells as a backing obtained growth of colonies consisting of myeloid cells from the mouse bone marrow and maintained hemetopoietic tissue in such cultures for 12 days. Judging from the published data [12], each such colony arises from one single hematopoietic stem cell. Our previous investigations showed that hematopoiesis is maintained in organ cultures of embryonic liver on millipore filters and in the same cultures of adult bone marrow grown on a bone stroma for 26 and 16 days, respectively [2].

Data on the composition of the hematopoietic tissue in embryonic liver culturies (including stem cells) are given in this paper.

EXPERIMENTAL METHOD

Organ cultures were set up and described previously [1]. Pieces of liver from 17 and 20 day mouse embryos of line CBA (measuring 2 mm) were placed on the surface of millipore filters (pore size 0.6-0.9μ). The under surface of the filters was in contact with the liquid phase of the culture medium consisting of 70% medium No. 199, 20% bovine serum, and 10% chick embryonic extract. To 100 ml of medium the following were added: 7 mg vitamin C, 400 mg glucose, 20 mg Na-β-glycerophosphate, 20 mg L-glutamic, and 50000 units each of streptomycin and penicillin. The gas phase was air. The medium was changed every 2-3 days. Impressions were made after 3-24 days from the cultures and stained by the May–Gruenwald–Giemsa method, or the cultures were fixed with alcohol–formol and stained with hematoxylin as total preparations or series of paraffin sections. Cell suspensions also were prepared from 7-23 day cultures by trypsinization. Films were made from these suspensions and they were also used for determining the number of colony-forming units (CFU) [9]. For this purpose, $4 \cdot 10^4$-$6 \cdot 10^4$ cells were injected intravenously into syngenic mice irradiated 1-2 days beforehand in a dose of 850 R. No cells were injected into control irradiated mice.

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TABLE 1. Composition of Hematopoietic Tissue (in %) in Organ Cultures of Liver of 17-day Mouse Embryo

Days of cultivation	Hemocytoblasts	Myeloblasts	Promyelocytes	Myelocytes	Metamyelocytes	Polymorphonuclear leukocytes	Proerythroblasts	Basophilic erythroblasts	Polychromatophilic erythroblasts	Oxyphilic erythroblasts
0	7	2	1	1	—	—	10	11	12	46
5	1	5	11	15	23	27	11	8	1	1
10	5	6	19	18	31	18	1	1	1	—
14	1	6	23	16	21	29	1	2	1	—
24	2	6	24	26	30	12	—	—	—	—

TABLE 2. Colony-Forming Units in Embryonic Liver Cultures

Duration of cultivation (in days)	Number of viable nucleated cells injected (10^3)	Number of colonies per spleen	Number of CFU/ 10^5 cells
7	66	8, 13, 16, 20, 21,	23,6
7	132	25, 28, 31, 32, 32	22,3
12	54	17, 20, 21, 21, 22, 22, 23	38,7
13	38	10, 14, 18, 19, 20, 22, 25, 25, 25	51,5
23	16,5	0, 0, 2, 3, 5,	11,4
	0	0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,	

The mice were sacrificed on the 9th-10th day, and the number of hematopoietic colonies in their spleens, fixed in Bouin's fluid, counted by means of a stereoscopic microscope. Some spleens were examined histologically.

EXPERIMENTAL RESULTS

During the first few days of cultivation, degeneration of the central areas of the explants took place. Hematopoietic and liver tissue was preserved at the periphery of the fragments. After 6-8 days the filters around the fragments were covered with stratified epithelial membranes on which were extensive zones of hematopoietic cells consisting of erythroid and myeloid elements at different stages of differentiation, including young proliferating hematopoietic cells. Numerous mitoses and groups of 4-10 megakaryocytes were visible among these cells. The zones of hematopoietic tissue were not clearly enough defined to enable separate hematopoietic foci to be distinguished at that time in the culture. On the 14th day these zones of hematopoiesis contained several tens of thousands of cells. Within the explant the zone of hematopoiesis persisted at its periphery. The width of this zone depended on the original dimensions of the fragment. The cultures remained in this form for more than 3 weeks, the number of hematopoietic cells in them gradually falling and the composition of these cells changing. In impressions taken from cultures grown for 5-24 days the composition of the hematopoietic cells was as given in Table 1. Until the 10th day of cultivation both the erythroid and myeloid branches of hematopoiesis persisted, but starting on the 15th day, only myeloid hematopoiesis was maintained in the cultures (including megakaryocytes).

Cells from 7-23 day cultures, after injection into mice, caused the formation of typical large hematopoietic colonies in the spleen. These results are given in Table 2. In their composition and structure, the foci were typical hematopoietic colonies characteristic of the spleen of irradiated mice injected with hematopoietic cells; erythroid foci were predominant.

Hence, differentiation of hematopoietic tissue was maintained for 3 weeks at least in organ cultures of embryonic liver. Not only were the proliferation and maturation of hematopoietic cells observed in such cultures, but the line of colony-forming cells, presumably hematopoietic stem cells, also was maintained. The morphology and composition of the colonies formed by the cells cultivated in vitro were not significantly different from those arising after transplantation of cells obtained directly from hematopoietic tissue.

Judging by the number of colonies, the number of CFU in the explants of embryonic liver was relatively large. The problem of how hematopoietic stem cells are maintained and utilized in the culture requires further study. The results described indicate that the maintenance of differentiation of hematopoietic tissue in vitro does in fact require interaction between its tissues and other tissues which are its natural neighbors

in vivo. Embryonic liver epithelium is one such tissue. It may be assumed that the positive results of these experiments are linked with the fact that differentiation of liver cells was maintained in the organ culture and their contact with hematopoietic cells were not disturbed.

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