PROLIFERATIVE ACTIVITY OF HEMATOPOIETIC STEM CELLS IN LONG-TERM ORGANOTYPICAL CULTURES OF EMBRYONIC MOUSE LIVER

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It was shown previously that hematopoietic cells can be repeatedly removed from organotypical cultures of mouse embryonic liver by washing from the surface of the cultures. Cells washed off 2 weeks or more after the beginning of culture contained cells of the granulocytic series at all stages of maturation, monocytes and macrophages, lymphoid and blast cells, and also pluripotent and comitted (granulocytic-macrophagal) precursors [1, 3]. If washing was carried out twice a week the surface layer of the culture was largely restored and the cultures differed only a little from intact, as regards both the total number of hematopoietic cells and the number of CFU-S (mean ratio 0.75 and 0.83, respectively) [2]. After 4 weeks, however, the number of CFU-S in the washings fell rapidly. Meanwhile the deep layers of the culture (remainder) were practically identical in the experimental and intact cultures as regards both the number of cells and the number of CFU-S. The problem arises of how regeneration of the washed layer takes place.

In the investigation described below the sensitivity of CFU-S located in the washed layer and in the remainder to ^{3}H-thymidine was studied.

EXPERIMENTAL METHOD

The liver of 17-day CBA or (CBA × C57BL)F_1 mouse embryos was used for culture. The methods of culture, of washing off the cells, and of removing the cells from the remainder of the cultures and counting them were described previously [2]. Hematopoietic stem cells were determined by cloning in the spleen of irradiated mice (CFU-S) [6]. Proliferative activity of the CFU-S was estimated by the "thymidine suicide" method [3]. At each time of the investigation cells from at least two cultures were pooled. The washed-off test cells were suspended in Hanks' solution and divided into three equal portions. One was left on ice until injected into irradiated mice. On the basis of the results obtained after injection of cells from this group, the number of CFU-S in the cultures was determined. The other two portions were treated with 100 μCi/ml of ^{3}H-thymidine (specific activity not less than 14 Ci/mmole), and in addition, "cold" thymidine was added to one of these tubes in a final concentration of 100 μg/ml as a control of nonspecific toxicity of the radioactive thymidine preparation. After incubation for 30 min at 37°C, in a volume of 0.4 ml, the cells were washed into cold medium containing 100 μg/ml thymidine, and injected intravenously into irradiated mice (1200 rads, ^{137}Cs). The percentage of CFU-S inactivated by ^{3}H-thymidine was calculated from the difference in the two incubated groups. The significance of the difference between the number of colonies counted was determined by Student's t test.

EXPERIMENTAL RESULTS

Proliferative activity of the CFU-S in the cultures was studied at different times for 47 days. As Table 1 shows, CFU-S in the washings showed significant proliferative activity only in two experiments at different periods of culture (on the 3rd and 9th days), whereas in the remainder of the cultures the CFU-S proliferated for 3-4 weeks. The percentage of CFU-S engaged in DNA synthesis, determined by this method, varied within wide limits, i.e.,

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TABLE 1. Sensitivity of CFU-S from Embryonic Liver Organ Cultures to ^{3}H-Thymidine

Expt. No.	Period of culture, days	Number of cells per culture ($\cdot 10^5$)		Number of CFU-S per culture (M ± m)		Percent of CFU-S killed by ^{3}H-thymidine			
		washings	remainder	washings	remainder	washings	P	remainder	P
1	2	19,6	68,6	155±14	1145±69	0		32	<0,001
2	3	12,3		209±8,6		47	<0,001		
2	7	8,4		260±20		14,3	>0,1		
3	9	5,85	17,6	570±30	1680±122	23,4	<0,001	18,9	<0,05
3	16	5,7	10,9	339±30	878±65	17,5	>0,05	36,6	<0,01
1	16	8,7	6,3	576±53	453±35	19	>0,1	89	<0,001
1	23	6,6	5,4	190±42		0			
3	23	13,4	6,7	413±20	1180±72			0	
4	23	10,7	11,7	189±18,3	599±70	0		30	≈0,05
4	30	10,5	11,5	90±14,8	353±37	23,5	>0,05	8,1	>0,1
1	30	4	4,2	134±18,4	2380±108	0		12,5	>0,2
3	30	9	16,4		1630±84			26,4	<0,01
1	44	0,45	4,1	85±7,5	607±101			0	
5	47	11,8	7,4	193±18	183±10	10,8	>0,2	12,5	>0,1

from 19 to 89. The initial sensitivity to ^{3}H-thymidine of CFU-S from the liver of 17-day CBA mouse embryos averaged 39.9 ± 0.75% in five experiments. This value is close to data in the literature for CFU-S for mouse liver in the late stage of pregnancy [3].

Hematopoiesis in the mouse liver virtually ceases after the first week of postnatal life and switches to the bone marrow and spleen [5]. The microenvironment in the liver *in vivo* may perhaps change at this time because of the beginning of diversification of liver function. The possibility cannot be ruled out that stimuli and demands connected with age changes in the newborn organism are absent in culture, and that the microenvironment of embryonic liver tissue persists for a long time, thus maintaining prolonged proliferation of hematopoietic stem cells and hematopoiesis.

Since CFU-S in the washed layer did not proliferate after 2 weeks of culture, restoration of their number in the upper layer of the culture can be explained only by migration from the deep layers. It is not yet clear what leads to loss of proliferative activity of the CFU-S which migrated into the washings: whether selection of nonproliferating CFU-S takes place in the upper layer, because of their greater mobility for example, or whether the CFU-S which have migrated here lose their ability to divide because of changed microenvironmental conditions or nutrition, or contact with the aerial phase. It is interesting to note that in a different system of culture, in which hematopoiesis is maintained for a long time, namely in liquid culture of bone marrow on a substrate of bone marrow stromal cells, no difference was observed in the proliferative activity of CFU-S located among stromal cells adherent to the bottom, and CFU-S located among the hematopoietic cells in suspension [4, 7]: it must be recalled that in Dexter's system all the cells were in virtually identical conditions of nutrition. It likewise is not yet clear why proliferation of CFU-S decreases in the cultures after 3-4 weeks. In experiment No. 1 (Table 1) cessation of active proliferation was observed on the 30th day, when the maximal number of CFU-S, corresponding to the initial number for the whole liver of the 17-day embryo, had accumulated in the remainder of the culture. It might be postulated that this accumulation causes inhibition of further proliferation of CFU-S. However, judging from the total number of cells and the fall in the number of CFU-S in the later stages, it is more likely that either the conditions of culture did not support viability and functional activity of the culture tissue any longer, or that the same differentiation of liver tissue as that which leads to cessation of hematopoiesis in the liver *in vivo* also takes place *in vitro*, although more slowly.

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