DIFFERENTIATION OF ERYTHROID PRECURSORS IN ORGAN CULTURES OF MOUSE EMBRYONIC LIVER

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Hematopoiesis with proliferation of polypotent hematopoietic stem cells (HSC), giving rise to growth of colonies in three distinct directions – erythroid, granulocytic, and megakaryocytic – on transplantation into irradiated mice [1, 2], can be maintained for a long time in organ cultures of mouse embryonic liver. Meanwhile erythropoiesis cannot be maintained during culture under these conditions, and morphologically recognizable erythroid precursors can be observed to be present in significant numbers only for 5-10 days [5, 7]. Termination of erythropoiesis cannot evidently be explained entirely by the fact that a sufficient concentration of erythropoietin is absent in the nutrient medium, for addition of serum from anemic animals to it was not followed by activation of erythropoiesis [4]. Taking these facts into consideration, it was decided to investigate whether differentiation of polypotent HSC into early erythroid precursors takes place in embryonic liver culture.

The writers showed previously [3] that normal mouse bone marrow contains erythropoietin-independent erythroid precursors which, during culture in a plasma clot in the presence of mouse serum as stimulator, give rise to colonies of two types: small colonies of hemoglobin-containing cells detectable on the 3rd day of culture, and large colonies, unicentric or multicentric, consisting of erythroid cells detectable on the 5th day of culture. The 3-day colony-forming units (CFU_{ei}) are later erythroid precursors than the 5-day burst-forming units (BFU_{ei}).

In the investigation described below the content of CFU_{ei} and BFU_{ei} was determined in long-term organ cultures of mouse embryonic liver.

EXPERIMENTAL METHOD

The liver of 17-day CBA or (CBA × C57BL)F_1 mouse embryos was cultured as described previously [6] on the phase boundary between nutrient medium and a mixture of air with 5% CO_2. The composition of the nutrient medium was: a clinically defined medium (Waymouth's MB 752/1 or Serumless Medium), to which glucose and ascorbic acid were added to final concentrations of 500 and 7.5 mg % – 67%, calf serum – 20%, mouse serum – 2%, chick embryonic extract – 10%, L-glutamine 200 mM – 1%, 100 IU penicillin and 50 μg streptomycin to 1 ml of nutrient mixture. Every 7 days cells were washed out of the cultures and the number of erythroid precursors among them was determined. Culture ended after 16 to 53 days, and on those days the precursors were determined both in washed out cells and among cells isolated from the remains of the cultures. CFU_{ei} and BFU_{ei} were determined by the method described previously [3] in plasma cultures with 10% normal mouse serum without the addition of erythropoietin. The morphological composition of hematopoietic cells from the organ cultures was calculated after examination of films stained by Romanovsky's method.

EXPERIMENTAL RESULTS

Data on the number of CFU_{ei} and BFU_{ei} in hematopoietic cells from organ cultures of embryonic liver are given in Tables 1 and 2. In only one of the 11 experiments were no CFU_{ei} and BFU_{ei} found at any stage

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TABLE 1. Concentration of CFU_{ei} and BFU_{ei} in Hematopoietic Cells of Embryonic Liver Cultures (mean and standard deviation)

Experimental conditions	$CFU_{ei}/10^5$ cells		$BFU_{ei}/10^5$ cells
	in washings	in residues	in washings
Culture			
7-9 days	14,72±14,45 (9)	—	1,77±28 (4)
14-16 days	29,8±23,0 (10)	7 (1)	5,5 (1)
18-25 days	35,86±29,45 (7)	34 (1)	2,0 (1)
28-32 days	23,4±35,4 (3)	15,4±11,3 (3)	2,5 (1)
46-56 days	12,5±9,0 (3)	8 (1)	
Uncultured embryonic liver	—	47,5±15,6 (3)	4,75±1,06 (2)
Uncultured bone marrow	—	23,22±10,27 (23)	3,1±2,82 (10)

Legend. Number of experiments given in parentheses.

TABLE 2. Concentration of CFU_{ei} and BFU_{ei} and Morphological Composition of Hematopoietic Cells in Embryonic Liver Cultures

Source of cells	Expt. No.	Duration of culture, days	Number of erythroid precursors per 10^5 cells		Morphological composition of hematopoietic cells, %			
			BFU_{ei}	CFU_{ei}	unidentifiable blast and lymphoid cells	erythroid cells	granulocytic cells	macrophages
Washings	1a	14	—	10,5	32,3	4	31	32,7
"	2	16	—	87	6,5	0,25	80,7	12,5
Residue	"	"	—	7	15	3	68,5	13,5
Washings	1b	16	5,5	28	21,8	1	58,2	17
"	3	28	2,5	—	14,3	1	56,6	28
"	4a	29	2,6	39	22	0	45	33
Residue	"	"	0	122	14	0	68	18
Washings	4b	29	2,2	80,5	28	0	33	39
Residue	"	"	1,6	153	19	0	65	16
Washings	5a	29	0	0	12	0	35	53
"	5b	29	0	0	4	0	44	53
"	1c	32	0,2	63,6	30,4	0,7	50,9	18
Residue	"	"	1,6	26	18,9	0,5	61	19,6
Washings	1b	46	—	20	18	0,2	46,2	34,4
Residue	1b	46	—	8	3,6	0	86,6	9,6
Washings	3	46	—	15	10,4	0,2	67,2	22,2
"	3	46	—	2,5	5,5	0	54,5	38

Legend. a, b, c) Different samples of calf serum added to nutrient medium.

(from the 8th to the 53rd day). In all other experiments both types of erythroid precursors were present in the cultures throughout the period of culture (until the 56th day); their average concentration and the ratio between the numbers of earlier and later precursors remained the same as in bone marrow and the original embryonic liver. Morphologically recognizable, although atypical, erythroid cells were present after 2 weeks in culture in low concentration only in those cultures which were growing on Waymouth's MB 752/1 medium (Table 2, experiments Nos. 1-3). Comparison of the morphological composition of the hematopoietic cells and the number of CFU_{ei} and BFU_{ei} among them shows that a low content or absence of morphologically identifiable nucleated erythroid cells did not correlate with the number of CFU_{ei} and BFU_{ei}. An average level of correlation was observed between CFU_{ei} and the percentage of morphologically unidentifiable cells, including blast cells and lymphocyte-like cells ($r = 0.482$, which is the same as r_{min} for a 95% level of probability).

During organ culture prolonged differentiation of both early and later erythroid precursors can thus take place in embryonic liver explants. Comparison of the results with data in the literature on differentiation of erythroid precursors in another long-term hematopoietic culture (a liquid culture of bone marrow by Dexter's method) is not easy because the test culture which we used identifies erythropoietin-independent precursors, whereas in bone marrow cultures erythropoietin-dependent erythroid precursors were tested. It must be pointed out that erythropoietin-independent mono- and polypotent erythroid precursors were found in mouse embryonic liver and bone marrow by other workers in other test systems, for example, in agar culture stimulated by medium conditions with mouse spleen cells, cultured with pokeweed mitogen [11, 12]. The concentration of early

erythropoietin-independent erythroid precursors in 17-18-day mouse embryonic liver, according to data given by these workers [13], was close to the concentration of BFU_{ei} which we found in the liver of 17-day embryos. It is not yet known whether erythropoietin-independent and erythropoietin-dependent BFU and CFU are the same precursors, which can proliferate and differentiate into erythroid cells under the influence of various stimulators, depending on as yet unidentified conditions of culture, or whether they are independent populations, each of which responds to its own stimulus [9, 13]. All workers who have studied liquid bone marrow cultures have observed prolonged differentiation of early erythroid precursors (BFU_e), but their further differentiation into CFU_e ended early or was not observed at all [10, 14]. Under these circumstances the addition of erythropoietin to the nutrient medium may promote differentiation of CFU_e only after special changes in the conditions of culture [8]. Nevertheless, irrespective of the solution to this problem of identity of the tested precursors, it must be pointed out that differentiation in the erythroid direction in organ cultures of embryonic liver progressed as far as more mature precursors (FEU_{ei}), and in some cultures prolonged (more than 4 weeks), although on a very small scale, differentiation to morphologically identifiable nucleated erythroid cells also was observed (Table 2).

The results show that the block to erythropoiesis in organ cultures of embryonic liver is due, not to a disturbance of differentiation of HSC into early erythroid precursors (BFU_{ei} and CFU_{ei}), but to events taking place at the level of more mature precursor cells than CFU_{ei}. Differentiation of more mature erythroid cells perhaps does not take place because of the insufficient level of erythropoietin and (or) absence of the necessary cell contacts as has been shown in cultures of adult bone marrow [8].

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