EFFECT OF THYMIC FEEDER ON KINETICS OF HEMATOPOIETIC STEM CELLS IN A SUSPENSION OF MOUSE EMBRYONIC LIVER

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The kinetics of hematopoietic stem cells was studied in organ culture of a suspension of mouse embryonic liver grown directly on filters and on a previously grown embryonic thymic feeder. In the first two weeks of culture, hematopoiesis in the suspension was virtually indistinguishable from hematopoiesis in fragments of the same age and the same time of culture, whereas later hematopoiesis was more active in the suspension grown on thymic feeder. The possible role of the thymus in hematopoiesis is discussed.

KEY WORDS: hematopoietic stem cells; embryonic liver; thymic feeder.

Normal hematopoiesis has been successfully maintained for about 70 days in fragments of mouse embryonic liver by the method of organ culture.

In the investigation described below, preservation of hematopoietic stem cells was studied in a suspension of embryonic mouse liver in organ culture. To investigate interaction between two or more tissues, a system consisting of a tissue feeder and a thick suspension of hematopoietic tissue, explained on it, and providing a larger area of contact between interacting cells and tissue than in the fragments, was created. The effect of a thymic feeder on the kinetics of hematopoietic stem cells in a suspension of embryonic mouse liver was studied in such a system.

EXPERIMENTAL METHOD

Mice of line CBA aged 6-12 weeks were used. A thick suspension [2] ($1 \cdot 10^8$–$2 \cdot 10^8$ cells/ml) was prepared from the liver of 17-day embryos and explanted on AA Millipore filters (pore diameter 0.8 μ) at the boundary between the liquid and gaseous phases [1] at the rate of 1-2 livers per dish. For combined cultures, the thymus of 17-day embryos was used as the feeder; from five to seven thymus glands, separated into two lobes, were placed in one dish, and after cultivation for 7-10 days, the suspension of mouse embryonic liver cells was explanted on to the resulting feeder. The number of stem cells in the cultures was determined by the method of Till and McCulloch on mice irradiated with Cs^{137} γ-rays (1200 rad). Under these conditions the number of endogenous colonies did not exceed 0.5 per spleen. A control of the number of stem cells and of all living cells in the cultures of the embryonic thymic feeder was set up for the cultures of embryonic liver suspension on the feeder. The number of living cells was counted with the aid of trypan blue.

EXPERIMENTAL RESULTS

The morphological characteristics of cultures of the mouse embryonic liver cell suspension on filters and on thymic feeder for three weeks of cultivation were given previously [2].

The total number of colony-forming units (CFU) and of living cells in the cultures of embryonic liver suspension on the filters was determined during cultivation for 32 days. As Table 1 shows, in the first

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TABLE 1. Number of CFU and Living Cells in Culture of Mouse Embryonic Liver Suspension* (M ± m)

Time of culture (in days)	Number of living cells per liver (x 10^6)			Number of CFU per 10^6 cells		Number of CFU per liver		P
	without feeder	on feeder	in feeder without liver	without feeder	on feeder	without feeder	on feeder	
0	16,2±0,74			15,6±0,99		2513±104		
3	2,33±0,33	5,5±0,95	2,6±1,01	30±0,50	16±1,01	705±107	814±29	<0,05
4	2,03±0,99	3,1±0,55	0,9	35±9,89	28±8,0	539±52	817±80	>0,05
7	3,52±1,08	4,2±0,68	2,4±0,75	27±4,95	21±1,6	799±213	850±103	<0,05
10—11	0,70±0,18	3,5±0,11	2,0±0,80	29±4,04	15±3,0	178±40	492±46	>0,01
14	0,70±0,14	1,9±0,14	2,9±0,10	24±6,7	15±3,5	160±28	278±67	<0,05
20—21	0,12±0,01	2,3±0,20	1,5	39±5,1	5,6±0,8	49,3±13	136±18	>0,02
30—32	0,12±0,02			40±8,8		49,7±23		

*At all periods of observation the feeder cells (thymus) did not contain CFU when injected into irradiated mice in a dose of up to $1.5 \cdot 10^6$ per mouse.

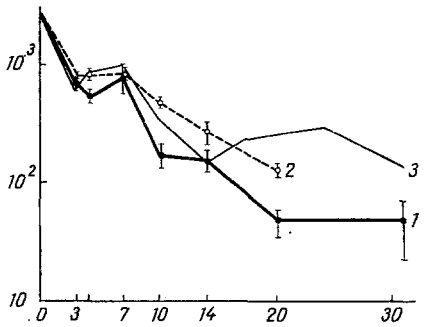

Fig. 1. Kinetics of CFU in organ culture of mouse embryonic liver cell suspension with or without thymic feeder: 1) number of CFU in embryonic liver cell suspension without feeder; 2) number of CFU in embryonic liver cell suspension on thymic feeder; 3) number of CFU in fragments of embryonic liver (data taken from paper by Rudneva and Samoilina [3]). Abscissa, time of cultivation (in days) starting from moment of explanation; ordinate, absolute number of CFU calculated per liver.

three days there was a sharp decrease both in the total number of cells (by 8 times) and in the number of CFU (on the average by 3-4 times). Later, in the course of cultivation for a month, there was a gradual decrease in the intensity of hematopoiesis, as shown by the morphology of total preparations, the decrease in the total number of cells in the culture, and the number of residual hematopietic stem cells. Throughout the period of observation the total number of cells fell faster than the number of CFU; for that reason, the relative number of CFU in the cultivated suspension was substantially higher than in the original embryonic liver.

Comparison of the cultures of embryonic liver cell suspension with the cultures of fragments of embryonic liver of the same age and the same period of cultivation (Fig. 1) shows that during the first two weeks of cultivation, hematopoiesis in the suspension was indistinguishable by all parameters from hematopoiesis in the fragments; later hematopoiesis in the suspension was inferior to that in the fragments. Partial destruction of the tissue structures, inevitable during preparation of suspensions, and also disturbance of interaction between hematopoietic cells and the epithelial stroma of the organ can evidently induce a decrease in hematopoiesis in the later stages of cultivation. In addition, by contrast with the fragments, in the suspension dynamic equilibrium was not restored, possibly on account of the relatively short periods of observation.

The number of stem cells and of all living cells in the mouse embryonic liver suspension explanted on thymic feeder was studied during 20 days of cultivation (Table 1). In the first 3-4 days, as in the suspension without feeder, a sharp decrease was observed in the number of hematopoietic stem cells, followed by a gradual decline of hematopoiesis, although this was slower to develop than in the suspension grown directly on filters. Accordingly, the number of CFU in the cell suspension on the thymic feeder, especially

after 10 days of cultivation, was higher than in the cell suspension without the feeder (differences statistically significant) (Fig. 1). Stimulation of hematopoiesis in a suspension of thymic feeder was observed previously in total preparations [2]. The increased number of living cells in the suspension of thymic feeder compared with the suspension without feeder was due entirely to contamination with feeder cells (Table 1). At all times of observation, no hematopoietic stem cells were found among the feeder cells. Presumably the stimulation of hematopoiesis in the system of embryonic liver cells–thymic feeder, compared with the cell suspension grown without the feeder, took place as a result of the stimulating action of the thymic feeder on hematopoiesis. The stimulant action of thymocytes on hematopoiesis has been observed in vivo in a syngeneic system [4, 7] and in a semiallogeneic system [6]. There is evidence that thymocytes, in a system in vitro, promote granulocytopoiesis in cultures of adult mouse bone marrow, although in that case no positive effect on stem cells could be found [5].

On the whole, the results show that during cultivation of a mouse embryonic liver cell suspension, hematopoiesis is maintained with preservation of the hematopoietic stem cells in culture for at least a month. Hence, it follows that preservation of the initial cytoarchitectonics of the hematopoietic tissue is not absolutely necessary for the maintenance of hematopoiesis in it. Improvement of hematopoiesis in combined cultures possibly indicates participation of the thymus in the regulation of hematopoiesis. A system consisting of an embryonic liver cell suspension on a thymic feeder is a convenient object with which to study this problem.

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