HEMATOPOIETIC STEM CELL PRODUCTION IN VITRO: WASHINGS FROM EMBRYONIC LIVER ORGAN CULTURES

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UDC 612.41-085.23

Hematopoietic cells, including splenic colony-forming units (CFUs) can be periodically washed off an embryonic liver organ culture in the course of 4 weeks. Under the conditions of culture used this operation does not substantially reduce the numer of CFUs in the culture. The washings can thus be used to increase the yield of CFUs from the culture.

KEY WORDS: hematopoietic tissue culture; hematopoietic stem cells; embryonic liver.

The organ culture method as applied to embryonic mouse liver enables hematopoiesis to be maintained in vitro for a long time. Under these circumstances, although erythropoiesis gradually ceases, hematopoietic stem cells (splenic colony-forming units – CFUs) are maintained in the culture and can be observed to proliferate and to differentiate into granulocytes and megakaryocytes [1, 2]. Possession of this equilibrium hematopoietic system in vitro provides an approach to the study of its responses to various disturbing factors.

In this investigation the possibility of obtaining hematopoietic cells, including stem cells, from a tissue in culture without disturbing the culture itself was studied. Cells were washed off the surface of the explants and the number of CFUs in them was determined. After this operation had been repeated several times the cultures were tested for their number of cells and CFUs and the results compared with control, intact cultures.

EXPERIMENTAL METHOD

The method of organ culture in [2] with the following modification was used. The filter with a pore diameter of 0.8 μ (AAWP, Millipore) was placed on a sieve made from fine Kapron thread stretched over a plastic ring. The ring was placed in a plastic Petri dish 50 mm in diameter, containing nutrient medium. The liver of 17-day CBA or (CBA × C57BL)F_1 mouse embryos was cut into 10 to 12 fragments and placed on the filter. The dish with the culture was placed in a humid chamber and incubated at 37°C. The nutrient mixture consisted of medium No. 199 with the addition of 20% calf serum, 10% chick embryonic extract, 1% L-glutamine (200 mM), 400 mg % glucose, 7.5 mg % Na ascorbinate, and antibiotics (100 units/ml penicillin and 50 μg/ml streptomycin). Half of the nutrient medium was changed for fresh every 2-3 days. Cells were washed off the surface of the explants with a jet of nutrient medium. At the end of culture the explants were scraped off the filter, cut into small pieces, and used to prepare homogeneous cell suspensions. The living cells were counted with trypan blue in a counting chamber. The number of CFUs in the cell suspensions was determined by the method of Till and McCulloch [4]. Syngeneic mice were irradiated in a dose of 1200 rad (^{137}Cs), the test cells were injected intravenously into them, and 8 days later the number of surface colonies was counted in the spleen.

EXPERIMENTAL RESULTS

The results of one of the experiments are given in Table 1. The cultures were washed off 5 times between the 4th and 16th days; next, 1, 2, 3, 5, and 8 days after the last washing, two dishes were taken at each time and the number of cells and CFUs remaining in the culture was determined. As Table 1 shows, the number of CFUs washed off each time was much less than their number in the culture; between the 17th and 24th days, moreover, an increase in the number of CFUs was observed in both the experimental and the control cultures, and this led to an increase in the total yield of CFUs (washings + final removal). In this experiment the washings gave an additional yield of 3.13×10^6 cells and 853 CFUs.

Laboratory of Bone Marrow Culture and Transplantation, Central Research Institute of Hematology and Blood Transfusion, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 3, pp. 281-282, March, 1979. Original article submitted February 16, 1978.

0007-4888/79/8703-0279$07.50

TABLE 1. Determination of Number of Cells and CFUs in Washed-Off Cultures

Period of culture, days	Source of cells	Cells (x 10^6)	CFUs (M ± m)	Total yield of	
				cells (x 10^6)	CFUs
4	Washing	1,3	200±21	—	—
7	»	0,48	314±21	—	—
10	»	0,23	60±3,6	—	—
14	»	0,90	202±30	—	—
16	»	0,22	77±9	—	—
17	Culture undisturbed	0,80 (1,0)	635±59 (906)	3,93	1488
18	» »	0,79 (0,82)	1080±54 (990)	3,92	1933
19	» »	0,85 (0,91)	838±79 (1006)	3,98	1691
21	» »	0,82 (1,7)	974±69 (1666)	3,95	1827
24	» »	0,99 (0,83)	1525±119 (1410)	4,12	2378

Legend. Here and in Table 2, numbers for control cultures given in parentheses.

TABLE 2. Total Yield of Cells and CFUs from Washed Off Cultures

Expt. no.	Days of washing off	Day of removal of cultures	Total yield of	
			cells (x 10^6)	CFUs
1	4, 7, 11th	16th	2,8 (1,0)	901 (415)
2	2, 4, 7, 9, 11, 14th	16th	4,17 (1,41)	650 (501)
3	3, 6, 9, 13, 16, 20, 23, 27, 30th	34th	3,79 (0,63)	934 (560)
4	2, 6, 9, 18, 22, 25, 29, 32, 36, 39th	43th	5,06 (0,41)	1850 (454)
5	23, 27, 30, 34th	37th	1,59 (1,24)	1143 (384)

In five other experiments (Table 2) the time of beginning washing off and the frequency and total number of washings were varied, as also were the times at which the cultures were removed. If the washings began after the first days in culture and continued at intervals of 3 days or more, the total yield of cells and CFUs increased with an increase in the total number and duration of the washings; however, too frequent repetition of the washings – at intervals of 2-3 days – could lead under these conditions to exhaustion of the culture (see experiment No. 2, in which the experimental culture on removal contained far fewer CFUs, namely 115 compared with 501 in the control). Regardless of the time of beginning washing, after 4 weeks in culture the washed off cells contained few CFUs; it is therefore advisable to start washing off during the first few days of culture, after the explants have become adherent to the substrate, and not to continue them after 4-5 weeks. The time of removal of the culture in order to obtain the maximal yield of CFUs is determined by the time when their content of CFUs is greatest (3-4 weeks in the case of cultures under the conditions described above).

These results thus show that washing off does not cause irreversible damage to the culture and that the operation can be repeated over a long but limited period of time, and they are evidence of the ability of the washed layer to regenerate. Although a considerable number of CFUs are washed off, especially initially, the culture as a whole is not exhausted and its CFU content is comparable with that observed in control cultures of the same age which have not been washed off. This fact means that repeated washing off can be used to accumulate hematopoietic stem cells, for subsequent preservation, for example. At the same time, the results raise a number of new problems for study. In particular, it is not clear what mechanisms ensure the stability of the system and its ability to regenerate, nor do we know what factors are responsible for exhaustion of the culture in the later stages.

LITERATURE CITED

1. N. V. Latsinik, E. A. Luriya, N. L. Samoilina, et al., Byull. Éksp. Biol. Med., No. 7, 88 (1969).
2. E. A. Luriya and I. E. P'yanchenko, Byull. Éksp. Biol. Med., No. 6, 81 (1967).
3. E. A. Luriya, N. L. Samoilina, Yu. A. Gerasimov, et al., Byull. Éksp. Biol. Med., No. 4, 103 (1971).
4. J. E. Till and E. A. McCulloch, Radiat. Res., 14, 213 (1961).