PROTECTIVE POTENTIAL OF FETAL LIVER HEMATOPOIETIC CELLS IN LONG-TERM CULTURE

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Investigations have shown that hematopoiesis and proliferation of CFUs can be maintained for several weeks in an organ culture of mouse embryonic liver (EL) [4, 7]. It has been shown by the use of chromosome markers that hematopoietic chimeras can be obtained in principle after transplantation of cultured syngeneic EL cells [3]. However, it is not yet clear how culture and, in particular, its duration, affects the magnitude of the protective potential of EL and whether the number of CFUs in the culture may reflect its protective potential.

To estimate the value of long-term culture of EL for the purpose of subsequent transplantation, the writers undertook a quantitative comparison of the protective potential of native and cultured EL.

EXPERIMENTAL METHOD

Recipient mice, which were female (CBA × C57BL/6)F_1 mice (CBF_1), were irradiated with γ-rays in a dose of 10.8 Gy (^{137}Cs, dose rate 0.2 Gy/min). After irradiation the mice received antibiotics with their food and acidified water. The protective efficacy of the transplanted cells was determined on the basis of their 3-week survival. Cells from EL of 17-day embryos (the day of discovery of a vaginal plug was taken as day 0) were removed by homogenization in the cold in medium 199 and washed once, after which nucleated cells with the leukocytic fluid were counted and diluted in appropriate concentrations. Fragments of EL were cultured by the organ culture method [1]. After the viable cells had been counted (with the aid of trypan blue) series of cell doses were injected intravenously into mice 3-5 h after irradiation. CFUs were determined by the method in [10]. Chimerism was established by the use of the C-banding method of staining the chromosomes: in stained films the Y chromosome was easily identified by the absence of a centromeric block [5].

EXPERIMENTAL RESULTS

Various doses of EL, cultured for different times (from 4 to 62 days) were injected into 217 mice, irradiated on average with a dose of LD 76/21 (150 control mice), and a series of doses of native EL cells was injected into 79 mice. The data on 3-week survival of the recipients were systematized depending on the dose of injected cells or the number of CFUs and the duration of culture (Tables 1 and 2). Analysis of these data showed that on transplantation of native or cultured cells of native EL or of EL cultured for 4-20 days, the fraction of surviving recipients was observed to bear a strictly quantitative relationship to the number of cells injected and also the number of CFUs; a close to maximal effect was achieved, moreover, by the use of quite low doses of injected cells and CFUs. After longer periods of culture the protective effect was weaker and less stable, and the maximal protective effect was observed only in individual experiments. The mean data on survival of the recipients after transplantation of EL cultured for a long time (24-62 days) showed no quantitative dependence on the number of injected CFUs.

After a few months some of the receipients were tested for chimerism. Since the EL fragments were randomized before transplantation into culture, it might be expected that the

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TABLE 1. Three-Week Survival of Irradiated Mice after Transplantation of Native or Cultured EL Cells: Dependence on Dose of Cells and Duration of Culture

Duration of culture, days	Number of cells injected ($\cdot 10^5$)					
	0	0,25—0,5	0,6—1,1	2—2,2	3,9—5,4	8—23
0	14/59	3/6	11/17	13/14	22/22	19/20
4	2/2	3/5		4/4		3/4
8	6/6				3/4	2/2
10	3/13					4/5
11	2/10	12/16		8/8	2/2	
13	1/5		4/4		3/3	3/3
18	6/16	6/10	3/4	4/4	3/3	3/3
20	2/3		6/6	2/3	3/3	
Pooled data						
4—20	22/55	21/31	13/14	18/19	14/15	15/17
24	0/7			3/4	0/8	
27	0/3		2/3	3/3	2/2	
29	0/9	2/10	0/2			
31	0/20	1/3		3/9	2/4	4/9
34	0/8		0/10		3/5	
38	1/11					4/4
41	0/5		2/5	2,5	4/5	
48	4/10		4/5		1/5	
55	4/14		4/6			
62	2/8		2/5	3/5	1/4	
Pooled data						
24—62	11/95	3/13	14/36	14/26	13/33	8/13
Average data, %						
0	24	50	65	93	100	95
4—20	40	68	93	95	93	88
24—62	12	23	39	54	39	62

Legend. Here and in Table 2: numerator gives number of surviving mice, denominator gives number of mice irradiated

TABLE 2. Three-Week Survival of Irradiated Mice after Transplantation of Native or Cultured EL Cells: Dependence on Number of Injected CFUs and Duration of Culture (pooled data)

Duration of culture, days	Number of CFUs injected						
	0	1	3—10	11—20	21—50	51—100	101—1389
0	14/59 (24)	1/10 (10)	14/23 (61)	12/12 (100)	14/14 (100)	10/10 (100)	55/56 (98)
4—20	22/55 (40)		10/14 (71)	21/27 (78)	17/19 (89)	13/14 (93)	11/11 (100)
24—62	11/95 (12)		13/26 (50)	8/18 (44)	7/16 (44)	6/11 (55)	16/38 (42)

Legend. Percentage of surviving mice shown in parentheses.

receipients would receive a mixture of cells derived from male and female embryos, and that their relative proportion would depend on the ratio between males and females among the donors. The results of analysis of chimerism after transplantation of native EL are given in Table 3. In the experiments of series I the number of cells with male karyotype was from 36 to 70% in the recipients' bone marrow and from 27 to 62% in the spleen. In the experiments of series II the number of cells with male karyotype among cells of the hematopoietic tissues did not exceed 6% in one mouse, and in the other none whatever were found. It can be tentatively suggested that whereas in the experiments of series I the ratio between female and male donors was about equal, in series II the donors were predominantly females, although the method used cannot rule out the possibility of reversion of the recipient's hematopoietic

TABLE 3. Karyologic Analysis of Hematopoietic Cells in Recipients of Native EL

Series of experiments	Number of cells injected (· 10^6)	Time after transplantation, months	Percentage of cells containing Y chromosome		
			bone marrow	thymus	spleen
I	0,4	11,7	64	—	62 (50)
	0,4	11,7	36	—	27
	2,4	11,7	54	—	42 (50)
	2,4	11,7	68	—	56
	22,4	11,7	70	—	58
II	0,2	8,3	3	6 (50)	0 (50)
	0,2	8,3	0	0 (20)	0 (50)

Legend. Here and in Table 4: number of metaphases analyzed shown in parentheses, in all other cases 100 metaphases were analyzed.

TABLE 4. Karyologic Analysis of Hematopoietic Cells in Recipients of Cultured EL

Series of experiments	Number of cells injected (·10^6)	Duration of culture, days	Time after transplantation, months	Percentage of cells containing Y chromosomes		
				bone marrow	thymus	spleen
I	2,43	8	9,3	53	—	28 (50)
	1,0	18	9,1	85	—	64
	0,39	18	9,1	82	—	68 (50)
	0,39	18	11,5	11	60 (38)	34 (50)
	0,39	18	11,5	78	80 (40)	60 (50)
II	1,0	10	5,5	10	5 (40)	12 (50)
	1,0	10	5,5	8	10 (30)	4 (50)
	0,21	24	6,4	6	72 (50)	8
	0,21	24	6,4	4	0 (30)	0 (50)
	0,5	31	5,1	0		0
	0,5	31	5,1	0	0 (30)	0

cells. Similar data were obtained on analysis of chimerism of the recipients of cultured EL (Table 4). In the experiments of series I, in recipients of EL cultured for 8-18 days, cells of male karyotype were present for a long time — at the last time of analysis (11.5 months). The high percentage of these cells is indirect evidence of complete chimerism and of a high ratio of males to females among the donor embryos. It will be noted that in some cases there were sharp differences between the organs in the proportion of cells with male and female karyotypes: in one case cells of female karyotype predominated in the bone marrow, in another case, although the proportion of cells of male karyotype was low in all the experiments of this series, the number of cells containing a Y chromosome in the thymus of one recipient was 72% (Table 4). This disproportion is not evidence in support of uniform production of cells from a large pool of early precursors — if that were the case constant representation of cells of female and male karyotypes would be expected.

Quantitative analysis showed on the whole that the protective potential of cultured EL, calculated as the number of injected cells, remained virtually at its initial level for about 20 days. After longer culture the protective activity was preserved only in some cultures, and for that reason the average effect for 24-62-day cultures was lower and did not show the same precise quantitative dependence on the number of injected hematopoietic cells as was observed for hematopoietic tissue initially and after a short period in culture. When the protective potential was calculated as the number of injected CFUs, some decrease in its value could be observed in the case of EL cultured for only a short time: whereas the maximal effect from native EL was observed after injection of 11-20 CFUs, the effect of cultured EL was maximal starting with 21 CFUs or more. In long-term cultures of EL the protective action did not depend on the number of injected CFUs, although the percentage of surviving mice, with all doses of CFUs (from 3 to 1389) was on average higher than in the control group. These data are evidence that the presence of a large number of CFUs in transplanted hematopoietic tissue does not guarantee the ability to protect lethally irradiated mice. Recourse to CFUs that are polypotent hematopoietic precursors is widely used for testing the suitability of hematopoietic cells for transplantation [8, 9]. It has been shown by the method of limiting dilutions [6] that totipotent hematopoietic precursors, capable of replacing defective hematopoiesis of anemic W/Wv mice, are present in normal bone marrow

in a much lower concentration than CFUs. According to recent experimental data, hematopoiesis in a bone marrow culture is maintained by significantly earlier precursors than CFUs [2]. The results of the present experiments, together with data in the literature, lead to the conclusion that CFUs are unable to restore hematopoiesis of irradiated animals, i.e., they are not totipotent stem cells. A parallel between the number of totipotent stem cells in hematopoietic tissues with CFUs or other precursors, and also with the total number of hematopoietic cells, can evidently be strictly guaranteed only for stable systems in vivo (normal bone marrow, EL, and so on). In cases when the hematopoietic system is subjected to disturbing influences, and also during manipulations with it in vitro, the relative proportions of the different categories of precursor cells may be disturbed. The protective potential of the hematopoietic cells or, in other words, the presence of totipotent stem cells among them in these cases can be judged at present only by direct testing.

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