SEMISYNGENEIC TRANSPLANTATION OF HEMATOPOIETIC CELLS FROM NATIVE OR CULTURED FETAL LIVER

N. L. Samoilina

UDC 616.36-003.971-018.1-053.13-089.843

KEY WORDS: hematopoietic stem cell, protective potential, secondary disease, transplantation of fetal liver, organ culture

It has become evident that the production of polypotent hematopoietic CFUs precursors in fetal liver (FL) [2] and bone marrow (BM) [6] cultures means the production of hematopoietic stem cells (HSC) capable, on transplantation, of protecting lethally irradiated recipients. It has been suggested that culture of BM be used as a method of reducing its graft verus host (GVH) activity, in order to avoid secondary disease (SD) following incompatible transplantation [7].

Transplantation of FL, unlike that of adult BM, does not necessarily induce lethal SD even when differences relative to the principal histocompatibility locus are present. However, data in the literature on delayed death of mice receiving FL cells cover a wide range — from complete absence to high values of mortality [4, 9, 10, 16-18]. The causes of these disparities have not been established.

The aim of this investigation was to compare quantitatively the protective activity of hematopoietic cells from native FL and FL cultured for different periods of time, and their ability to cause delayed death after transplantation of various doses of cells from mice of the parental line into F_1 hybrids.

EXPERIMENTAL METHOD

C57BL/6 (B6) and (CBA × C57BL/6)F_1 (CBF_1) mice were used. FL cells from 17-day B6 fetuses were obtained by homogenization in medium 199 in the cold. After washing the cells were counted and diluted for intravenous injection. The method of culture of the cells and their removal from the cultures was described previously [1]. To determine 8-11-day CFUs the cells were injected into B6 mice irradiated with a dose of 8.8 Gy (^{137}Cs, dose rate 0.2 Hy/min). The recipients for semisyngeneic transplantation were female CBF_1 mice aged 4-8 months, irradiated in a dose of 10.8 Gy. All the irradiated mice received acidified water, polymyxin M sulfate, and monomycin with their food. The protective effect of transplanted cells was judged from the 3-week survival rate of the recipients, and the presence of SD was judged from mortality after the 3rd week until the end of the 3rd month. The presence of donor's cells in the recipients was determined by the immunocytotoxic test, using strain-specific CBA-anti-B6 and B6-anti-CBA sera [5].

EXPERIMENTAL RESULTS

After injection of hematopoietic cells from native FL the protective effect was observed to be dependent on the dose of cells injected (Table 1). With doses of under $0.5 \cdot 10^6$ nucleated cells virtually no protective action was observed. Increasing the dose above $5 \cdot 10^6$ did not increase the survival rate of the recipients. For semisyngeneic transplantation under the conditions used, the average maximal protective effect was about 80%. Short-term (up to 15 days) culture did not change the quantitative dependence of the effect on survival on the dose of cells significantly. However, during longer culture, a certain protective effect was observed only in some of the experiments, whereas in others there was no such effect; for that reason no quantitative dependence on dose of cells was observed as a whole. A similar although unexpected result was obtained on analysis of dependence of the

Cell Engineering Laboratory, 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 A. I. Vorob'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 2, pp. 228-231, February, 1988. Original article submitted March 5, 1987.

0007-4888/88/0002-0269$12.50 © 1988 Plenum Publishing Corporation

TABLE 1. Dependence of Survival of Irradiated CBF_1 Mice on Dose of Injected B6 FL Cells and on Duration of Its Culture

Duration of culture, days	Number of cells injected (x 10^6)				
	0	0,05—0,25	0,3—0,5	0,6—1,9	2—5,5
0	19/68 (28%)	9/34 (26%)	9/20 (45%)	22/30 (73%)	77/94 (82%)
4—15	18/75 (24%)	4/8 (50%)	9/16 (56%)	7/10 (70%)	25/28 (89%)
17—35	8/125 (6,4%)	5/15 (33%)	9/26 (35%)	8/33 (24%)	7/25 (28%)

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

TABLE 2. Dependence of Survival of Irradiated CBF_1 Mice on Number of Injected CFUs and Duration of Culture of B6 FL

Duration of culture, days	Number of CFUs injected					
	0	2—14	18—33	60—99	102—195	202—1116
0	19/68 (28%)	9/34 (26%)	17/30 (57%)	23/30 (77%)	31/40 (77,5%)	64/84 (76%)
4—15	18/75 (24%)	4/8	2/4	6/11 (55%)	9/12 (75%)	25/30 (83%)
17—31	8/125 (6,4%)	—	—	4/8	6/25 (24%)	14/49 (29%

protective effect on the number of transplanted CFUs. A dependence was clearly revealed when native FL or FL after a short period of culture was transplanted, but none was found after transplantation of FL previously cultured for a long period (Table 2). Injection of more than 1000 CFUs (disregarding the settling factor) from a long-term culture was ineffective, whereas injection of fewer than 100 CFUs from native FL had the maximal protective action.

Analysis of survival in the late stages of the investigation showed that it depends inversely on the dose of transplanted native FL cells (Table 3). However, appreciable mortality was observed only after the use of doses greater than those sufficient to give the maximal protective effect: $5 \cdot 10^6$-$25 \cdot 10^6$ cells. Cultured FL, when doses of cells giving the maximal protective action were transplanted ($2 \cdot 10^6$-$4.6 \cdot 10^6$) were unable to induce delayed death of recipients from SD.

Under the conditions of irradiation used in these experiments (mean $LD_{83/21}$) marked hematopoietic reversion could be expected in the recipients. Testing for chimerism showed that the duration of chimerism depends on the dose of FL cells injected: after injection of the minimal dose of 10^5 cells partial chimerism was observed in one recipient, and it was absent in the other (Table 4). After injection of $0.25 \cdot 10^6$ to $25 \cdot 10^6$ cells all 12 recipients studied 9-15 months after transplantation contained hematopoietic cells of the donor's phenotype. Survival of hematopoietic cells of cultured B6 FL depended mainly on the dose of cells: no donor's cells could be found in mice receiving fewer than $0.4 \cdot 10^6$ cells when tested 8-13 months after transplantation. After injection of larger doses the presence of cells with the donor's phenotype depended on the time of investigation of the chimeras. For instance, 1.5-5 months after transplantation all six recipients studied were found to be complete or partial chimeras, but when tested after 8-13 months, only eight of the 17 recipients were chimeras.

Confirmation of survival of hematopoietic cells from the native FL of a parental line in F_1 hybrids indicates that death of some recipients before 2-3 months can be interpreted as death from SD. The causes of this considerable mortality at these same times in the control irradiated mice and recipients of FL after long-term culture were evidently different, and could be associated with exhaustion of the hematopoietic potential of totipotent hematopoietic stem cells (TPHSC) still preserved after irradiation or transplanted in an insufficient dose.

The quantitative approach used in this investigation enables the ability of hematopoietic tissue to protect irradiated animals, by restoring hematopoiesis, to be assessed by the direct route. Just as in the case of syngeneic transplantation of FL [19], after semisyn-

TABLE 3. Delayed Death of CBF_1 Recipients of FL from Parental Line B6

Cells injected	Number of cells ($\times 10^6$)	Number of mice surviving 3 weeks	Percentage of animals dying after	
			3 weeks-2 months	3 weeks-3 months
—	0	45	42	47
FL	0,1—1,0	40	17	17
FL	2,0—3,26	40	15	27
FL	5,0—8,0	48	46	46
FL	25	16	62	62
Cultured FL				
4—15 days	0,05—1,9	16	19	19
17—31 days	0,05—0,5	14	50	57
	0,74—2,3	15	53	60

TABLE 4. Presence of B6 Donors' Cells in Hematopoietic Organs of CBF_1 Recipients

Transplanted cells	Number of cells ($\times 10^6$)	Time after transplantation, months	Chimerism								
			complete			partial *			absent		
			BM	thymus	spleen	BM	thymus	spleen	BM	thymus	spleen
FL	0,5; 2; 5; 25	5—7,9	10	8	10	0	0	0	0	0	0
FL	0,1	9	n.d.	0	0	n.d.	1	1	n.d.	1	1
FL	0,25; 1; 2,5; 5; 25	9—15	8	9	11	1	1	1	0	0	0
Cultured FL											
10 days	2	1,5	1	1	1	0	0	0	0	0	0
10, 17, 31 days	0,74—3,3	4,4—5	4	2	4	1	3	1	0	0	0
4, 8, 10 days	0,4—0,5	8—13	1	3	3	0	1	2	0	3	3
4, 8, 10 days	2—4,4	8—13	2	1	2	0	2	1	0	6	6
4, 11, 17 days	0,025—0,32	8—10	0	0	0	0	0	0	2	5	7

Legend. Asterisk indicates that among hematopoietic cells of thymus or spleen there were more than 30% and fewer than 70% of donor's cells, and in BM there more than 30 and fewer than 50% of donor's cells; n.d.) no data.

geneic transplantation of FL the protective effect depended on the dose of cells injected, but this was not necessarily parallel to the number of injected CFUs — cells from long-term cultures possessed weak protective activity, even though they had a high concentration of CFUs, including some aged 11 days. It is thus evident that TPHSC cannot be regarded as identical to CFUs, and in order to estimate the repopulating ability of hematopoietic tissue intended for transplantation, the number of CFUs it contains cannot be relied upon, as has been the case until now [11]. Hematopoietic precursors which have progressed even further in differentiation, and form colonies in vitro, are even less suitable for this purpose. To estimate the protective properties of hematopoietic cells that have been subjected to any kind of treatment, only the direct method of assessment of survival after transplantation of a series of cell doses appears to be reliable at the present time [3].

GVH dependence of FL of the parental line during transplantation into F_1 hybrids is likewise a dose-dependent phenomenon, as was shown previously for BM [3]. The essential difference with FL, however, is the fact that doses sufficient to achieve the maximal protective effect were not large enough to induce SD, whereas BM caused lethal SD in doses just exceeding ED_{31}. Thus one cause of the disparities as regard the activity of FL in the induction of SD, noted previously in the literature, may be that different doses of cells were used.

During assessment of the repopulating ability on the basis of 3-week survival, no difference was found between short-term cultures and native FL. However, tests of chimerism in the later stages showed that native FL cells have a definite advantage. Under conditions of competitive repopulation, TPHSC of native FL in most cases had the advantage over previously irradiated recipient's cells, whereas TPHSC from cultured FL had the advantage in only half of the cases.

CFUs were maintained for a long time in liquid BM cultures and also in FL organ cultures, but it was noted that the repopulating ability of BM from the cultures was reduced [12]. GVH activity of BM cultures remained high in allogeneic transplantation, despite rapid loss of T cells [12, 13], but when it was absent, survival of the graft was not recorded [7, 15]. Only when the donor's and recipient's lines differed with respect to the minor histocompatibility barrier could reduction of GVH activity and the protective action of a 3-day BM culture be demonstrated [14]. Comparison of the two systems of hematopoietic tissue culture shows that as regards its possible use for transplantation, the system of organ culture of FL has advantages with respect both to preservation of repopulating ability and absence of GVH activity.

LITERATURE CITED

1. O. I. Gan and N. L. Samoĭlina, Byull. Éksp. Biol. Med., No. 7, 94 (1982)
2. N. L. Samoilina, Byull. Éksp. Biol. Med., No. 3, 281 (1979).
3. N. L. Samoilina, Hematopoietic Precursor Cells in Mechanisms of Injury and Compensation of the Blood System Associated with Exposure to Extremal Factors [in Russian], Chelyabinsk (1986), p. 9.
4. D. W. H. Barnes, P. L. T. Ilbery, and J. F. Loutit, Nature, 181, 488 (1958).
5. J. L. Chertkov, I. M. Gelfand, O. A. Gurevitch, et al., Proc. Natl. Acad. Sci. USA, 76, 2955 (1979).
6. T. M. Dexter, T. D. Allen, and L. G. Lajtha, J. Cell. Physiol., 91, 335 (1977).
7. T. M. Dexter and E. Spooncer, Nature, 275, 135 (1978).
8. E. Jones-Villeneuve and R. A. Phillips, Exp. Hematol., 8, 65 (1980).
9. A. Lengerova, Transplant. Bull., 5, 69 (1958).
10. B. Lowenberg, H. M. C. de Zeeuw, K. A. Dicke, and D. W. van Bekkum, J. Natl. Cancer. Inst., 58, 959 (1977).
11. B. Lowenberg, Biology of Bone Marrow Transplantation, New York (1980), p. 417.
12. P. Mauch, J. M. Lipton, B. L. Hamilton, et al., Blood, 63, 1112 (1984).
13. P. Mauch, J. M. Lipton, B. L. Hamilton, et al., Blood, 66, 542 (1985).
14. M. A. McMillen and R. L. Simmons, Br. J. Haematol., 48, 171 (1981).
15. E. Spooncer and T. M. Dexter, Transplantation, 35, 624 (1983).
16. O. Tulunay, R. A. Good, and E. J. Yunis, Proc. Natl. Acad. Sci. USA, 72, 4100 (1975).
17. D. E. Uphoff, J. Natl. Cancer Inst., 20, 625 (1958).
18. I. S. Urso, Radiat Res., 9, 197 (1958).