NUMBER OF FIBROBLAST COLONY-FORMING CELLS IN MOUSE BONE MARROW

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UDC 611.419-018.15-085.23+612.419.014.2:612.6]-085.23

KEY WORDS: bone marrow; stroma; colony formation; feeder.

Stromal or fibroblast colony forming cells (FCFC) are identified by the formation of fibroblast colonies in monolayer cultures of cells from hematopoietic and lymphoid organs [3, 4, 6-8].

The number of colonies growing in cultures reflects the FCFC population in the original bone marrow. However, it must be recalled that during disaggregation of cells by various methods a different fraction of the FCFC contained in the bone marrow is liberated, and that the part of the FCFC, present in the cell suspension, that will form colonies will depend on the conditions of culture [1, 8].

We give below data on extraction of an additional number of FCFC from mouse bone marrow tissue by trypsinization, over and above the number released by mechanical disaggregation of the hematopoietic tissue, and on the effect of feeder cells on the efficiency of fibroblast colony formation (EFCF).

EXPERIMENTAL METHOD

Bone marrow cells from CBA or (CBA × C57BL/6)F_1 mice were used for explantation. The contents of the medullary cavity of the femora were expelled with a syringe into DMEM medium and single-cell suspensions were prepared by mechanical disaggregation (by passing the bone marrow through syringes with needles of decreasing diameter) or by trypsinization. For trypsinization, before the bone marrow was passed through syringes, it was placed in a 0.25% solution of trypsin and agitated on a magnetic mixer at 18°C for 30 min. All the cell suspensions were filtered through a four-layered kapron filter, centrifuged for 10 min at 400g, and the cell residues were resuspended in complete medium (α-MEM or DMEM) with 20% embryonic calf serum.

For the colony formation test [2] the cell suspensions were explanted into glass flasks with an area of 40 cm^2 and cultured in a CO_2 incubator. Feeder cells were added to some of the cultures 2 h after explantation: suspensions of bone marrow or spleen cells from guinea pigs or micre, irradiated in a dose of 60 Hz (^{60}Co). In some experiments the feeder cells were freed from adhesive and phagocytic cells before irradiation by means of iron carbonyl [5]. The number of colonies consisting of 50 fibroblasts or more was counted on the 10th-12th day in cultures fixed with ethanol and stained by Giemsa's method.

EXPERIMENTAL RESULTS

The effect of additional feeder on the efficiency of colony formation was determined in experiments with explantation of suspensions of bone marrow cells with the addition of different numbers of feeder cells (Table 1). The results of experiments Nos. 1, 2, and 3 show that, compared with cultures without feeder, addition of $0.5 \cdot 10^6$ to $20 \cdot 10^6$ irradiated guinea pig bone marrow cells increased EFCF, by a degree which was increased by an increase in the number of feeder cells, and the lower the initial density of the bone marrow cells subjected to explantation, the greater the increase in EFCF. In experiment No. 3, in cultures without feeder, no linear dependence of the number of colonies on the number of bone marrow cells could be achieved, for EFCF increased with an increase in the density of explantation. Meanwhile, on addition of the feeder EFCF became constant, i.e., a linear relationship was estab-

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0007-4888/86/0004-0505$12.50

TABLE 1. Effect of Additional Feeder on EFCF in Mouse Bone Marrow Cultures

Expt. No.	Number of explanted cells ($\cdot 10^5$)	Number of feeder cells ($\cdot 10^6$)	Number of colonies in flasks	EFCF ($\cdot 10^{-4}$)
1	3	—	2, 4	0,10
	3	1	3, 10	0,22
	3	3	11, 19	0,50
	3	10	60, 73	2,17
2	20	—	42, 46	0,24
	20	0,5	76, 81, 109	0,44
	20	3	112, 117, 127	0,60
	20	20	219, 222	1,10
3	5	—	1, 1, 2	0,03
	10	—	1, 5	0,03
	20	—	11, 11, 14	0,07
	40	—	44, 68, 85	0,17
	80	—	159, 179, 188	0,22
	5	20	77, 95, 99	1,81
	10	20	188, 191, 212	1,97
	20	20	328, 365, 397	1,82
4	20	—	0, 0, 0	0
	20	20	248, 256, 281	1,31
	20	10*	267, 273, 296	1,38
	20	20*	321, 354	1,69
5	20[a]	—	0, 0, 0	0
	20[b]	—	47, 79, 117	0,4
	20[c]	—	86, 106	0,5
	20[d]	—	66, 156, 181	0,7
	20[a]	20	0, 0, 0	0
	20[b]	20	367, 398, 411	2,0
	20[c]	20	359, 371	1,8
	20[d]	20	361, 372, 369	1,9

Legend. Explanted cells: explanted mouse bone marrow cells, mechanically disaggregated by passage through syringes, were used in cultures. Feeder cells obtained from suspension of irradiated guinea pig bone marrow cells. *) Suspension of feeder cells treated with iron carbonyl. a, b, c, d) Culture medium contained 0, 5, 10, or 20% embryonic calf serum.

TABLE 2. Effect of Different Feeder Cells on EFCF in Mouse Bone Marrow Cultures

Feeder			EFCF in presence of feeder/EFCF without feeder
Donor	Source of cells	Number of cells ($\cdot 10^6$)	
Guinea pig	Bone marrow	20	13,3
	Spleen	3	4,5
	» »	20	5,4
Mouse	Bone marrow	3	4,4
	The same	20	4,0
	» »	40	3,5
	Spleen	3	4,5
	» »	20	6,2

Legend. Into each culture $2 \cdot 10^6$ mouse bone marrow cells, mechanically disaggregated by passage through syringes, were explanted.

lished between the number of colonies and the number of explanted cells. The aim of experiment No. 5 was to determine whether the action of the feeder on EFCF depends on the concentration of serum. Clearly addition of the feeder cells did not compensate for the absence of serum, and 5% of serum was sufficient for the stimulating action of the feeder to be manifested ful-

TABLE 3. Effect of Trypsinization of Bone Marrow Tissue on EFCF

Expt. No.	Treatment with trypsin	Number of explanted cells ($\cdot 10^4$)	Feeder	Number of colonies in flasks	EFCF ($\cdot 10^{-4}$)
1	−	5	−	0, 0, 0	0
	−	5	+	6, 8, 12	1,8
	+	1	−	0, 0, 0	0
	+	1	+	8, 9, 11	9,3
	+	3	−	0, 0, 0	0
	+	3	+	26, 27, 31	9,3
2	+	3	+	0, 2, 4	0,7
	+	0,3	+	2, 4, 6	13,3
	+	1	+	10, 14, 18	14,0
3	+	25	+	9, 17, 17	0,6
	+	2	+	32, 45, 61	23,0

Legend. The cell suspension from the bone marrow of the left femur from three to five donors was prepared by mechanical disaggregation, and from the bone marrow of the right femur by trypsinization. Irradiated guina pig bone marrow cells in a dose of $2 \cdot 10^7$ were used as the feeder.

TABLE 4. Dependence of EFCF on Time of Trypsinization of Bone Marrow During Preparation of Suspension

Expt. No	Treatment with fragments of bone marrow with trypsin	Treatment of suspension of mechanically disintegrated cells with trypsin	Number of explanted cells ($\cdot 10^4$)	Number of colonies in flasks	EFCF ($\cdot 10^{-4}$)
1	+	−	5	25, 30, 38	6,2
	+	−	20	118, 122, 131	6,2
	−	+	50	45, 57	1,0
	−	+	50	39, 40, 48	0,8
	−	+	200	144, 169, 172	0,8
	−	+	200	139, 157, 172	0,8
2	+	−	5	49, 52, 74	11,6
	−	−	50	16, 21, 24	0,4
	−	+	50	16, 17, 19	0;3
	−	+	200	77, 84, 84	0,4
	−	+	200	72, 78, 84	0,4

Legend. Mouse bone marrow cells, disintegrated by passage through syringes or by trypsinization were explanted with addition of $2 \cdot 10^7$ irradiated guinea pig bone marrow cells as feeder.

ly. To determine whether feeder activity of irradiated bone marrow depends on adhesive and phagocytic cells, bone marrow cells treated with iron carbonyl were used as the feeder. The results of experiment No. 4 show that feeder activity was independent of adhesive and phagocytic bone marrow cells.

Table 2 gives the results of experiments in which the feeder activity of bone marrow and spleen cells was compared. These experiments showed that guinea pig bone marrow cells induced a stronger feeder effect than guinea pig or mouse spleen cells and mouse bone marrow cells.

A similar number of nucleated cells ($15 \cdot 10^6$ to $18 \cdot 10^6$) was liberated from the contents of the medullary cavity of one femur when cell suspensions were prepared by mechanical disaggregation or by trypsinization of the bone marrow. Values of EFCF in three experiments with parallel explantation of cells of the trypsinized and mechanically disaggregated bone marrow

from the same donors are given in Table 3. It will be clear from Table 3 that EFCF for trypsinized bone marrow was significantly higher than for mechanically disaggregated bone marrow. The presence of the additional feeder increased EFCF, and for cells of trypsinized and nontrypsinized bone marrow it was ($\cdot 10^{-4}$), 11.3 ± 1.9 and 0.7 ± 0.1, respectively.

Table 4 gives the results of experiments in which the effects of trypsinization of bone marrow fragments or of cell suspensions obtained by mechanical disaggregation were compared. The bone marrow from the left femur was first trypsinized, then passed through syringes, whereas marrow from the right femur of the same donors was passed first through syringes and, later, half of the resulting suspension was trypsinized. According to the results of these experiments EFCF for cells obtained by trypsinization of bone marrow fragments was significantly higher and for cells isolated by mechanical disaggregation; subsequent treatment of the mechanically disaggregated bone marrow by trypsin, moreover, did not increase EFCF.

The increase in EFCF during trypsinization of mouse bone marrow was thus not connected with activation by trypsin of the colony-forming properties of the already disaggregated cells, but with additional release of FCFC, which either are not released by mechanical disaggregation of bone marrow, or are injured. The experiments showed that the presence of hematopoietic cells has a stimulating action on the development of mouse stromal colonies, and their colony-stimulating activity is still preserved after irradiation in a dose of 60 Gy, i.e., it evidently is independent of proliferation of feeder cells. By trypsinization and the use of an irradiated feeder it is possible to increase EFCF for mouse bone marrow substantially by comparison with EFCF values achieved previously [2].

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TRANSPLACENTAL EFFECT OF METHYLCOBALAMIN ON GROWTH OF MOUSE EMBRYONIC KIDNEY TISSUE IN ORGAN CULTURE

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UDC 616.46:612.646.014.46:615.356:577.164.16

KEY WORDS: methylcobalamin; organ culture of the kidney; transplacental action

The effect of methylcobalamin (MC) on growth of embryonic tissues is due to its functional role as coenzyme for methionine synthetase (EC 2.1.1.13), which controls the formation of active forms of folate required for the intensive turnover of C_1 compunds in proliferating cells in mammals and man [11]. The cellular level of cobalamin-dependent methionine synthetase in embryonic tissues is evidently an important parameter determining their rate of growth. During human embryogenesis the highest level of methionine synthetase is observed in the fetal tissues and the serum level of MC (the main transport form of the cobalamins) is high [9, 12]. The necessary supply of MC to the fetal tissues is ensured by means of placental receptors and different classes of transcobalamin in the blood serum. Intensive uptake of cobalamins by em-

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 101, No. 4, pp. 471-474, April, 1986. Original article submitted April 25, 1985.

0007-4888/86/0004-0508$12.50