CLONING OF STEM CELLS IN THE BONE MARROW OF IRRADIATED MICE

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Various numbers of cells of intact or irradiated bone marrow from syngeneic donors were injected into mice irradiated with a lethal dose. A linear relationship was found between the number of 8-9 day colonies growing in the femoral marrow and the number of injected cells, and an exponential relationship was found with the dose of irradiation. The pattern of cloning of the stem cells in the bone marrow was similar to that in the spleen. Differences were found in the radiosensitivity of colony-forming units (CFU) depending on the organ (spleen, bone marrow) in which they formed the colonies. CFUs settling in the bone marrow were more resistant (D_0 160-200 R) than CFUs settling in the spleen (D_0 80-100 R). Differences in the radiosensitivity of the CFUs are due, it is postulated, to the presence of a heterogeneous population of stem cells and also to specific features of the organ (spleen, bone marrow) in which the colonies are formed.

KEY WORDS: colony-forming units; bone marrow; spleen; radiosensitivity.

Transplantation of intact or irradiated bone marrow cells leads to the formation of colonies of cells of the erythroid, myeloid, and megakaryocytic types in the spleen of irradiated recipient mice [3], and the number of colonies bears a linear relationship to the number of injected cells but falls exponentially if the donors of the cells are irradiated with doses of between 100 and 600 R. Besides in the spleen, colonies can be formed in the bone marrow [2, 6]. However, the pattern of cloning of both intact and irradiated hematopoietic cells in the bone marrow has not been studied.

The objects of the present investigation were as follows: 1) to study the relationship between the number of injected cells and the number of colonies growing in the bone marrow; 2) to investigate the effect of the dose of irradiation of the injected cells on their ability to form colonies in the femoral marrow.

EXPERIMENTAL METHOD

Male C57BL mice and F_1 (CBA × C57BL) female mice weighing 20-22 g were used. The conditions of irradiation of the recipient animals and of the bone marrow cells of the donors are given in Table 1.

The bone marrow was taken from the femur immediately (C57BL) or 24 h after irradiation (F_1). Cells irradiated in vitro were injected immediately after irradiation. On the 8th-9th day after transplantation of hematopoietic cells the femur and spleen were removed from the syngeneic recipients. The material was fixed in Bouin's fluid. The bones were decalcified in 5% HNO_3 solution and embedded in paraffin wax. Sections 5-7 μ in thickness were cut in a continuous series. The number of colonies was counted in histological specimens stained with hematoxylin and eosin, and subdivided into erythroid, myeloid, megakaryocytic, and mixed. The equation of the regression lines was calculated by the method of least squares. The radiosensitivity of the stem cells was characterized at the mean lethal dose (D_0).

EXPERIMENTAL RESULTS

It will be clear from Fig. 1a (curve 1) that a linear relationship exists between the number of colonies

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TABLE 1. Conditions of Irradiation of Recipient Mice and of Donor's Bone Marrow Cells

Mice	Dose of irradiation (in R)		Source of γ-rays	Dose rate (in R/min)
	of bone marrow cells	of recipients		
F_1 (CBA × C57BL)		900 (86)	Cs^{137}	37
C57BL	400, 500, 600, 700 (in vivo)	800 (40)	Co^{60}	289
F_1 (CBA × C57BL)	100, 200, 300, 400, 500, 600 (in vivo)	875 (71)	Co^{60}	289
F_1 (CBA × C57BL)	74, 148, 222, 296, 407, 555, 703 (in vitro)	900 (94)	Cs^{137}	37

Legend. Number of recipients given in parentheses.

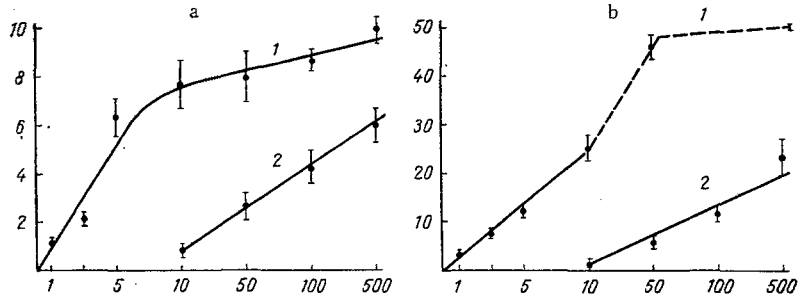

Fig. 1. Number of colonies growing in femoral marrow (a) and spleen (b) as a function of number of injected cells af intact (1) bone marrow or bone marrow irradiated in a dose of 400 R (2) from F_1 hybrid mice (8th-9th day after transplantation of cell). Abscissa, number of cells injected ($\times 10^4$); ordinate, mean number of colonies in bone marrow (a) or spleen (b). Broken line denotes conjectural number of colonies.

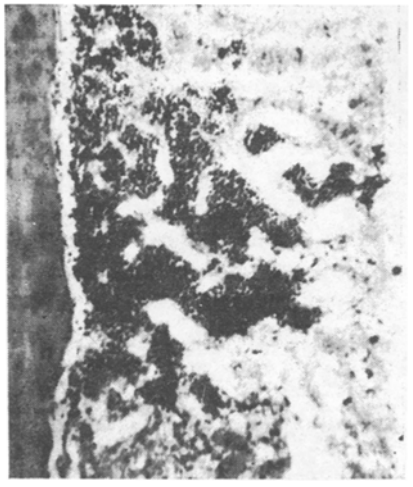

Fig. 2. Formation of hematopoietic colony in femoral medullary cavity of radiochimera (8th day after transplantation of cells). Hematoxylin-eosin, objective 10, Gomal 6 ×.

growing in the femur of the irradiated (900 R) mice and the number of intact bone marrow cells injected. The curve showing the relationship between the dose of injected cells and the number of colonies consists of two parts: linear and plateau. The linear function was found to hold good if small numbers of cells were transplanted ($1 \cdot 10^4 - 5 \cdot 10^4$), but with an increase in the number of cells ($1 \cdot 10^5$ or more) the number of colonies in the femur remained unchanged (plateau). A similar pattern of colony formation was found in the spleen (Fig. 1b; curve 1).

Transplantation of large numbers of intact cells (from $1 \cdot 10^5$ to $5 \cdot 10^6$) led to the formation of the same number of colonies in the bone marrow of the irradiated recipients (the number of colonies "comes out onto a plateau"). Since in the case of transplantation of cells from irradiated donors it was necessary to work (see below) with just this number of cells, correlation between the number of growing colonies and the number of injected irradiated cells was investigated additionally. The results showed that after transplantation of $1 \cdot 10^5 - 5 \cdot 10^6$ bone marrow cells from irradiated (400 R) donors, the number of colonies in the femur of F_1 hybrid recipients irradiated in a dose of 900 R increased as a linear function of the number of injected cells (Fig. 1a, curve 2). A similar relationship was found for cells forming colonies in the spleen (Fig. 1b, curve 2).

Whereas the number of colonies in the bone marrow and spleen was constant after transplantation of $1 \cdot 10^5 - 5 \cdot 10^6$ intact bone marrow cells, if the same number of irradiated cells was injected, the number of colonies arising in these organs lay on the linear part of the dose–effect curve.

TABLE 2. Relationship Between Number of Colonies Growing in Femur and Number of Bone Marrow Cells from Irradiated F_1 Hybrid Donors Injected (M ± m)

Dose (in R)	Number of cells injected (·10⁵)	Type of hematopoietic colonies				Mean number of colonies per femur
		erythroid	myeloid	megakaryocytic	mixed	
0	0,5	22,0±6,0	38,0±7,0	12,0±2,0	2,0±0,4	74,0±16,0 (25)
100	1,0	4,0±0,8	8,0±1,0	9,0±2,0	0	21,0±4,0 (12)
200	2,5	2,5±0,4	2,8±0,5	1,7±0,3	0,6±0,3	7,6±0,8 (13)
300	5,0	0,8±0,2	1,2±0,3	2,4±0,6	0	4,4±1,0 (11)
400	5,0	0,8±0,3	2,0±0,4	1,6±0,4	0	4,4±0,9 (10)
500	10,0	1,6±0,5	1,8±0,5	1,2±0,2	0	4,6±1,2 (15)
600	20,0	0,23±0,02	0,37±0,03	0,26±0,04	0	0,86±0,04 (12)

Legend. Calculated per 10^6 transplanted cells, irradiation control deducted. Number of recipients tested on 9th day after transplantation of cells given in parentheses.

TABLE 3. Radiosensitivity of Bone Marrow Cells Forming Colonies in Spleen and Bone Marrow (M ± m)

Organ	Donor mice	D_0 and extrapolation number (n)			
		erythroid colonies	myeloid colonies	megakaryocytic colonies	total number of colonies
Spleen	C57BL *	85±5	80±6	90±5	82±5 n=2,1
	F_1 (CBA × C57BL) *	115±5	108±7	110±6	110±5 n=1,1
	F_1 (CBA × C57BL) †	95±6	95±6	103±8	100±7 n=1,1
Bone marrow	C57BL *	170±6	160±8	165±7	165±7 n=1,4
	F_1 (CBA × C57BL) *	197±18	217±20	180±12	195±18 n=0,9
	F_1 (CBA × C57BL) †	167±10	170±11	170±12	170±12 n=0,85

*Irradiation of donors.
†Irradiation of cell suspension.

Morphological analysis of the colonies (Fig. 2) showed that, unlike in the spleen, colonies of myeloid type predominated in the bone marrow, being 1.5-2 times more numerous than the erythroid colonies, whereas in the spleen there was an equal preponderance of erythroid colonies. Differences in differentiation of stem cells proliferating in the bone marrow and spleen are now regarded as a manifestation of the effect of the microenvironment of the stroma. According to some workers, various factors inducing hematopoiesis exist in the stroma of the spleen and bone marrow, and they direct differentiation of the stem cells in the erythroid (in the spleen) or myeloid (in the bone marrow) direction [5, 6]. The ability of the stroma of hematopoietic organs to regulate differentiation of the transplanted stem cells had not yet been completely proved. However, the results obtained in these experiments can be interpreted as support for this hypothesis.

Experimental data on the ability of transplanted irradiated bone marrow cells to form colonies in the femoral marrow are given in Table 2.

Since no differences were found in colony formation by cells irradiated under different conditions (in vivo or in vitro), the experimental results will be described together. The investigation was carried out on C57BL and F_1 (CBA ×C57BL) mice. On the 9th day after transplantation of the corresponding number of irradiated bone marrow cells, an exponential relationship was found between the dose of irradiation and the number of colonies in the femur and at the same time in the spleen of the syngeneic recipient. Irrespective of the dose of irradiation of the stem cells the mean size of the erythroid, myeloid, and megakaryocytic colonies in the bone marrow (0.4-0.5, 0.5-0.7, and 0.2-0.3 mm, respectively) was indistinguishable from the size of the homonymous cell colonies obtained by transplantation of intact unirradiated cells. The mean diameter of these types of colonies in the spleen was 0.8-0.9, 0.4-0.5, and 0.2-0.3 mm, respectively. The erythroid colonies thus grew to the largest size in the spleen, and the myeloid colonies in the bone marrow. Whereas in the spleen under the influence of radiation the transplanted cells formed significantly smaller colonies of the three main types, in the bone marrow under the same conditions of irradiation the dimensions of the colonies were unchanged.

Quantitative analysis of the colony counts in the femur after transplantation of irradiated bone marrow cells made it possible to estimate the value of the effective dose leading to a decrease in the number of stem cells by 63% (D_0). In this case lower radioresistance of the cells forming colonies in the bone marrow than of the cells forming colonies in the spleen was found (Table 3).

Characteristically, the value of the extrapolation number for cells forming colonies in the bone marrow was below one, whereas for cells forming colonies in the spleen the extrapolation number was over one.

It can be postulated on the basis of these results that the population of stem cells is heterogeneous, consisting of groups differing in radiosensitivity and extrapolation number. One group of stem cells possibly may form colonies in the spleen – spleen colony-forming units (CFU_S). For this fraction of cells D_0 is 80-100 R and $n > 1$. The other group of stem cells forms colonies in the bone marrow – bone marrow colony-forming units (CFU_{bm}). For this fraction of cells D_0 is about 160-200 R and $n < 1$. This agrees to some extent with the results of investigations showing that stem cells settling in the spleen or bone marrow possess different kinetic parameters, rate of growth [1], and hemoglobin production [4]. On the other hand, the radiosensitivity of the stem cells may be due to specific features of the organ (spleen, bone marrow) in which the colonies are formed. In this connection it may be postulated that irradiated (partly injured) stem cells, on entering the bone marrow, exhibit a higher capacity for repair than cells settling in the spleen. As a result the cloning efficiency in the bone marrow is much higher than in the spleen. By cloning efficiency is meant the manifestation of ability to form colonies either by all stem cells settling in a given order or by some of them. The possibility cannot therefore be ruled out that in both organs what is determined is the effect of radiation on the ability of stem cells to undertake effective cloning rather than colony formation.

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