RADIOSENSITIVITY OF BONE MARROW CELLS FORMING FIBROBLAST COLONIES IN MONOLAYER CULTURES

G. N. Kuz'menko,* A. F. Panasyuk, A. Ya. Fridenshtein, and N. N. Kulagina

UDC 612.419.014.482-085.23

The radiosensitivity of guinea-pig bone marrow cells forming colonies consisting of fibroblast clones in monolayer cultures was determined. The cells were irradiated in suspension in vitro before explantation. Colony formation was suppressed virtually completely after irradiation in doses of 600 R or more. Suppression of colony formation in the suspensions after irradiation depended on its action on the colony-forming cell and not on the bone marrow cells which play the role of natural "feeder cells" in the cultures. The mean lethal dose or 37% dose $D_0 = 178 \pm 15$ R and the extrapolation number $n = 1.44$.

Colonies consisting of clones of fibroblasts are formed in monolayer cultures of bone marrow cells. The efficiency of cloning is about $1:10^5$ for guinea-pig and human bone marrow [3-5]. Growth of fibroblasts in the form of isolated colonies evidently takes place on account of the considerable dissociation of the colony-forming cells and because of the presence of large numbers of cells in the bone marrow which do not participate in colony formation but which condition the medium (natural feeder cells). Colony-forming cells can maintain themselves for a long time, which follows from the high proliferative pool in the colonies [1, 3] and the possibility of repeated subculture with retention of the diploid set of chromosomes [2, 3]. Transplantation of fibroblasts from bone marrow cultures to in vivo conditions in a diffusion chamber demonstrates their osteogenic potential [4-6, 8], i.e., shows that they are stromal cells, precursors of bone marrow. The nature of these cells requires further study.

This paper describes the results of a study of the radiosensitivity of colony-forming cells of guinea-pig and human bone marrow after irradiation in vitro.

EXPERIMENTAL METHOD

Bone marrow cells were washed out with medium no. 199 from the femora of adult guinea-pigs and from the resected ribs of hematologically healthy adult persons. The cell suspensions were pipetted and filtered through four layers of Kapron, after which their concentration was adjusted to 1×10^7-2×10^7 cells/ml. Each cell suspension was then poured into several tubes, one of which was used as the control while the rest were irradiated at 4°C from a cobalt source with a dose rate of 166 rad/min.

The irradiated and control suspensions were explanted in equal quantities and in identical volumes of medium in Roux flasks. In some experiments mixtures of irradiated and unirradiated cells used was too small to allow the formation of fibroblast colonies on explantation in flasks with a standard surface. The medium either was changed once, 48 h after explantation, or was unchanged throughout the period of cultivation. On the 8th-12th day the cultures were fixed with 96° alcohol, stained with azure-eosin, and the number of colonies of fibroblasts was counted under a binocular loupe.

*Deceased.

Laboratory of Immunomorphology, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 10, pp. 94-97, October, 1972. Original article submitted January 17, 1972.

TABLE 1. Effect of Irradiation on Number of Colonies Growing in Cultures of Guinea-pig Bone Marrow

	Dose of irradiation (in R)	Number of explanted cells	No. of growing colonies	Mean ECF per 10^5 cells
Explantation into 250-ml flasks with base area about 78 cm^2	—	$1,04 \times 10^7$	129; 132	
			166; 157	1,4
	150	$1,04 \times 10^7$	103; 149	1,2
	300	$1,04 \times 10^7$	26; 35	0,29
	600	$1,04 \times 10^7$	7; 10	0,08
Explantation into 250-ml flasks with base area about 78 cm^2	—	1×10^7	128; 83	1,06
	150	1×10^7	74; 58	0,66
	300	1×10^7	36; 37	0,37
	400	1×10^7	13; 20	0,17
	500	1×10^7	7; 7	0,07
	600	1×10^7	1; 3; 0; 2; 0	0,01
Explantation into 50-ml flasks with base area about 28 cm^2	—	$2,5 \times 10^6$	102; 93	3,91
	100	$2,5 \times 10^6$	60; 67; 83	2,81
	200	$2,5 \times 10^6$	54; 38	1,84
	450	$2,5 \times 10^6$	7; 12; 13	0,43
Explantation into 50-ml flasks with base area about 28 cm^2	—	$2,6 \times 10^6$	48; 30	1,42
	300	$2,6 \times 10^6$	6; 15	0,4
	600	$2,6 \times 10^6$	1; 4	0,08
	1200	$2,6 \times 10^6$	0; 0	—
Explantation into 50-ml flasks with base area about 28 cm^2	—	$1,3 \times 10^6$	20; 21; 16	1,46
	Control + 600	$1,3 \times 10^6$ $2,6 \times 10^6$	21; 24; 33	0,67

TABLE 2. Effect of Irradiation on Number of Colonies of Fibroblasts Growing in Cultures of Human Bone Marrow

	Dose of irradiation (in R)	No. of explanted cells	No. of growing colonies	Mean ECF per 10^5 cells
Explantation into 50-ml flasks with base area about 28 cm^2	—	$3,4 \times 10^6$	124; 145	3,95
	600	$3,4 \times 10^6$	0; 0	—
	1200	$3,4 \times 10^6$	0, 0	—
	2400	$3,4 \times 10^6$	0; 0	—
Explantation into 50-ml flasks with base area about 28 cm^2	—	3×10^6	23; 8	0,53
	150	3×10^6	15; 5	0,33
	300	3×10^6	2; 0	0,04
	600	3×10^6	0; 0	—

The proportion of surviving colony-forming cells after irradiation in each particular dose was determined by comparing the number of colonies growing in the control and irradiated cultures. The survival curve was plotted and from it the dose required to reduce the surviving fraction to 0.37 on the exponential part of the curve (D_0 or D_{37}) and the extrapolation number (n) for the colony-forming cells of guinea-pig bone marrow were obtained from it.

EXPERIMENTAL RESULTS

The morphology of the colonies growing in the irradiated and control cell suspension was similar on the whole and has been described previously [5]. Meanwhile, the number of colonies formed in the cultures of irradiated cells decreased progressively with an increase in the dose of irradiation. The results for guinea-pig bone marrow are given in Table 1 and those for human bone marrow in Table 2.

It will be noted that the efficiency of colony formation (ECF) of the unirradiated bone marrow from individual guinea-pigs varied from 1.06 to 3.91 per 10^5 explanted cells, while the ECF of the human marrow varied from 0.53 to 3.95. The effect of irradiation on the ECF was approximately the same in cultures of

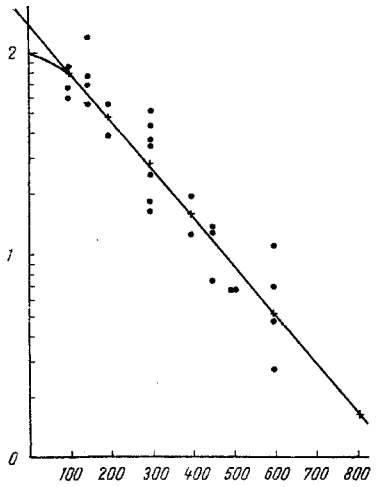

Fig. 1. Curve of survival of colony-forming guinea-pig bone marrow cells on irradiation in vitro. Abscissa, dose of irradiation (in R); ordinate, log of percentage of surviving colony-forming units. $D_0 = 178 \pm 15$ R; $n = 1.44$.

guinea-pig and human bone marrow: virtually complete suppression of colony formation was observed after irradiation in doses of 600 R or more; smaller doses led to partial suppression.

When bone marrow irradiated in a dose of 600 R was mixed with unirradiated bone marrow the number of colonies which grew was approximately the same as that expected from the unirradiated part of the suspension. The expected ECF was 0.56 per 10^5 explanted cells, while its actual value in the experiment was 0.67.

The curve of survival of the colony-forming cells is plotted in Fig. 1.

The fact that after explantation of a mixture of bone marrow cells irradiated in a dose of 600 R and of unirradiated cells the ECF of the mixture corresponded to the ECF of the unirradiated cells shows that suppression of colony formation in the irradiated suspensions depends on the action of irradiation on the colony-forming cells and not on the bone marrow cells which play the role of feeders.

The degree of inhibition of colony formation in irradiated suspensions of bone marrow cells is thus an index of the radiosensitivity of the precursor cells of the fibroblasts with respect to their ability to form colonies in vitro.

It is estimated from the results obtained that for colony-forming guinea-pig bone marrow cells $D_0 = 178 \pm 15$ R and $n = 1.44$; the radiosensitivity of the colony-forming cells on human bone marrow is evidently similar.

It is noteworthy that the radiosensitivity of the hematopoietic stem cells of mouse bone marrow, which form hematopoietic colonies in the spleen of irradiated recipients ($D_0 = 108$ R; $n = 1.34$) [7], differs from the radiosensitivity obtained in the present experiments for bone marrow cells forming colonies of fibroblasts, and belonging to the category of stromal precursor cells. This could reflect qualitative differences between cells of these two categories and the relatively lower radiosensitivity of the stromal cells.

LITERATURE CITED

1. I. V. Keilis-Borok et al., Tsitologiya, 11, 1402 (1971).
2. É. D. Miskarova et al., Byull. Éksperim. Biol. i Med., No. 9, 78 (1970).
3. A. F. Panasyuk et al., Probl. Gematol., No. 8 (1972).
4. A. Ya. Fridenshtein et al., Tsitologiya, 12, 1147 (1970).
5. R. K. Chailakhyan, A. Ya. Fridenshtein, et al., Tsitologiya, 12, 1147 (1970).
6. R. K. Chailakhyan, A. Ya. Fridenshtein, A. V. Vasil'ev, et al., Byull. Éksperim. Biol. i Med., No. 2, 94 (1970).
7. R. K. Chailakhyan and K. S. Lalykina, Dokl. Akad. Nauk SSSR, 187, No. 2, 473 (1969).
8. E. A. McCulloch et al., Radiat. Res., 16, 822 (1962).
9. A. J. Friedenstein et al., Cell Tissue Kinet., 3, 393 (1970).