RADIOSENSITIVITY AND DIRECTION OF DIFFERENTIATION OF COLONY-FORMING CELLS IN THE SPLEEN

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The radiosensitivity of splenic colony-forming units (CFUs) settling in the bone marrow and spleen does not differ significantly. The value of D_0 for CFUs forming colonies in the spleen varies between 105 and 120 R. D_0 for CFUs forming colonies in bone marrow is 120-135 R. It is postulated that two fractions for CFUs are represented in bone marrow, one radiosensitive, the other radioresistant. In the spleen mainly the radiosensitive fraction of CFUs is found.

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

Since Till and McCulloch [5] used the method "splenic colonies" to study the kinetics and properties of hematopoietic stem cells, the radiosensitivity of these cells has been a frequent subject of study. It is generally accepted that the value of D_0 for colony-forming units (CFUs) of the bone marrow and spleen varies between 80 and 120 R. These figures were obtained by counting colonies distinguishable macroscopically in spleens. In a previous investigation [2] the writer showed that the principles governing cloning of bone marrow stem cells in the femur and spleen are similar. The number of colonies growing in the bone marrow or spleen is a linear function of the number of cells injected and an exponential function of the dose of irradiation. The value of D_0 for bone marrow CFUs forming colonies in the spleen or femur differs significantly. CFUs settling in bone marrow are more radioresistant (D_0 = 160-200 R) than CFUs settling in the spleen. Heterogeneity of the population of bone marrow stem cells differing in their sensitivity to radiation has been postulated. If it is assumed that the bone marrow contains two fractions of stem cells, one of which (radiosensitive) settles in the spleen whereas the other (radioresistant) settles in the marrow, it ought therefore to follow that the spleen contains mainly the radiosensitive fraction of stem cells. Consequently, splenic CFUs ought evidently to have equal radiosensitivity regardless of the organ (spleen or bone marrow) which they colonize.

The radiosensitivity and direction of differentiation of splenic CFUs settling in bone marrow and spleen were studied.

EXPERIMENTAL METHOD

Female (CBA × C57BL)F_1 mice or a ready-made suspension of their spleen cells were irradiated with Cs^{137} γ rays in doses of between 100 and 600 R (dose rate 37 R/min). Immediately after irradiation the spleen was removed from the donors and a suspension of its cells prepared and injected intravenously into irradiated (900 R) mice of the same strain. On the eighth day after transfusion of the cells the recipients' spleens and femurs were removed. The method of treatment of the material was described previously [2].

EXPERIMENTAL RESULTS

It will be clear from Table 1 that after transplantation of spleen cells irradiated in vivo or in vitro the number of colonies in the femur decreased exponentially with an increase in the dose of irradiation. The character of differentiation of the transplanted intact or irradiated cells in the medullary cavity was similar to that in the spleen. The ratio between the number of colonies of erythroid and myeloid types was

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TABLE 1. Number of Colonies of Different Types in Femur of Mice after Transplantation of Spleen Cells Irradiated in vivo or in vitro (M ± m)

Conditions of irradiation	Dose of irradiation (in R)	Number of cells injected ($\times 10^7$)	Type of hematopoietic colonies			Mean number of colonies per femur
			erythroid	myeloid	megakaryocytic	
In vivo	0	0,1	18,0±1,0	12,0±0,6	10,0±0,4	40,0±3,0 (15)
	100	0,5	7,0±0,5	5,2±0,4	4,3±0,3	16,5±1,0 (15)
	200	0,5	3,2±0,3	2,0±0,2	1,8±0,1	7,0±0,6 (15)
	300	1,0	1,7±0,1	1,2±0,1	0,6±0,05	3,5±0,3 (15)
	400	1,5	1,0±0,02	0,7±0,01	0,3±0,01	2,0±0,1 (14)
	500	3,0	0,4±0,01	0,25±0,01	0,2±0,01	0,85±0,05 (14)
	600	3,0	0,18±0,01	0,12±0,01	0,1±0,01	0,4±0,02 (14)
In vitro	0	0,1	16,0±2,0	14,0±2,0	7,0±0,8	37,0±4,0 (17)
	100	0,2	6,9±0,5	4,4±0,5	2,5±0,2	13,8±0,7 (19)
	200	0,5	3,5±0,4	3,5±0,4	1,1±0,1	8,1±0,6 (15)
	300	1,0	1,2±0,1	1,0±0,1	0,5±0,05	2,7±0,1 (15)
	400	2,0	0,27±0,03	0,23±0,02	0,17±0,01	0,67±0,05 (15)
	500	5,0	0,42±0,05	0,32±0,04	0,2±0,02	0,94±0,05 (11)

Legend: 1) number of recipients in parentheses. Number of colonies calculated per 10^7 transplanted cells; 2) combined results of two experiments.

TABLE 2. Radiosensitivity of Splenic CFUs Forming Colonies in the Femoral Marrow and Spleen (M ± m)

Organ	Conditions of irradiation of transplanted spleen cells	D_0 and extrapolation number (n)				
		erythroid colonies	myeloid colonies	megakaryocytic colonies	total number of colonies in organ	number of macroscopically visible colonies in spleen
Spleen	In vivo	110±10 n=1,0	109±10 n=0,93	117±12 n=0,9	110±10 n=0,98	108±8 n=1,1
	In vitro	105±10 n=0,93	105±9 n=0,92	108±15 n=0,9	106±10 n=1,0	96±7 n=1,0
Bone marrow	In vivo	134±6 n=0,97	133±7 n=0,98	128±6 n=0,97	133±8 n=0,97	—
	In vitro	122±9 n=0,94	122±9 n=0,92	134±13 n=0,91	125±10 n=0,94	—

1:1.6 in the marrow and 1:3 in the spleen. The dimensions of the colonies of erythroid, myeloid, and megakaryocytic types in the bone marrow were not significantly changed by the action of radiation and their mean diameter was 0.4-0.6, 0.25-0.4, and 0.15-0.25 mm, respectively. The mean diameter of all types of colonies in the spleen decreased significantly with an increase in the dose of irradiation. The results of these experiments show that after transplantation of spleen cells they settle in the spleen and bone marrow where they form colonies mainly of erythroid type. The dimensions of the erythroid colonies in both organs are greater than those of the colonies of other types of cells.

Data on the radiosensitivity of the spleen cells forming colonies in the bone marrow and spleen are given in Table 2.

The values of D_0 for CFUs forming colonies in bone marrow and for CFUs settling in the spleen do not differ significantly. The difference was 10-25 R. The extrapolation number varied within limits of 1 ± 0.1 regardless of the organ in which the splenic CFUs proliferated. The results of the previous [2] and present experiments thus suggest that two populations of stem cells exist in the bone marrow, one of them radiosensitive, the other radioresistant. The population located in the spleen consists chiefly of radiosensitive cells.

However, this conclusion must be regarded as purely conventional, for when the radiosensitivity of stem cells is determined several factors must be taken into account, including the efficiency of cloning, the specific effect of the organ itself, and the value of the index of colonization (f):

$$f = \frac{N_2}{N_1} \times 100\%$$

where N_2 is the number of CFUs settling in the organ, and N_1 the number of CFUs injected. As the results in Table 2 show, the probability of a difference between the values of D_0 for CFUs settling in the spleen and bone marrow varies within the range of significance $0.5 < P \geq 0.05$, i.e., in certain cases it is significant; in the writer's view, this may evidently point to an effect of these factors on the determination of radiosensitivity of the cells.

The view that two populations of CFUs exist is also supported by the character of differentiation of CFUs in the spleen and bone marrow. It must be emphasized that the potential capability of splenic CFUs of differentiating into cells of the three basic types of hematopoiesis is the same both in the spleen and in the bone marrow: In these organs colonies of erythroid type predominate. Bone marrow CFUs form colonies mainly of erythroid type in the spleen and mainly of myeloid type in the bone marrow [2]. The choice of direction of differentiation of the stem cells is evidently controlled not only by the stroma (microenvironment) of the organ in which these cells proliferate [6, 7], but also by various other factors embodied in the cells themselves. In recent years new evidence has been obtained in support of the view that the realization of the potential ability of stem cells to differentiate takes place through cooperation between stem cells and lymphocytes [1]. In the light of what has been said above it may be postulated that the similarity of differentiation of splenic CFUs in different regions of hematopoietic tissue is the result of interaction between the CFUs and splenic lymphocytes.

How important an effect parameters such as cloning efficiency and the value of f may have on the determination of radiosensitivity of the CFUs is not yet known. Nevertheless, it can be concluded that splenic and bone marrow CFUs differ not only in the character of their differentiation and in their sensitivity to radiation, but also in certain other features [3, 4]. The final conclusion regarding the causes of these differences in radiosensitivity and in the direction of differentiation of CFUs of different sources must await further experiments.

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