DISTRIBUTION OF COLONY-FORMING CELLS IN THE BONE MARROW AND SPLEEN OF IRRADIATED MICE

V. N. Shvets

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Two hours after transplantation of 2×10^5 bone marrow cells, 1.7-2.8% and 14-16% of colony-forming units (CFU) were retained respectively in the femoral bone marrow and spleen of the recipients. After 24 h, 1-2% and 21-26% of CFU respectively remained in these organs. If 2.5×10^6-1.7×10^7 bone marrow cells were transplanted, the fraction f in the bone marrow and spleen of the recipients (under saturation conditions) was unchanged during the 24-h period. The value for CFU settling in the spleen was 20-22%, whereas for CFU settling in the femoral marrow the value of f was 2.4-2.7%. It is postulated that the same number of CFU settles in the whole volume of the bone marrow as in the whole spleen. The total number of CFU settling in the spleen and in the whole volume of the bone marrow averages 40-50%. The fate of the remaining CFU is unknown.

KEY WORDS: fraction f; bone marrow; spleen; stem cells.

Bone marrow cells contain colony-forming units (CFU) which, if transplanted into lethally irradiated recipients, settle in the spleen, proliferate, and form cell colonies [10]. Fraction f of the injected CFU, which settles in the recipients' spleen, can be measured by the retransplantation method [9].

Several workers [5, 7-9] have shown that 17-25% of CFU of the total number transfused are retained in the spleen. By the method of "spleen" colonies it is possible to determine the value of f for CFU settling in the spleen. However, the value of f in other regions of hematopoietic tissue, such as in bone marrow, has so far received little study.

The object of this investigation was to determine the value of f for CFU settling in the femoral bone marrow and spleen after transplantation of different numbers of bone marrow cells and in relation to the time between injection and retransplantation of the cells.

EXPERIMENTAL METHOD

The method of isolating fraction f of the injected CFU, which settles in the spleen and produces colonies, was introduced by Siminovitch et al. [9]. The method includes retransplantation of spleen or bone marrow cells of the primary recipient, into which the test cells were injected, into the secondary, irradiated recipient. The number of cell colonies in the spleen or bone marrow of the secondary recipient is counted on the 9th day. The fraction f can be represented as follows:

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

i.e., as the ratio between the number of CFU (N_2) settling in the organ of the secondary recipient and actually forming colonies, and the number of CFU (N_1) found in the primary transfusion material and taken as 100%. A review of examples of calculation of the value of f is given in [9]. The value of f was determined by two methods, based on the fact that during transplantation of the test cells they settle in the spleen and in the medullary cavity of the femur, where they form cell colonies. Recording colonies in the spleen or in the femur has been named the method of "splenic" and of "bone-marrow" colonies, respectively.

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TABLE 1. Value of f for CFU of Bone Marrow Settling in Medullary Cavity of Femur (in %)

Number of bone marrow cells transplanted into 1st recipient	Time (in h) of retransplantation of bone marrow cells into 2nd recipient	Fraction f	
		method of "splenic colonies"	method of "bone marrow colonies"
2×10^5	2	2,6	4,0
2×10^5	2	2,2	3,5
2×10^5	24	1,0	2,0
2×10^5	24	1,0	2,0
$2,5\times10^6$	2	1,7	2,4
$1,7\times10^7$	2	2,4	2,5
$3,25\times10^6$	24	1,3	2,7
$1,7\times10^7$	24	2,0	2,4

TABLE 2. Value of f for CFU of Bone Marrow Settling in Spleen (the "splenic" colonies method)

Number of bone marrow cells transplanted into 1st recipient	Time (in h) of retransplantation of bone marrow cells into 2nd recipient	Fraction f (in %)
2×10^5	2	16
$2\times.0^5$	2	15
2×10^5	4	14
2×10^5	6	24
2×10^5	24	22
2×10^5	24	21
$2,5\times10^6$	2	22
$4,5\times10^6$	2	20
$1,7\times10^7$	2	21
$3,25\times10^6$	24	21
$1,7\times10^7$	24	22

In the present experiments the value of f was determined by a modification of the method suggested by Siminovitch et al. [9]. Different numbers of isologous bone marrow cells of intact animals were injected intravenously into lethally irradiated (1000 R, ^{137}Cs γ-rays, dose rate 37 R/min) (CBA × C57BL) F_1 mice 24 h after irradiation. The spleen and femoral marrow were removed from the (intermediate) recipients 2-24 h after injection of the cells. A known number of cells was injected intravenously into the second (final) recipient, previously irradiated in a dose of 950 R, so as to determine the number of colonies formed in the spleen and bone marrow on the 9th day. The method of histological analysis of the material was described earlier [3].

EXPERIMENTAL RESULTS

Values of f for CFU settling in the bone marrow are given in Table 1. Clearly 2 h after transplantation of 2×10^5 bone marrow cells, 2.2-2.6% of CFU discovered by the method of "splenic" colonies and 3.5-4% tested by the method of counting colonies in the femoral marrow had settled in the recipients' femur. The number of CFUs found by both methods 24 h after transplantation of the same number of cells into the femur was 1-2%. Conversely, during the first 2-4 h 14-16% of the total number of CFU injected was retained in the recipients' spleen. After 6 h and until the end of the 1st day the number of CFU settling in that organ increased to 21-26% (Table 2).

During the first few hours after injection of a small number of CFU, most of these cells enter the bone marrow and fewer the spleen. Later the number of CFU in the marrow decreases, whereas the number in the spleen increases. This distribution of the number of CFU in different parts of the hematopoietic tissue is evidently determined by their migration. The effect of repopulation of stem cells from the bone marrow and spleen is well documented [1, 4]. A somewhat different character of distribution of CFU in different parts of the hematopoietic tissue was found after transplantation of 2.5×10^6-1.7×10^7 cells. In that case the value of f was unchanged during the 24 h in both the bone marrow and the spleen (Tables 1 and 2); this result evidently depended on the saturation effect.

The experimental results show that only one tenth as many CFU settle in the femoral marrow of lethally irradiated primary recipients as in the spleen. Remembering that the volume of the femoral marrow is 10% of the total volume of marrow in the body, this means that the same number of CFU settles in the whole marrow as in the whole spleen. The total number of CFU settling in the spleen and in the whole volume of the bone marrow averages 40-50%. The fate of the remaining CFU is unknown. After transplantation of a smaller number of cells (2×10^5) the distribution of CFU in different parts of the hematopoietic tissue is unequal, whereas after injection of a large number of cells these differences are smoothed out because of the rapid saturation of the body with stem cells.

Several workers have shown that after transplantation of 2×10^6 to 2×10^7 bone marrow cells the value of f for CFU settling in the spleen 2 h after injection of the cells is 0.13-0.25 [5-9, 11], with a mean value of 0.2 (20%). The value of f obtained in the present experiments with transplantation of the same numbers of bone marrow cells was indistinguishable from the figures given by the authors cited above: The mean value of f was 0.21 (21%). The value of f for CFU settling in the whole volume of bone marrow did not differ significantly from that for CFU settling in the spleen. The available data indicate the absence of affinity of CFU for either organ and they are in agreement with the results obtained by other workers [2]. At the same time, it must be pointed out that after transplantation of a small number of cells organ-specific affinity of the CFU is exhibited, but it evidently cannot be detected under saturation conditions.

LITERATURE CITED

1. R. M. Khaitov, "Experimental analysis of practically important problems in transplantation of hematopoietic tissues into irradiated recipients," Author's Abstract of Doctoral Dissertation, Tashkent (1972).
2. I. L. Chertkov, L. N. Lemenova, and O. A. Mendelevich, Probl. Gematol., No. 2, 37 (1972).
3. V. N. Shvets, Byull. Éksp. Biol. Med., No. 5, 40 (1975).
4. D. R. Boggs and P. A. Chervenick, Transplantation, 11, 191 (1971).
5. S. S. Fred and W. W. Smith, Proc. Soc. Exp. Biol. (New York), 128, 364 (1968).
6. Y. Gidali, I. Feher, and S. Antal, Blood, 43, 573 (1974).
7. S. K. Lahiri and L. M. Putten, Cell Tissue Kinet., 2, 21 (1969).
8. J. Schooley, J. Cell. Physiol., 68, 249 (1966).
9. L. Siminovich, E. A. McCulloch, and J. E. Till, J. Cell Comp. Physiol., 62, 327 (1963).
10. J. E. Till and E. A. McCulloch, Radiat. Res., 14, 213 (1961).
11. J. E. Till and E. A. McCulloch, Ser. Haemat., 5, 15 (1972).