PROLIFERATION AND DIFFERENTIATION OF HEMATOPOIETIC STEM CELLS DURING HYPOKINESIA

V. V. Portugalov* and V. N. Shvets

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Exogenous cloning of hematopoietic stem cells of bone marrow and spleen in the femur and spleen of recipient mice showed that during hypokinesia the kinetics of the stem cell population differs in the two organs (spleen and bone marrow). The character of differentiation of the transplantation stem cells from the different sources was undisturbed in the recipients' spleen. Bone marrow stem cells, settling in the femur, changed their character of differentiation toward an increase in erythropoietic function, whereas the direction of differentiation of the splenic stem cells was unchanged.

KEY WORDS: hematopoietic stem cells; hypokinesia; bone marrow; spleen.

A previous investigation showed that in rats with limited movements besides a decrease in their body weight, the number of cells in the hematopoietic organs also decreased [7]. To study the kinetics of the population and the direction of differentiation of the hematopoietic stem cells in mice during hypokinesia, a method [10] based on the ability of hematopoietic cells when transplanted into lethally irradiated recipients to form colonies in the spleen [10, 11] and bone marrow [8, 12] was used.

EXPERIMENTAL METHOD

Female (CBA × C57BL) F_1 mice weighing 20-22 g were kept in special small containers. On the first, third, seventh, 15th, 30th, and 45th days of hypokinesia the femoral bone marrow and spleen were removed from the mice. Cell suspensions were prepared from them and injected intravenously into the same mice after irradiation in a dose of 900 R with Cs^{137} γ rays (dose rate 37 R/min). On the ninth day after transplantation of the cell suspension the spleen and femora of the recipients were removed. The material was fixed in Bouin's fluid and embedded in paraffin wax. The number of colonies was counted in sections cut at intervals of 50 μ and stained with hematoxylin-eosin. Colonies were identified as erythroid, myeloid, megakaryocytic, and mixed.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that after transplantation of bone marrow cells from mice after a period of hypokinesia, colonies of erythroid, myeloid, megakaryocytic, and mixed types formed in the femoral marrow of the recipients. The ratio between the numbers of erythroid and myeloid colonies (E/M) was increased up to 2, whereas after transplantation of bone marrow cells of intact animals the value of E/M was below 1. When spleen cells of experimental mice were transplanted into the femoral marrow of recipients, the same types of colonies were formed (Table 2) as after transplantation of bone marrow cells. However, the E/M ratio was the same for colony-forming units (CFU) in the spleen of the experimental mice as for the control animals. The kinetics of the CFU population from different sources (bone marrow or spleen), settling and proliferating in the femora of the recipients, was the same in both intact and experimental mice at all times of investigation.

*Corresponding Member, Academy of Medical Sciences of the USSR.

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TABLE 1. Character of Colonies in Femoral Marrow of Irradiated Mice Receiving $5 \cdot 10^6$ Bone Marrow Cells from "Hypokinetic" Mice (M ± m)

Day of expt.	No. of recipients	Type of hematopoietic colonies				E/M	Mean number of colonies per femur
		E	M	Mk	mixed		
0	20	1,3±0,2	2,0±0,3	1,3±0,2	0,1±0,1	0,6	4,7±0,6
1	10	1,5±0,1	1,0±0,2	0,2±0,1	0,3±0,1	1,5	3,0±0,4
3	11	2,2±0,2	1,6±0,2	1,6±0,2	0	1,4	5,4±0,5
7	9	2,0±0,1	1,0±0,1	0,1±0,1	0	2,0	3,1±0,2
15	10	1,6±0,2	1,0±0,1	0,4±0,1	0	1,6	3,0±0,3
30	12	1,7±0,3	1,4±0,2	1,1±0,2	0,1±0,1	1,2	4,3±0,3
45	13	1,8±0,1	1,4±0,2	1,0±0,1	0,2±0,1	1,3	4,4±0,4

Legend. Here and in Table 2: E) erythroid, M) myeloid, MK) megakaryocytic.

TABLE 2. Character of Colonies in Femoral Marrow of Irradiated Mice Receiving 10^6 Spleen Cells from "Hypokinetic" Mice (M ± m)

Day of expt.	No. of recipients	Type of hematopoietic colonies				E/M	Mean number of colonies per femur
		E	M	Mk	mixed		
0	25	1,2±0,1	0,8±0,1	1,1±0,1	0,2±0,06	1,5	3,3±0,2
1	10	2,4±0,3	1,2±0,2	1,0±0,1	0,4±0,1	2,0	5,0±0,5
3	11	2,3±0,2	1,4±0,2	1,0±0,2	0,1±0,1	1,6	4,8±0,3
7	9	2,6±0,3	1,3±0,3	0,8±0,1	0	2,0	4,7±0,4
15	10	2,0±0,2	1,1±0,2	0,9±0,2	0	1,8	4,0±0,3
30	12	2,4±0,2	1,3±0,1	1,2±0,2	0,2±0,1	1,7	5,1±0,4
45	13	1,8±0,1	1,1±0,2	1,0±0,1	0,1±0,1	1,6	4,0±0,3

In mice with restricted movements the ability of hematopoietic stem cells isolated from different sources to form colonies in the femoral marrow was thus undisturbed. The character of differentiation was changed only for CFU of the bone marrow, whereas the direction of differentiation of the splenic CFU of the experimental mice remained unchanged. Somewhat different results were obtained for CFU of the marrow and spleen forming colonies in the recipients' spleen. In experiments carried out at all times of hypokinesia, the CFU (from the different sources) settling in the recipients' spleen retained their powers of differentiation. Changes in the number of CFU (the "splenic colonies" test) in the bone marrow of the experimental mice were fluctuating in character: The number of CFU reached a maximum on the first to third and 30th-45th days of hypokinesia, whereas the number of CFU in the spleen decreased exponentially.

Many workers [4, 5] regard limitation of movement as a special kind of stress. One indication of the development of a stress response is atrophy of the thymicolymphatic apparatus [6]. The decrease in the number of lymphocytes was due to two causes: death of the cells and inhibition of their reproduction [6], and migration of cells into other organs, especially into bone marrow [3]. In other words, during stress the permeability (barrier function) of bone marrow for lymphocytes is reduced. In this connection it can be assumed that the test suspension of bone marrow cells from the experimental animals with stress differed from the suspension of bone marrow cells from the control animals in that it contained lymphocytes of thymus origin (T lymphocytes). This suggests that the change in direction of differentiation of the bone marrow CFU settling in the femur was due to the arrival of T lymphocytes in the bone marrow of the experimental mice. Indirect evidence in support of this view is given by the fact that after transplantation of spleen cells, these proliferated in the recipients' bone marrow, where they formed colonies chiefly of erythroid type. The mouse spleen is known to contain about 35% of T lymphocytes [9]. Direct proof of the influence of T lymphocytes on the choice of direction of differentiation of the stem cells is given by the writers' unpublished data, obtained after transplantation of a mixture of syngeneic bone marrow cells and thymocytes (F_1 b.m. + F_1 thy → F_1). Such a mixture apparently simulates the population of spleen cells. After transplantation of a mixture of syngeneic bone marrow cells and thymocytes, colonies chiefly of erythroid type are formed in the bone marrow of the irradiated recipients, i.e., differentiation of stem cells of bone marrow origin under the influence of T lymphocytes is of the "splenic type." In this case the E/M ratio in the spleen was unchanged by transplantation of bone marrow cells, either alone or mixed with thymocytes.

It can accordingly be concluded from these results that during restriction of movement, evidently as a result of a redistribution of lymphocytes and, possibly, of stem cells, the relations between these two

types of cells are such that they lead to a change in the character of differentiation of the stem cells and in their population in different hematopoietic organs.

The biological significance of this phenomenon is evidently that, because of the deficiency of muscular function and the accompanying reduced demand of oxygen by the skeletal muscles, the surface of the erythron is reduced through inhibition of the erythroplastic function of the bone marrow [1, 2] and, possibly, hemolysis of erythrocytes. The role of the T lymphocytes coupled with the unchanged hematopoiesis may be to compensate for the deficiency of precursors of erythropoiesis and to increase the nonspecific resistance of the organism.

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