AN INCREASED NUMBER OF HEMATOPOIETIC STEM CELLS IN MICE IN THE INITIAL PERIOD OF THE STRESS REACTION

Yu. I. Zimin

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A nonspecific response of the bone marrow, consisting of an increase in the number of lymphocytes and a decrease in the number of mature granulocytes, takes place in F_1(CBA × C57BL) mice in a state of stress. In the bone marrow 9-12 h after the beginning of the stress reaction to immobilization for 3 h the number of cells capable of forming colonies in the spleen when transplanted into lethally irradiated recipients is increased by 45-50%. This increase is interpreted as the result of division of stem cells as a result of a stress-induced change in the microstructure of the bone marrow.

KEY WORDS: bone marrow; hematopoietic cells; stress.

Changes in the cell composition of the circulating blood [4, 10, 11], the bone marrow [2, 4, 5], and lymphoid tissues [6, 7] arising in the early period of the stress reaction are evidently a source of information leading to the activation of compensatory mechanisms affecting the hematopoietic stem cells.

Of all the methods that can be used to study stem cells, the most popular is that of Till and McCulloch [17], which, with some qualifications, gives some idea of the number of these cells in the hematopoietic tissue.

The object of this investigation was to study the number of colony-forming cells in the bone marrow in the initial period of the stress reaction.

EXPERIMENTAL

F_1(CBA × C57BL) hybrid mice weighing 25 g were obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR. To produce a state of stress the mice were immobilized on their backs for 3 h between 10 a.m. and 1 p.m. The number of colony-forming cells [17] in a mixture of bone marrow cells from 3-6 animals was determined 3, 6, 9, 12, and 24 h after the beginning of exposure. For this purpose, $1 \cdot 10^5$ viable cells from the femoral marrow were transplanted into lethally irradiated (900 R) syngeneic mice. The recipients were irradiated with Cs^{135} on a Gamma Cell apparatus with a dose rate of 710 R/min. On the 9th day after transplantation the number of colonies in the spleen was counted visually.

Parallel with the preparation of cell suspensions, the number of nucleated cells in the femur of the same animals was determined [4]. An idea of the absolute number of cells in the femur and, consequently, in the graft was obtained from an examination of the myelogram obtained from squash preparations.

The numerical results were subjected to statistical analysis using Student's parametric criterion.

RESULTS

During the stress reaction to immobilization in mice, a stereotyped nonspecific response of the bone marrow as described by the writer previously [1, 3, 5] was observed – an increase in the number of lymphocytes accompanied by a decrease in the number of mature cells of the myeloid series (Table 1).

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TABLE 1. Number of Myelokaryocytes ($\times 10^6$) in the Femur during 12 h after the Beginning of Immobilization for 3 h (M ± m)

Type of cell	Control	Time after beginning of exposure			
		3 h	6h	9h	12h
Lymphocytes	7,7±0,65	9,8±0,23*	10,0±0,64*	10,3±0,88*	8,7±0,56
	(8)	(6)	(6)	(6)	(3)
Polymorphs	6,4±0,41	4,1±0,21*	3,4±0,36*	4,6±0,21*	4,2±0,78

Note: Number of animals shown in parentheses. Values differing significantly from the control ($P \leq 0.05$) are marked by an asterisk.

TABLE 2. Number of Colonies in Spleen of Lethally Irradiated Mice after Transplantation of $1 \cdot 10^5$ Bone Marrow Cells Taken at Different Times after the Beginning of Immobilization for 3 h from Syngeneic Donors (M ± m)

	Control	Time after beginining of exposure				
		3 h	6 h	9h	12h	24 h
Number of colonies	14,2±0,89	15,8±0,67	16,4±0,95	21,3±1,15	20,6±2,29	15,0±1,44
Number of recipients	9	11	5	10	5	6
P				<0,05	<0,05	

The total number of cells in the femur remained unchanged. By the mechanism of its onset this reaction was regarded as migration of the lymphocytes into the bone marrow from extramedullary sources and mobilization of the granulocyte reserve of the bone marrow [4, 5]. Parallel with and, in particular, after these changes (24-72 h later), there was a sharp increase in the number of blast cells of the myeloid series [4, 5].

The authors of some recent publications [12,16] state that the group of stem cells is relatively isolated from the regulatory influences of the intact organism. Regulation of the rate of proliferation and differentiation takes place at the tissue level. In other words, in the strictly determined process of proliferation and differentiation of the stem cell certain conditions must be observed, of which the most important are cellular cooperation and interaction. It is difficult to imagine that the changes in cell composition mentioned above are not reflected in the microarchitectonics of the bone marrow. Consequently, during the stress reaction the basic conditions exist for the receipt of regulatory stimuli by the stem cell from the surrounding microenvironment.

As Table 2 shows, 9-12 h after the beginning of the stress reaction the number of cells in the bone marrow of the donor mice capable of forming colonies in the spleen of lethally irradiated recipients was increased by 45-50%.

During the stress reaction there is thus an increase in the pool of hematopoietic stem cells, produced by division of stem cells in situ rather than by migration, for their number in the extramedullary sources was 10-100 times smaller [13]. It is difficult at present to say what the stimulus for this division could be, for in these experiments significant changes occurred in two parameters – the number of lymphocytes and the number of mature myeloid cells. According to some workers [8, 9, 14], there is a parallel between the regenerative accumulation of stem cells in the bone marrow and the number of lymphocytes. It was accordingly concluded that the lymphoid population of the bone marrow correlates closely with the stem cell population.

It seems logical to suppose that under these experimental conditions the stem cell receives two stimuli: a stimulus to homoplastic division from the lymphoid cell population and a stimulus to transformation into cells sensitive to neuropoietic hormones as a result of the release of mature myeloid cells. These processes lead ultimately to the activation of myelopoiesis and hyperplasia of the bone marrow [2, 5].

The increase in proliferative activity of the myeloid tissue arising in the stress reaction plays an important role in the increase of the nonspecific resistance of the organism, at least to factors in whose action an important role is played by elements of cellular and humoral defense.

LITERATURE CITED

1. O. I. Belousova and M. I. Fedotova, Radiobiol. Radioter., 9, 343 (1968).
2. P. D. Gorizontov and I. A. Rudakov, Pat. Fiziol., No. 2, 17 (1964).
3. P. D. Gorizontov, Arkh. Pat., No. 9, 3 (1973).
4. Yu. I. Zimin, Pat. Fiziol., No. 3, 36 (1969).
5. Yu. I. Zimin, Byull. Éksperim. Biol. i Med., No. 7, 19 (1969).
6. Yu. I. Zimin, Byull. Éksperim. Biol. i Med., No. 6, 21 (1971).
7. Yu. I. Zimin, Izvest. Akad. Nauk SSSR, Seriya Biol., No. 4, 517 (1973).
8. E. N. Shcherbova et al., Radiobiol. Radioter., 9, 463 (1968).
9. G. Cudkowicz et al., Ann. New York Acad. Sci., 114, 571 (1964).
10. C. Harlow and H. Selye, Proc. Soc. Exp. Biol. (New York), 36, 141 (1937).
11. L. Kumagai and T. Dougherty, Endocrinology, 55, 90 (1954).
12. E. McCulloch and J. Till, Am. J. Path., 65, 601 (1971).
13. H. Micklem et al., Proc. Roy Soc. B., 165, 78 (1966).
14. A. Pisciotta et al., J. Lab. Clin. Med., 63, 445 (1964).
15. L. Siminovitch et al., J. Cell. Physiol., 62, 327 (1963).
16. J. Trentin, Am. J. Path., 65, 621 (1971).
17. J. Till and E. McCulloch, Radiat. Res., 14, 213 (1961).