ROLE OF THE STROMAL MICROENVIRONMENT IN REGULATION OF MEDULLARY HEMATOPOIESIS DURING STRESS

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UDC 616.419-003.971-092:616.419-018.2/-02:613.863

KEY WORDS: microenvironment; stroma; stress; precursor cells.

In the modern view an important role in the regulation of function of hematopoietic precursor cells (HPC) is ascribed to the stromal microenvironment in hematopoietic tissue [4]. The chief components of the parenchyma of hematopoietic organs are fibroblasts which, in conjunction with other stromal cells, form specific territories where the ancestral cells of the blood realize their proliferative and differential potential in accordance with the demands of the body as a whole [2, 6]. The most adequate model for the study of the integrative properties of the hematopoietic microenvironment in vivo is the method of ectopic bone marrow transplantation [7]. Meanwhile, despite definite progress in the study of the role of stromal cells in the regulation of hematopoiesis in vivo and in vitro, the concrete mechanisms lying at the basis of interaction between stroma and HSC in situ (in particular, when the body is exposed to extremal conditions) remain far from clear [4].

The aim of this investigation was to study the role of the stromal microenvironment in the regulation of medullary hematopoiesis during stress.

EXPERIMENTAL METHOD

Experiments were carried out on 500 male (CBA × C57Bl)F_1 mice weighing 18-20 g (from the Rappolovo Nursery, Academy of Medical Sciences of the USSR) and seven male Chinchilla rabbits weighing 2.5-3.3 kg. The mice were immobilized in the supine position for 10 h. At various times after immobilization animals were killed by cervical dislocation. The total number of myelokaryocytes in the bone marrow was determined and the myelogram counted on films. Ectopic bone marrow transplantation was carried out by the method in [7], for which purpose a column of bone marrow from the femur was grafted beneath the renal capsule of intact recipients. Some of the recipients were killed seven days later and the "primary" focus of hematopoiesis which had formed was retransplanted beneath the renal capsule of intact animals. The cell composition and weight of the "primary" and "secondary" (retransplanted) foci of hematopoiesis were determined in all cases on the 30th day after transplantation [4]. The colony- and cluster-forming ability of the bone marrow cells was determined by the diffusion chamber method, as described previously [5], in a plasma clot after incubation for seven days in the peritoneal cavity of mice (24 h before implantation of the chambers, cyclophosphamide was injected intraperitoneally into the recipients in a dose of 200 mg/kg). The optimal concentration of bone marrow cells, chosen as a result of a study of dose dependence between the number of hematopoietic cells transplanted into culture and the number of colonies formed (Fig. 1), was 0.5×10^9/liter of medium of the following composition: 10% embryonic calf serum, 40% medium RPMI-1640, 20% plasma from intact rabbits, 30% Alsever's solution, 290 mg/liter of L-glutamine, and 500 mg/liter $CaCl_2$. Foci of hematopoiesis containing more than 50 cells were classed as colonies, those containing from three to 50 cells as clusters. The qualitative composition of the colonies was determined on thin-layer preparations of plasma clot after staining with azure II–eosin or by Lepen's hemoglobin marker reaction [3]. The numerical results were subjected to statistical analysis by parametric (Student's) and nonparametric (Wilcoxon–Mann–Whitney) tests [1].

EXPERIMENTAL RESULTS

Immobilization for 10 h is accompanied by a significant increase in the number of colony-forming and cluster-forming units (CFU-dc, CLFU-dc) on the 5th-6th day, followed by an increase on the 6th-

Research Institute of Pharmacology, Tomsk Scientific Center, Academy of Medical Sciences of the USSR. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 3, pp. 270-272, March, 1988. Original article submitted May 15, 1987.

TABLE 1. Time Course of Total Number of Myelokaryocytes (THC), CFU-dc, CLFU-dc, Cell Count and Weight of "Primary" and "Secondary" Ectopic Foci of Hematopoiesis from Bone Marrow of (CBA × C57Bl)F_1 Mice Immobilized for 10 h

Time after immobilization, days	THC ($\times 10^6$)	CFU-dc ($\times 10^3$)	CLFU-dc ($\times 10^3$)	Ectopic focus			Weight, mg
				"primary," cell count ($\times 10^6$)	weight, mg	"secondary" cell count ($\times 10^6$)	
Before immobilization	19,2	4,2	91,6	3,6	1,2	2,1	1,0
3	19,9	4,7	98,5	8,6*	0,9	4,5*	1,1
5	19,8	16,4*	186,7*	7,9*	2,0	3,3*	0,5
6	24,8*	8,5*	113,0*	—	—	—	—
7	29,1*	7,0	101,0	3,8	1,1	2,5	0,1*
8	20,5	4,1	92,1	—	—	—	—

*$p < 0.05$.

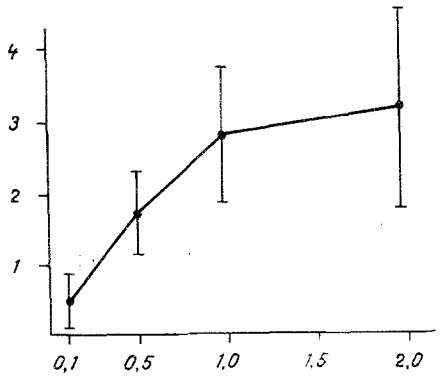

Fig. 1. Dose dependence between number of hematopoietic cells transplanted into tissue culture and number of colonies formed. Abscissa, number of bone marrow cells transplanted into culture (myelokaryocytes, $\times 10^6$); ordinate, number of colonies (per chamber).

7th day in the total number of myelokaryocytes (Table 1). Whereas during culture of bone marrow from intact animals in diffusion chambers, growth of colonies of granulocyte-macrophage type alone is observed (GM-CFU-dc), on cloning of hematopoietc cells obtained on the 5th day after the beginning of immobilization, besides myeloid colonies, aggregates consisting of erythroid hemoglobin-containing cells (E-CFU-dc) were found (up to 12.5 ± 1.7%). The increase in release of CFU-dc and CLFU-dc (on the 5th day) from the bone marrow of the stressed animals preceded in time the development of marked hyperplasia of medullary erythropoiesis (from $1.8 \times 10^6 \pm 0.4 \times 10^6$ to $5.4 \times 10^6 \pm 0.5 \times 10^6$ cells; $p < 0.05$) and of granulocytopoiesis (from $11.1 \times 10^6 \pm 0.7 \times 10^6$ to $14.8 \times 10^6 \pm 0.3 \times 10^6$ cells; $p < 0.05$).

A fact of fundamental importance is that the development of the changes described above in the blood system was preceded by activation of cells carrying an hematopoiesis-inducing microenvironment. For instance, bone marrow taken on the 3rd day after immobiliation formed an ectopic focus of hematopoiesis with a cell count 2.4 times higher than that in intact animals (Table 1). Whereas under normal conditions the ratio of the weight of the ectopic focus to its cell count was about 1:3, on the 3rd day after the beginning of immobilization the ratio was 1:10. No less interesting results were obtained by repeated transfer of heterotopic foci of hematopoiesis. Stem cells that are stromal precursors, responsible for transfer of the hematopoiesis-inducing microenvironment, also acquire the ability, under the conditions described above, to form a secondary focus with an increased cell count. The ratio of the weight of ectopic focus of hematopoiesis to its cell count varied from 1:2 (intact animals) to 1:7 (5th day after immobilization).

Proof of the important role of bone marrow stromal cells in the regulation of hematopoiesis during stress was thus obtained.

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ROLE OF CARDIOVASCULAR NEURONS OF THE BULBAR CARDIOVASCULAR CENTER IN ADAPTIVE RESPONSES OF THE CIRCULATORY SYSTEM TO CHANGES IN COMPOSITION OF THE INSPIRED AIR

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UDC 612.13.014.49-06:
612.282-06:612.223

KEY WORDS: cardiovascular neurons; bulbar cardiovascular center; changes in blood gas composition.

Changes in composition of the inspired air lead to appreciable changes in cardiac activity, vascular tone, and the respiratory system, and in the case of prolonged exposures, changes in hematopoiesis also. Most attention in the study of mechanisms of adaptive responses is currently being paid to the respiratory centers [2, 7, 9, 11], whereas the role of the cardiovascular neurons of the cardiovascular center in these responses has not yet been explained.

The aim of the present investigation was to study the latent period and character of changes in the firing pattern of the cardiovascular neurons of the bulbar cardiovascular center during changes in the O_2 and CO_2 concentrations in the inspired air.

EXPERIMENTAL METHOD

Experiments were carried out on 48 rabbits weighing 3-4 kg under pento-barbital anesthesia (60 mg/kg intraperitoneally). Electrical activity of the bulbar cardiovascular neurons was recorded extracellularly with glass microelectrodes with a tip 1 μ in diameter, and filled with 2.5 M KCl. The neurons were identified by means of stereotaxic coordinates in the region of the nucleus of the tractus solitarius (2 mm rostrally and caudally to the obex). The functional group to which the cardiovascular neurons belonged was determined by means of criteria described previously [3, 4, 6, 12]. The ECG in standard lead I or II, and the blood pressure in the carotid artery, by a direct method using the ÉMT-35 electromanometer, were recorded continuously during all experiments. The parameters recorded were amplified by means of an M-42 four-channel myograph.

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 0007-4888/88/0003-0314$12.50