Reactions of Hemopoietic Granulocytic Stem in Hypoxia of Different Severity

G. N. Zyuz'kov, L. A. Gur'yantseva, N. I. Suslov, A. M. Dygai, and E. D. Gol'dberg

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 10, pp. 379-382, October, 2002
Original article submitted May 8, 2002

We studied the reactions of granulocytic hemopoietic stem after acute hypoxia and during the development of posthypoxic encephalopathy. Damage to brain structures was associated with intensification of the bone marrow hemopoiesis due to activation of hemopoiesis-inducing microenvironment and more intense formation of hemopoietic islets, despite reduced proliferative capacity of granulocytic precursors.

Key Words: *granulocytopoiesis; hypoxia; encephalopathy*

Hypoxia is one of the main factors limiting energy metabolism. Some specific features of metabolism in nervous tissue explain extreme sensitivity of the brain to hypoxia and maximum severity of structural and functional changes at all levels of the CNS preceding damage to other organs maintaining homeostasis. Damage to structures responsible for compensatory and adaptive reactions determines progressive development of disease because of decompensation of adaptive systems during the posthypoxic period [5,7]. The important role of the blood system in the maintenance of homeostasis and crucial importance of the central (neuroendocrine) regulation of hemopoietic processes prompted us to investigate hematological shifts in hypoxia.

Here we studied the reactions of the hemopoietic granulocytic stem and their mechanisms in hypoxia of different severity.

MATERIALS AND METHODS

Experiments were carried out on 540 male CBA/CaLac mice (18-20 g). First-category conventional inbred mice were obtained from Animal Breeding Center of Department of Experimental Biomedical Simulation, Institute of Pharmacology. Two variants of hypoxic exposure (in a sealed 500-ml pressure chamber) were used. The animals were removed from the chamber 10-15 sec after the end of generalized seizures and/or respiration arrest. No significant changes in the psychoneurological status were detected after single short-term hypoxic exposure. Repeated exposure (two sessions with a 10-min interval) led to the formation of encephalopathy starting from day 1 of experiment. Encephalopathy was diagnosed by amnesia in retrieval of conditioned passive avoidance test and disorders in the orientation and exploratory behavior in an open field on days 1-10 [1,8].

Parameters of peripheral blood and bone marrow hemopoiesis [6], content of granulomonocytic precursors (CFU-GM) in the bone marrow, their proliferative activity and intensity of CFU-GM differentiation, production of humoral hemopoietic factors by individual fractions of hemopoiesis-inducing microenvironment (HIM), and structure and function of the bone marrow were evaluated on days 1-10 [3].

The data were statistically analyzed by Student's test using standard.

RESULTS

Hypoxia led to hyperplasia of the hemopoietic granulocytic stem. The number of immature neutrophilic

Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences

0007-4888/02/1344-0325$27.00

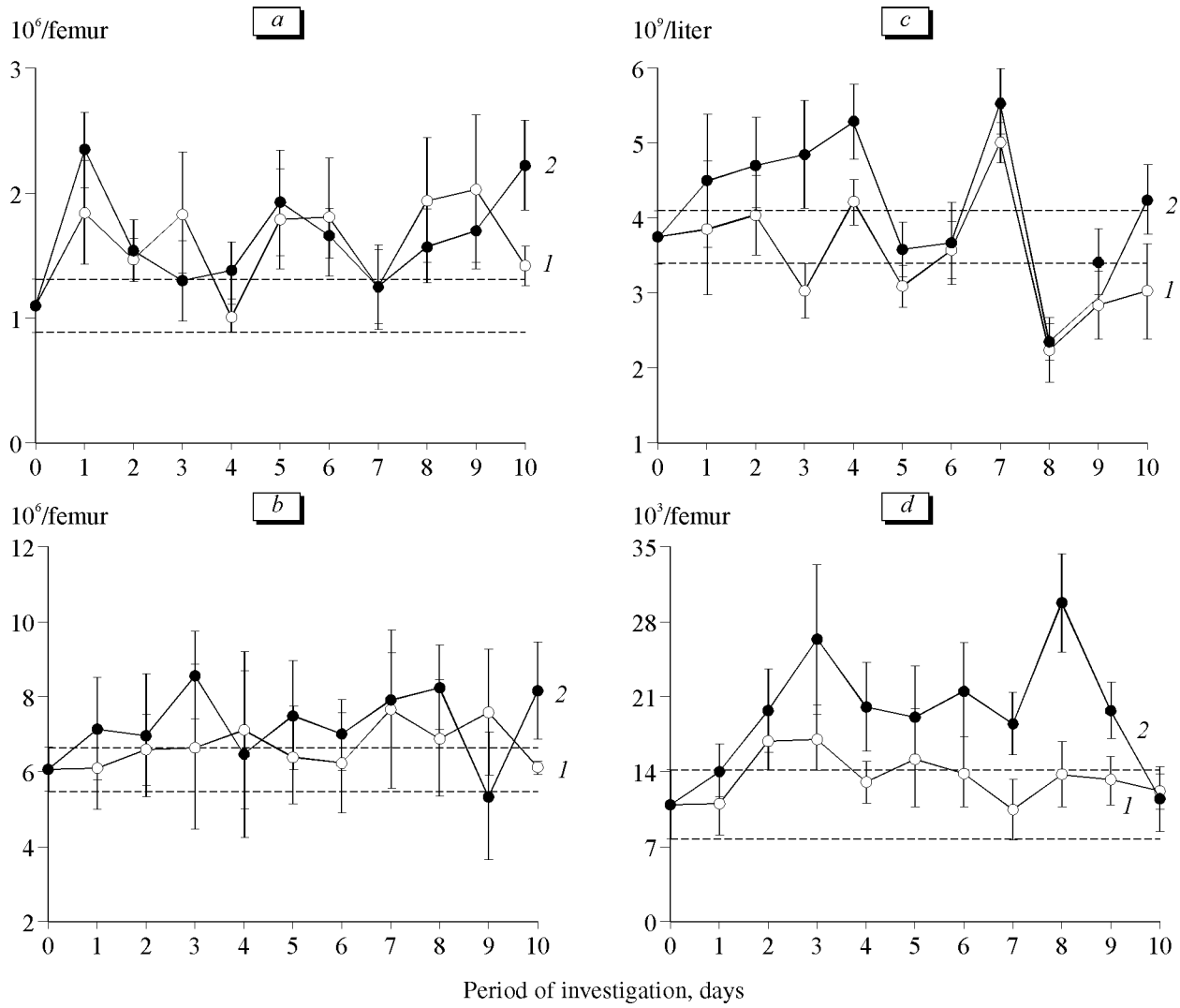

Fig. 1. Time course of immature neutrophilic granulocytes in the bone marrow (*a*), mature neutrophilic granulocytes (*b*) and segmented neutrophils (*c*) in the peripheral blood, and granulocytic hemopoietic islets in the bone marrow (*d*) in male CBA/CaLac mice after acute hypoxia (*1*) and during developing posthypoxic encephalopathy (*2*). Baseline values correspond to the zero on the abscissa. Here and in Figs. 2 and 3: dotted lines show confidence intervals at $p<0.05$.

granulocytes in the bone marrow increased (days 1-3, 5, 6, 8, 9), while the content of mature forms did not surpass the initial level and the number of segmented neutrophils in the peripheral blood decreased (days 3, 5, 8, 9) (Fig. 1). These changes resulted from increased colony-forming capacity of hemopoietic tissue, stimulation of CFU-GM division, and disorders in the realization of the differentiation potentials of hemopoietic precursors throughout the observation period (Fig. 2). Production of humoral regulators by adherent bone marrow cells and colony-stimulating activity of the serum increased despite decreased level of colony formation in 7-day cultures in test systems with supernatant of nonadherent myelokaryocytes on days 7 and 8 (Fig. 3).

Study of bone marrow structure and function showed increased yield of cell complexes associated with fibroblast-like elements, the number of the granulocytic hemopoietic islets (HI) increased only on days 2 and 3 (Fig. 1, *d*). This probably resulted from accumulation of committed precursor cells in hemopoietic tissue. These reactions of the blood system in general corresponded to changes characteristic of the general adaptation syndrome and resulted from activation of the stress-realizing systems [4]. Dissociation of proliferation and differentiation processes during these reactions can be explained by specific nature of the damaging factor and the absence of a direct stimulatory effect on granulomonocytopoiesis.

The existence of a universal system regulating activity of the hemopoietic tissue and including local and distant (neuroendocrine) regulatory mechanisms, is now proven [2]. The study of the possible effects of posthypoxic encephalopathy on hemopoiesis re-

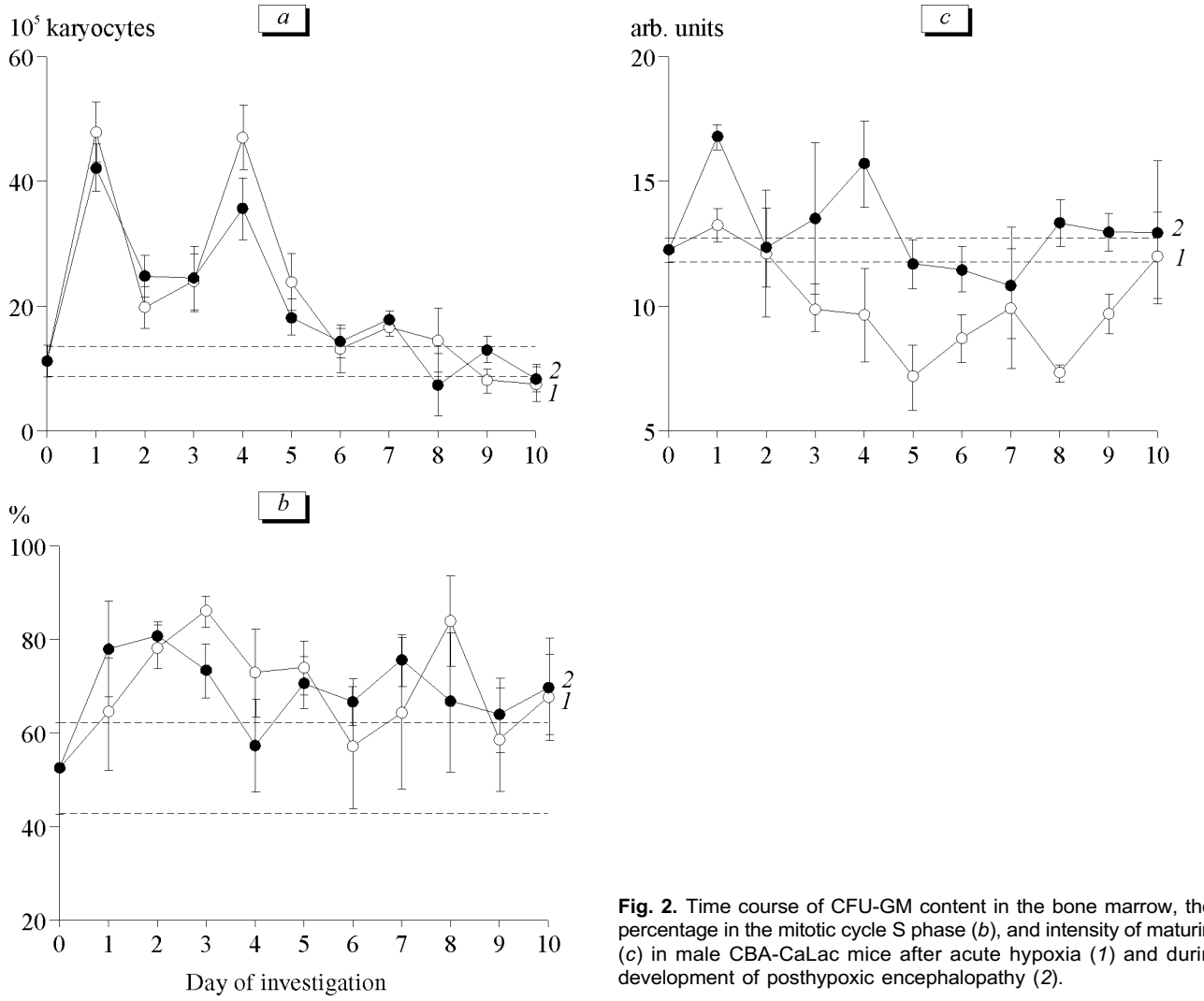

Fig. 2. Time course of CFU-GM content in the bone marrow, their percentage in the mitotic cycle S phase (*b*), and intensity of maturing (*c*) in male CBA-CaLac mice after acute hypoxia (*1*) and during development of posthypoxic encephalopathy (*2*).

vealed some interesting peculiarities. We observed inhibition of CFU-GM division, which was significant on day 3 of the experiment, and compensatory acceleration of maturation of hemopoietic precursors on days 3, 5, 6, 8 after hypoxic exposure. These changes were preceded by increased secretory activity of adherent and nonadherent bone marrow nuclears and increased serum colony-stimulating activity (Fig. 3). In addition, we observed a pronounced increase in the number of granulocytic cell associations, which ensured accumulation of mature neutrophilic granulocytes in the bone marrow and promoted the development of peripheral blood neutrophilic leukocytosis (Fig. 1).

Hence, hypoxia damaging brain structures was associated with decreased proliferative activity of committed granulomonocytic precursor cells in the posthypoxic period against the background increased secretory activity of HIM cells and their increased cooperation with hemopoietic precursors, which eventually led to extension of the bone marrow granulomonocytopoiesis and to more intense release of white blood cells into the circulation. Hence, the severity of oxygen deficiency determines the type of adaptive reactions of the blood system, which, in turn, can influence the course of repair processes in tissues after hypoxic exposure.

The study was supported by the Russian Foundation for Basic Research (grant No. 00-04-48745).

REFERENCES

1. J. Buresh, O. Bureshova, and J. P. Houston, *Methods and Basic Experiments for Studies of Brain and Behavior*, Ed. A. S. Batuev [in Russian], Moscow (1991), p. 398.
2. E. D. Gol'dberg, A. M. Dygai, and V. V. Zhdanov, *Role of Hemopoiesis-Inducing Microenvironment in Hemopoiesis Regulation in Cytostatic Myelosuppressions* [in Russian], Tomsk (1999), p. 81.
3. E. D. Gol'dberg, A. M. Dygai, and V. P. Shakhov, *Tissue Culture Methods in Hematology* [in Russian], Tomsk (1992).

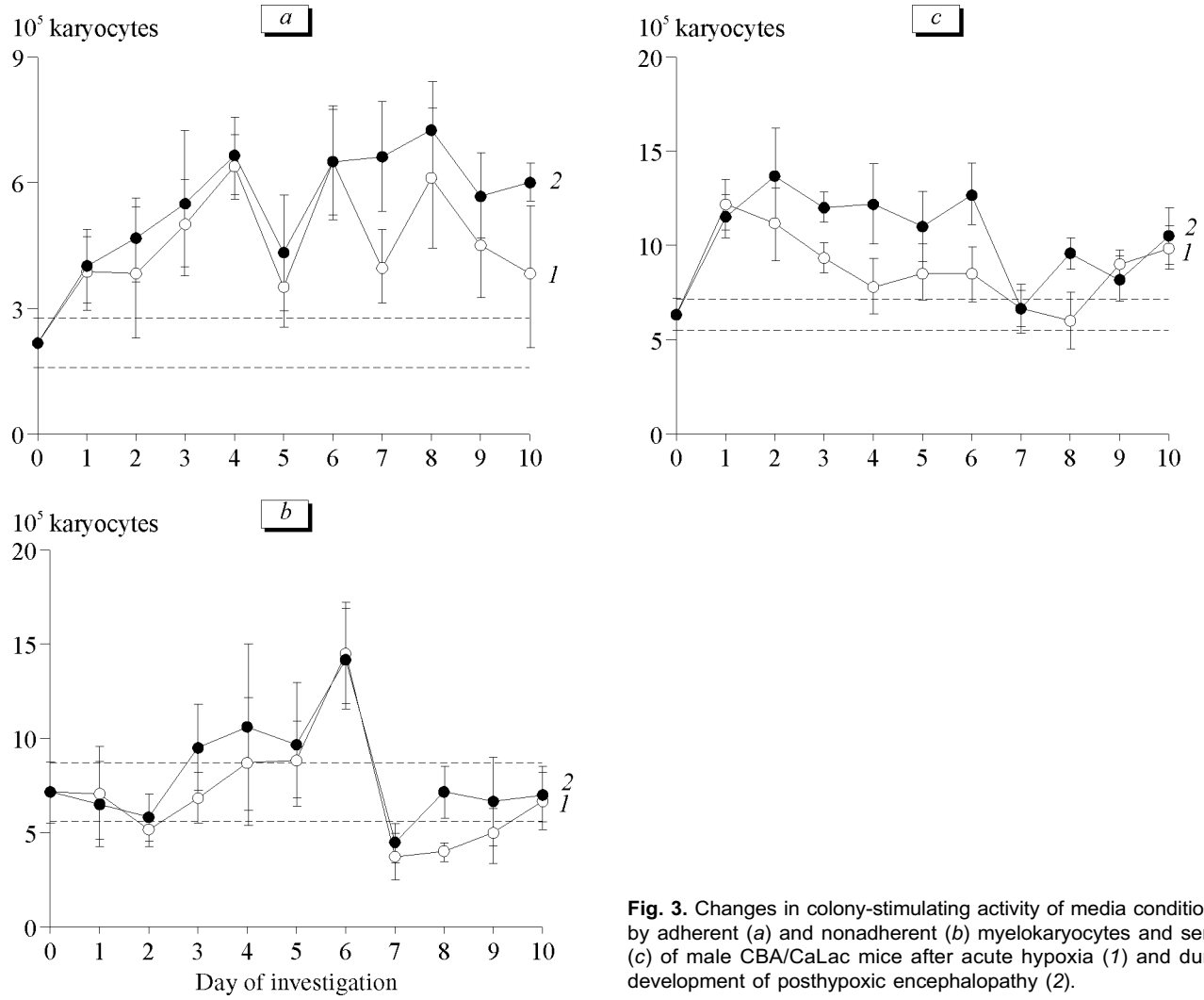

Fig. 3. Changes in colony-stimulating activity of media conditioned by adherent (*a*) and nonadherent (*b*) myelokaryocytes and serum (*c*) of male CBA/CaLac mice after acute hypoxia (*1*) and during development of posthypoxic encephalopathy (*2*).

4. E. D. Gol'dberg, A. M. Dygai, and I. A. Khlusov, *Role of the Autonomic Nervous System in Hemopoiesis Regulation* [in Russian], Tomsk (1997), pp. 65-66.
5. A. M. Gurvich, G. V. Alekseeva, and V. V. Semchenko, *Post-reanimation Encephalopathy* [in Russian], Omsk (1996), p. 10.
6. *Laboratory Methods of Investigation in Clinical Practice: Handbook*, Ed. V. V. Men'shikov [in Russian], Moscow (1987).
7. G. S. Krause, B. C. White, S. D. Aust, *et al.*, *Crit. Care Med.*, **1**, 726 (1988).
8. R. N. Walsh and R. A. Cummins, *Psychol. Bull.*, **83**, 482-504 (1976).