Changes in Granulocytic Hemopoietic Stem and Their Mechanisms during Hypoxia of Different Genesis

G. N. Zyuz'kov, A. M. Dygai, and E. D. Gol'dberg

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 3, pp. 254-258, March, 2005
Original article submitted November 9, 2004

We studied reactions of the granulocytic hemopoietic stem during hypoxia of different genesis and severity. Stimulation of granulocytopoiesis was determined by an increase in functional activity of granulomonocytic precursors due to changes in feeder capacity of cells in the hemopoiesis-inducing microenvironment and colony-stimulating activity of the plasma. The development of encephalopathy caused by oxygen deficiency was accompanied by a decrease in the number of bone marrow granulomonocytic precursors due to reduction of proliferative activity (despite the increase in secretory activity of microenvironmental cells and increase in plasma colony-stimulating activity). Severe hypoxia accelerated maturation of hemopoietic cells and produced neutrophilic leukocytosis.

Key Words: *granulocytopoiesis; hypoxia; encephalopathy*

Inhibition of aerobic oxidation in the brain tissue during hypoxia modulates function of the central nervous system (CNS) and contributes to variations in integrative and triggering activity of cells. Moreover, it is followed by a variety of pathological processes leading to progressive changes in metabolic and functional activity of internal organs during decompensation of adaptive mechanisms [1,8]. Pathogenic influences on the brain during hypoxia produce dysfunction of various organs (*e.g.*, hemodynamic disturbances and immune or hemostasis disorders) [1,3]. The blood system maintains homeostasis and plays a role in the development of compensatory and adaptive reactions during oxygen deficiency. Red blood cells play a key role in the adaptation to oxygen deficiency. Published data show that phlogogenic substances and inflammatory effectors (neutrophils and monocytes) significantly modulate the course of recovery processes in the ischemic tissue [11,12]. The mechanisms of regulation of granulomonocytopoiesis under hypoxic conditions remain unknown.

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

Here we studied changes in the granulomonocytic hemopoietic stem during hypoxia of different genesis and severity.

MATERIALS AND METHODS

Experiments were performed on 1546 CBA/CaLac mice (class I conventional mouse strain) weighing 18-20 g and obtained from the nursery of Experimental Biomedical Modeling Department (Institute of Pharmacology, Tomsk Research Center). Hypoxic hypoxia and two variants of hemic hypoxia served as experimental models.

Hypoxic hypoxia was modeled by placing the animal (once or twice with 10-min interval) in a 500-ml sealed chamber. The mice were removed from this chamber after termination of generalized convulsions and/or visual respiratory arrest for 10-15 sec.

Hemic hypoxia was induced by intraperitoneal injection of phenylhydrazine hydrochloride (single doses 30 and 150 mg/kg) or blood withdrawal. Blood loss was induced by puncture of the retroorbital sinus and withdrawal of 30% circulating blood volume (CBV) through a graduated Pasteur pipette washed with heparin (series I). In series II, 70% CBV were repeatedly

0007-4888/05/1393-0279 © 2005 Springer Science+Business Media, Inc.

withdrawn over 2-3 h (3 procedures). The volume of withdrawn blood was estimated taking into account the fact that CBV in rodents corresponds to $^1/_{13}$ of body weight.

Peripheral blood indexes and intensity of bone marrow hemopoiesis were estimated by routine blood tests on days 1-10 [9]. We studied the number of granulomonocytic precursors (CFU-GM) in the bone marrow, proliferative activity and intensity of differentiation of these cells, colony-stimulating activity (CSA) of individual fractions in the hemopoiesis-inducing microenvironment (HIM), plasma CSA, and structural and functional characteristics of the bone marrow [7].

The results were analyzed by methods of variational statistics (Student's *t* test, nonparametric Mann—Whitney *U* test). Integral parameters that were numerically equal to normalized means were calculated on days 1-10 [10]. Statistical treatment involved correlation and factor analyse.

RESULTS

Single hypoxic exposure in a sealed chamber, administration of the hemolytic poison in a dose of 30 mg/kg, and effusion of 30% CBV did not produce significant changes in the psychoneurological status. Serious oxygen deficiency (2-fold hypoxia in a sealed chamber, administration of the hemolytic poison in a dose of 150 mg/kg, and effusion of 70% CBV) was followed by the development of encephalopathy recorded by amnesia in conditioned passive avoidance paradigm and disturbances in the orientation and exploratory activity in the open field [2].

Hypoxia of different genesis was followed by hyperplasia of the granulocytic hemopoietic stem. The number of immature and mature neutrophilic granulocytes in the bone marrow significantly increased under various conditions of hypoxia. The number of immature cells increased (up to 184.5% of the baseline level, day 9), while the count of mature cells remained unchanged during hypoxia in a sealed chamber. Other changes were observed under both regimens of hemic hypoxia. Accumulation of immature cells was revealed in the late period after phenylhydrazine administration (up to 185.33% of the baseline level, day 7) and blood loss (up to 160.87% of the baseline level, day 10). The content of mature neutrophilic granulocytes in the hemopoietic tissue increased 3 and 7 days after administration of phenylhydrazine (to 142.5% of baseline on day 7). It should be emphasized that the number of these cells increased after effusion of 30% CBV and peaked on day 3 (141.2% of baseline). The number of band neutrophils in the peripheral blood increased and peaked on day 1 after hypoxia in a sealed chamber (293.75% of the baseline level) and on day 4 after administration of the hemolytic poison and blood loss (309.1 and 354.54% of the baseline level, respectively). Hypoxic hypoxia decreased the number of segmented neutrophils, which was minimum on day 8 after treatment (59.7%). These changes were probably associated with rapid efflux of leukocytes in tissues (Table 1).

The observed changes in granulocytopoiesis were preceded by an increase in colony-forming activity of the bone marrow. A variety of changes developed after oxygen deficiency of different genesis. The number of CFU-GM in methylcellulose increased and peaked on day 1 after hypoxic hypoxia, phenylhydrazine administration, and blood loss (428.83, 727.2, and 375.0%, respectively). The division rate for these cells increased 6-8 days after hypoxic hypoxia, phenylhydrazine administration, and blood loss (157.8, 258.9, and 189.35%, respectively). The rate of maturation of granulocyte-macrophage precursors increased and peaked on days 4 and 9 after phenylhydrazine administration (184.2%) and blood loss (137.4%), respectively. However, this parameter decreased after hypoxia in a sealed chamber (minimum value 58.78%, day 5; Table 1). Activity of clonogenic cells mainly depends on production of various humoral hemopoietic factors by HIM cells [4]. CSA of conditioned media from adherent cells increased and peaked on days 6, 8, and 4 after hypoxia in a sealed chamber (299.5%), modeling of hemolytic anemia (188%), and blood loss (250.4%), respectively. Administration of the hemolytic poison induced an increase in CSA of nonadherent myelokaryocytes (up to 388% of the baseline level, day 5). Hypoxic hypoxia (day 8) and blood loss (day 4) decreased CSA of nonadherent bone marrow cells to 51.6 and 61.3% of the baseline level, respectively. The content of hemopoietically active substances in the plasma from mice of different groups increased at all terms of observations. These changes were most pronounced under conditions of phenylhydrazine-produced erythrodieresis (increase to 317% of the baseline level on day 5). We studied direct cell-cell interaction between stromal cells of HIM and hemopoietic cells. The ability of fibroblastoid cells to form cell complexes increased. These changes were accompanied by a significant increase in the content of granulo-cytic hemopoietic islets during hypoxia in a sealed chamber (154.2 and 155.7% on days 2 and 3, respectively) and blood loss (140% of the baseline level, day 1; Table 1).

The correlation matrix remained unchanged in mice of various groups. Changes in the bone marrow tissue were accompanied by a significant increase in plasma CSA. These results illustrate greater contribution of long-range humoral mechanisms in the regulation of granulomonocytopoiesis during oxygen defi-

TABLE 1. Integral Parameters of Individual Granulomonocytopoietic Elements during Hypoxia of Different Genesis (%)

Treatment	Neutrophilic granulocytes		Segmented neutrophils	CFU-GM	CFU-GM in S-phase of the cell cycle	ClFU-GM/ CFU-GM	CSA			Granulocytic hemopoietic islets
	immature	mature					adherent myelokaryocytes	nonadherent myelokaryocytes	blood plasma	
Not producing encephalopathy										
hypoxia	149.0	111.06	93.12	199.24	134.72	81.42	218.85	98.35	140.52	124.95
anemia	149.0	117.74	132.85	305.39	207.74	156.78	158.21	145.56	206.0	94.45
blood loss	119.11	124.86	91.49	195.65	152.71	106.31	149.1	93.69	162.31	111.55
Producing encephalopathy										
hypoxia	153.23	120.91	112.13	184.55	133.82	107.4	263.55	113.82	170.51	183.08
anemia	104.43	77.42	292.19	169.54	141.98	199.56	232.84	160.34	200.6	121.25
blood loss	96.39	70.52	117.14	178.35	103.16	162.13	198.05	112.86	309.4	97.07

ciency of different genesis. The degree of functional coordination between individual cells of HIM remained low under these conditions.

These reactions of the granulomonocytic hemopoietic stem are consistent with changes typical of the general adaptive syndrome. They are mainly determined by activation of the stress-realizing system [6]. Intergroup differences in uncoupling of proliferation and differentiation of granulomonocytic precursors during hypoxia in a sealed chamber and blood loss are probably related to different severity of treatment. The observed differences were associated with variations in the production of phlogogenic substances in hypoxic tissues [11,12].

The general system of hemopoiesis regulation includes local and distant (neuroendocrine) interrelated regulatory mechanisms [4]. The study of the effect produced by encephalopathy (mechanism for dysregulation of hemopoiesis) on granulocytopoiesis under conditions of hypoxia revealed several blood phenomena. For example, brain disorders of different genesis were accompanied by a decrease in the content of CFU-GM in the bone marrow. Cell count decreased under conditions of hypoxia in a sealed chamber (day 4), hemolytic anemia (days 2, 4-7, and 10), and posthemorrhagic anemia (days 9 and 10). Proliferative activity of cells decreased under conditions of hypoxic hypoxia (day 3), phenylhydrazine administration (days 2, 3, 5, 7, and 10), and posthemorrhagic anemia (days 2, 6, 9, and 10). Maturation of precursor cells was accelerated in various periods of observations. It was accompanied by an increase in CSA in study biological fluids (Table 1). Precursor cells underwent most pronounced changes in animals with encephalopathy produced by administration of the hemolytic poison in the high dose. Hypoxia in a sealed chamber and massive hemolysis were followed by an increase in the number of granulocyte associations in the bone marrow. The number of granulocytic hemopoietic islets increased by 48.13 and 26.8%, respectively. However, the count of these islets decreased by 14.48% after withdrawal of 70% CBV. These changes were accompanied by a decrease in the count of immature and mature neutrophilic granulocytes in hemopoietic tissue during the posthemorrhagic period (days 2, 7, and 9) and under conditions of induced hemolysis (days 4 and 5). Mature neutrophilic granulocytes were accumulated in the bone marrow 10 days after hypoxic hypoxia. We observed neutrophilic leukocytosis in the peripheral blood, which was particularly pronounced in animals with phenylhydrazine-produced encephalopathy (292.19%). These changes were probably associated with the impaired ability of toxically damaged leukocytes to migrate in tissues, but not with rapid maturation of CFU-GM.

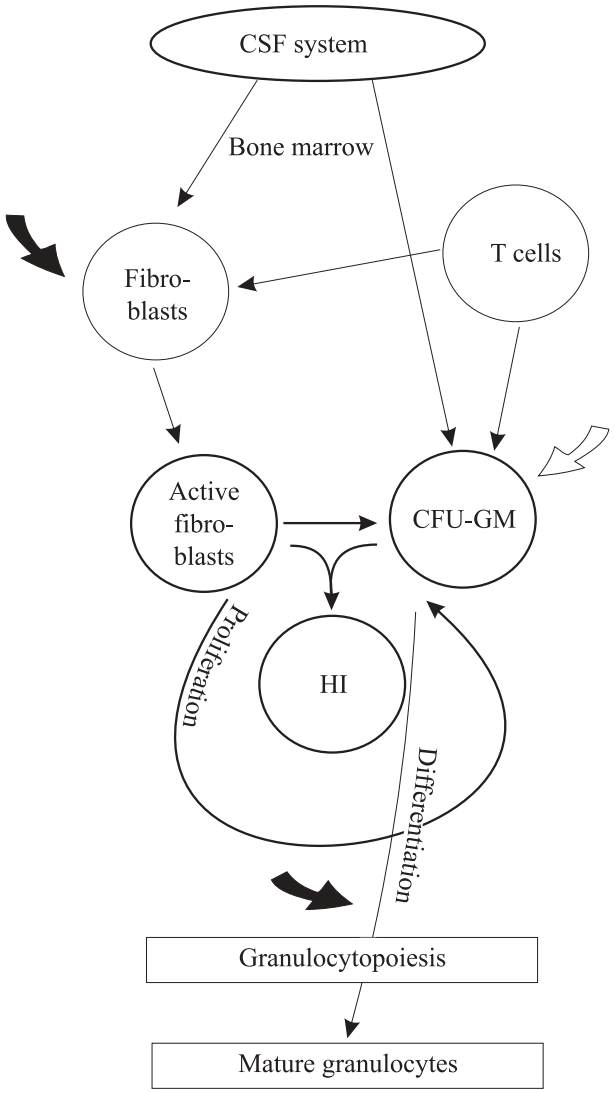

Fig. 1. Regulation of granulocytopoiesis during oxygen deficiency. CSF, colony-stimulating factor; CFU-GM, granulocyte-macrophage colony-forming units; HI, hemopoietic islets. Thin lines: oxygen deficiency can produce different changes. Thick lines: activating effect. Black and white arrows: activating and inhibitory effect produced by damage to CNS, respectively.

Thus, hypoxia produces damage to the brain structures and significant changes in the granulocytic stem, which is followed by an increase in the number of peripheral blood segmented neutrophils.

Analysis of rank correlation coefficients revealed an increase in the number of signal relations between individual elements of the granulomonocytic stem. These changes illustrate increased strain of the system, which was most pronounced during hemolysis-induced encephalopathy. Reduction of variables (correlation relationships) by means of factor analysis revealed an increase in the contribution of humoral regulators from HIM stromal cells in blood changes. Plasma hemopoietins play a minor role in the development of CNS disorders after hypoxia in a sealed chamber and blood loss. Dysregulation of granulocytopoiesis during severe oxygen deficiency is associated with damage to the central neuroendocrine system under the influence of "aggressive" hypoxic factors. Our previous studies revealed a direct relationship between damage to hemopoietic precursors accompanying the increase in secretory activity of adherent myelokaryocytes and development of encephalopathy in the post-hypoxic period [5,9]. Rapid maturation of CFU-GM during severe oxygen deficiency is probably related to serious hypoxic alteration of tissues (Fig. 1). This is accompanied by the release of destruction cell products and secretion of antiinflammatory cytokines [11,12].

These data indicate that the type of compensatory and adaptive reactions in the blood system depends on the severity of oxygen deficiency and brain injury. These reactions determine adaptation of the organism to hypoxia.

REFERENCES

1. G. V. Alekseeva, A. M. Gurvich, and V. V. Semchenko, *Post-resuscitation Encephalopathy (Pathogenesis, Clinics, Prevention, and Therapy)* [in Russian], Omsk (2003).
2. Ya. Buresh, O. Bureshova, G. P. Houston, *Methods and Main Experiments in Studies of the Brain and Behavior* [in Russian], Ed. A. S. Batuev, Moscow (1991), p. 398.
3. *Hypoxia. Adaptation, Pathogenesis, and Clinics* [in Russian], Ed. Yu. L. Shevchenko, St. Petersburg (2000).
4. E. D. Gol'dberg, A. M. Dygai, and V. V. Zhdanov, *Role of the Hemopoiesis-Inducing Microenvironment in Cytostatic-Induced Myelosuppressions* [in Russian], Tomsk (1999).
5. E. D. Gol'dberg, A. M. Dygai, and G. N. Zyuz'kov, *Eksper. Klin. Med.*, No. 2, 30-35 (2003).
6. E. D. Gol'dberg, A. M. Dygai, and I. A. Khlusov, *Role of the Autonomic Nervous System in Regulation of Hemopoiesis* [in Russian], Tomsk (1997).
7. E. D. Gol'berg, A. M. Dygai, and V. P. Shakhov, *Tissue Culture Methods in Hematology* [in Russian], Tomsk (1992).
8. *Dysregulation Pathology* [in Russian], Ed. G. N. Kryzhanovskii, Moscow (2002).
9. G. N. Zyuz'kov, L. A. Gur'yantseva, N. I. Suslov, *et al.*, *Byull. Eksp. Biol. Med.*, **136**, No. 10, 379-382 (2002).
10. V. V. Novitskii, V. A. Fokin, and V. E. Gol'dberg, *Method for Integral Analysis of the Toxic Effect of Antitumor Preparations on the Blood System: Methodical Recommendations* [in Russian], Ed. E. D. Gol'dberg, Moscow (1990).
11. *Problems of Hypoxia: Molecular, Physiological, and Medical Aspects* [in Russian], Eds. L. D. Luk'yanova and I. B. Ushakov, Moscow, Voronezh (2004).
12. C. Li and R. M. Jackson, *Am . J. Physiol. Cell Physiol. (USA)*, **282**, No. No. 2, 227-241 (2002).