Role of Thy-1,2$^+$ Cells in the Regulation of Hemopoiesis during Severe Hypoxia

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We studied the state of the bone marrow Thy-1,2$^+$ cell pool under conditions of severe hypoxia. T cell mechanisms of hemopoiesis regulation are preserved under conditions of severe oxygen deficiency due to changes in functional properties of Thy-1,2$^+$ cells. We revealed an indirect (mediated through cooperation with adherent myelokaryocytes) stimulating effect of Thy-1,2$^+$ cells on erythroid precursors as well as direct and indirect feeder effects of these cells on granulocyte-monocyte precursors.

Key Words: *hypoxia; encephalopathy; hemopoiesis; hemopoiesis-inducing microenvironment; T lymphocytes*

Regulation of hemopoiesis is realized via close cooperation of local and distant regulatory mechanisms. Migration of Thy-1,2$^+$ cells to the hemopoietic tissue can induce hyperplasia of the bone marrow hemopoiesis under extreme conditions, including oxygen deficiency [2,5]. Our previous studies showed that Thy-1,2$^+$ cells primarily stimulate erythropoiesis under conditions of oxygen deficiency. This effect is most pronounced during the interaction of Thy-1,2$^+$ cells with adherent bone marrow cells [4]. It remains unclear whether these cells of the hemopoiesis-inducing microenvironment (HIM) play a role in disadaptation of the hemopoietic tissue associated with dysfunction of the central nervous system (CNS) during severe hypoxia [2,3,7].

Here we studied the role of Thy-1,2$^+$ cells in hematological shifts during severe oxygen deficiency.

MATERIALS AND METHODS

Experiments were performed on 372 CBA/CaLac mice (class I conventional mouse strain) weighing 18-20 g and obtained from the nursery of the Department of Experimental Biomedical Modeling (Institute of Pharmacology, Tomsk Research Center). Hypoxic hypoxia and 2 regimens of hemic hypoxia served as the experimental models. For hypoxic hypoxia modeling, the animals were placed in a 500-ml sealed chamber (twice at a 10-min interval). 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 single intraperitoneal injection of 150 mg/kg phenylhydrazine hydrochloride. Otherwise, blood loss was induced by puncture of the retroorbital sinus and withdrawal of 70% circulating blood volume (CBV) through a graduated Pasteur pipette washed with heparin solution. The blood was withdrawn over 2-3 h (3 procedures). The volume of the withdrawn blood was estimated taking into account the fact that CBV in rodents corresponds to $^1/_{13}$ of body weight. Oxygen deficiency was accompanied by encephalopathy. It was confirmed by the development of amnesia during performance of a conditioned passive avoidance response [1] and changes in orientation and exploratory activities of animals in the open field test [1].

The number of bone marrow Thy-1,2$^+$ cells was estimated in the complement-dependent cytotoxic test with monoclonal anti-Thy-1,2$^+$ antibodies

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(clone 5A-8 CL 8600A, Sigma) on days 1-5 and 7 after treatment [6]. The role of Thy-1,2$^+$ cells in the regulation of hemopoiesis was studied by culture methods on days 2 and 4. The intensity of colony formation, *i.e.* the number of erythroid (E) and granulocyte-monocyte colony-forming units (CFU) was compared in cultures of nonadherent bone marrow cells and after culturing of the nonadherent fraction on adherent feeder layer. These cultures included Thy-1,2$^+$ cells or these cells were removed by treatment with monoclonal anti-Thy-1,2$^+$ antibodies (clone 5A-8 CL 8600A, Sigma) [6]. Pharmacological protection of the brain during experimental hypoxia was induced by intraperitoneal injection of sodium hydroxybutyrate (Virion Scientific and Production Association) in a single dose of 500 mg/kg. Control animals received an equivalent volume (0.2 ml) of physiological saline under similar conditions. The data were subjected to variational statistical analysis (Student's *t* test and nonparametric Wilcoxon—Mann—Whitney *U* test).

RESULTS

Hypoxia of different genesis accompanied by the development of encephalopathy had little effect on the number of Thy-1,2$^+$ cells in the hemopoietic tissue. During various periods of the study the number of these cells in treated mice did not differ from that in intact animals. The number of Thy-1,2$^+$ cells increased only on day 3 after the development of experimental encephalopathy due to hypoxic hypoxia (Fig. 1). These data contradict the results of our previous experiments [4]. We previously showed that the number of bone marrow Thy-1,2$^+$ cells significantly increases during oxygen deficiency of different genesis not accompanied by dysfunction of CNS. Contradictory data on the state of T cells

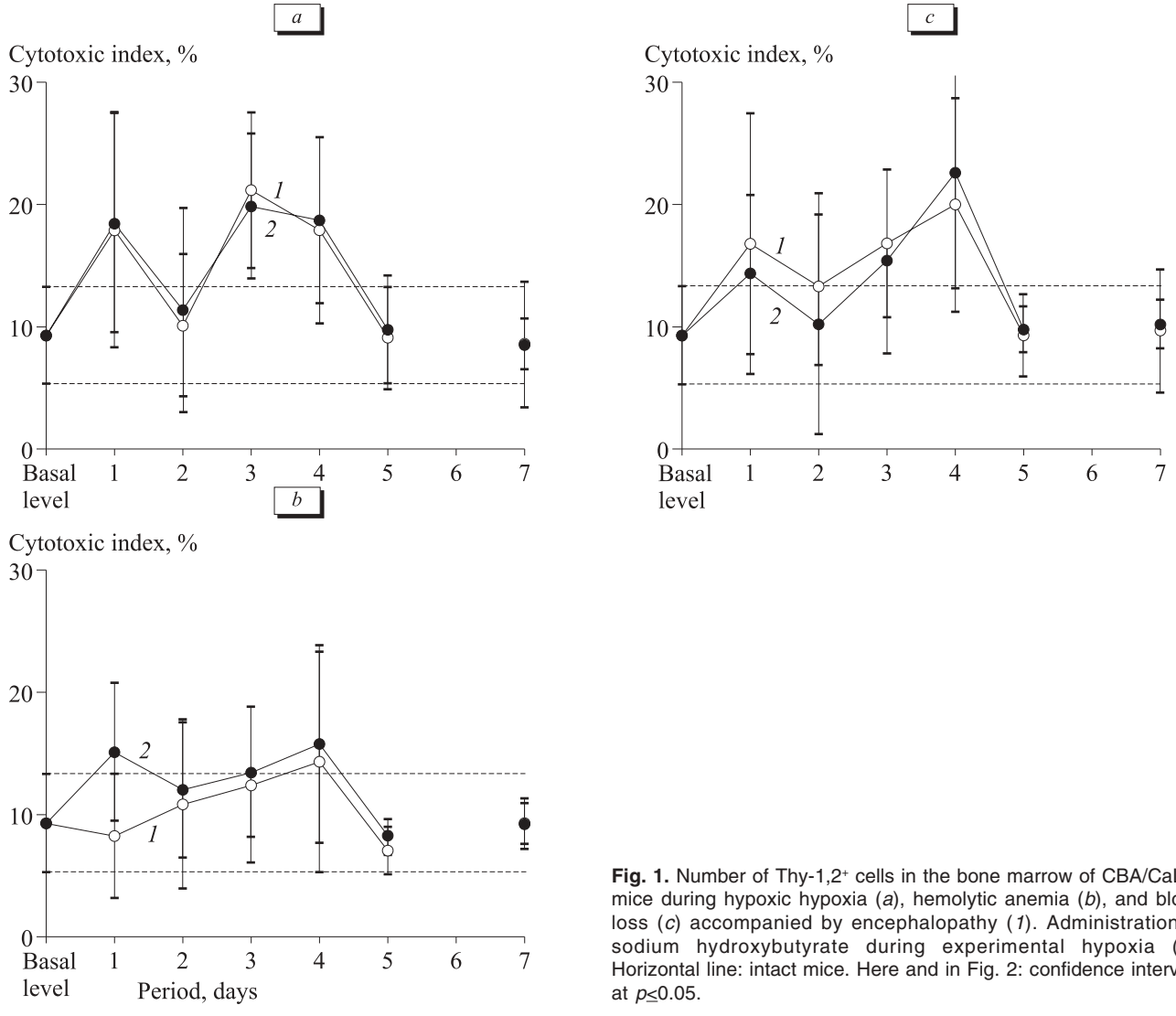

Fig. 1. Number of Thy-1,2$^+$ cells in the bone marrow of CBA/CaLac mice during hypoxic hypoxia (*a*), hemolytic anemia (*b*), and blood loss (*c*) accompanied by encephalopathy (*1*). Administration of sodium hydroxybutyrate during experimental hypoxia (*2*). Horizontal line: intact mice. Here and in Fig. 2: confidence intervals at $p \leq 0.05$.

are probably associated with an increase in the severity of hypoxia.

Although quantitative characteristics of T cells remained practically unchanged under these conditions, the presence of even small number of Thy-1,2$^+$ cells in the culture had a strong feeder effect on the growth of CFU-E during severe oxygen deficiency. The effect was observed only during interaction of these cells with adherent myelokaryocytes (Fig. 2, 3). Published data show that compensated hypoxia is followed by an increase in not only indirect, but also direct erythropoiesis-stimulating activity of HIM cells [4]. Experimental encephalopathy is accompanied by a significant increase in the capacity of Thy-1,2$^+$ cells to stimulate the growth of granulocyte-monocyte precursors. These

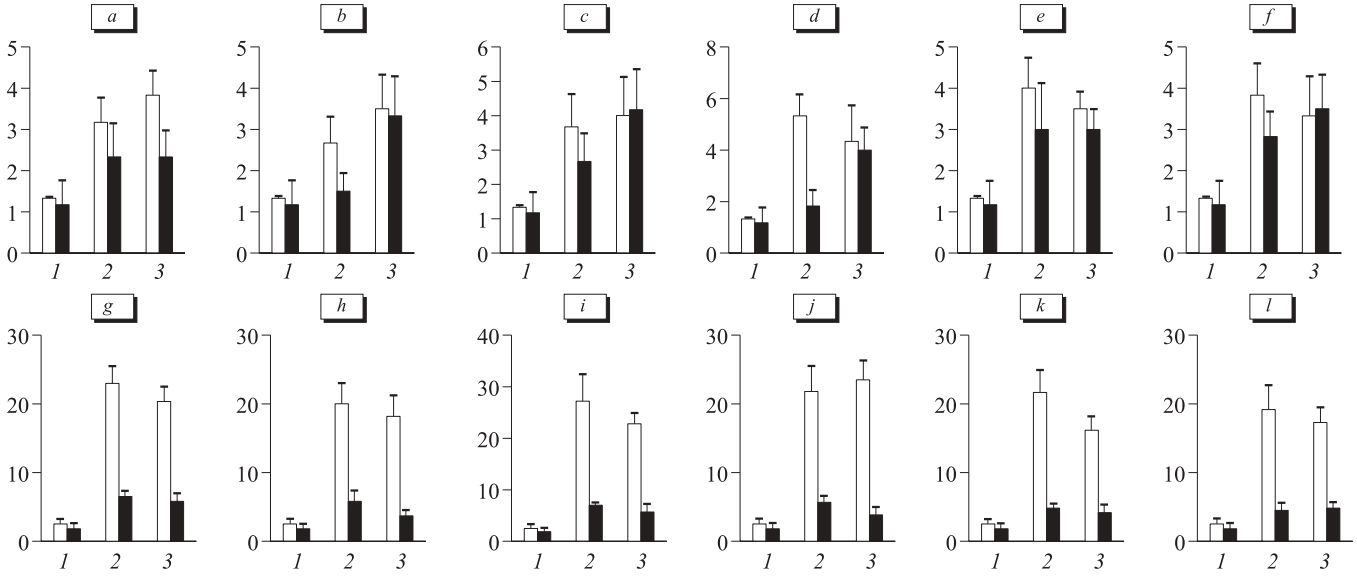

Fig. 2. Growth of erythroid colonies from nonadherent myelokaryocytes (*a-f*) and nonadherent bone marrow cells of CBA/CaLac mice on adherent feeder layer (*g-l*) during hypoxic hypoxia (*a*, *g*), hemolytic anemia (*c*, *i*), and blood loss accompanied by encephalopathy (*e*, *k*). Administration of sodium hydroxybutyrate during experimental hypoxic hypoxia (*b*, *h*), hemolytic anemia (*d*, *j*), and blood loss (*f*, *l*). Here and in Fig. 3: ordinate, colony-forming activity of the bone marrow (per 10^5 myelokaryocytes). Basal level (*1*); days 1 (*2*) and 4 of study (*3*). Light bars: suspension of complement-treated nonadherent cells (control). Dark bars: suspension of nonadherent cells deprived of Thy-1,2$^+$ cells.

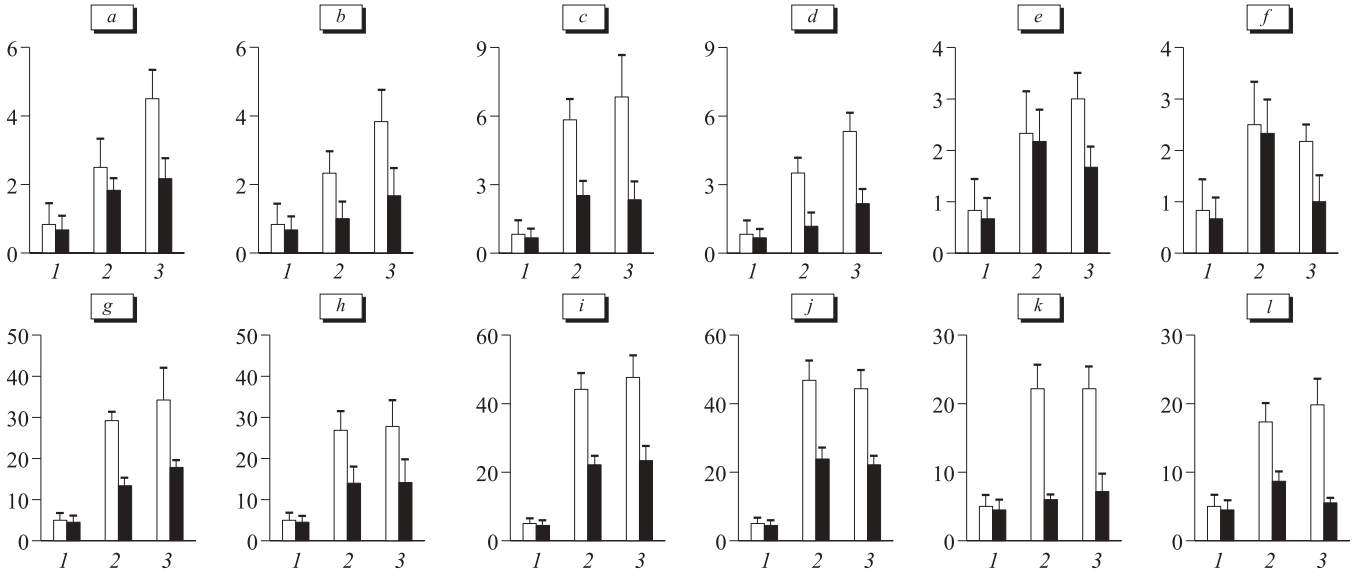

Fig. 3. Growth of granulocyte-monocyte colonies from nonadherent myelokaryocytes (*a-f*) and nonadherent bone marrow cells of CBA/CaLac mice on an adherent feeder layer (*g-l*) during hypoxic hypoxia (*a*, *g*), hemolytic anemia (*c*, *i*), and blood loss accompanied by encephalopathy (*e*, *k*). Administration of sodium hydroxybutyrate during experimental hypoxic hypoxia (*b*, *h*), hemolytic anemia (*d*, *j*), and blood loss (*f*, *l*).

changes were observed not only during culturing of bone marrow cells on adherent feeder layer, but also after growth of only nonadherent cells (as distinct from the exposure not accompanied by brain dysfunction [4]; Figs. 2, 3). Our findings attest to changes in the vector of the direct feeder effect of Thy-1,2$^+$ cells: from primary influence on erythroid precursors under conditions of compensated hypoxia [4] to stimulation of granulomonocytopoiesis during encephalopathy. The effect of Thy-1,2$^+$ cells on hemopoietic precursors due to the interaction with adherent myelokaryocytes under conditions of CNS dysfunction was more pronounced than during similar hypoxic states not accompanied by CNS disorders.

For evaluation of the contribution of CNS injury and hypoxia to variations in the T cell regulatory mechanisms during severe oxygen deficiency the animals received single injection of sodium hydroxybutyrate. Pharmacological protection of the brain completely prevented changes in the psychoneurological status. However, this antihypoxant had no effect on the number of Thy-1,2$^+$ cells in the hemopoietic tissue (Fig. 1). Feeder capacity of these cells for hemopoietic precursors remained practically unchanged under conditions of neuroprotection. These results suggest that hypoxia has a direct effect (not mediated by CNS structures) on the T-cell mechanisms of hemopoiesis regulation during severe oxygen deficiency. The only exception was an increase in the stimulatory effect of T cells on CFU-E in the early period after administration of sodium hydroxybutyrate under conditions of hypoxic and hemolytic anemia (day 2, Fig. 2). These results attest to possibility of using antihypoxants for modification of the role of Thy-1,2$^+$ cells in the regulation of hemopoiesis.

We conclude that migration of T cells to the bone marrow during severe hypoxia [2,5] is determined by not quantitative changes in the bone marrow Thy-1,2$^+$ cell population, but changes in functional activity of these cells depending on the severity of oxygen deficiency in the organism, but not depending on injury to CNS structures.

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