GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

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

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We studied the role of Thy-1,2$^+$ cells in the regulation of hemopoiesis during oxygen deficiency of different genesis. These cells of the hemopoiesis-inducing microenvironment play an important role in the compensatory and adaptive reactions of the blood system to hypoxia. Thy-1,2$^+$ cells directly or indirectly (via interaction with adherent myelokaryocytes) stimulated hemopoietic precursors. The effect of these cells on committed erythroid precursors was most pronounced.

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

The blood system plays a key role in the maintenance of homeostasis and adaptation of the organism to oxygen deficiency. According to current views T cells constitute one of the most active and labile fractions of the hemopoiesis-inducing microenvironment. Qualitative and quantitative composition of these cells in hemopoietic tissue easily changes under extreme conditions. T cells regulate proliferation and differentiation of hemopoietic precursors via the release of humoral regulators and interaction with other cells of the microenvironment [5]. Migration of T cells expressing membrane Thy-1,2 antigen into hemopoietic tissue often serves as a trigger mechanism of hyperplasia of bone marrow hemopoiesis under the influence of extreme factors [3,5]. The mechanisms of hemopoiesis regulation by T lymphocytes under conditions of oxygen deficiency are poorly understood.

Here we studied the role of Thy-1,2$^+$ cells in blood changes during hypoxia of different genesis.

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MATERIALS AND METHODS

Experiments were performed on 136 CBA/CaLac mice weighing 18-20 g (class I conventional mouse strain 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 modeling hypoxic hypoxia the animals were placed in 500-ml sealed chamber. The mice were removed from this chamber after termination of generalized convulsions and/or visual cessation of breathing within 10-15 sec. Hemic hypoxia was induced by intraperitoneal injection of phenylhydrazine hydrochloride in a single dose of 30 mg/kg or letting of 30% circulating blood (puncture of the retroorbital sinus with a graduated Pasteur pipette washed with heparin). The volume of withdrawn blood was estimated taking into account that the circulating blood volume in rodents corresponds to $^1/_{13}$ of their body weight. The number of bone marrow Thy-1,2$^+$ cells was estimated in the complement-dependent cytotoxic test with monoclonal

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anti-Thy-1,2$^+$ antibodies (clone 5A-8 CL 8600A, Sigma) on days 1-5 and 7 after treatment [4]. The role of Thy-1,2$^+$ cells in the regulation of hemopoiesis was studied on days 2 and 4. The intensity of colony formation (number of erythroid and granulocyte-monocyte colony-forming units, CFU) was compared in cultures of nonadherent bone marrow cells under basal conditions and during culturing on an adherent sublayer. These cultures included Thy-1,2$^+$ cells or were deprived of these cells by treatment with monoclonal anti-Thy-1,2$^+$ antibodies (clone 5A-8 CL 8600A, Sigma) [4].

The results were analyzed by Student's *t* test and nonparametric Wilcoxon—Mann—Whitney *U* test.

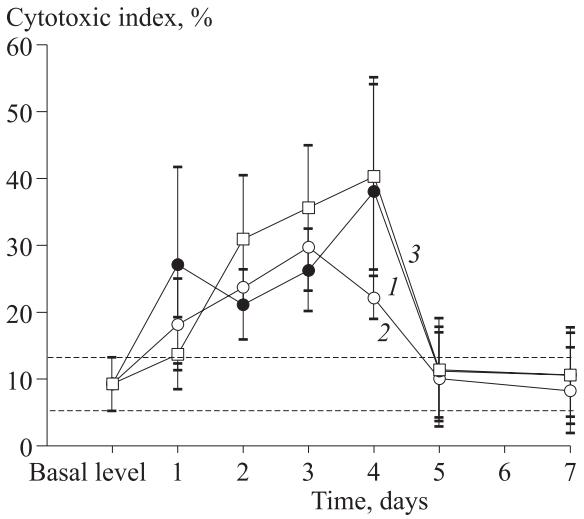

Fig. 1. Dynamis of the content of Thy-1,2$^+$ cells in the bone marrow of CBA/CaLac mice during hypoxic hypoxia (*1*), hemolytic anemia (*2*), and blood loss (*3*). Dotted lines: upper and lower limits of the confidence interval in intact mice, $p \leq 0.05$.

RESULTS

Under conditions of balanced hemopoiesis Thy-1,2$^+$ cells practically did not modulate function of hemopoietic precursors. Colony-forming activity of intact bone marrow cells remained unchanged after the removal of this fraction. Under conditions of oxygen deficiency of different genesis, the content of Thy-1,2$^+$ cells in the bone marrow underwent similar changes. The number of these cells in hemo-

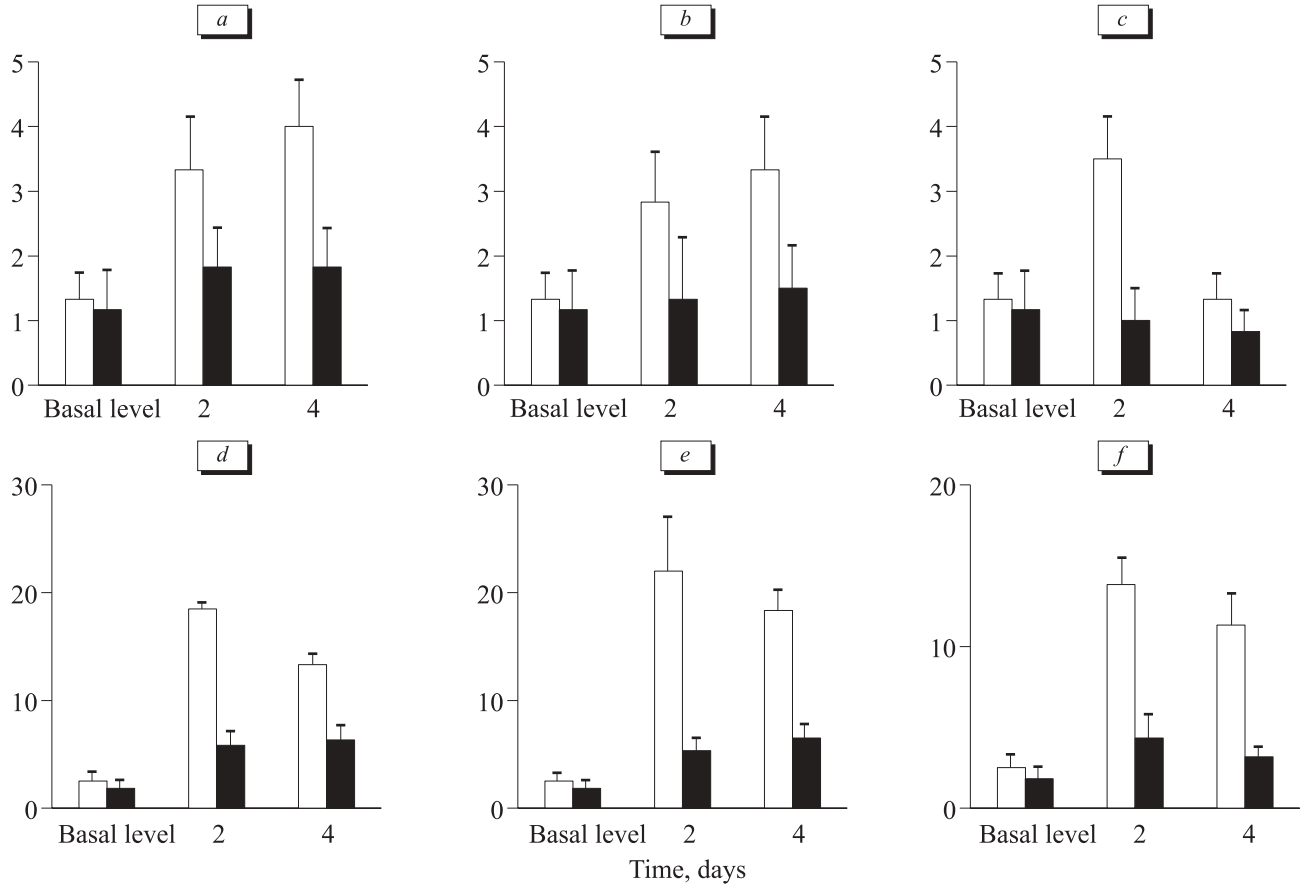

Fig. 2. Growth of erythroid colonies from nonadherent myelokaryocytes (*a*, *b*, *c*) and adherent myelokaryocytes on adherent sublayer (*d*, *e*, *f*) of bone marrow cells of CBA/CaLac mice during hypoxic hypoxia (*a*, *d*), hemolytic anemia (*b*, *e*), and blood loss (*c*, *f*). Here and in Fig. 3: ordinate, colony-forming activity of the bone marrow (per 10^5 myelokaryocytes). Light bars: suspension of complement-treated nonadherent cells (control). Dark bars: suspension of nonadherent cells deprived of Thy-1,2$^+$ cells. Confidence intervals at $p \leq 0.05$.

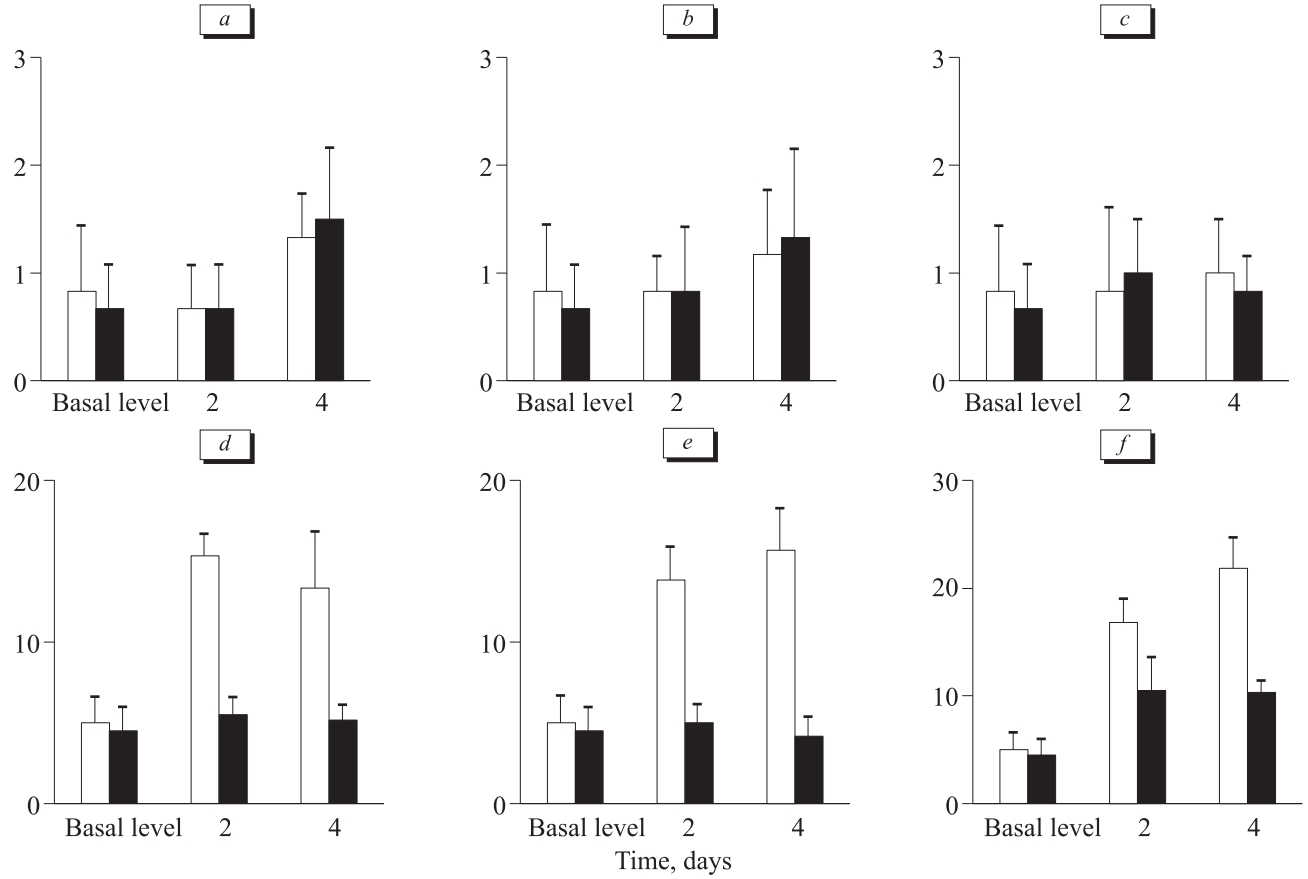

Fig. 3. Growth of granulocyte-monocyte colonies from nonadherent myelokaryocytes (*a*, *b*, *c*) and adherent myelokaryocytes on the adherent sublayer (*d*, *e*, *f*) of bone marrow cells of CBA/CaLac mice during hypoxic hypoxia (*a*, *d*), hemolytic anemia (*b*, *e*), and blood loss (*c*, *f*).

poietic tissue significantly increased on days 2-4 after hypoxic hypoxia and blood loss. This parameter peaked on days 3 and 4 after hypoxic hypoxia (320.3% of the basal level) and blood withdrawal (434.3% of the basal level), respectively. The number of Thy-1,2$^+$ cells increased on days 1-4 of hemolytic anemia and peaked on day 4 (410% of the basal level, Fig. 1).

Against the background of increased content of T cells in the bone marrow under various conditions of hypoxia, we observed considerable stimulation of functional activity of hemopoietic precursors under the influence of these cells. Irrespective on hypoxia model, Thy-1,2$^+$ cells produced a strong stimulatory effect on proliferation and differentiation of erythroid precursors (days 2 and 4). Thy-1,2$^+$ cells of the bone marrow produced a direct activating effect on committed erythroid precursors under conditions of hypoxic hypoxia, hemolytic anemia (days 2 and 4), and blood loss (day 2). The indirect effect of Thy-1,2$^+$ cells in various periods of the study (days 2 and 4) was related to the interaction with adherent myelokaryocytes. The stimulatory effect on granulocytes and macrophages was revealed only during the interaction of Thy-1,2$^+$ cells with stromal cells of the microenvironment (Figs. 2 and 3).

Our results indicate that migration of Thy-1,2$^+$ cells to the bone marrow plays an important role in hyperplasia of hemopoietic tissue under various extreme conditions [1,3,5]. These cells of the hemopoiesis-inducing microenvironment determine the adequate response of the blood system to hypoxia of different genesis. Under conditions of oxygen deficiency Thy-1,2$^+$ cells stimulate primarily the erythroid hemopoietic stem responsible for oxygen supply to tissues. Similarly to other extreme conditions [1,3,5], feeder properties of Thy-1,2$^+$ cells mainly concern committed precursor cells interacting with adherent cells of the bone marrow.

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