GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Role of Humoral Factors in the Regulation of Hemopoiesis during Immobilization Stress

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Experiments were performed on CBA/CaLac mice with hyperplasia of hemopoiesis induced by 10-h immobilization. Erythropoietic and colony-stimulating activity increased under the influence of hemopoietic growth factors and other humoral substances, which plays a major role in the increase in bone marrow cellularity during immobilization stress. Erythropoietin stimulated proliferation of erythroid colony-forming units, while granulocyte colony-stimulating factor accelerated maturation of granulocyte precursors.

Key Words: *hemopoiesis; stress; erythropoietin; granulocyte colony-stimulating factor*

Immobilization stress serves as a standard model for studying functional activity of hemopoietic tissue during hyperplasia [2,6]. Much attention was given to this problem. However, the regulation of hemopoiesis under conditions of its activation requires further investigations. Previous studies showed that immobilization is followed by activation of subsequent stages in the cascade mechanism of hemopoiesis regulation [1,4]. The adaptive response of hemopoietic tissue is determined by general stress-realizing systems (pituitary-adrenal and sympathoadrenal systems) via central neuroendocrine mechanisms. Activation of these systems is accompanied by hyperplasia of the hemopoietic tissue in the bone marrow (primarily due to stimulation of erythropoiesis and granulomonocytopoiesis) and increase in the count of peripheral blood cells. Stimulation of hemopoiesis is associated with intensive migration of regulatory T lymphocytes in the bone marrow. Cells of the hemopoiesis-inducing microenvironment (macrophages and stromal cells) and T lymphocytes determine proliferation and differentiation activity of precursor cells. This effect is related to initiation of production of humoral regulators (cytokines and glycosaminoglycans) and stimulation of cell-cell interactions, which leads to the increased formation of cell associations and accelerated maturation of hemopoietic cells.

It should be emphasized that immobilization stress is accompanied by activation of distant mechanisms of hemopoiesis regulation. These changes are accompanied by an increase in erythropoietic (EPA) and colony-stimulating activity (CSA) in the plasma [2,4]. Fine mechanisms for the regulation of hemopoiesis should be determined in further studies. It is necessary to evaluate the role of individual humoral factors in the regulation of proliferation and differentiation of hemopoietic cells. According to current concepts, erythropoietin acts as the major lineage-restricted regulator of erythropoiesis, while granulocytopoiesis is regulated by granulocyte colony-stimulating factor (G-CSF) [1,7]. Both hemopoietins can stimulate proliferation and maturation of hemopoietic precursors depending on conditions [11,12]. The concentrations of erythropoietin and G-CSF in the blood are extremely low during normal hemopoiesis. Under the influence of extreme factors these compounds are intensively pro-

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duced by various cell types and their content in the peripheral blood markedly increases [10].

Here we evaluated the role of erythropoietin and G-CSF in the regulation of hemopoiesis during immobilization stress.

MATERIALS AND METHODS

Experiments were performed on 105 CBA/CaLac mice (class I conventional mouse strain) aging 2 months and obtained from nursery of the Institute of Pharmacology

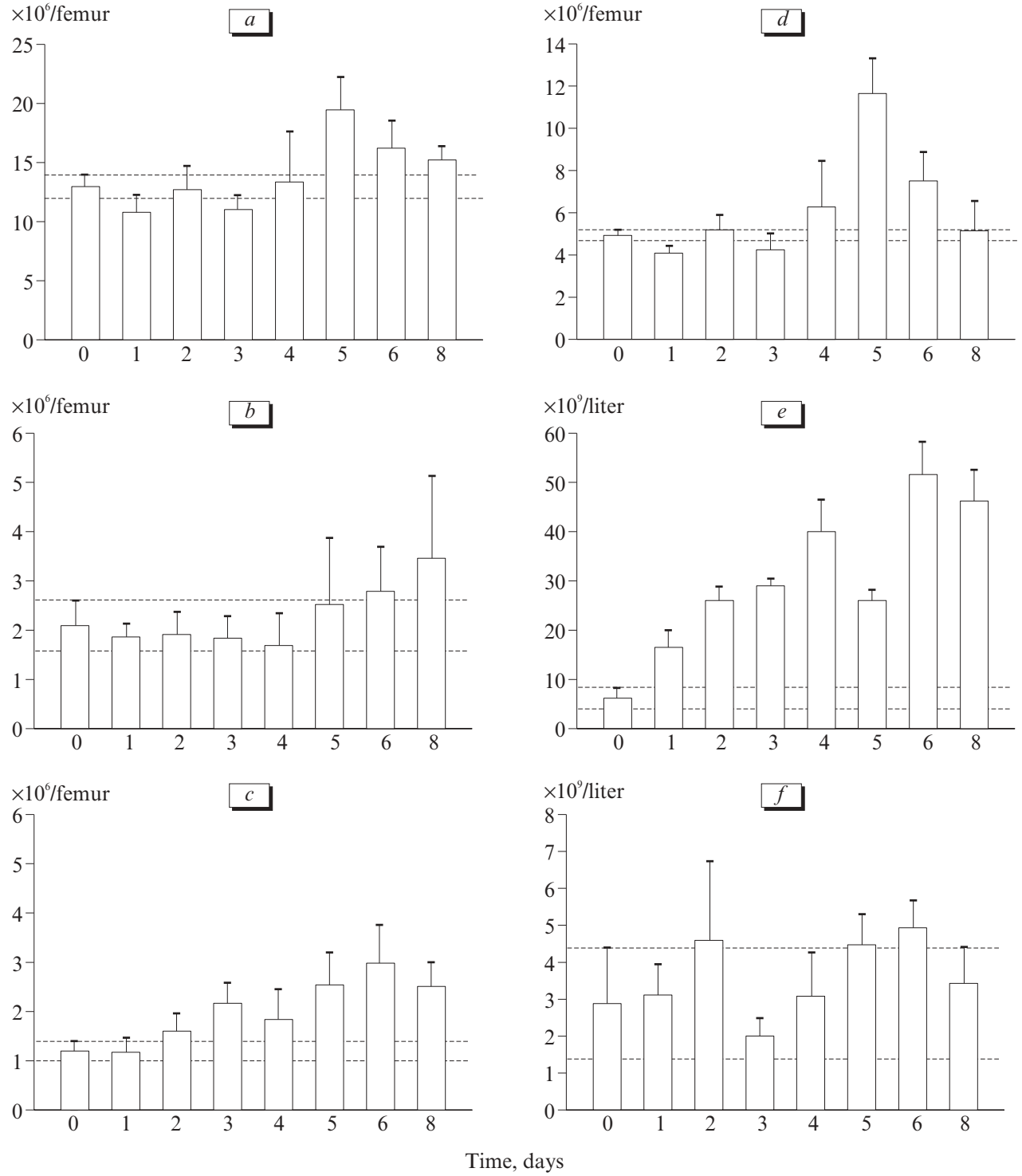

Fig. 1. Total count of myelokaryocytes (*a*), erythrokaryocytes (*b*), immature (*c*) and mature neutrophilic granulocytes in the bone marrow (*d*), and content of reticulocytes (*e*) and segmented neutrophils in the peripheral blood (*f*) from CBA/CaLac mice after 10-h immobilization. Here and in Figs. 2 and 3: confidence intervals at p=0.05.

(Tomsk Research Center). Experimental mice were immobilized on the back for 10 h. The animals were decapitated on days 1-8 under ether anesthesia. The number of peripheral blood leukocytes, erythrocytes, and reticulocytes, white blood count, and indexes of bone marrow hemopoiesis (total number of myelokaryocytes and myelogram) in treated and control mice were determined routinely [8]. The content of committed erythroid (CFU-E) and granulomonocytic precursors (CFU-GM) in the bone marrow was evaluated *in vitro* by cloning nonadherent myelokaryocytes in a semisolid culture medium [3]. EPA and CSA of the plasma from treated and control animals were determined in biological tests [3]. The role of endogenous erythropoietin and G-CSF in the formation of EPA and CSA was evaluated by suppression of erythroid and granulocyte-macrophage colony growth during treatment of peripheral blood plasma with monoclonal antibodies against mouse erythropoietin (MABE, PharMingen) or G-CSF (R&D Systems). The contents of erythropoietin and G-CSF in blood plasma were measured by enzyme immunoassay with Sangui Bio Tech Inc. and R&D Systems kits, respectively, according to manufacturer's recommendations. The intensity of sample staining was determined on an AIFR-01 Uniplan device (PIKON).

The results were analyzed by Student's *t* test [9].

RESULTS

Immobilization of mice for 10 h led to stimulation of erythropoiesis and granulomonocytopoiesis in the bone marrow. On days 5-8 total karyocyte count in the bone marrow of immobilized animals was much higher than in the control. The total cell count peaked on day 5 (150% of the baseline level, Fig. 1). These changes were associated with a considerable increase in the number of mature neutrophilic granulocytes in the hemopoietic tissue (stab and segmented neutrophils, days 5-6), immature neutrophils (myeloblasts, promyelocytes, myelocytes, and metamyelocytes), monocytes and macrophages (days 5-8), and erythronormoblasts (day 8).

The study of blood leukocytes and individual cells showed that white blood count corresponds to the state of bone marrow hemopoiesis. The number of circulating segmented neutrophils in stressed mice increased 6 days after immobilization (Fig. 1). Examination of the peripheral erythron in various periods after treatment revealed reticulocytosis.

Immobilization of mice for 10 h increased colony-forming activity of the bone marrow (Fig. 2). The number of CFU-E in hemopoietic tissue increased to 188-532% on days 2, 4, and 6. The content of CFU-GM 2 days after immobilization increased more than 2-fold, but on day 6 this parameter decreased. These temporal changes were probably related to accelerated maturation of precursor cells.

Comparative study of myelogram and colony-forming activity of the bone marrow indirectly characterizes proliferation and differentiation of hemopoietic precursors [5]. The increase in the count of morphologically identified erythroid cells and immature neutrophils coincides with the decrease in the number of precursors. These data suggest that accelerated maturation of hemopoietic precursors follows the increase in proliferative activity and plays a major role in the development of hemopoietic hyperplasia during stress.

Plasma EPA increased to 242, 290, and 468% of the baseline level on days 1, 2, and 6 after immobilization, respectively (Fig. 3). MABE added to the

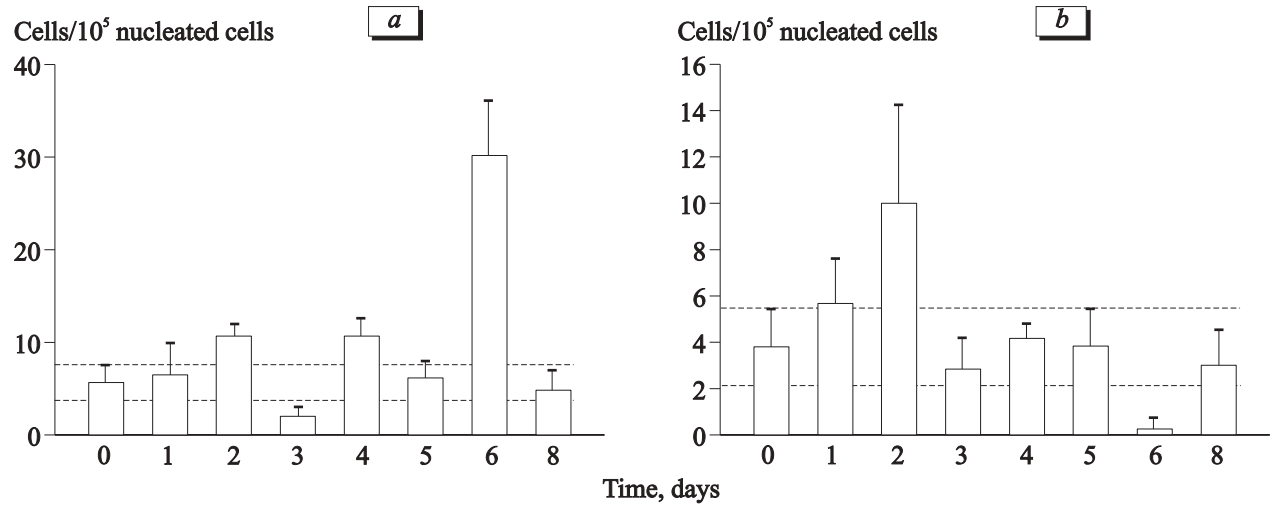

Fig. 2. Content of committed precursor cells for erythropoiesis (*a*) and granulomonocytopoiesis (*b*) in the bone marrow of CBA/CaLac mice after 10-h immobilization.

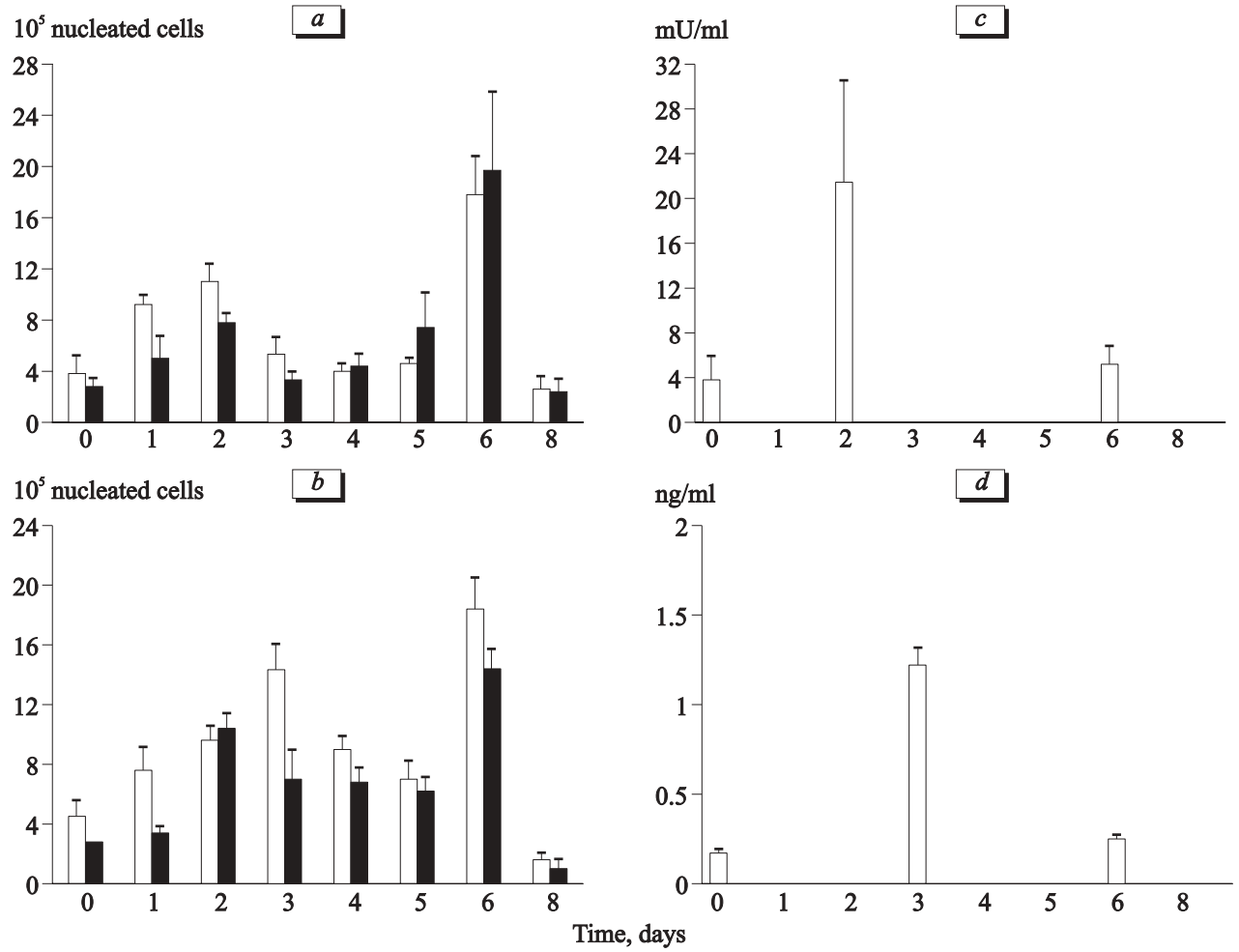

Fig. 3. Erythropoietic (*a*) and colony-stimulating activity (*b*) and concentrations of erythropoietin (*c*) and granulocyte CSF (*d*) in peripheral blood plasma from CBA/CaLac mice after 10-h immobilization. Dark bars: plasma treated with monoclonal antibodies against mouse erythropoietin (*a*) and granulocyte CSF (*b*).

culture medium markedly decreased EPA of the plasma from immobilized mice on days 1 and 2 (to 54 and 71%, respectively). MABE did not abolish the increase in plasma EPA on day 6 after immobilization. Therefore, some factors differing from erythropoietin determine EPA in this period.

CSA of the plasma increased after 10-h immobilization and reached maximum on days 3 and 6 (Fig. 3). Treatment of the plasma obtained on days 1, 3, 4, and 6 with monoclonal antibodies against G-CSF considerably decreased CSA (more than by 2 times in the early period). However, this procedure did not completely abolish the increase in CSA. These results indicate that various neuroendocrine factors are involved in the formation of plasma EPA under extreme conditions.

The concentrations of erythropoietin and G-CSF in the peripheral blood were estimated by means of enzyme immunoassay. These measurements were performed in the period of most significant changes in plasma EPA and CSA, respectively. The mean content of erythropoietin in the blood markedly increased and surpassed the baseline level by 5.66 times on day 2 (Fig. 3). Despite the considerable increase in blood EPA on day 6 after immobilization, the concentration of endogenous erythropoietin did not differ from that in intact mice. The data suggest that other humoral substances are involved in the regulation of erythropoiesis during this period. Plasma G-CSF content increased and surpassed the baseline level by 1.05 ng/ml on day 3 after stress. Similar results were obtained when studying neutralization of G-CSF with monoclonal antibodies (Fig. 3). The concentration of this cytokine in the blood from stressed mice decreased on day 6, but remained higher than in intact animals.

We conclude that the increase in functional activity of hemopoietic precursors during immobilization is determined by the increase in plasma EPA and CSA. Erythropoietin stimulates proliferation of CFU-E, while G-CSF accelerates maturation of granulocyte

precursors. Therefore, humoral factors of the peripheral blood play a major role in the regulation of hemopoiesis during stress.

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