Hemopoietic Precursors: Mechanisms of Regulation under Conditions of Chronic Stress

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We studied the content of hemopoietic precursor cells in the bone marrow, production of IL-1, IL-3, and colony-stimulating and erythropoietic activities by bone marrow cells under conditions of chronic stress. It was shown that in mice subjected to 15-h immobilization no hyperplasia of the bone marrow developed against the background of increased content of committed erythro- and granulomonocytopoietic precursors and enhanced production of short-distant humoral regulators of hemopoiesis, which reflects dysregulation of hemopoietic cells proliferation and differentiation processes in stressed animals.

Key Words: *hemopoietic precursors; cytokines; chronic stress*

The key role in the regulation of proliferation and differentiation of hemopoietic precursor cells under extreme conditions is played by hemopoiesis-inducing microenvironment (HIM) [4,9]. The important role in the mechanisms of hemopoiesis regulation during stress is played by HIM cells releasing some humoral factors (erythropoietin, IL-1, IL-3, IL-6, IL-11, CSF) essential for adequate response of the hemopoietic tissue [5,7]. Additional need for mature cells developing in some pathologies leads to increased strain in the blood system, in particular, massive consumption of hemopoietic precursors, which are required for replenishment of cell death in hierarchically lower populations [2,9]. However, long-term stress can exhaust the compensatory reserve of the hemopoietic tissue, which is accompanied by dysregulation of the pool of hemopoietic precursors and suppression of all hemopoietic lineages [8]. The mechanism of this phenomenon remains unclear.

Here we studied regulation of proliferation and differentiation of hemopoietic precursor cells under conditions of chronic stress.

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MATERIALS AND METHODS

Experiments were carried out on 2-2.5-month-old male (n=140) CBA/CaLac mice weighting 18-20 g. The animals were subjected to repeated 15-h immobilization (with a forceps by the skin fold on the neck) over 10 days. Intact CBA/CaLac mice (n=6) served as the control. The animals were obtained from nursery of Institute of Pharmacology, Tomsk Research Center (certified animals). The mice were sacrificed by cervical dislocation under ether narcosis at different terms of the experiment. The number of myelokaryocytes in the bone marrow was determined routinely; their qualitative composition was evaluated on smears stained after Nocht—Maksimov [6].

Hemopoietic islets were isolated, the content of erythro- and granulomonocytopoiesis precursor cells (CFU-E and CFU-GM) was determined, IL-1, IL-3, colony-stimulating and erythropoietic activities of media conditioned by adherent and nonadherent bone marrow cells were assayed [6]. The number of Thy-1,2$^+$ cells was determined by the method of complement-dependent cytolysis using anti-Thy-1,2$^+$ monoclonal antibodies (clone 5A-8 CL 8600A, Cedarlane) [6]. Feeder

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activity of adherent bone marrow cells was evaluated by their ability to maintain colony-formation [1].

The data were processed statistically using Statsoft software. Student *t* and Wilcoxon—Mann—Whitney *U* tests were applied [3].

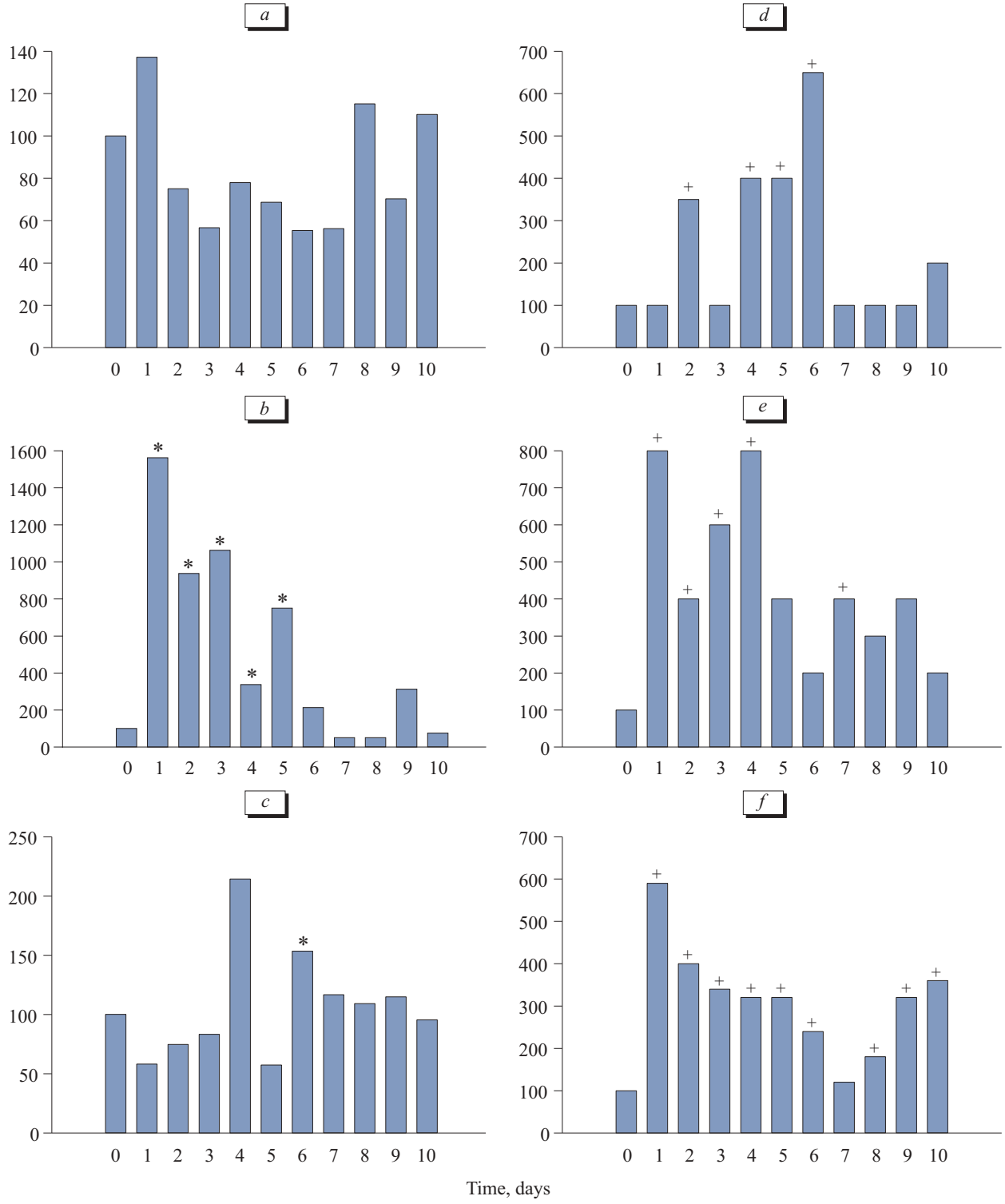

Fig. 1. Content of erythroid cells (*a*), CFU-E (*b*), and erythroid islets (*c*) in the bone marrow and production of erythropoietic activity (EPA) by adherent (*d*), nonadherent (*e*) myelokaryocytes and in blood serum (*f*) of CBA/CaLac mice subjected to repeated 15-h immobilization. Ordinate: total number of erythroid cells per femur, % of baseline (*a*); number of CFU-E per femur, % of baseline (*b*); number of erythroid islets per femur, % of baseline (*c*); EPA, % of baseline (*d-f*). Here and Fig. 2: $^{*}P_t<0.05$, $^{+}P_U<0.05$ compared to the control.

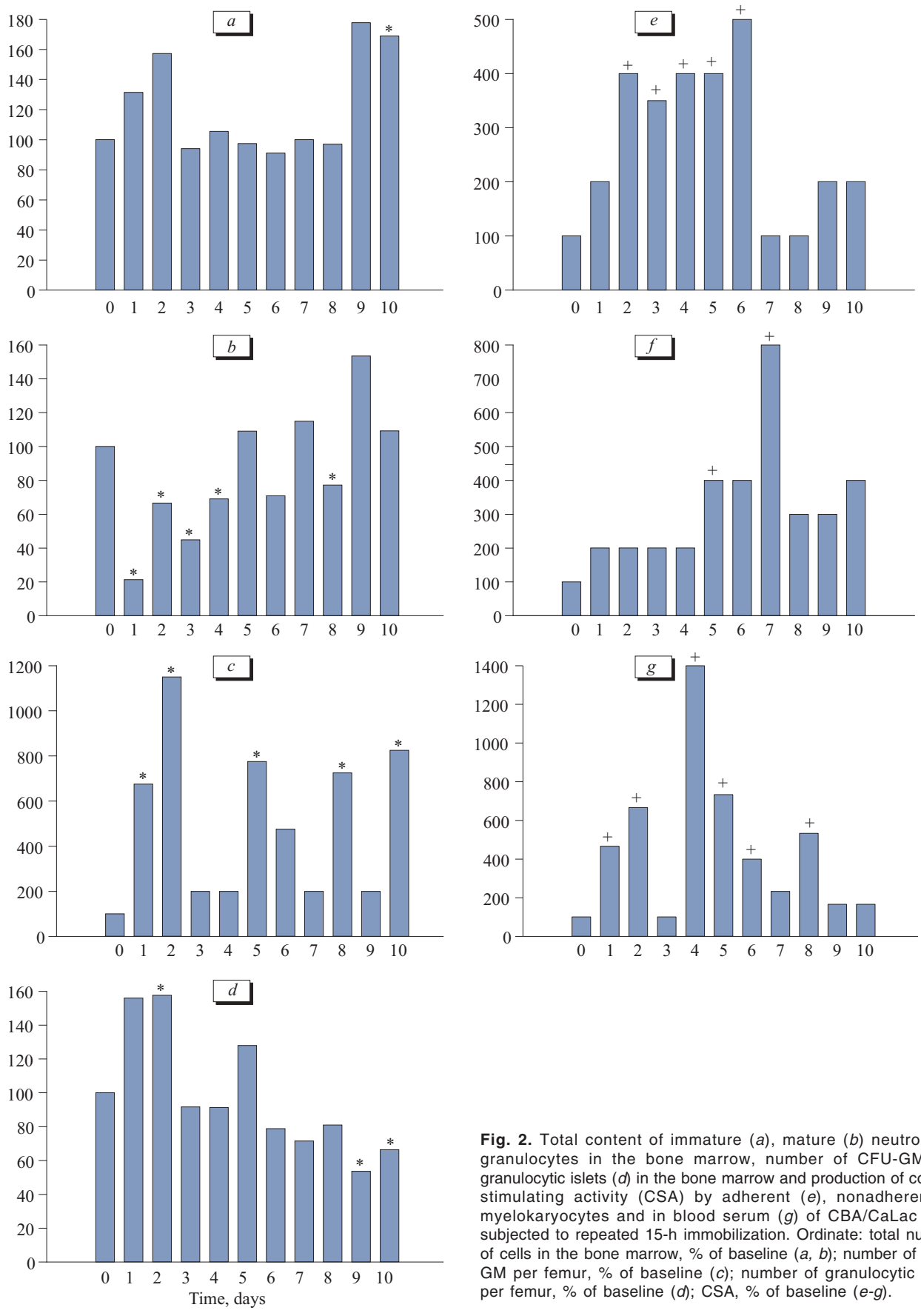

Fig. 2. Total content of immature (*a*), mature (*b*) neutrophilic granulocytes in the bone marrow, number of CFU-GM (*c*), granulocytic islets (*d*) in the bone marrow and production of colony-stimulating activity (CSA) by adherent (*e*), nonadherent (*f*) myelokaryocytes and in blood serum (*g*) of CBA/CaLac mice subjected to repeated 15-h immobilization. Ordinate: total number of cells in the bone marrow, % of baseline (*a, b*); number of CFU-GM per femur, % of baseline (*c*); number of granulocytic islets per femur, % of baseline (*d*); CSA, % of baseline (*e-g*).

RESULTS

The production of erythroid precursors sharply increased at early terms of the experiment (days 1-3); their number increased by more than 10 times compared to the control (Fig. 1, *b*). The formation of granulomonocyte-macrophage colonies was also increased from the first days of the experiment. The yield of CFU-GM was significantly increased on days 1-2, 5, 8, and 10 of the experiment (675-1150, 775, 725, and 825%, respectively, from baseline level; Fig. 2, *c*).

Analysis of the morphological composition of the bone marrow in stressed animals revealed decreased number of erythrokaryocytes (except day 1, Fig. 1, *a*) and slightly increased content of erythroid islets (significant on day 6, Fig. 1, *c*). This can result from disturbed differentiation of erythroid cells, because, according to modern views, maturation of hemopoietic cells from precursor to more mature forms occurs in hemopoietic islets [10]. In the bone marrow of mice subjected to repeated immobilization the content of immature neutrophilic granulocytes did not increase throughout the experiment, except day 10 (Fig. 2, *a*). The content of mature neutrophilic granulocytes after a transient decrease (days 1-4) gradually recovered and attained the initial level on day 9 of the experiment (Fig. 2, *b*). Analysis of structural and functional organization of the bone marrow revealed considerably increased content of granulocytic islets only on day 2 of the experiment (157.7% from the initial level), but then this parameter decreased (significant decrease was observed on days 9-10; Fig. 2, *d*).

The content of CFU-E in the bone marrow increased against the background of enhanced production of erythropoietic activity by nonadherent (days 1-4) and adherent (days 4-6) myelokaryo-

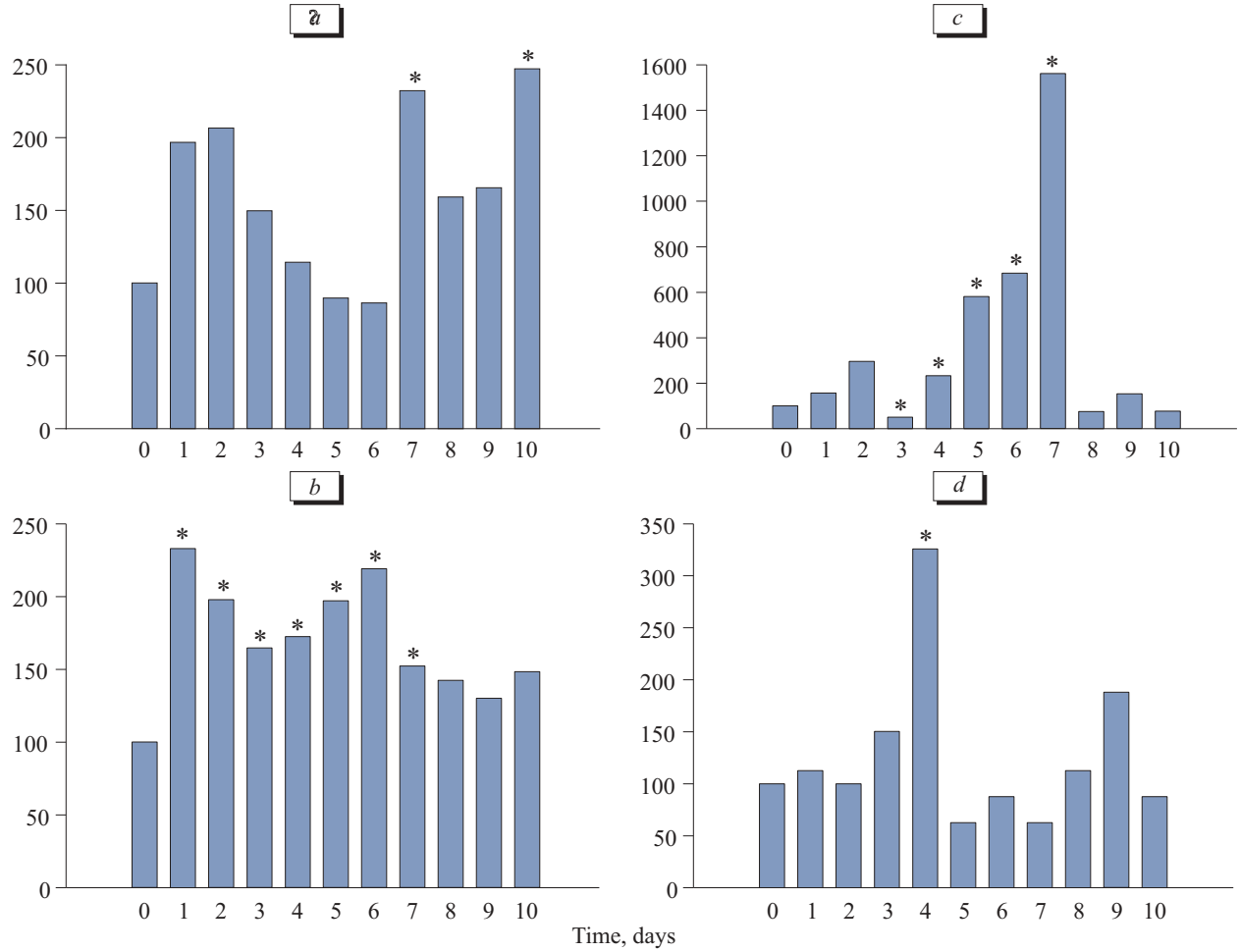

Fig. 3. Content of Thy-1,2$^+$ cells in the bone marrow (*a*), production of IL-1 activity by adherent (*b*), IL-3 activity by nonadherent (*c*) myelokaryocytes and feeder activity of adherent bone marrow cells (*d*) from CBA/CaLac mice subjected to repeated 15-h immobilization. Ordinate: content of Thy-1,2$^+$ cells, % of baseline (*a*); IL-1 activity, % of baseline (*b*); IL-3 activity, % of baseline (*c*); feeder activity, % of baseline (*d*). *P_t<0.05 compared to the control.

cytes (Fig. 1, *d*, *e*); serum erythropoietic activity also increased (Fig. 1, *f*). Colony-stimulating activity increased in both media conditioned by adherent myelokaryocytes (significant increase was observed on days 2-6) and supernatants of nonadheon days 5 and 7 respectively, from baseline level). The level of colony-stimulating activity in the serum of experimental animals on days 1-2, 4-6, and 8 of the experiment significantly increased compared to the control (Fig. 2, *e-g*).

Repeated stress exposure was not associated with accumulation of Thy-1,2$^+$ cells in the bone marrow at early terms of the experiment against the background of pronounced thymus involution, although previous experiments demonstrated increased content of T cells in the bone marrow under conditions of immobilization stress [9]. Significant accumulation of Thy-1,2$^+$ cells in the bone marrow was noted at later terms: on days 7 and 10 (232.2 and 247.2% compared to the initial level; Fig. 3, *a*). This cell population is probably responsible for enhanced production of IL-3 (maximum on day 7; Fig. 3, *c*) in supernatants of nonadherent bone marrow cells from stressed mice. Production of IL-1 by adherent myelokaryocytes was enhanced starting from the first day of the experiment and remained increased throughout the observation period (Fig. 3, *b*), being a manifestation of nonspecific response of the organism [11]. Feeder activity of adherent bone marrow cells from stressed animals was considerably increased only on day 4 of the experiment and did not differ from the control at other terms (Fig. 3, *d*).

Thus, the absence of hyperplasia of the bone marrow hemopoiesis together with increased yield of hemopoietic precursors, enhanced production of short-distant regulators of hemopoiesis, and minor increase in the number of hemopoietic islets attested to imbalance in hemopoietic cell proliferation and differentiation under conditions of chronic stress.

REFERENCES

1. I. V. Bogdashin, E. V. Sycheva, V. V. Zhdanov, *et al., Byull. Eksp. Biol. Med.,* **113**, No. 3, 282-284 (1992).
2. R. P. Gale and A. Butturini, *Gematol. Transfuziol.*, **39**, No. 6, 3-6 (1994).
3. V. E. Gmurman, *Probability Theory and Mathematical Statistics* [in Russian], Moscow (2001).
4. E. D. Gol'dberg, A. M. Dygai, and V. V. Zhdanov, *Role of Hemopoiesis-Inducing Environment in Regulation of Hemopoiesis in Cytostatic Myelosuppression* [in Russian], Tomsk (1999).
5. E. D. Gol'dberg, A. M. Dygai, and I. A. Khlusov, *Role of Autonomic Nervous System in Regulation of Hemopoiesis* [in Russian], Tomsk (1997).
6. E. D. Gol'dberg, A. M. Dygai, and V. P. Shakhov, *Methods of Tissue Culture in Hematology* [in Russian], Tomsk (1992).
7. E. D. Gol'dberg, A. M. Dygai, and E. Yu. Sherstoboev, *Mechanisms of Local Regulation of Hemopoiesis* [in Russian], Tomsk (2000).
8. P. D. Gorizontov, O. I. Belousova, and M. I. Fedotova, *Stress and Blood System* [in Russian] Moscow (1983).
9. A. M. Dygai and V. P. Shakhov, *Role of Cell-Cell Interactions in Regulation of Hemopoiesis* [in Russian], Tomsk (1989).
10. Yu. M. Zakharov and A. G. Rassokhin, *An Erythroblastic Islet* [in Russian, Moscow (2002).
11. C. A. Dinarello, *Blood*, **87**, 2095-2147 (1996).