GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Local Regulation of Proliferation and Differentiation of Hemopoietic Precursors during Experimental Neuroses

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 1, pp. 17-22, January, 2002
Original article submitted November 14, 2001

Intensive formation of hemopoietic islets and secretion of humoral hemopoiesis stimulators by cells of the hemopoietic microenvironment underlie activation of proliferation and differentiation of erythroid and granulocyte-macrophage precursors in conflict situation. Stimulation of division and maturation of committed granulomonocytic precursors during paradoxical sleep deprivation is primarily related to intensive secretion of humoral factors by hemopoietic elements. The inhibition of proliferative activity and differentiation of erythroid precursors is associated with the impaired formation of erythroid hemopoietic islets and suppressed secretion of humoral erythropoiesis stimulators by nonadherent fractions of the hemopoietic microenvironment.

Key Words: *experimental neuroses; hemopoietic precursors; hemopoietic islets; hemopoiesis-inducing microenvironment*

Our previous studies revealed the mechanisms underlying adaptive reconstruction of the hemopoietic tissue in experimental animals with neuroses of various geneses [5,6]. Conflict situations are accompanied by hyperplasia of the bone marrow hemopoiesis. Paradoxical sleep deprivation is followed by stimulation of granulocytopoiesis and inhibition of erythropoiesis [5]. Various neurotransmitters, including norepinephrine, dopamine, serotonin, and acetylcholine are involved in plastic reconstructions of the hemopoietic tissue during neuroses [3,9,10]. The regulatory effects of neurotransmitters on hemopoiesis are realized via the sympathetic nervous system [8]. Erythropoiesis and granulocytopoiesis are associated with activity of serotoninergic and dopaminergic systems, respectively [9]. During experimental neuroses adrenergic receptors localized on elements of the hemopoiesis-inducing microenvironment (HIM) and hemopoietic precursors transfer information to hemopoietic target cells [2,8]. The mechanisms underlying local regulation of hemopoiesis during experimental neuroses are poorly understood.

Here we studied the role of HIM in the regulation of proliferation and differentiation of committed precursors in conflict situation and paradoxical sleep deprivation.

MATERIALS AND METHODS

Experiments were performed on 150 CBA/CaLac mice aging 2-2.5 months (Laboratory of Experimental Biological Modeling, Institute of Pharmacology, Tomsk Research Center). Conflict situation (10 min) [7] and paradoxical sleep deprivation (48 h) [14] served as the models of experimental neuroses. On days 1-7 the animals were euthanized by cervical dislocation under ether anesthesia. Unfractionated bone marrow cells (2×10^5 nuclear cells/

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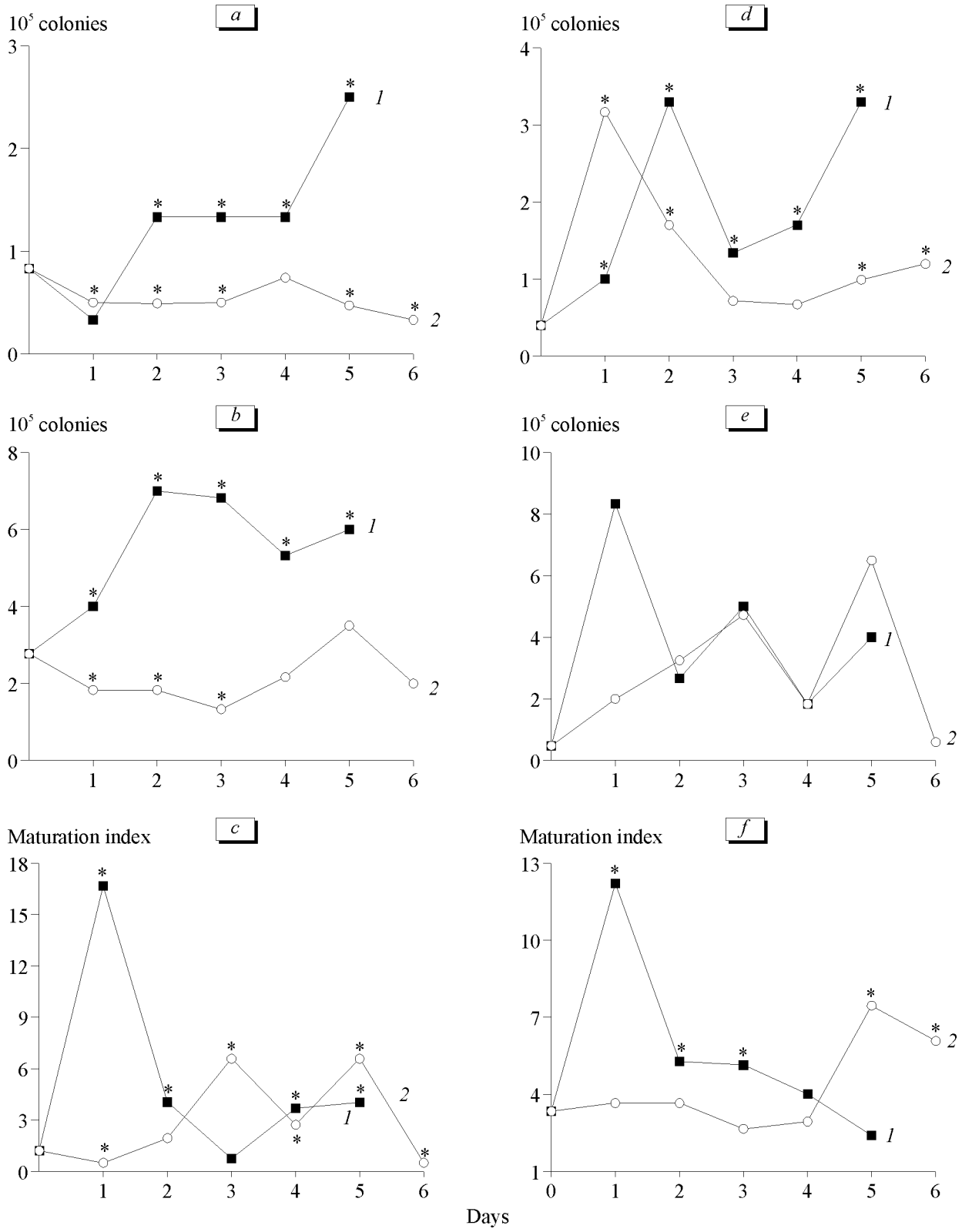

Fig. 1. Contents of erythroid (*a*) and granulocyte-macrophage CFU (*d*), erythroid (*b*) and granulocyte-macrophage cluster-forming units (*e*), and intensity of maturation of erythroid (*c*) and granulocyte-macrophage CFU (*f*) in the bone marrow of CBA/CaLac mice. Here and in Figs. 2 and 3: conflict situation (*1*) and paradoxical sleep deprivation (*2*).

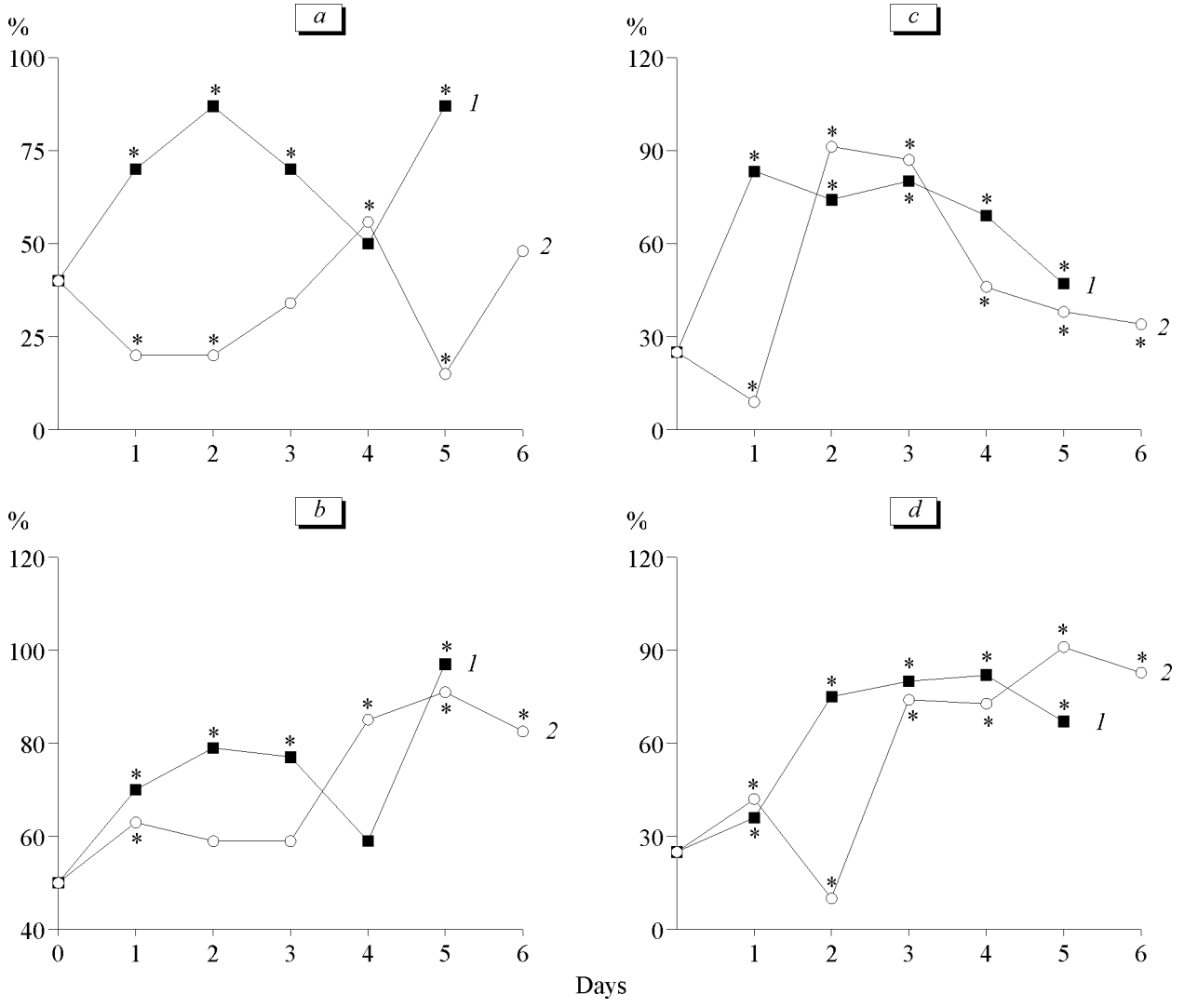

Fig. 2. Counts of erythroid (*a*, *c*) and granulocyte-macrophage (*b*, *d*) CFU (*a*, *b*) and cluster-forming units (*c*, *d*) in S-phase of the cell cycle in the bone marrow of CBA/CaLac mice.

ml) were cultured for 3 and 7 days in methylcellulose tissue culture for obtaining colony- (CFU) and cluster-forming units (ClFU) of erythro- (CFU-E and ClFU-E) and granulomonocytopoiesis (CFU-GM and ClFU-GM), respectively [1]. The intensity of hemopoietic precursor differentiation was estimated by the index of maturation (ratio between the counts of clusters and colonies grown in the same well). Proliferative activity of precursors was evaluated by the method of cell suicidal using hydroxyurea [1]. Structural and functional organization of the bone marrow was determined by enzymatic isolation of hemopoietic islets and their counting in a Goryaev chamber. The quantitative composition of hemopoietic islets was estimated by examination of cytological preparations. Erythroid, granulocytic, and mixed (erythrogranulocytic) hemopoietic islets were isolated by estimating morphological characteristics of cells associated with central elements [1]. Erythropoietic and colony-stimulating activities in media conditioned by adherent and nonadherent HIM cells were measured in a semisolid culture on intact mouse myelokaryocytes [1].

The results were analyzed by standard methods of variational statistics. The significance of differences was evaluated by Student's *t* test and Wilcoxon nonparametric rank test.

RESULTS

Conflict situation led to a significant increase in the count of bone marrow CFU-E and ClFU-E on days 2-5 and 1-5, respectively. The number of CFU-GM and ClFU-GM increased on days 1-5 (Fig. 1). After paradoxical sleep deprivation the content of hemopoietic precursors in mice underwent other changes. The

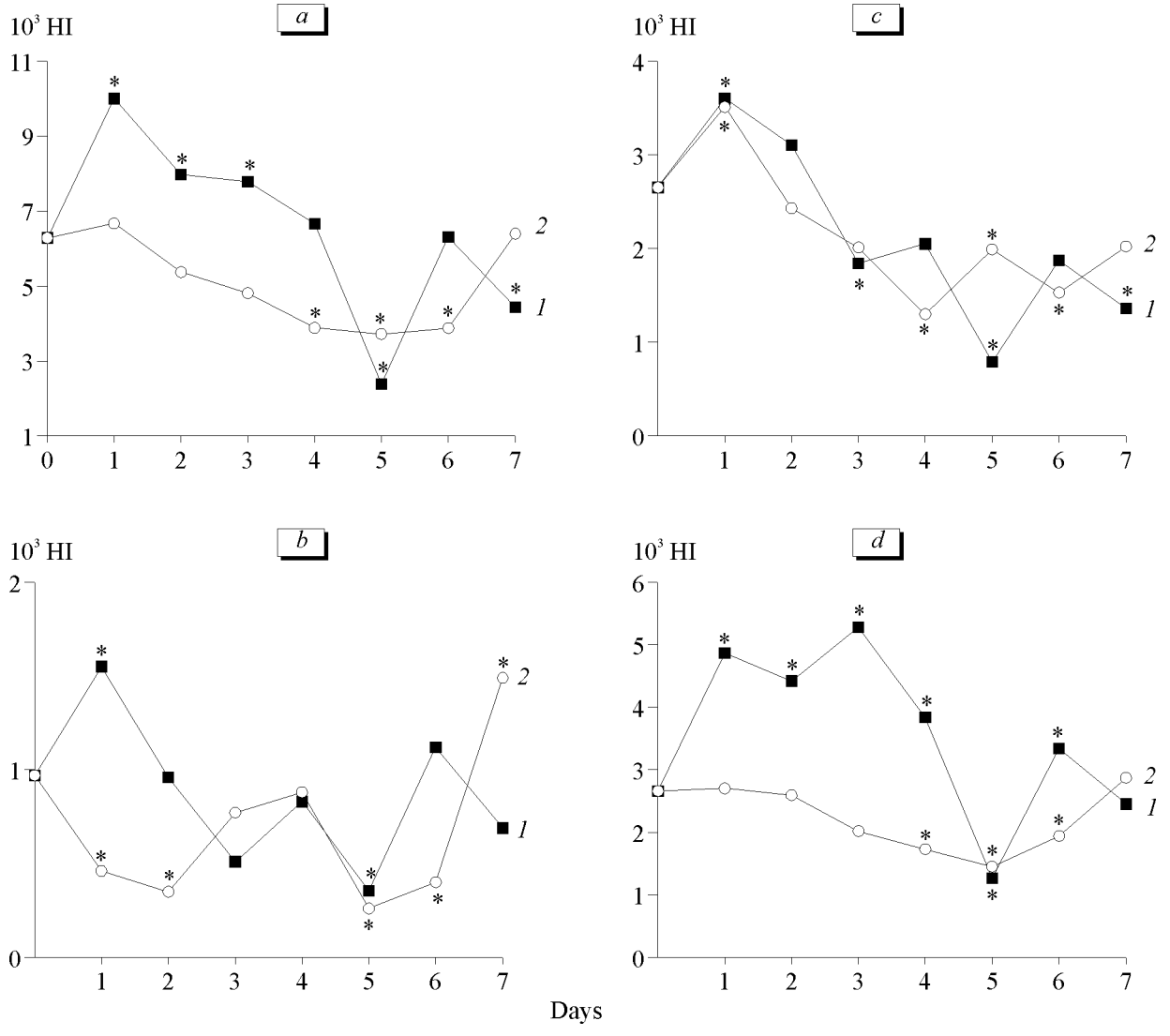

Fig. 3. Total count of hemopoietic islets (HI, *a*) and number of erythroid (*b*), granulocytic (*c*), and mixed HI (*d*) in the bone marrow of CBA/CaLac mice.

increase in the count of bone marrow CFU-GM (days 1, 2, 5, and 6) and ClFU-GM (days 1-5) was accompanied by a decrease in the number of CFU-E (days 1-3, 5, and 6) and ClFU-E (days 1-3).

Proliferative activity of erythroid and granulocyte-macrophage precursors markedly increased in conflict situation (Fig. 2). These changes were accompanied by a significant increase in the maturation index for erythroid (days 1, 2, 4, and 5) and granulocyte-macrophage precursors (days 1-3, Fig. 1). The count of granulocyte-macrophage precursors in S-phase of the cell cycle increased after paradoxical sleep deprivation (CFU-GM on days 1 and 4-6; ClFU-GM on days 1 and 3-6, Fig. 2), while activation of differentiation of granulomonocytic precursors was initiated only by the end of the observation period (days 5 and 6). The count of DNA-synthesizing bone marrow CFU-E decreased, while the number of erythroid clusters increased. The intensity of maturation of erythroid precursors underwent wave changes. The rate of differentiation decreased on days 1 and 6, but increased on days 3-5 (Fig. 1).

After conflict situation the total number of hemopoietic islets in mouse bone marrow increased due to accumulation of mixed (erythrogranulocytic) structures (Fig. 3). It should be emphasized that the count of erythroid and granulocytic hemopoietic islets increased 1 day after conflict situation, but decreased on days 5 and 3, 5, and 7, respectively. By contrast, the count of hemopoietic islets was low at all terms after paradoxical sleep deprivation.

Proliferation and differentiation of hemopoietic cells is regulated not only by cell-cell interactions, but also by humoral factors released from HIM cells [4]. Conflict situation intensified secretion of humoral factors by adherent and nonadherent HIM cells regu-

lating erythropoiesis and granulomonocytopoiesis. Paradoxical sleep deprivation was followed by an increase in colony-stimulating activity in supernatants of adherent (days 1, 2, and 4-7) and nonadherent HIM cells (days 2, 3, and 5-7); erythropoietic activity of nonadherent fractions also increased (days 2-7). However, erythropoietic activity in adherent HIM cells decreased on days 1-3 and 5-7.

Our results indicate that the activation of proliferation and differentiation of hemopoietic precursors associated with the formation of hemopoietic islets and secretory activity of HIM elements underlies hyperplasia of erythro- and granulomonocytopoiesis in the bone marrow. However, during paradoxical sleep deprivation activation of proliferation and differentiation of granulocyte-macrophage precursors is related only to intensive secretion of short-distance humoral regulators. These changes are not accompanied by an increase in the count of hemopoietic islets, in which committed hemopoietic precursors develop into mature cells [13]. The decrease in proliferative activity of erythroid precursors and inhibition of differentiation of erythropoietic precursors are associated with impaired formation of erythroid (macrophage-positive) and mixed hemopoietic islets and suppressed production of humoral erythropoiesis stimulators by adherent HIM cells.

These findings should be analyzed with respect to the regulation of hemopoiesis by neurotransmitters during neuroses [2,3,8-10]. Plastic reconstruction of the hemopoietic tissues in conflict situation and paradoxical sleep deprivation is a complex multifactor process, which can be analyzed in terms of the theory of functional systems [11,12]. According to this theory, neurotransmitters, sympathetic nervous system, adrenoceptors, and HIM elements are the constituents of this functional system.

This work was supported by the Russian Foundation for Basic Research (grant No. 00-04-48745).

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