Specific Features of the Erythroid Hemopoietic Stem in CBA/CaLac Mice with Neuroses Demonstrating Good and Poor Learning Capacities

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We studied changes in the erythroid hemopoietic stem in CBA/CaLac mice with experimental neuroses demonstrating good and poor learning capacities (conflict situation and paradoxical sleep deprivation followed by training in a 3-arm T-maze). The animals with different learning capacities exhibited pronounced hyperplasia of the erythroid hemopoietic stem in response to neurosis. Activation of the erythron in good learners was related to acceleration of division and maturation of erythroid precursors and enhanced formation of cell complexes containing central macrophage. In poor learners hyperplasia of the erythroid hemopoietic stem under conditions of conflict situation manifested in activation of proliferation and differentiation (against the background of decreased count of erythroid and mixed complexes in the bone marrow), while after paradoxical sleep deprivation followed by T-maze training this hyperplasia was associated with increased formation of additional hemopoietic islets (against the background of desynchronization of division and maturation of erythroid precursor cells).

Key Words: *erythropoiesis; individual reactivity; experimental neurosis; hemopoiesis-inducing microenvironment; regulation*

The blood system plays a role in adaptation to neuroses [3,4]. Proliferation and differentiation of hemopoietic cells during experimental neuroses are regulated by a complex multilevel system consisting of distant (neurotransmitters) and local mechanisms [1]. Transduction of the signals from the central nervous system to hemopoietic cells is realized via α- and β-adrenergic receptors on cells of the hemopoiesis-inducing microenvironment and hemopoietic precursors.

The stress response and neurotic reaction mainly depend on specific features of cognitive behavior. Specific functions of the neurotransmitter, neurohormonal, bioenergetic, and other systems differ in animals exhibiting different behavioral reactions to stress and emotional load [5,6,8]. The search and synthesis of new drugs are based on the dependence of their effects on individual behavioral characteristics (higher nervous activity) [5,8].

At the same time, the responses of the blood system to emotional and stress factors in animals with different behavioral characteristics remain unknown. The study of neuroses is of particular importance, since the reaction in humans depends on individual evaluation of the neurotic factor.

This work was designed to study the role of individual characteristics of cognitive behavior in reactivity of the erythroid hemopoietic stem and local mechanisms of regulation of the erythron during experimental neuroses.

MATERIALS AND METHODS

Experiments were performed on 120 CBA/CaLac mice (class I conventional mouse strain) aging 2-2.5 months and obtained from the collection of the Laboratory of

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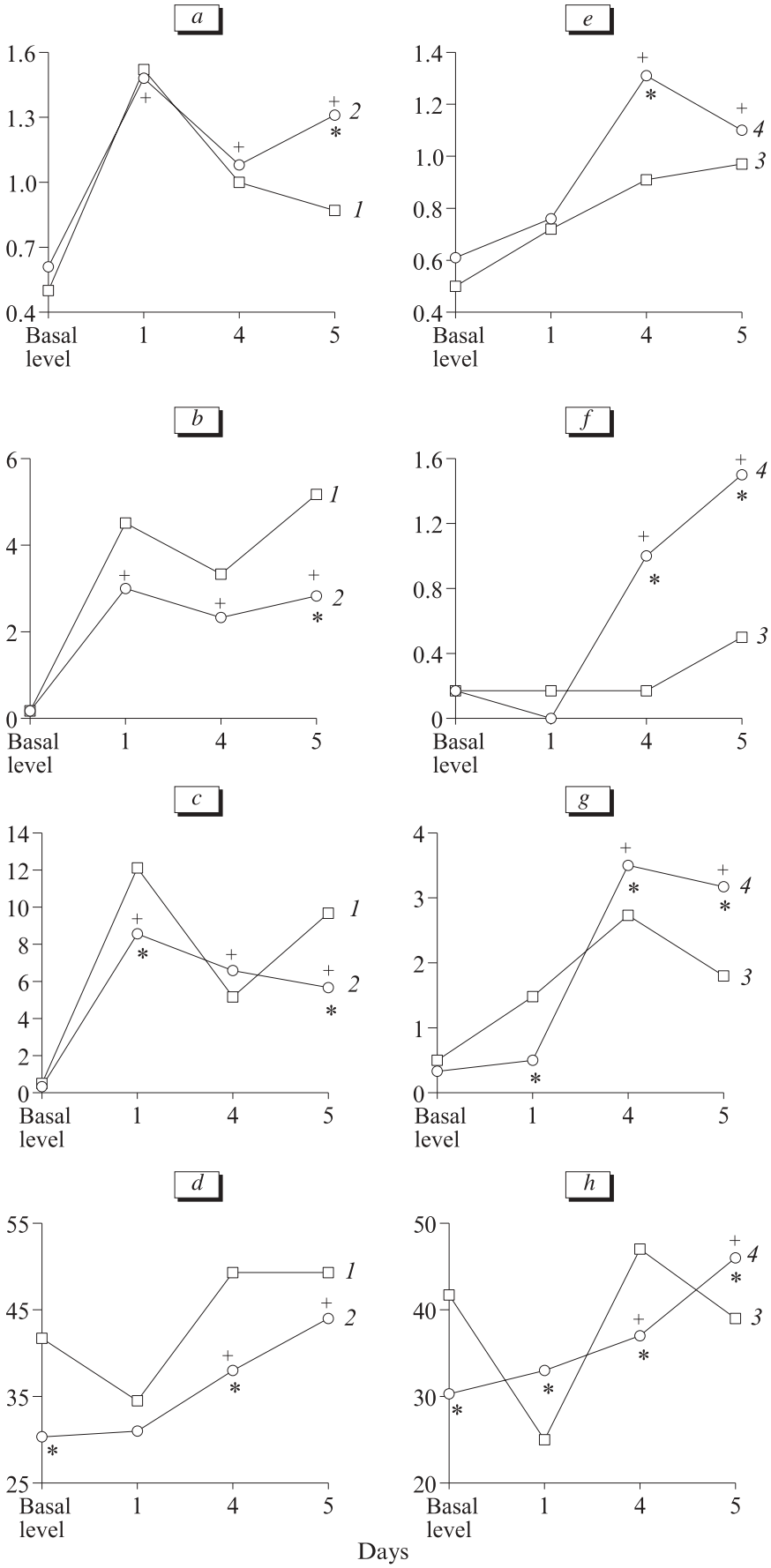

Fig. 1. Count of bone marrow erythrokaryocytes (*a*, *e*), CFU-E (*b*, *f*), and CIFU-E (*c*, *g*) and number of peripheral blood reticulocytes (*d*, *h*) in good (*1*, *3*) and poor learned CBA/CaLac mice (*2*, *4*) subjected to conflict situation (*1*, *2*) or paradoxical sleep deprivation and training in a 3-arm T-maze (*3*, *4*). Ordinate: number of erythrokaryocytes ($\times 10^6$ cells in femur, *a*, *e*) CFU-E (*b*, *f*), CIFU-E (*c*, *g*), colonies and clusters ($\times 10^5$ cells in bone marrow), and peripheral blood reticulocytes (‰, *d*, *h*). Here and in Figs. 2 and 3: $p<0.05$: *compared to good learned animals; +compared to the basal level.

Experimental Biological Modeling (Institute of Pharmacology, Tomsk Research Center).

Conflict situation (10 min) and paradoxical sleep deprivation (PSD, 48 h) [9] followed by training in a 3-arm T-maze (2 days) served as the models of experimental neuroses. Seven days before the tests the animals were divided into groups of good and poor learners (GL and PL, respectively) depending of their maze behavior [7]. Peripheral blood reticulocytes were counted 24 h before (basal level) and 1, 4, and 5 days after treatment. The mice were euthanized by cervical dislocation under ether anesthesia. The number of bone marrow erythrokaryocytes was estimated [2]. The content of erythroid colony-forming (CFU-E) and cluster-forming units (ClFU-E) in the bone marrow was determined by *in vitro* cloning of myelokaryocytes in methylcellulose [2]. Proliferative activity of erythroid precursors was evaluated by the method of cell suicide using hydroxyurea. The intensity of erythroid precursor differentiation was estimated by the index of maturation. It was calculated as the ratio between the numbers of clusters and colonies in a well [2]. For evaluation of the structural and functional organization of the bone marrow, hemopoietic islets were isolated by enzymatic treatment their quantitative and qualitative characteristics were studied.

The results were analyzed by standard methods of variational statistics. The significance of differences

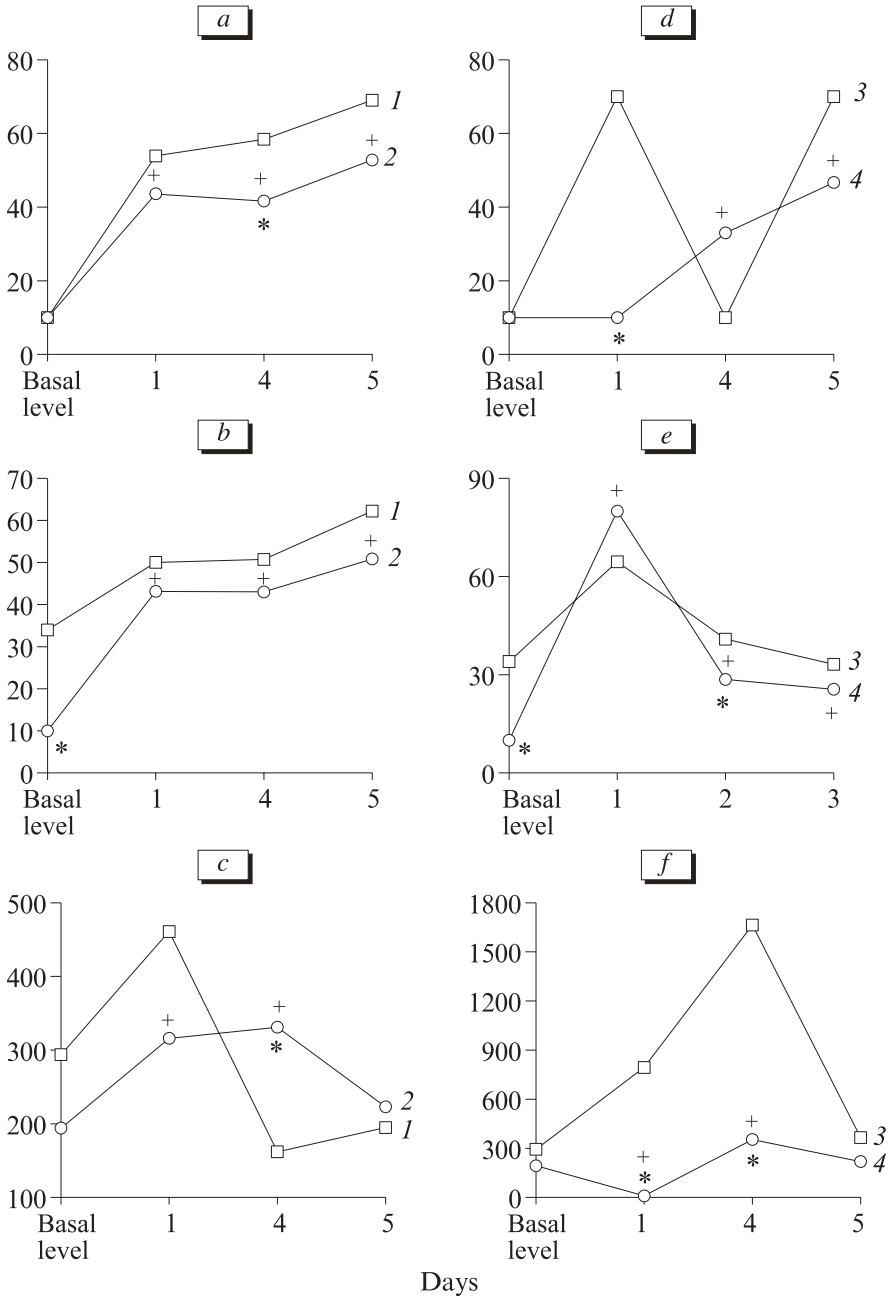

Fig. 2. Ratio of CFU-E (*a*, *d*) and ClFU-E in S-phase of the mitotic cycle (*b*, *e*) and maturation of erythroid cells in the bone marrow (*c*, *f*) of good (*1*, *3*) and poor learned CBA/CaLac mice (*2*, *4*) subjected to conflict situation (*1*, *2*) or paradoxical sleep deprivation and training in a 3-arm T-maze (*3*, *4*). Ordinate: ratio of precursor cells (*a*, *b*, *d*, *e*) in S-phase of the mitotic cycle (%) and index of maturation (ratio between the numbers of clusters and colonies in a well, *c*, *f*).

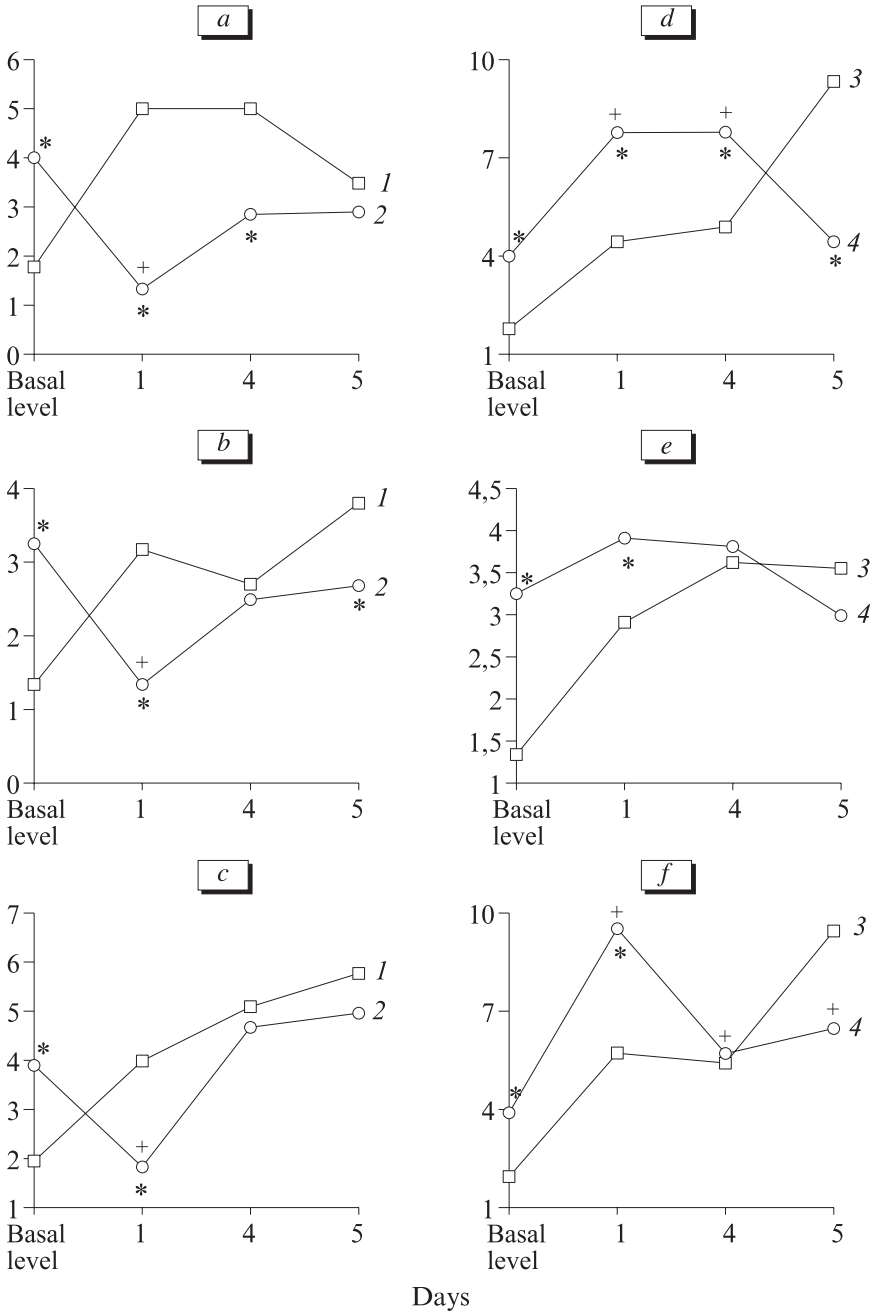

Fig. 3. Number of macrophage-positive (*a*, *d*), erythroid (*b*, *e*), and erythrogranulocytic hemopoietic islets (*c*, *f*) in the bone marrow of good (*1*, *3*) and poor learned CBA/CaLac mice (*2*, *4*) subjected to conflict situation (*1*, *2*) or paradoxical sleep deprivation and training in a 3-arm T-maze (*3*, *4*). Ordinate: number of hemopoietic islets in the bone marrow, $\times 10^3$.

was evaluated by parametric Student's *t* test and nonparametric Wilcoxon—Mann—Whitney *U* test.

RESULTS

Conflict situation was accompanied by stimulation of the erythroid hemopoietic stem in GL and PL. The increase in the number of bone marrow erythrokaryocytes (days 1, 4, and 5) and count of erythroid precursors in the methylcellulose culture (days 1, 4, and 5) was accompanied by the development of peripheral blood reticulocytosis (days 4 and 5, Fig. 1). The number of bone marrow erythrokaryocytes in PL mice was much higher than in GL animals (day 5). However, the intensity of erythroid colony formation (days 1 and 5) and degree of reticulocytosis (day 4) in GL mice surpassed these parameters in PL animals ($p<0.05$).

After PSD and T-maze training the number of bone marrow erythroid cells in GL was lower than in PL (days 4 and 5, Fig. 1). Peripheral blood reticulocytosis was observed only in PL on days 4 and 5 after treatment. By contrast, GL were characterized by reticulocytopenia (day 1). The pool of erythroid precursors underwent different changes in mice subjected to PSD followed by maze training: in PL the number of CFU-E and ClFU-E in the bone marrow increased on

days 4 and 5, while in GL only the count of ClFU-E increased on days 1, 4, and 5.

Evaluation of proliferative activity and differentiation of erythroid precursors in mice revealed the following regularities. Conflict situation increased proliferative activity of erythroid precursors in both groups on days 1, 4, and 5 (especially in GL) and stimulated maturation of erythroid precursors on day 1 in GL (day 1) and on days 1 and 4 in PL animals (Fig. 2). However, in GL we observed inhibition of maturation of erythroid cell on days 4 and 5.

PSD and T-maze training were followed by activation of erythroid precursor proliferation (CFU-E, days 1 and 5; ClFU-E, day 1) and erythroid cell differentiation in GL mice (days 1 and 4, Fig. 2). PL animals exhibited a strong proliferative response of CFU-E (days 4 and 5) and ClFU-E (various stages), which was most pronounced 1 day after treatment. Maturation of erythroid precursors underwent phasic changes. Differentiation of erythroid precursors was suppressed on day 1, but activated on day 4.

The study of structural and functional organization of the bone marrow showed that the number of macrophage-associated cell complexes in GL mice increases on days 1 and 4 after conflict situation. Qualitative study showed that the count of erythroid and erythrogranulocytic hemopoietic islets in GL increases in various stages of observations (Fig. 3). The content of cell complexes in PL decreased on day 1, but returned to normal (macrophage-positive cell complexes and erythroid hemopoietic islets) or significantly exceeded the basal level on days 4 and 5 (erythrogranulocytic hemopoietic islets).

Experimental neurosis produced by PSD and T-maze training was accompanied by an increase in the number of macrophage-positive hemopoietic islets in GL (days 1, 4, and 5) and PL (days 1 and 4, Fig. 3). It should be emphasized that the number of islets in GL far surpassed the corresponding parameter in PL (days 1 and 4). We observed intensive formation of erythroid and mixed hemopoietic islets in animals of both groups. However, accumulation of erythroid complexes was not statistically significant in PL animals.

The state of erythroid hemopoiesis during neuroses is regulated by a variety of neurotransmitter systems [1]. There are behavioral, biochemical, and EEG markers for the reaction to emotional stress (freezing response and active behavior) [6]. The observed differences between GL and PL mice are probably related to specific activity of central regulatory systems (*e.g.*, neurotransmitter system) that depend on neurochemical and neurotransmitter mechanisms of neuroses.

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