Reactions of the Erythroid Hemopoietic Stem and Their Mechanisms during Blood Loss

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We studied changes in the erythroid hemopoietic stem during blood loss of different severity. Stimulation of erythropoiesis during the posthemorrhagic period was related to functional activation of erythroid precursors, which resulted from changes in feeder capacity of cells from the hemopoiesis-inducing microenvironment and erythropoietic activity of the plasma. The development of encephalopathy under conditions of massive blood loss was accompanied by a reduction of erythroid hyperplasia due to a decrease in the number of proliferating erythroid precursor cells, despite high secretory activity of adherent myelokaryocytes, rise in erythropoietic activity of the plasma, increased formation of erythroid hemopoietic islets, and accelerated maturation of hemopoietic cells.

Key Words: *erythropoiesis; blood loss; anemia; encephalopathy*

Bleeding and blood loss are most common and serious complications. Massive blood loss activates complex compensatory and adaptive reactions, while in case of insufficiency or incompetence of these adaptation mechanisms it leads to impairment of vital functions of the organism. Specific features of the metabolism of nerve cells determine high susceptibility of the brain to hypoxia and contribute to the development of severe structural and functional changes during compensatory hypovolemia in the central nervous system (CNS) [1]. Damage and subsequent reorganization of brain structures responsible for the regulation of internal organs lead to dysregulation pathology manifesting in dysfunction of various visceral systems [3,8]. Changes in hemopoiesis modify the course of recovery processes during the posthemorrhagic period. However, the mechanisms of local and distant regulation of hemopoiesis under conditions of blood loss remain unclear. The influence of posthemorrhagic encephalopathy on the blood system is poorly understood. Previous studies showed that disturbances in CNS play an important role in disadaptation of the hemopoietic tissues during hypoxic hypoxia and hemolytic anemia [4,5,10].

Here we studied the mechanisms of regulation of erythropoiesis during acute blood loss of different severity.

MATERIALS AND METHODS

Experiments were performed on 546 CBA/CaLac mice (class I conventional mouse strain) weighing 18-20 g and obtained from the nursery of the Department of Experimental Biomedical Modeling (Institute of Pharmacology, Tomsk Research Center). Acute blood loss of different severity was produced by puncture of the retroorbital sinus and loss of 30% circulating blood volume (CBV); otherwise, a specific volume of blood was repeatedly withdrawn through a graduated Pasteur pipette washed with heparin solution over 2-3 h (70% CBV, 3 procedures). The specific volume of blood was estimated taking into account that CBV in rodents corresponds to $^1/_{13}$ of body weight. The loss of 30% CBV did not produce significant changes in the psychoneurological status. Serious blood loss (70% CBV) was followed by the development of encephalopathy. It was estimated by amnesia during conditioned pas-

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sive avoidance performance [2] and disturbances in orientation and exploratory activity of animals in the open field [2,14]. Peripheral blood indexes were recorded on days 1-10 using an ABACUS automatic blood analyzer (Diatron) under veterinary conditions. The state of bone marrow hemopoiesis was estimated by routine hematological methods [11]. We studied the number of erythroid precursor cells (CFU-E) in the bone marrow, their proliferative activity and intensity of differentiation, production of erythropoietic substances by different fractions of the hemopoiesis-inducing microenvironment (HIM), erythropoietic activity (EPA) of the plasma, and structural and functional organization of the bone marrow [7].

The results were analyzed by methods of variational statistics (Student's *t* test, nonparametric Wilcoxon—Mann—Whitney *U* test) and correlation and factor analyses.

RESULTS

The loss of 30% CBV decreased the number of erythrocytes (days 1-5), reduced hematocrit (days 1-5) and hemoglobin content in the peripheral blood (days 1-5, 7, and 8). The count of bone marrow erythrokaryocytes significantly increased throughout the observation period (266.03% of the basal level, day 9). The number of peripheral blood reticulocytes also increased (to 354.15% from the basal level, day 6). Qualitative analysis of blood cells revealed an increase in the mean corpuscular concentration of hemoglobin on days 1 and 3, which can be explained by the release (from stores) of erythrocytes formed under conditions of balanced hemopoiesis, intensive synthesis of hemoglobin, and slow maturation of erythroid precursors (Fig. 1).

Changes in the bone marrow compartment of erythron depended on activity of committed precursors. Effusion of 30% CBV increased the yield of CFU-E in methylcellulose culture and the rate of cell division (days 1-7, 3-5, and 7-9) and accelerated maturation of erythroid precursors (days 2, 4, 5, 7-9, Fig. 2). The observed changes in proliferation and differentiation of clonogenic cells resulted from enhanced production of erythropoietic substances by adherent (days 4 and 8) and nonadherent myelokaryocytes (days 1 and 5), high erythropoietic activity of the plasma (days 1, 2, 7, and 9; Fig. 3), and high ability of stromal hemopoietic cells to form new structural and functional bone marrow units during the posthemorrhagic period (erythroid hemopoietic islets; Fig. 1, *f*).

These results show that blood loss not accompanied by CNS injury was followed by significant compensatory and adaptive changes in the blood system. It was manifested in severe hyperplasia of the erythroid hemopoietic stem [9,12,13] due to increased feeder capacity of HIM cells and high content of erythropoietic substances in the plasma.

Analysis of rank correlation coefficients (*r*) revealed a considerable increase in the number of signal relationships between individual compartments of the blood system reflecting complementarity of erythropoiesis-stimulatory activities of regulatory systems (erythropoietin system and HIM). The central (neuroendocrine) regulatory compartment probably coordinated changes in the hemopoietic tissue. The reduction of relationships by using factor analysis showed that stimulation of erythropoiesis during blood loss primarily depends on the formation of additional hemopoietic foci.

CNS plays an important role in the regulation of hemopoietic activity under extreme conditions [6]. In series II we studied the effect of posthemorrhagic encephalopathy on changes in the blood system under conditions of severe blood loss.

The animals with brain disorders produced by the loss of 70% CBV had several hematological phenomena. Damage to CNS decelerated the reduction of hyperplasia in the erythroid hemopoietic stem (days 4 and 7-9), which was related to a decrease in the number of erythroid precursor cells in the bone marrow and proliferative activity of CFU-E (Fig. 1, *a*; 2, *a*, *b*). However, we revealed compensatory activation of CFU-E differentiation on days 2-6 and 8. These changes were associated with an increase in secretory function of adherent myelokaryocytes (days 7 and 9), erythropoietic activity of the plasma (days 4, 6, and 8-10), and ability of stromal cells to form erythroid colonies (Fig. 1, *f*). The peripheral erythron system underwent significant changes. Massive blood loss resulted in severe and long-term anemia (decrease in the number of erythrocytes, hematocrit, and hemoglobin level on days 1-10). It was associated with not only the increase in the volume of withdrawn blood. Despite a well-defined reaction of reticulocytes, the number of erythrocyte did not returned to normal due to macrocytosis (days 3-8 and 10) and diaeresis of newly formed large red blood cells during passage in the microcirculatory bed. The higher was the volume of withdrawn blood, the greater was the increase in the average corpuscular concentration of hemoglobin in the early period of observations (Fig. 1).

The correlation and factor analysis revealed a significant decrease in the number of cause-effect relations between phenomenological characteristics, state of precursor cells, and functional activity of erythropoiesis-regulating systems upon changes in the number of factors in the correlation matrix reflecting reduced role of cell-cell interactions in the reaction of hemopoietic tissue decreased, while the contribution

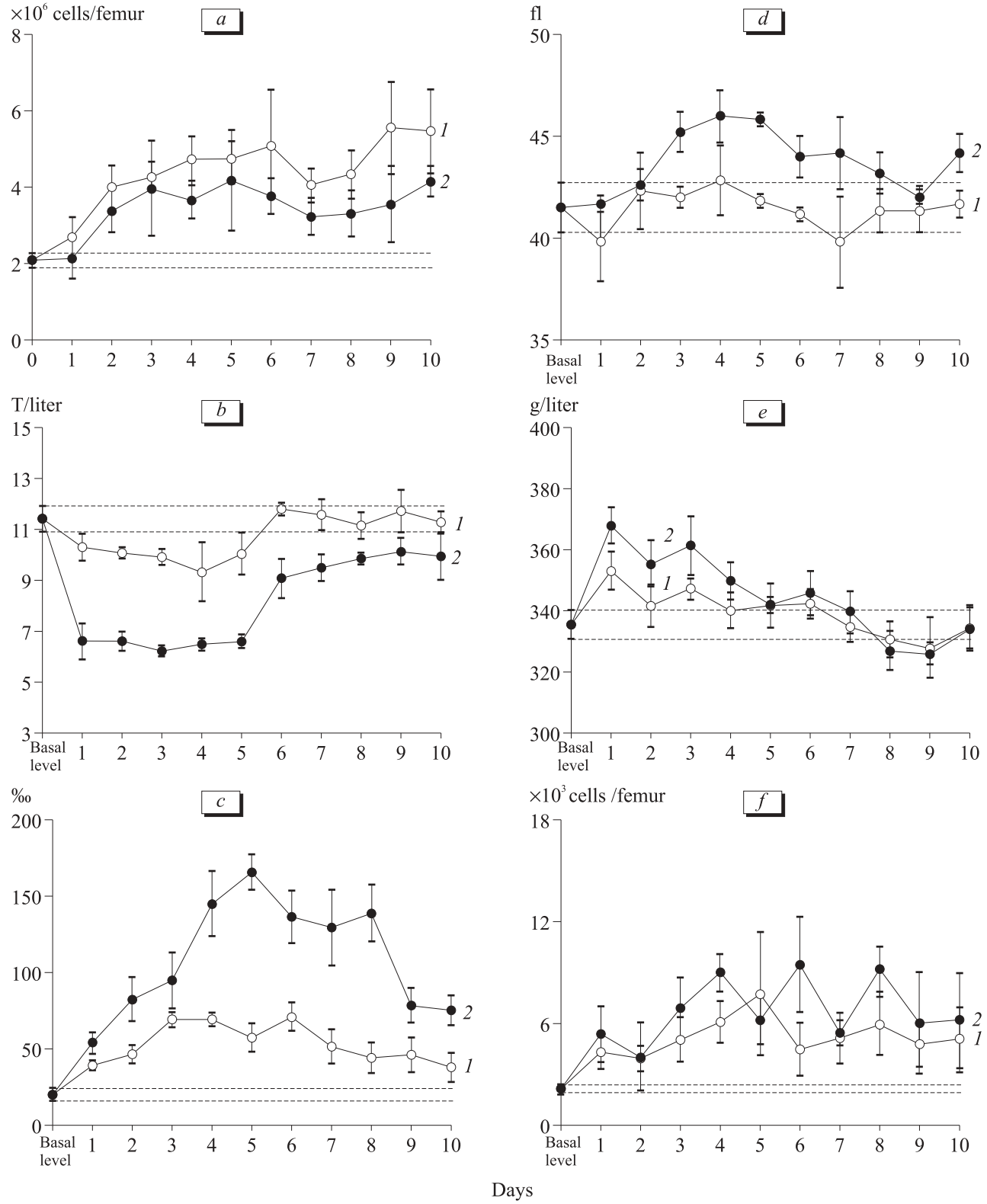

Fig. 1. Number of bone marrow erythrokaryocytes (*a*) and peripheral blood erythrocytes (*b*) and reticulocytes (*c*), volume of erythrocytes (*d*), mean corpuscular concentration of hemoglobin (*e*), and amount of erythroid hemopoietic islets in the bone marrow of CBA/CaLac mice (*f*) after loss of 30% circulating blood volume (CBV, *1*) and development of encephalopathy produced by loss of 70% CBV (*2*). Here and in Figs. 2 and 3: area between dotted lines shows confidence interval for intact animals at p=0.05.

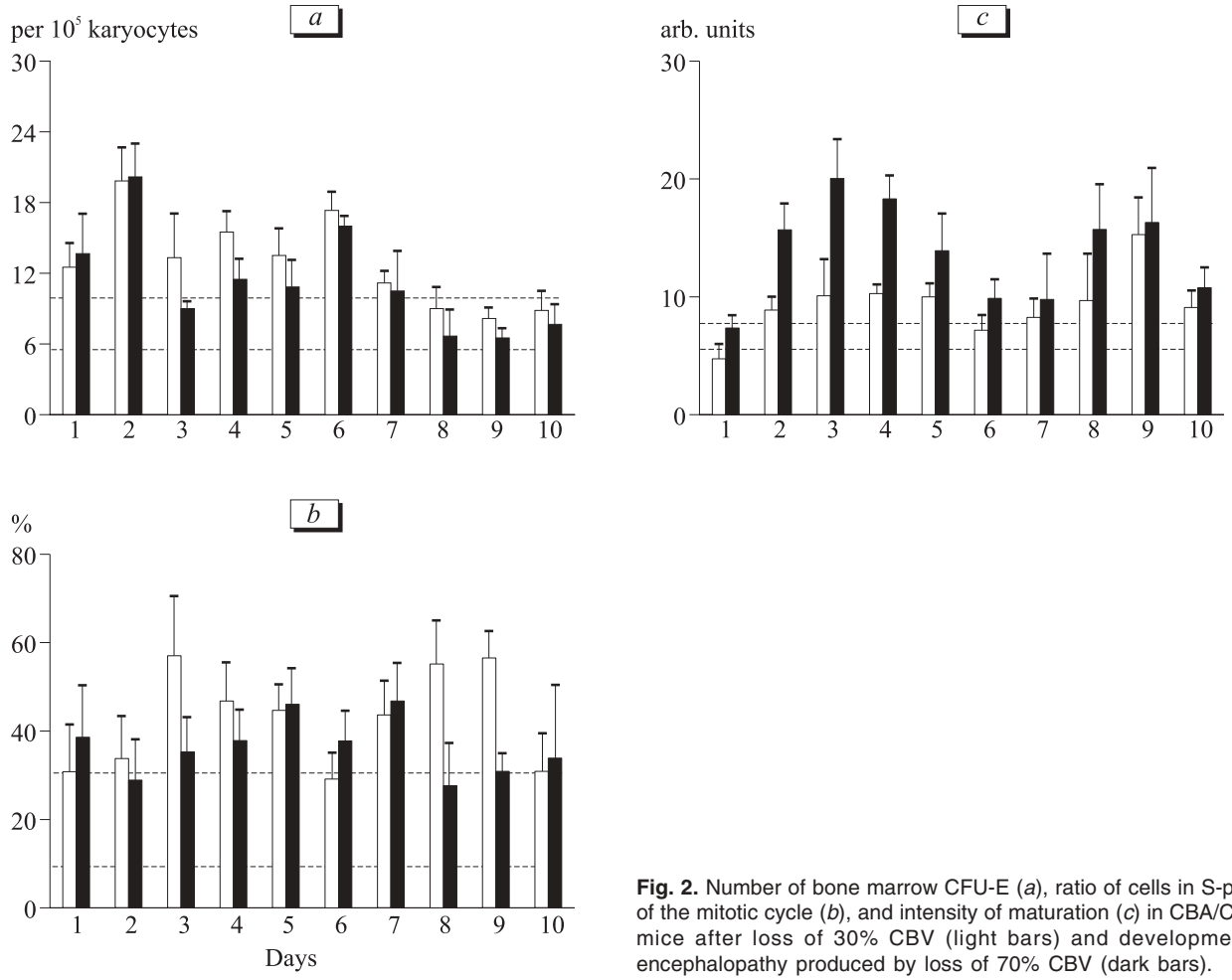

Fig. 2. Number of bone marrow CFU-E (*a*), ratio of cells in S-phase of the mitotic cycle (*b*), and intensity of maturation (*c*) in CBA/CaLac mice after loss of 30% CBV (light bars) and development of encephalopathy produced by loss of 70% CBV (dark bars).

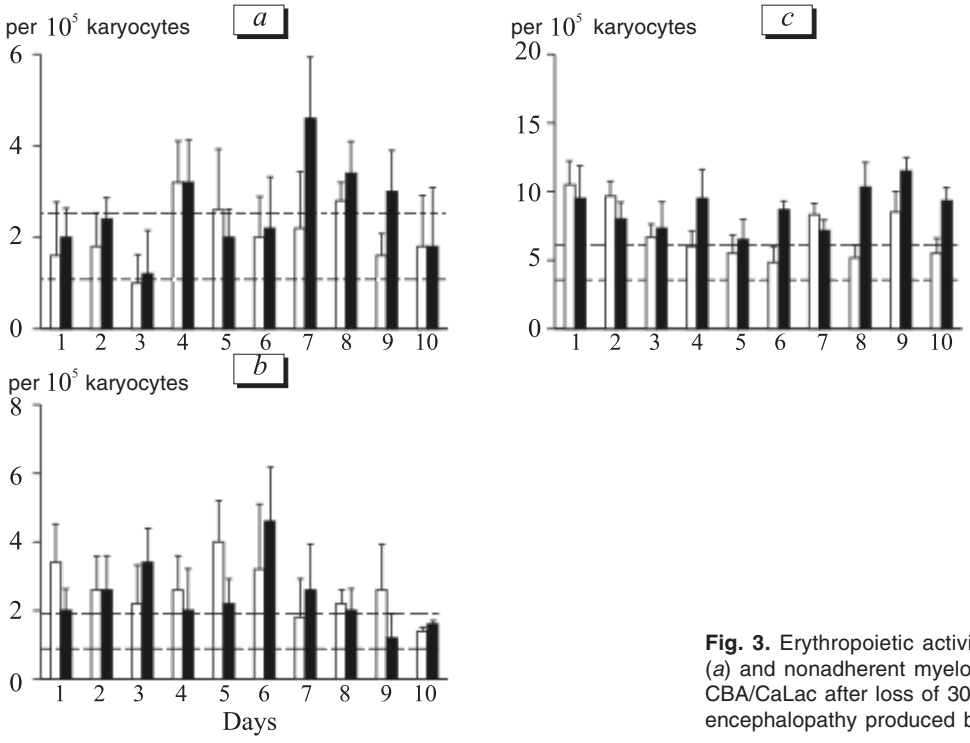

Fig. 3. Erythropoietic activity of conditioned media from adherent (*a*) and nonadherent myelokaryocytes (*b*) and blood plasma (*c*) in CBA/CaLac after loss of 30% CBV (light bars) and development of encephalopathy produced by loss of 70% CBV (dark bars).

of these relationships in erythropoietic activity of the plasma increased. These changes suggest dysregulation of hemopoiesis during blood loss accompanied by CNS injury. Hemopoietic changes in animals with posthemorrhagic encephalopathy were similar to blood disorders observed during hypoxic hypoxia and hemolytic anemia and accompanied by psychoneurological disturbances [4,5,10]. Experimental models of oxygen deficiency showed that damage to hemopoietic precursors under conditions of increased feeder capacity of stromal hemopoietic cells is directly related to the rise in plasma erythropoietic activity accompanied by brain injury.

On the whole, the state of the blood system determined by incompetence of compensatory and adaptive mechanisms of hemopoiesis during massive blood loss (hemic hypoxia) can be considered as erythropoietic distress. It manifested in disadaptation of the hemopoietic tissue and formation of abnormal erythrocytes. Damage to specific structures in CNS determines an inadequate response of the blood system.

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