Dopaminergic Regulation of Erythropoiesis in Modeled Myelosuppressions Induced by Cyclophosphamide and 5-Fluorouracil

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We studied the role of the dopaminergic system in the regulation of erythropoiesis in cytostatic myelosuppressions. During cyclophosphamide and 5-fluorouracil treatment, the state of erythropoiesis precursors, functional activity of cell elements of hemopoiesis-inducing mircroenvironment (secretion of erytropoietic activity, formation of hemopoietic islets) are controlled by dopaminergic structures of the brain. The regulatory effect of CNS is mediated via dopamine receptors on precursors and microenvironmental cells and via modulation of activity of the erythropoietin system. Specific dopaminergic control of hemopoiesis-inducing microenvironment and erythroid precursors is determined by peculiarities of the suppressive effects of the alkylating agent and antimetabolite on the blood system.

Key Words: *dopamine; erythropoiesis; hemopoietic microenvironment; precursors; cytostatics*

The principles of regulation of the blood system are the mail problem of modern hematology. The concept on role of local regulation of reproduction of the cell composition of the hemopoietic tissue and its functional activity under normal and pathological conditions (*e.g.* myelosuppression) was developed [1,7]. At the same time, activation of dopaminergic system leads to immunostimulation [4,5,9] accompanied by accumulation of $CD4^+$ T cells in the bone marrow [6], increase in the count of antibody-producing and rosette-forming cells in the spleen of experimental animals, abolition of the stress-induced suppression of the immune reaction [10], and enhancement of proliferative response of T cells to mitogens [13]. Taking into account the fact that neuroleptic haloperidol modulates the cellularity of the erythroid hemopoietic stem during neurotization [2], we hypothesized that brain dopamine can be involved into regulation of the blood system.

Here we studied the role of the dopaminergic system in the regulation of erythropoiesis in cytostatic myelosuppressions.

MATERIALS AND METHODS

The experiments were carried out on 2.0-2.5-month-old female CBA/CaLac mice (n=620, conventional mouse strain obtained from the nursery of Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences).

Cytostatic myelosuppression was modeled by single intraperitoneal injection of $^1/_3$ MTD alkylating agent cyclophosphamide (CP, 83 mg/kg) or fluoropyrimidine antimetabolite 5-fluorouracil (5-FU, 76 mg/kg). Experimental animals received single intraperitoneal injection of neuroleptic haloperidol (Gedeon Richter A.O.) in a dose of 3 mg/kg 30 min before cytostatic exposure. Controls

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received an equivalent volume (0.2 ml) of physiological saline. On days 1, 2, 3, 4, 5, 6, and 7 after cytostatic treatment the animals were sacrificed by cervical dislocation under ether narcosis and the number of erythrokaryocytes in the bone marrow was determined.

The content of erythroid colony- (CFU-E) and cluster-forming units (ClFU-E) in the bone marrow was studied by *in vitro* cloning of myelokaryocytes in methylcellulose culture medium [3]. Proliferative activity of hemopoietic precursors was studied by the method of cell suicide with hydroxyurea, the intensity of their differentiation was evaluated by the index of maturation (clusters to colonies ratio in the same well) [3]. Structural and functional organization of the bone marrow was evaluated by qualitative and quantitative composition of hemopoietic islets after their enzymatic isolation [8]. Erythropoietic activity (EPA) in conditioned media from adherent and nonadherent elements of hemopoiesis-inducing microenvironment (HIM) and in blood serum was measured on intact mouse myelokaryocytes [3].

The final concentrations of dopamine and erythropoietin in bone marrow culture were 10^{-8} M and 0.5 U/ml, respectively.

The data were processed using standard methods of variation statistics. Significance of differences was evaluated using parametric Student's *t* test or nonparametric Wilcoxon—Mann—Whitney *U* test [5].

RESULTS

CP decreased the number of erythrokaryocytes in the bone marrow (days 1 and 3-7) and reticulocytes in the peripheral blood (days 1-7; Fig. 1). Depression of erythropoiesis was caused by inhibition of the formation of erythroid and erythrogranulocytic hemopoietic islets (days 1-7), disturbances in EPA production by adherent and nonadherent HIM cells (days 1, 3, and 5), and low serum EPA level (days 1, 3, 5, and 7, Fig. 1; Table 1). In contrast, proliferative activity (days 1, 3, 5, and 7) and intensity of differentiation (days 1 and 3) of erythroid precursors considerably surpassed those in intact controls, which determined considerable yield of CFU-E (days 3 and 5) and ClFU-E (days 1, 3, 5, and 7; Fig. 2). Secretory activity of HIM cells markedly increased on day 7 (Table 1).

In case of 5-FU treatment, suppression of erythropoiesis (days 1-7) was more pronounced compared to treatment with the alkylating agent (Fig. 1). We observed inhibition of the formation of all types of hemopoietic islets (days 1-6), disturbances in EPA deficiency in supernatants of adherent (days

TABLE 1. Effect of Haloperidol on EPA ($\times 10^5$ cells) in Biological Fluids in CBA/CaLac Mice after CP and 5-FU Treatment ($X \pm m$; n=9)

Day of experiment		Supernatants from adherent myelokaryocytes	Supernatants from nonadherent myelokaryocytes	Blood serum
Intact		0.25±0.25	0.25±0.25	0.25±0.25
CP	1	0.25±0.25	0.25±0.25	0.25±0.25
		1.75±0.28*+	0.25±0.25	1.50±0.25*+
	3	0.25±0.25	0.25±0.25	0.25±0.25
		2.00±0.25*+	0.25±0.25	0.75±0.25
	5	0.25±0.25	0.25±0.25	0.25±0.25
		0.75±0.25	0.25±0.25	0.75±0.25
	7	1.50±0.25*	1.25±0.25*	0.25±0.25
		3.5±0.4*+	1.25±0.25*	0.50±0.28
5-FU	1	2.00±0.25*	2.75±0.25*	0.25±0.25
		1.50±0.28*	0.50±0.25+	0.25±0.25
	3	1.50±0.28*	0.25±0.25	0.25±0.25
		2.25±0.25*	0.25±0.25	0.50±0.28
	5	0.25±0.25	1.0±0.1	0.50±0.28
		0.25±0.25	0.25±0.25	0.50±0.28
	7	0.25±0.25	0.50±0.28	0.25±0.25
		0.25±0.25	0.25±0.25	0.50±0.28

Note. Numerator: control (physiological saline), denominator: experiment (haloperidol). $p<0.05$ compared to *intact mice, +control mice.

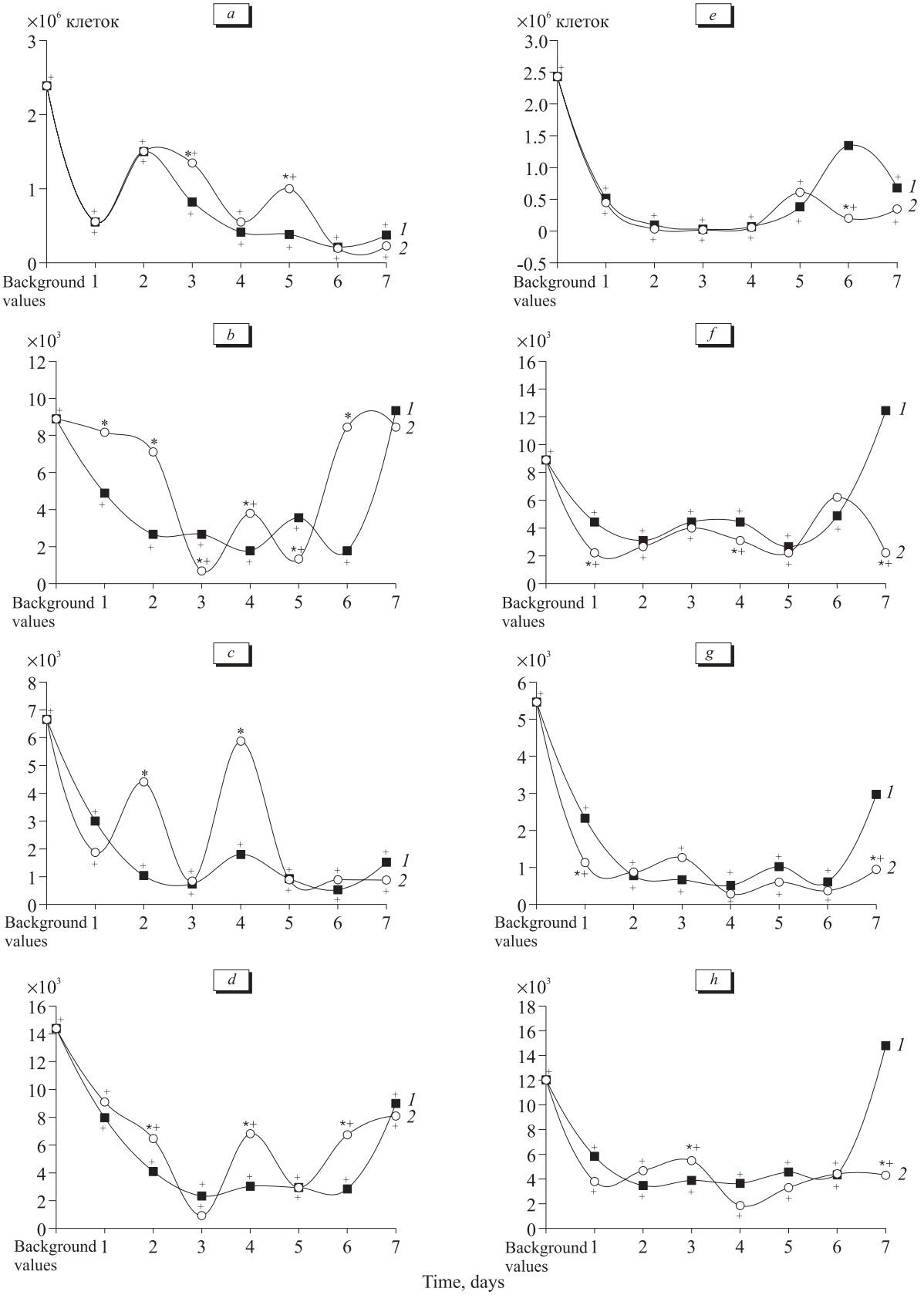

Fig. 1. Content of erythrokaryocytes (*a*, *e*), macrophage-positive (*b*, *f*), erythroid (*c*, *g*), and erythrogranulocytic (*d*, *h*) hemopoietic islets in bone marrow of CBA/CaLac mice after treatment with CP (*a-d*) and 5-FU (*e-h*). Here and on Fig. 2, 3: *1*) control (physiological saline), *2*) experiment (haloperidol). $p<0.05$ compared to *background values, +control.

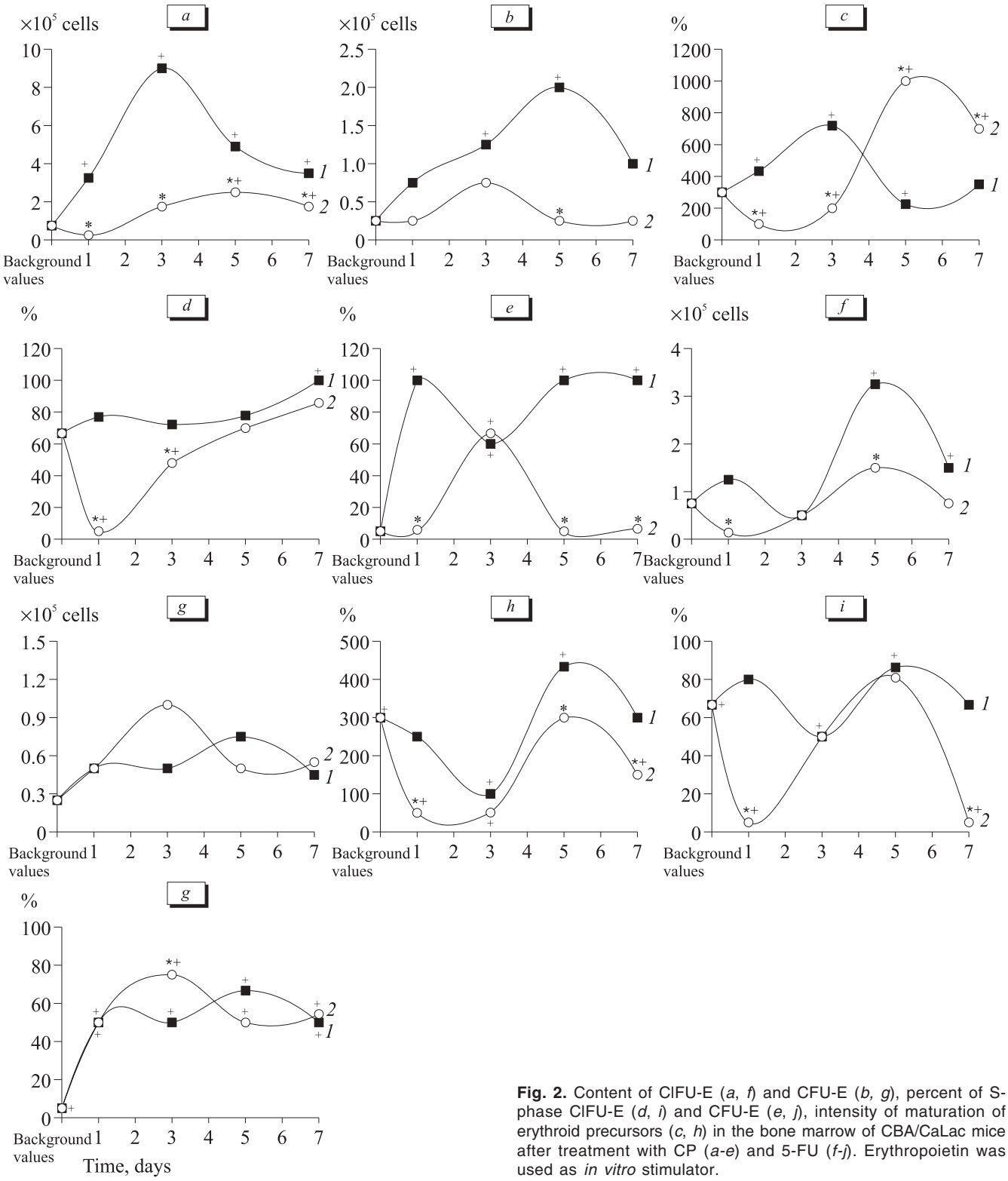

Fig. 2. Content of ClFU-E (*a*, *f*) and CFU-E (*b, g*), percent of S-phase ClFU-E (*d*, *i*) and CFU-E (*e*, *j*), intensity of maturation of erythroid precursors (*c*, *h*) in the bone marrow of CBA/CaLac mice after treatment with CP (*a-e*) and 5-FU (*f-j*). Erythropoietin was used as *in vitro* stimulator.

5 and 7) and nonadherent (days 3, 5 and 7) myelokaryocytes, and in blood serum (days 1, 3, 5, and 7, Fig. 1; Table 1). Functional activity of erythroid precursors was enhanced (except inhibition of maturation on day3, Fig. 2). Production of CFU-E growth factors by HIM cells increased at early terms: (by adherent cells on days 1 and 3, by nonadherent cells on day 1).

Thus, suppression of the erythron under conditions of cytostatic treatment was associated with

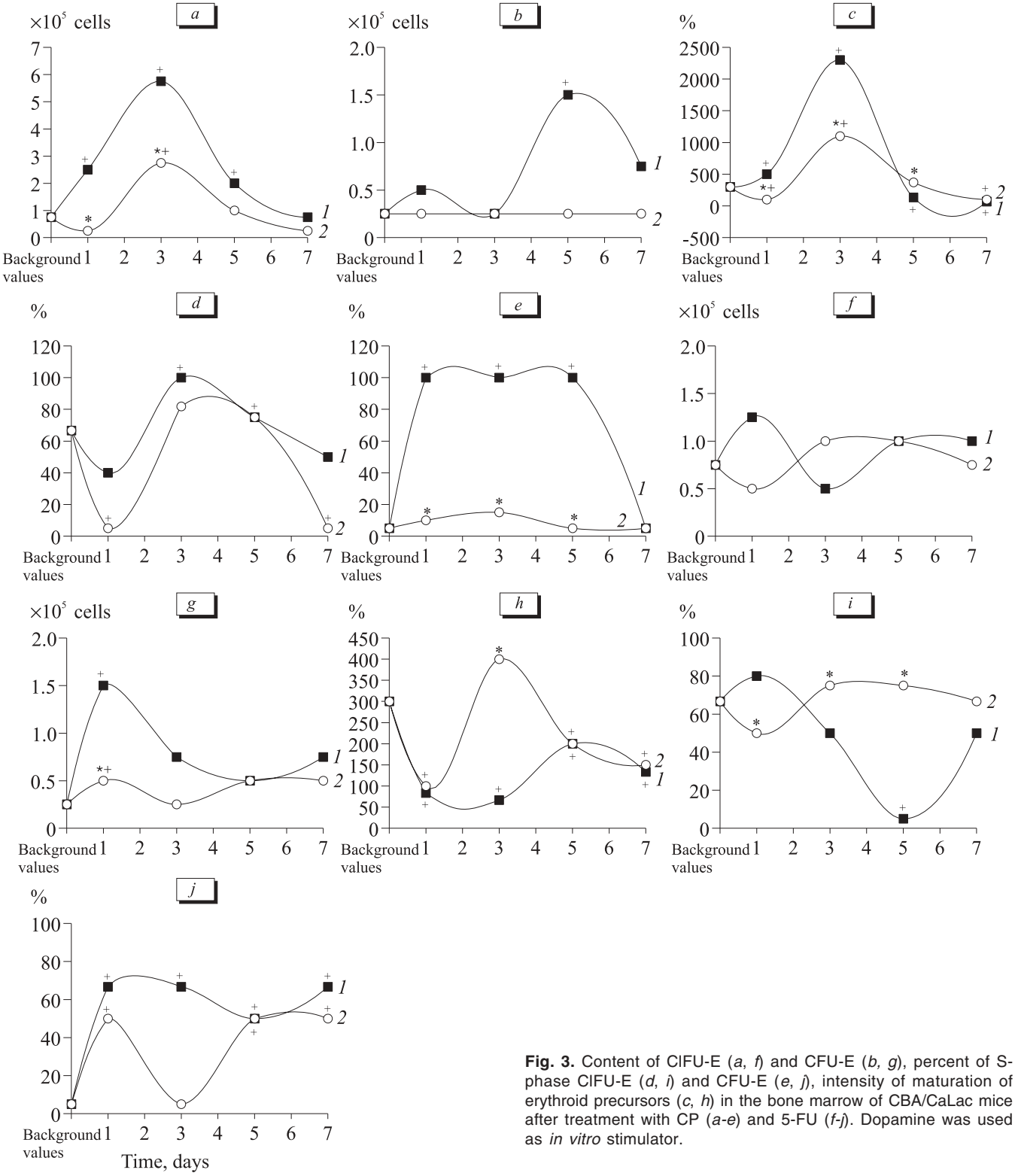

Fig. 3. Content of CIFU-E (*a*, *f*) and CFU-E (*b*, *g*), percent of S-phase CIFU-E (*d*, *i*) and CFU-E (*e*, *j*), intensity of maturation of erythroid precursors (*c*, *h*) in the bone marrow of CBA/CaLac mice after treatment with CP (*a-e*) and 5-FU (*f-j*). Dopamine was used as *in vitro* stimulator.

impaired function of HIM, in particular, formation of cell associations and EPA secretion (at specific terms for each model of myelosuppression). Active proliferation and differentiation of erythroid precursors was preserved in case of their culturing in the presence of erythropoietin (to a greater extent after treatment with CP).

Hemopoietic cells depend on not only hemopoietic growth factors and inhibitors, but also catecholamines (epinephrine and norepinephrine) pro-

duced by neurons and cells [1,2,7]. Dopamine was also found in short- and long-living bone marrow cultures [11,12], which suggests that this amine can produce a regulatory effect on multipotent stem cells and more mature precursors. In light of this it is interesting to study receptor dopamine mechanisms of regulation of proliferation and differentiation of erythroid precursors in mice after cytostatic treatment.

Under conditions of myelosuppression induced by CP treatment, dopamine accelerated *in vitro* division of CFU-E on day 1, 3, and 5 of the experiment, while the yield of colonies increased only on day 5 (Fig. 3). This phenomenon was associated with increased rate of maturation of precursors on days 1 and 3, which in turn increased the yield of ClFU-E during the specified terms (even against the background of reduced proliferative activity of cluster-forming units on day 1). In case of 5-FU treatment, stimulation of dopamine receptors increased the number of DNA-synthesizing CFU-E (days 1, 3, 5, and 7) on the one hand, and deceased the number of S-phase ClFU-E (day 5) and the rate of differentiation of precursors (days 1, 3, 5, and 7) on the other. These changes led to accumulation of precursors in the hemopoietic tissue on day 1 after injection of the antimetabolite.

These findings attest to involvement of peripheral dopaminergic mechanisms in the regulation of proliferation and differentiation of erythroid precursors during cytostatic myelosuppression. CP activated these processes, while 5-FU treatment led to discoordination of cell division and maturation.

When studying the role of postsynaptic brain dopaminergic D2 receptors in hemopoiesis regulation under conditions of cytostatic myelosuppression we found that haloperidol prevented depression of bone marrow erythropoiesis after CP treatment (days 3 and 5), but aggravated it after 5-FU treatment (day 6; Fig 1).

The effect of the neuroleptic on hemopoietic islets and hemopoietins corresponded to changes in the content of erythrokaryocytes. In mice treated with CP, haloperidol promoted accumulation of macrophage-positive (days 1, 2, 4, and 6), erythroid (days 2 and 4) and erythrogranulocytic (days 2, 4, and 6) cell associations, increased EPA in conditioned media of adherent HIM cells (days 1, 3, and 7) and in blood serum (day 1; Fig. 1; Table 1). In animals receiving 5-FU, haloperidol aggravated disturbances in the formation of hemopoietic islets of all types (days 1, 4, and 7) and abolished enhanced production of activities by nonadherent myelokaryocytes (day 1).

Reduced activity of the dopaminergic system before CP treatment decreased the content of erythropoietic precursors (Fig. 2, 3). The primary cause of inhibition of ClFU-E and CFU-E growth in methylcellulose medium was suppression of proliferative activity of precursors (more pronounced in the presence of dopamine in the culture). Decreased rate of cell maturation at the early terms of the experiment aggravated inhibition of CFU-and ClFU yield (dopamine>erythropoietin), while increased ClFU/CFU ratio at later terms had practically no effect on this process.

In mice treated with 5-FU and haloperidol, suppression of the growth of late precursors (days 1, 5, and 7) in case of erythropoietin correlated with the decrease in the number of S-phase ClFU-E (days 1 and 7) and activity of differentiation (days 1, 5, and 7; Fig. 2, 3). Under these conditions, the content of ClFU-E in bone marrow culture in the presence of dopamine on day 1 of the experiment decreased due to the decrease in ClFU-E proliferation. Further acceleration of maturation (day 3) and increase in the number of DNA-synthesizing ClFU-E (days 3 and 5) compensated inhibition of CFU proliferation (day 3), so that the number of precursors during the specified terms did not significantly differ from that in intact control.

Thus, dopaminergic system is involved into suppression of erythropoiesis during cytostatic treatment. The regulatory effect of CNS on proliferation and differentiation processes is mediated via dopamine receptors on erythroid precursors and HIM cells and via modulation of activity of the erythropoietic system. The opposite reaction of HIM to haloperidol (in the system of hemopoietic islets and hemopoietins) after treatment with the alkylating agent and antimetabolite reflects peculiarities of dopamine regulation of erythropoiesis under these conditions. After treatment with CP, the dopaminergic system aggravates disturbances in structural and functional organization of the bone marrow and EPA production by HIM cells (thus aggravating depression of erythropoiesis), on the contrary, after treatment with 5-FU the amine prevents the destructive processes. The differences between these models were also seen in CNS regulation of the pool of erythropoiesis precursors (most pronounced during culturing of myelokaryocytes with dopamine).

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