Role of Serotoninergic System in Regulation of Erythropoiesis during Cytostatic Myelosuppressions

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We studied the role of serotoninergic system in the regulation of erythropoiesis after treatment with cyclophosphamide and 5-fluorouracil. The state of erythropoiesis precursors and functional activity of cell elements of hemopoiesis-inducing mircroenvironment (secretion of hemopoietic activity, formation of hemopoietic islets) are controlled by serotoninergic structures of the brain. The regulatory effect of CNS is mediated via serotonin receptors on precursors and microenvironmental cells and via modulation of activity of the erythropoietin system. Specific shifts in the erythron are determined by peculiarities in the interaction between distant and local controlling structures and suppressive effect of cyclophosphamide and 5-fluorouracil on hemopoietic cells and regulatory systems.

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

The role of neurotransmitter systems in the regulation of hemopoiesis under normal and pathological conditions is poorly understood. The adrenergic system is best studied. Under extreme conditions (immobilization stress, blood loss, cytostatic and neurotizing exposures, hypoxia, *etc.*), the α- and β-adrenergic receptor mechanisms regulate activity of committed hemopoietic precursors and cells of the hemopoiesis-inducing microenvironment (HIM) [1-3]. The role of central regulatory elements is played by the sympathetic nervous system [2,3] and adrenergic brain structures [2]. At the same time, there is evidence that other neurotransmitters also affect the hemopoietic processes. For instance, serotonin increases the number and proliferative activity of hemopoietic stem cells forming colonies in the spleen (CFUs) of lethally irradiated mice [7]. The stimulating effect of the amine is mediated via serotonin S2 receptors on hemopoietic stem cells [8]. Recent studies demonstrated a modulating effect of antiserotonin drug cyproheptadine on the content of morphologically discernible bone marrow cells during experimental neurosis [2]. However, there is no clear-cut concept on the regulatory effect of brain serotonin on the blood system under pathological conditions.

Here we studied the role of the serotoninergic system in the regulation of bone marrow 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 antiserotonin prepara-

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tion cyproheptadine (Serva) in a dose of 30 mg/kg 30 min before cytostatic exposure. Controls 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 [4]. 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) [4]. Structural and functional organization of the bone marrow was evaluated by qualitative and quantitative composition of hemopoietic islets after their enzymatic isolation [9]. The number of CFU-E formed by nonadherent bone marrow cells from intact mice on a substrate of adherent myelokaryocytes precultured with CP for 1 h was evaluated [5]. The difference between the two obtained values reflected the capacity of cytostatic-treated adherent cells to bind intact erythroid precursors. Erythropoietic activity (EPA) in conditioned media from adherent and nonadherent elements of HIM and in blood serum was measured on intact mouse myelokaryocytes [4].

The final concentrations of serotonin, erythropoietin, and CP in bone marrow culture were 10^{-8} M, 0.5 U/ml, and 20 μg/kg, 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.

RESULTS

Cytostatics considerably decreased the content of erythrokaryocytes in the bone marrow and induced the development of reticulocytopenia persisting throughout the observation period (these shifts were more pronounced after 5-FU treatment, Fig. 1). At the same time, CP and 5-FU accelerated the growth of CFU-E (on days 3, 5, 7 and 5), intensity of proliferation (days 1, 3, 5, and 7) and differentiation (days 1, 3, and 5) of erythropoietic precursors in bone marrow culture (Fig. 2). Index of maturation of erythroid cells transiently decreased: on day 5 after CP treatment and on day 3 after 5-FU treatment.

Analysis of the system of local hemopoiesis regulation revealed a decrease in the number of hemopoietic islets of all types in the bone marrow

TABLE 1. Effect of Cyproheptadine 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
		3.00±0.25*+	0.25±0.25	1.00±0.25
	3	0.25±0.25	0.25±0.25	0.25±0.25
		0.75±0.25	0.25±0.25	0.25±0.25
	5	0.25±0.25	0.25±0.25	0.25±0.25
		0.50±0.28	0.25±0.25	0.50±0.28
	7	1.5±0.25*	1.25±0.25*	0.25±0.25
		5.00±0.35*+	1.25±0.25*	0.25±0.25
5-FU	1	2.00±0.25*	2.75±0.25*	0.25±0.25
		0.50±0.28+	0.75±0.25+	0.25±0.25
	3	1.50±0.28*	0.25±0.25	0.25±0.25
		0.25±0.25+	0.50±0.28	0.25±0.25
	5	0.25±0.25	1.0±0.1	0.50±0.28
		1.75±0.25*+	0.50±0.28	0.25±0.25
	7	0.25±0.25	0.50±0.28	0.25±0.25
		0.25±0.25	0.25±0.25	0.5±0.28

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

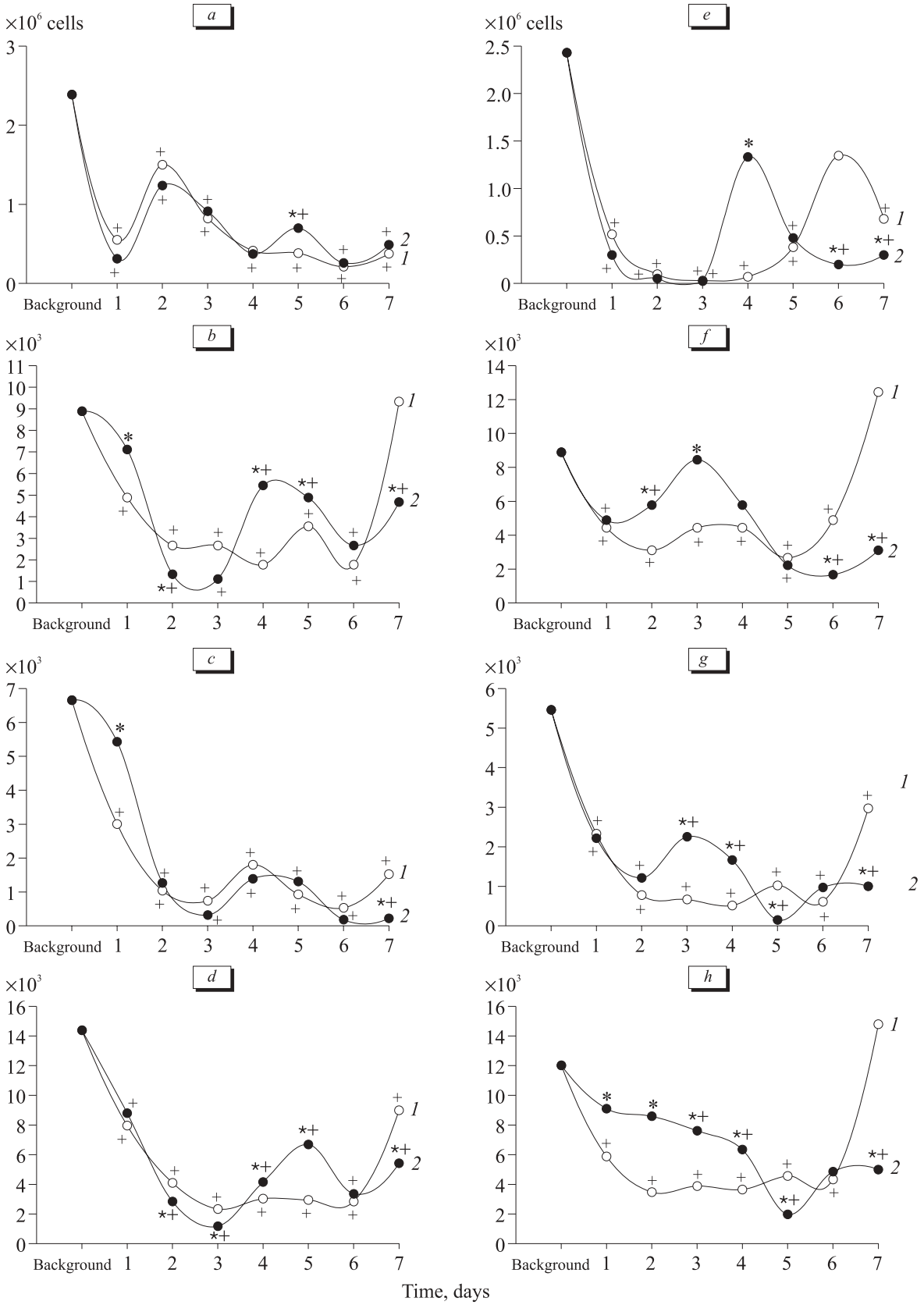

Fig. 1. Content of erythrokaryocytes (*a*, *e*), macrophage-positive (*b*, *f*), erythroid (*c*, *g*), and erythrogranulocytic (*d*, *h*) hemopoietic islets in bone marrow of CBA/CaLac mice after treatment with CP (*a-d*) and 5-FU (*e-h*). Here and on Fig. 2, 3: *1*) control (physiological saline), *2*)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

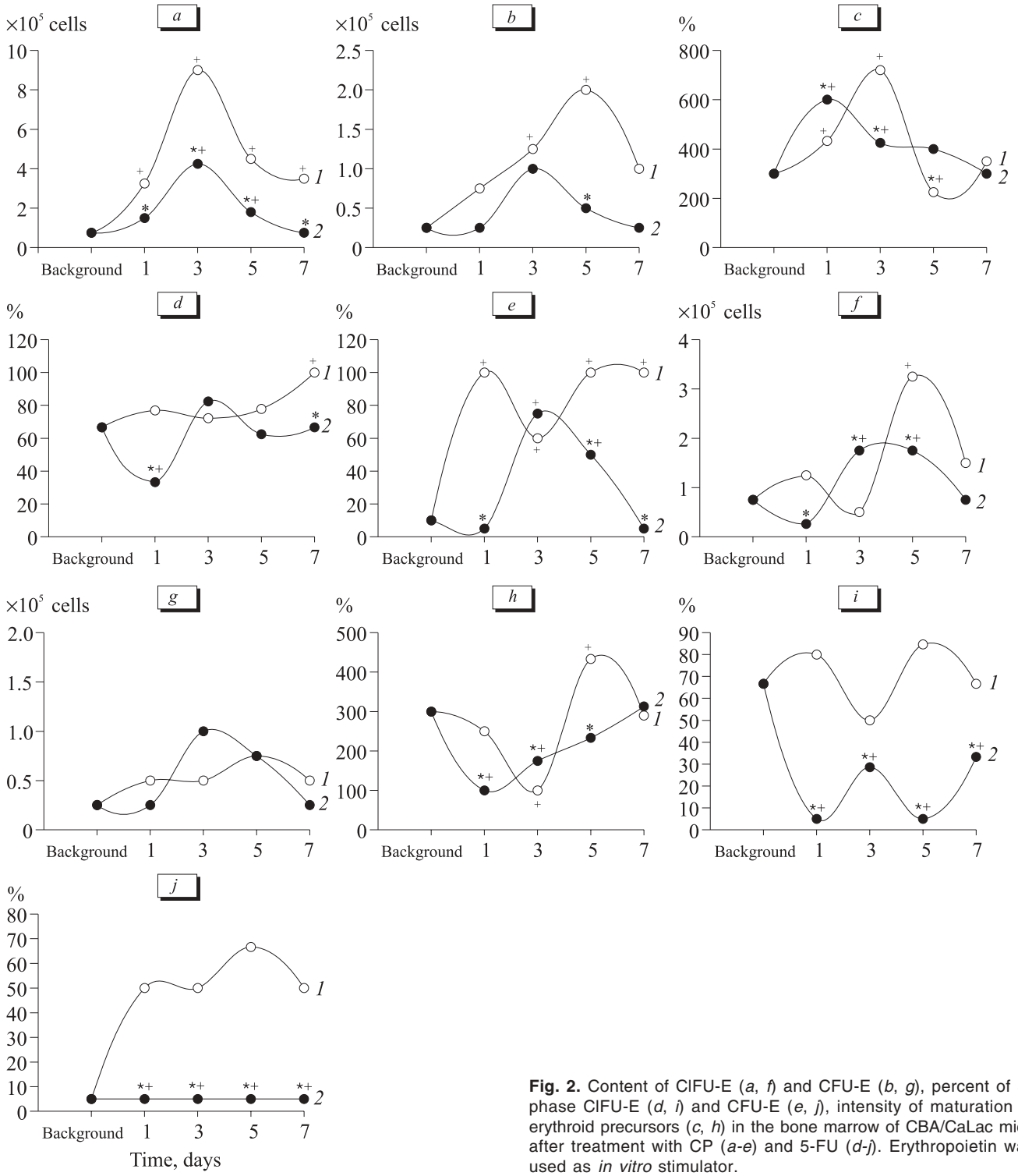

Fig. 2. Content of ClFU-E (*a*, *f*) and CFU-E (*b*, *g*), percent of S-phase ClFU-E (*d*, *i*) and CFU-E (*e*, *j*), intensity of maturation of erythroid precursors (*c*, *h*) in the bone marrow of CBA/CaLac mice after treatment with CP (*a-e*) and 5-FU (*d-j*). Erythropoietin was used as *in vitro*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

of mice with myelosuppression (Fig. 1). The most pronounced destructive changes were observed after CP treatment. Measurement of EPA in biological fluids revealed enhanced production of CFU-E growth factors by adherent and nonadherent cells of HIM only by the end of the experiment in case of CP (day 7) and at early terms in case of 5-FU (days 1 and 3; Table 1). Low activity in the serum was characteristic for both cytostatics.

Under conditions of myelosuppression induced by CP, acceleration of CFU-E division under the effect of serotonin observed throughout the experiment increased their number only on days 5 and 7 (Fig. 3). The absence of CFU accumulation at early

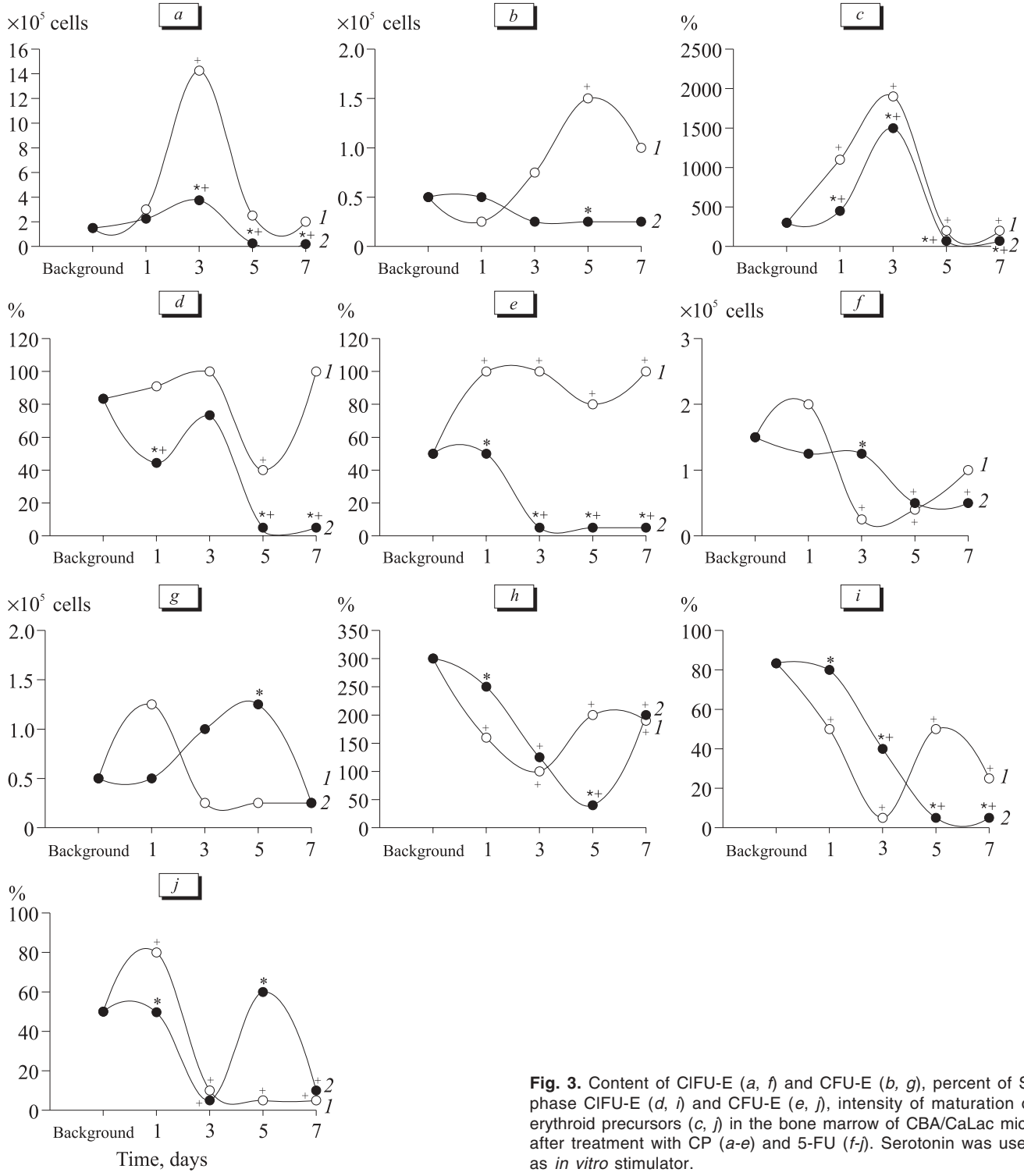

Fig. 3. Content of ClFU-E (*a*, *f*) and CFU-E (*b, g*), percent of S-phase ClFU-E (*d*, *i*) and CFU-E (*e*, *j*), intensity of maturation of erythroid precursors (*c*, *j*) in the bone marrow of CBA/CaLac mice after treatment with CP (*a-e*) and 5-FU (*f-j*). Serotonin was used as *in vitro* stimulator.

terms can be explained by considerably accelerated cell maturation manifested in increased yield of ClFU-E on day 3 of the experiment (despite their low proliferative activity). In experiments with 5-FU, the content of CFU-E did not differ from the initial level, while the number of ClFU-E (on days 3 and 5) was below the corresponding values in intact control. The observed phenomena were probably associated with inhibition of proliferation (on days 3, 5, and 7) and differentiation (days 1, 3, 5, and 7) of erythropoiesis precursors.

Serotonin considerably (by 88%) increased binding of erythroid precursors to adherent HIM cells compared to intact control. Pretreatment of macro-

phages with CP (for 1 h) followed by stimulation of serotonin receptors with the amine suppressed binding of intact precursors to adherent cells.

These findings suggest that disturbances in cell-cell interactions (serotonin mechanisms of hemopoietic islet formation) and EPA production by HIM cells and deficiency of activities in the serum play an important role in inhibition of erythropoiesis induced by CP treatment. Under these conditions erythroid precursors retain high proliferative activity and intensity of differentiation. We believe that suppression of the erythron after 5-FU treatment was caused by not only inhibition of the formation of erythroid and erythrogranulocytic cell associations and low EPA in blood serum and supernatants of myelokaryocytes, but also by discoordination (associated with erythropoietin) and suppression (caused by serotonin mechanisms) of precursor proliferation and maturation.

Cyproheptadine prevented the development of erythron depression under conditions of CP treatment (on day 5). In case of 5-FU treatment, cyproheptadine earlier normalized the cellularity of the erythroid stem (by day 5 *vs.* day 6 in the control group). However, erythrokaryocytes were practically absent in the bone marrow at later terms (days 6 and 7).

Cyproheptadine suppressed the growth of CFU-E and ClFU-E under conditions of CP-induced myelosuppression, which was associated with decreased proliferative activity and intensity of differentiation of erythroid precursors (Fig. 2, 3). This regularity was noted after addition of erythropoietin and serotonin to the culture medium. Serotonin more markedly suppressed these process compared to erythropoietin increasing the rate of precursor maturation on days 1 and 3.

Treatment with cyptoheptadine and 5-FU and the use of erythropoietin *in vitro* suppressed functional activity of the pool of erythroid precursors throughout the experiment (Fig. 2). The only exception was day 3, when the rate of cell maturation led to an increase in ClFU-E number. Under these conditions, the effects on serotonin on erythroid precursors were more intricate. For instance, the increase in the index of differentiation and the number of DNA-synthesizing ClFU-E against the background of reduced number of S-phase CFU-E led to accumulation of more mature precursors and to a decrease in the percent of less mature cells in the bone marrow at early term of the experiment (Fig. 3). An opposite picture was observed at later terms.

In experiments with 5-FU, cyproheptadine restored structural and functional organization of the bone marrow, which was seen from the increase in the number of hemopoietic islets in the bone marrow on days 1-4 of the experiment (Fig. 1). However, at later terms the content of cell complexes decreased. In case of CP, cyproheptadine increased the content of hemopoietic islets on days 1, 4, and 5 and further suppressed the processes of formation of cell associations on days 2, 3, and 7.

Under conditions of low activity of the serotoninergic system and CP-induced myelosuppression, the secretion of EPA by adherent cell elements of HIM considerably increased (on days 1, 3, and 7; Table 1). In experiments with 5-FU, cyproheptadine abolished high activity in conditioned media from both myelokaryocytes fractions on days 1 and 3 of the experiment, but promoted EPA production by adherent cells on day 5.

Thus, under conditions of cytostatic treatment the state of the pool of erythropoiesis precursors and functional activity of HIM are regulated by serotonin of CNS. The regulatory effect of brain serotoninergic structures on proliferation and differentiation processes is mediated via serotonin receptors on erythroid precursors and HIM cells and via modulation of activity of the erythropoietin system. The pattern and magnitude of the effect of cyproheptadine on the rate of precursor proliferation and maturation, processes of formation of hemopoietic islets and erythropoietic component of HIM in the dynamics of myelosuppression are different even for one model. It can be hypothesized that serotonin can not only stimulate, but also inhibit processes in various compartments of the system of local erythron regulation. The differences between the models in the shifts of the studied parameters in response to cyproheptadine treatment can be explained by specific suppressive effects of the alkylating agent and antimetabolite on bone marrow cells and regulatory systems [1,3].

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