GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Role of Serotonin in the Regulation of Granulocytopoiesis during Cytostatic-Induced Myelosuppressions

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We studied the role of the serotoninergic system in the regulation of the granulocytic hemopoietic stem during cytostatic myelosuppressions caused by administration of cyclophosphamide or 5-fluorouracil. After cytostatic treatment, the state of granulocytopoietic precursors and functional activity of cells in the hemopoiesis-inducing microenvironment were regulated by serotoninergic structures of the brain. The regulatory influence of CNS was realized via serotonin receptors on precursors and microenvironment cells. The response of the granulocytic stem was determined by specific interaction between distant and local regulatory structures and inhibitory effect of cyclophosphamide and 5-fluorouracil on granulocytic cells and regulatory systems.

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

The role of the adrenergic system in the regulation of granulocytopoiesis during cytostatic-induced myelosuppressions is now beyond doubts [2,3]. It can be hypothesized that proliferation and differentiation of hemopoietic cells are regulated by a variety of neurotransmitter systems. For example, serotonin increases proliferative activity of hemopoietic stem cells in the bone marrow via serotonin 5-HT2 receptors [5,6]. Under conditions of experimental neuroses, cyproheptadine-induced blockade of postsynaptic serotonin 5-HT2 receptors in the brain considerably modulated the dynamics of bone marrow neutrophilic granulocyte count [4]. However, published data provides no clear insight into the role of serotonin in the development of cytostatic-induced myelosuppressions.

Here we studied the role of the serotoninergic system in the regulation of the granulocytic hemopoietic stem during cytostatic-induced myelosuppressions.

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MATERIALS AND METHODS

Experiments were performed on 620 female CBA/CaLac mice (class I conventional mouse strain) aging 2-2.5 months and obtained from the nursery of Institute of Pharmacology.

Cytostatic myelosuppression was induced by single intraperitoneal injection of alkylating agent cyclophosphamide (CP; $^1/_3$ of the maximum tolerated dose, MTD, 83 mg/kg; Verofarm) and fluoropyrimidine antimetabolite 5-fluorouracil (FU; 76 mg/kg; Darnitsa). The animals of treatment groups received intraperitoneal injection of antiserotonin drug cyproheptadine (single dose 30 mg/kg, Serva) 30 min before cytostatic treatment. Control animals received an equivalent volume of physiological saline (0.2 ml) under similar conditions. On days 1, 2, 3, 4, 5, 6, and 7 after cytostatic treatment, the

animals were euthanized by cervical dislocation under ether anesthesia. The count of neutrophilic granulocytes in the bone marrow was evaluated. The content of granulomonocytopoietic colony-forming (CFU) and cluster-forming units (ClFU) in the bone marrow was measured by *in vitro* culturing of myelokaryocytes in methylcellulose [1]. Proliferative activity of hemopoietic precursors was studied by the method of cell suicide using hydroxyurea. Cell differentiation was assayed by the index of maturation (cluster/colony ratio in a well) [1]. Structural and functional characteristics of the bone marrow were estimated by enzymatic isolation of hemopoietic islets and study of their quantitative and qualitative composition [7]. Colony-stimulating activity of conditioned media from adherent and nonadherent cells of the hemopoiesis-inducing microenvironment and blood plasma was tested with intact mouse myelokaryocytes [1].

The final concentrations of serotonin (Sigma) and granulocyte colony-stimulating factor (G-CSF, NIKTI BAV) in the bone marrow culture were 10^{-8} M and 5 ng/ml, respectively.

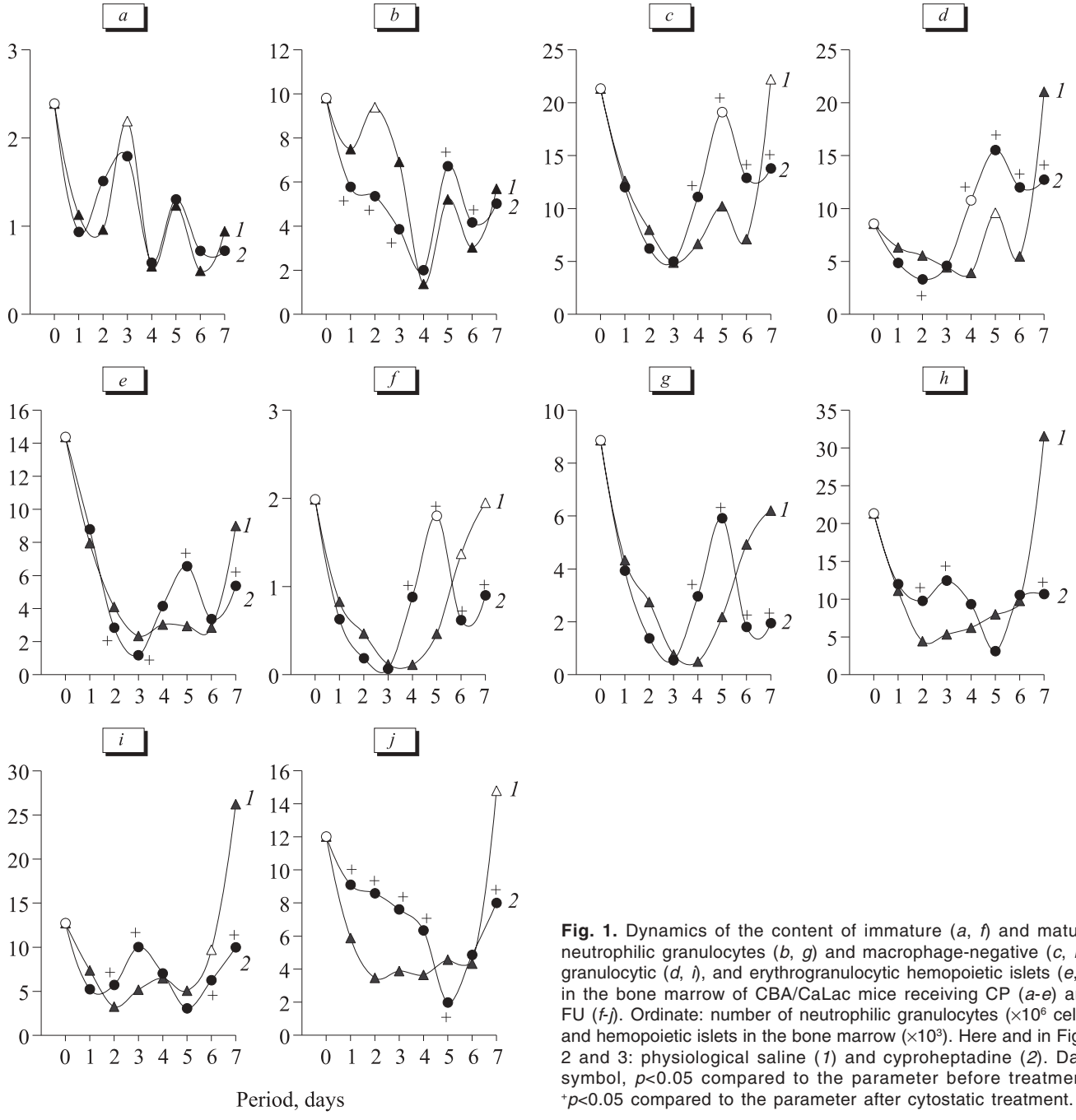

Fig. 1. Dynamics of the content of immature (*a*, *f*) and mature neutrophilic granulocytes (*b*, *g*) and macrophage-negative (*c*, *h*), granulocytic (*d*, *i*), and erythrogranulocytic hemopoietic islets (*e*, *j*) in the bone marrow of CBA/CaLac mice receiving CP (*a*-*e*) and FU (*f*-*j*). Ordinate: number of neutrophilic granulocytes ($\times 10^6$ cells) and hemopoietic islets in the bone marrow ($\times 10^3$). Here and in Figs. 2 and 3: physiological saline (*1*) and cyproheptadine (*2*). Dark symbol, $p<0.05$ compared to the parameter before treatment; $^+p<0.05$ compared to the parameter after cytostatic treatment.

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

RESULTS

CP decreased the number of immature (days 1, 2, and 4-7) and mature (days 1 and 3-7) neutrophilic granulocytes in the bone marrow, which was accompanied by neutrophilic leukopenia (days 1-5) and then neutrophilia in the peripheral blood (days 6 and 7, Fig. 1). Functional activity of granulocytic precursors was suppressed only on day 1 (Fig. 2). Further acceleration of precursor differentiation and maturation (days 3, 5, and 7) was accompanied by a significant increase in the content of granulomonocytic CFU (day 5) and ClFU in the methylcellulose medium (days 3, 5, and 7).

FU also decreased the number of granulocytic cells on days 1-5. However, the count of nucleated

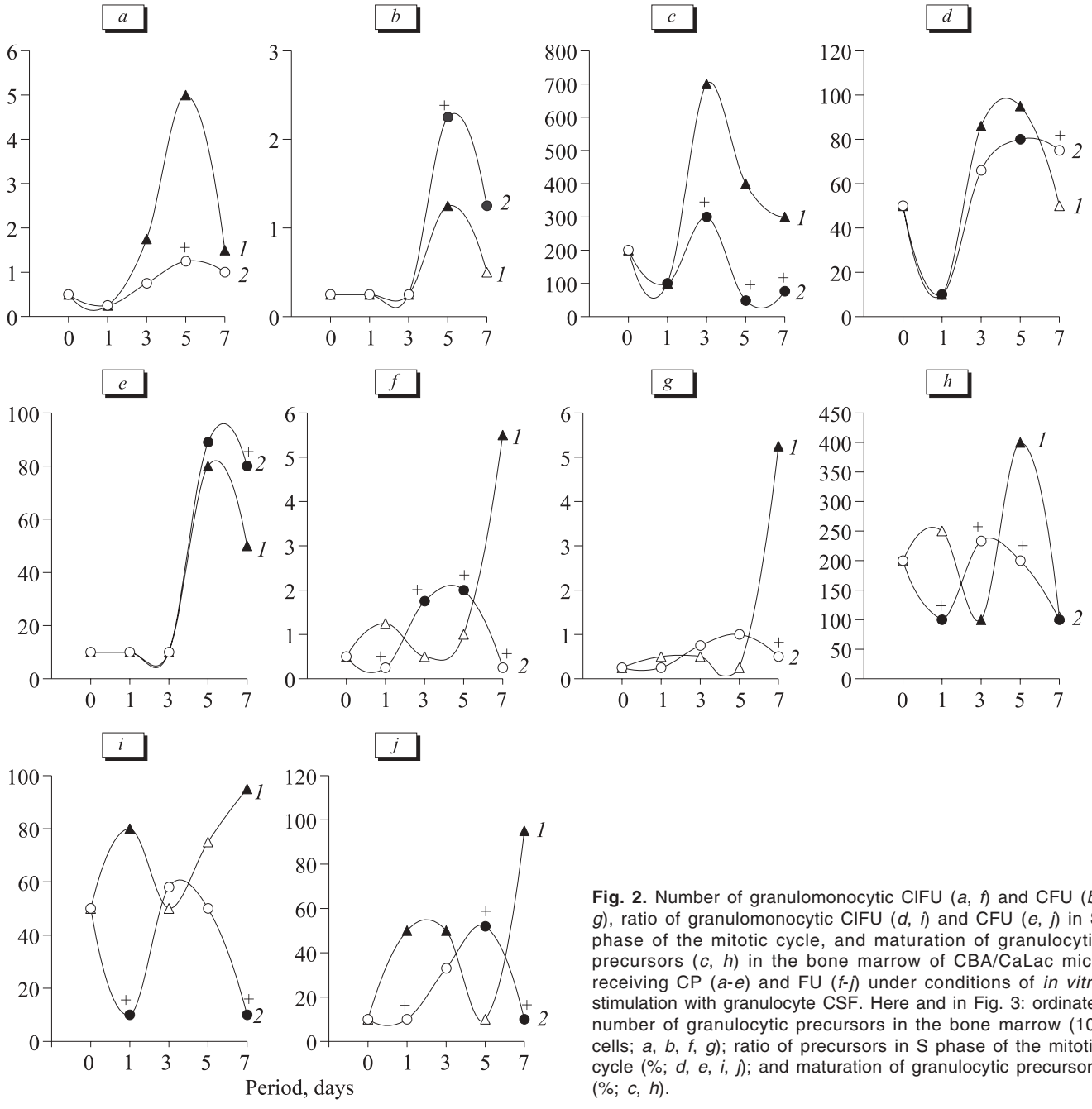

Fig. 2. Number of granulomonocytic ClFU (*a*, *f*) and CFU (*b*, *g*), ratio of granulomonocytic ClFU (*d*, *i*) and CFU (*e*, *j*) in S phase of the mitotic cycle, and maturation of granulocytic precursors (*c*, *h*) in the bone marrow of CBA/CaLac mice receiving CP (*a*-*e*) and FU (*f*-*j*) under conditions of *in vitro* stimulation with granulocyte CSF. Here and in Fig. 3: ordinate: number of granulocytic precursors in the bone marrow (10^5 cells; *a*, *b*, *f*, *g*); ratio of precursors in S phase of the mitotic cycle (%; *d*, *e*, *i*, *j*); and maturation of granulocytic precursors (%; *c*, *h*).

TABLE 1. Effect of Cyproheptadine on Colony-Stimulating Activity ($\times 10^5$ Cells) in Biological Fluids of CBA/CaLac Mice Receiving CP and FU ($X \pm m$)

Period of study, *in vivo* treatment			Supernatants		Blood plasma
			from adherent myelokaryocytes	from nonadherent myelokaryocytes	
Intact control		physiological saline (n=9)	0.25±0.25	0.25±0.25	0.25±0.25
CP	day 1	physiological saline (n=9)	0.25±0.25	0.25±0.25	1.50±0.28*
		cyproheptadine (n=9)	1.75±0.25*+	0.25±0.25	1.00±0.25
	day 3	physiological saline (n=9)	0.25±0.25	0.50±0.28	1.00±0.25
		cyproheptadine (n=9)	0.25±0.25	0.25±0.25	0.50±0.28
	day 5	physiological saline (n=9)	0.25±0.25	0.25±0.25	0.25±0.25
		cyproheptadine (n=9)	0.50±0.28	0.25±0.25	0.25±0.22
	day 7	physiological saline (n=9)	0.25±0.25	0.25±0.25	0.75±0.25
		cyproheptadine (n=9)	1.50±0.35*+	0.50±0.28	1.50±0.28*
FU	day 1	physiological saline (n=9)	0.25±0.25	0.25±0.25	1.50±0.28*
		cyproheptadine (n=9)	0.75±0.25	0.50±0.28	0.50±0.25+
	day 3	physiological saline (n=9)	0.25±0.25	0.50±0.28	0.25±0.25
		cyproheptadine (n=9)	1.50±0.25*+	0.25±0.25	0.25±0.25
	day 5	physiological saline (n=9)	2.25±0.25*	0.25±0.25	0.50±0.28
		cyproheptadine (n=9)	1.75±0.25*	0.25±0.25	0.50±0.28
	day 7	physiological saline (n=9)	0.25±0.25	0.25±0.25	0.25±0.25
		cyproheptadine (n=9)	0.75±0.25	0.25±0.25	0.25±0.25

Note. $p<0.05$: *compared to intact control; +compared to cytostatic-treated animals not receiving cyproheptadine.

cells in bone marrow tissue tended to increase on day 6. Hence, the number of immature neutrophils returned to normal on days 6 and 7 (Fig. 1). The number of peripheral blood neutrophils decreased on days 2 and 4-7. On days 1, 3, and 5, the content of granulomonocytic CFU and ClFU in the bone marrow did not exceed the initial level. These specific features were associated with discoordination of proliferation and differentiation of granulomonocytopoietic precursors (Fig. 2). Stimulation of cell division was accompanied by accumulation of granulomonocytic CFU and ClFU on day 7.

These data show that cytostatics in $^1/_3$ MTD induced a long-term suppression of granulocytopoiesis. Destructive changes in the bone marrow were most severe on days 4-7 (period II) and 1-3 (period I) after treatment with alkylating agent and fluoropyrimidine antimetabolite, respectively. After CP administration, functional activity of granulomonocytic precursors returned to normal more rapidly compared to that in experiments with FU.

In vitro addition of serotonin increased the release of granulomonocytic CFU and ClFU due to activation of precursor proliferation during CP-induced myelosuppression (day 3, Fig. 3). Accumulation of granulomonocytic precursors in the tissue culture after administration of FU and serotonin was due to acceleration of cell division and maturation (day 7).

Serotoninergic structures on granulomonocytic precursors are involved in the regulation of cell proliferation and differentiation during cytostatic-induced myelosuppressions. It should be emphasized that stimulation of these processes by serotonin was much less significant compared to that observed in experiments with granulocyte CSF (Figs. 2 and 3).

The study of the system of local hemopoiesis regulation showed that the number of cell complexes not associated with macrophage cells decreased in the bone marrow of CP-treated mice (days 1-6). Qualitative study revealed a decrease in the content of mixed (days 1-7) and granulocytic hemopoietic islets (days 2-4 and 6). FU impaired the formation of cell complexes of various types (days 1-6). The number of hemopoietic islets increased on day 7 after induction of myelosuppression.

The study of colony-stimulating activity in biological fluids showed that production of growth factors for granulocytic CFU by cells of the hemopoiesis-inducing microenvironment is impaired at various terms after CP treatment (Table 1). Colony-stimulating activity of myelokaryocytes was also abnormal after FU administration. The exception

was an increase in the number of granulomonocytic CFU after administration of supernatants from adherent cells (day 5) to the culture of intact myelokaryocytes. Growth factor deficiency in blood plasma on days 3, 5, and 7 after cytostatic treatment was preceded by their short-term activation (day 1).

Our results show that disturbances in the formation of bone marrow hemopoietic islets and production of colony-stimulating activity of adherent and nonadherent cells by hemopoiesis-inducing microenvironment as well as deficiency of plasma activities play an important role in inhibition of granulocytopoiesis induced by CP. Granulomonocytic precursors are capable of undergoing proliferation and differentiation under these conditions (especially after *in vitro* administration of granulocyte CSF). FU-induced inhibition of the granulocytic stem was associated with not only structural and functional disintegration of the bone marrow and decrease in colony-stimulating activity of blood plasma and myelokaryocyte supernatants, but also discoordination of precursor proliferation and maturation.

Evaluation of the role of brain postsynaptic serotonin 5-HT2 receptors in the regulation of hemo-

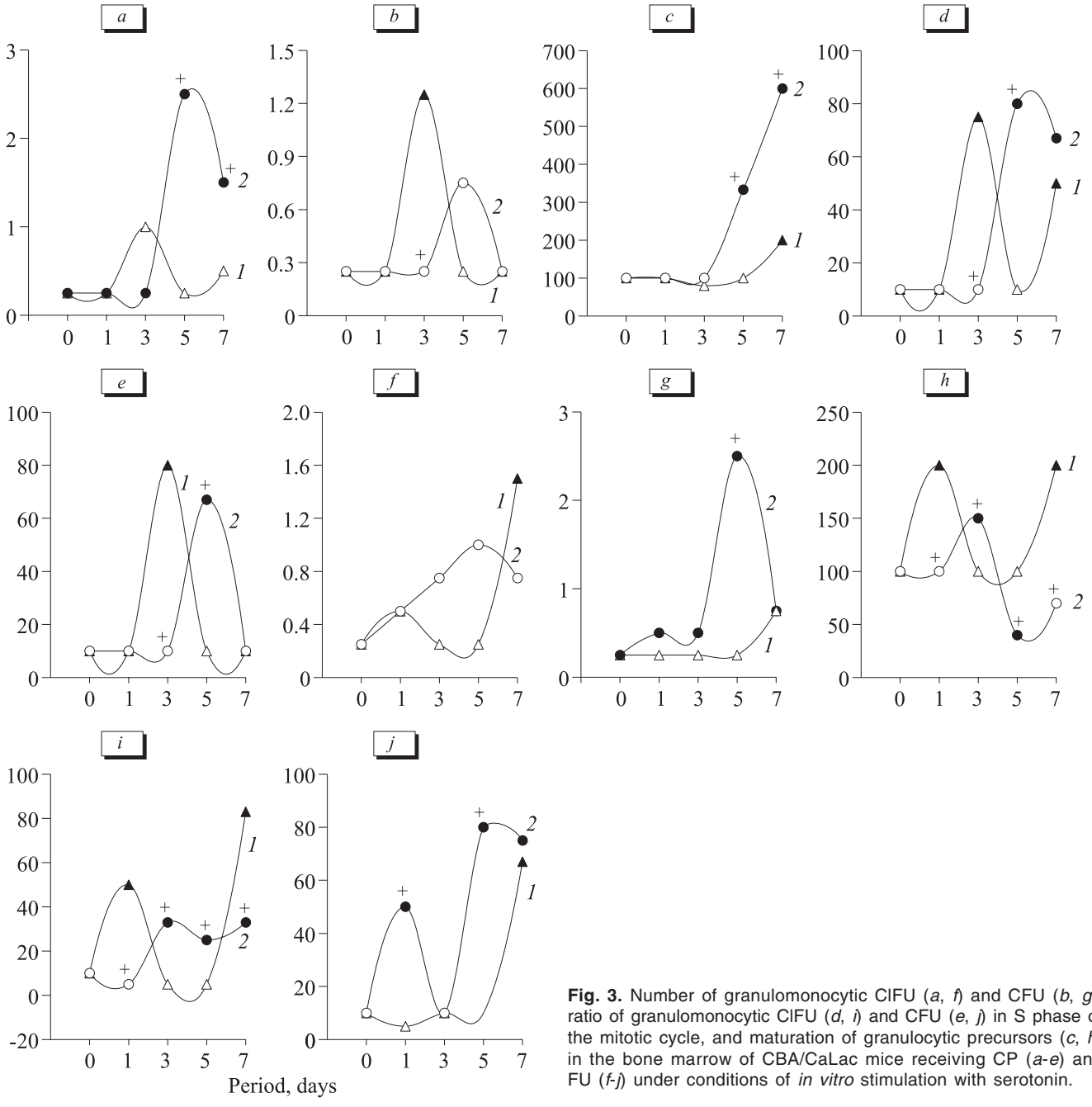

Fig. 3. Number of granulomonocytic ClFU (*a*, *f*) and CFU (*b*, *g*), ratio of granulomonocytic ClFU (*d*, *i*) and CFU (*e*, *j*) in S phase of the mitotic cycle, and maturation of granulocytic precursors (*c*, *h*) in the bone marrow of CBA/CaLac mice receiving CP (*a*-*e*) and FU (*f*-*j*) under conditions of *in vitro* stimulation with serotonin.

poiesis during experimental myelosuppressions revealed some regularities. Cyproheptadine increased the severity of granulocytopoiesis suppression in the early period after CP administration (days 1-3), but prevented its aggravation at later stages (day 5). After FU treatment, cellularity of the granulocytic stem returned to normal much more rapidly (on days 4-5 *vs*. 6-7 in the control), but then inhibition of the granulocytic stem developed again (days 6-7).

After consecutive treatment with cyproheptadine and CP, granulocyte CSF inhibited the growth of granulomonocytic ClFU (days 3 and 5) and stimulated the formation of granulomonocytic CFU (days 5 and 7) in the bone marrow culture. These changes were associated with inhibition of differentiation (days 3, 5, and 7) and activation of proliferation of granulomonocytopoietic precursors (day 7, Fig. 2). Serotonin *in vitro* decelerated precursor differentiation on day 3, which led to a decrease in the content of CFU and ClFU to the initial level (Fig. 3). Functional activity of granulomonocytopoietic precursors increased in days 5-7.

After combined treatment with antiserotonin drug and FU, granulocyte CSF decreased functional activity of granulomonocytic precursors (days 1 and 7, Fig. 2). The number of granulomonocytic ClFU increased in other periods of the study. It should be emphasized that this phenomenon was related to accelerated maturation on days 3 and enhanced proliferation (against the background of suppressed differentiation) on day 5. Serotonin had different effects on granulomonocytopoietic precursors. The increase in the number of DNA-synthesizing granulomonocytic ClFU (days 3 and 5) and CFU (day 5) and stimulation of precursor differentiation (day 3) led to accumulation of CFU and ClFU on days 3 and 5 (Fig. 3). These processes were uncoordinated on day 1 and suppressed on day 7.

Combined treatment with cyproheptadine and CP increased the degree of structural and functional disintegration in the bone marrow (period I and day 7). This conclusion was derived from the decrease in the number of granulocytic (days 2 and 7) and mixed hemopoietic islets (days 2, 3, and 7; Fig. 1). However, a typical increase in the content of cell complexes (granulocytic hemopoietic islets) was observed on days 4-6. In experiments with CP, the antiserotonin drug stimulated the formation of hemopoietic islets during the early period of the study (especially erythrogranulocytic islets), but inhibited the formation of cell complexes at later terms.

Colony-stimulating activity of adherent cells of the hemopoietic microenvironment considerably increased under conditions of blockade of serotoninergic system and CP-induced myelosuppression (days 1 and 7, Table 1). Administration of cyproheptadine before FU treatment reduced high level of activity in blood plasma (day 1) and enhanced production of colony-stimulating activity by adherent nucleated cells (day 3).

Our findings demonstrate that the state of granulomonocytic precursors and activity of hemopoietic microenvironment during cytostatic-induced myelosuppressions are regulated by the serotoninergic system. The regulatory influence of brain serotoninergic structures on proliferation and differentiation is realized via serotonin receptors on precursors and cells of the hemopoiesis-inducing microenvironment, as well as via modulation of CSF system activity. Cyproheptadine potentiates inhibition of granulocytopoiesis on days 1-3, but prevents its aggravation at later terms (days 4-7). The serotoninergic system probably has an ambiguous role in the pathogenesis of disturbances in the granulocytic hemopoietic stem under these experimental conditions: serotonin produced in CNS stimulates hemopoiesis in the early stage, but contributes to structural and functional disintegration of the bone marrow during the follow-up period. Under conditions of antimetabolite treatment, due attention should be given to the serotoninergic control of individual elements in the system for local regulation of granulocytopoiesis. Under the effect of antiserotonin agent, the increase in the formation of hemopoietic islets and production of growth factors by adherent cells of the hemopoiesis-inducing microenvironment is followed by the inhibition of these processes. However, functional activity of granulocyte-macrophage precursors decreases after addition of granulocyte CSF to tissue culture. *In vitro* treatment with serotonin is accompanied by discoordination of cell differentiation and maturation.

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