GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Role of Central Adrenergic Structures in the Regulation of Granulocytopoiesis during Cytostatic Treatment

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We studied the role of central adrenergic structures in the regulation of granulocytic hemopoietic stem during cytostatic treatment. It was found that brain norepinephrine promoted the decrease in functional activity of adherent mononuclears of the hemopoiesis-inducing environment, cytokine-dependent and α-adrenergic mechanisms of regulation of division and maturation of granulomonocytic precursors under conditions of cyclophosphamide treatment. Under conditions of 5-fluorouracil treatment, the effect of the adrenergic system was aimed at the formation of granulocytic and fibroblast-type cell complexes, activation of the production of colony-forming activity by microenvironmental cells, and acceleration of maturation of neutrophilic granulocytes associated with the system of granulocytic CSF and peripheral α-adrenergic structures.

Key Words: *adrenergic system; precursors; hemopoiesis-inducing microenvironment; granulocytopoiesis; cytostatics*

Control of hematological complications of anticancer chemotherapy is an urgent problem of modern oncology and hematology [3,4,8]. Myelosuppressive effects of cytostatics can be diminished by treatment with hemostimulating drugs. Nonspecific protectors of hemopoiesis (zymosan, splenin, group B vitamins, nucleic acid precursors, hormones, lithium salts) and new preparations created on the basis of endogenous hemopoiesis regulators (CSF, IL, erythropoietin, *etc.*) are used for these purposes. Extremely high activity of the latter preparations is determined by their key role in the regulation of the main hemopoietic stems *in situ* [1,2,10-12].

A promising approach to the creation of effective hemopoiesis stimulators is the search for agents modulating distant mechanisms of hemopoiesis regulation. Ample experimental material accumulated during recent years proved the important role of peripheral adrenergic structures in the mechanisms of regulation of blood system under pathological conditions [5,6]. Methods of treatment of cytostatic myelosuppressions with neuropharmacological drugs modulating activity of the sympathetic nervous system were proposed and tested in clinical practice [6]. However, the role of central adrenergic structures in cytostatic-induced abnormalities in the hemopoietic tissue remains little studied.

Here we evaluated the role of central adrenergic structures in the regulation of granulocytopoiesis under conditions of cytostatic-induced hemosuppressions.

MATERIALS AND METHODS

The experiments were carried out on 2-2.5-month-old female CBA/CaLac mice (n=650, conventional mouse strain obtained from the nursery of Institute

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Cytostatic myelosuppression was modeled by single intraperitoneal injection ($^1/_3$ MTD) of alkylating agent cyclophosphamide (83 mg/kg) or fluoropyrimidine antimetabolite 5-fluorouracil (76 mg/kg). The animals of experimental groups received single intraperitoneal injection of sympatholytic reserpine (Polfa) in a dose of 2 mg/kg 30 min before cytostatic treatment. Controls received an equivalent volume (0.2 ml) of physiological saline under the same conditions. On days 1, 2, 3, 4, 5, 6, and 7 after cytostatic treatment, the content of neutrophil leukocytes in the peripheral blood was determined, the animals were sacrificed by cervical dislocation under ether narcosis, and the number of mature and immature neutrophilic granulocytes in the bone marrow was evaluated. The content of granulocyte-macrophage CFU and cluster-forming units (CFU-GM and ClFU-GM) in the bone marrow was determined by *in vitro* cloning of myelokaryocytes in methylcellulose culture medium [7]. Proliferative activity of committed granulomonocytic precursor cells was evaluated by the method of cell suicide using hydroxyurea and the intensity of cell differentiation was determined by the index of maturation (ratio of clusters to colonies in the same well) [7]. Structural and functional organization of the bone marrow was studied after enzymatic isolation of hemopoietic islets followed by evaluation of their quantitative and qualitative composition [9]. The level of colony-stimulating activity in conditioned media from adherent and non-adherent cells of the hemopoiesis-inducing environment (HIM) and blood serum was tested using intact mouse myelokaryocytes [7].

Final concentration of a-adrenoceptor agonist mezaton (Experimental plant of Drug Research Center, Khar'kov) and β-adrenoceptor isadrine (Sigma) in the bone marrow culture was 10^{-8} M, final concentration of recombinant granulocytic CSF (G-CSF, Neupogen, Hoffman-La Roche) was 5 ng/ml.

The data were processed by standard methods of variation statistics. Reliability of differences was evaluated using parametric Student's *t* test and non-parametric Mann—Whitney *U* test.

RESULTS

Reserpine prevented the decrease in the content of neutrophilic granulocytes in the bone marrow at the early terms after cytostatic treatment (days 1 and 2, Fig. 1). A significant decrease in the count of immature neutrophils in the experimental group was observed later, on day 3 of the experiment,

TABLE 1. Effect of Reserpine on the Level of Colony-Stimulating Activity ($\times 10^5$ Cells) in Biological Fluids in CBA/CaLac Mice after Treatment with Cyclophosphamide or 5-Fluorouracil ($X \pm m$)

Day of experiment	*In vivo* preparation	Supernatants of adherent myelokaryocytes	Supernatants of non-adherent myelokaryocytes	Blood serum
Intact control	Physiological saline (n=9)	0.40±0.15	0.38±0.26	0.5±0.1
Cyclophosphamide				
1	Physiological saline (n=9)	0.25±0.15	0.4±0.23	1.50±0.25*
	Reserpine (n=9)	0.45±0.25	0.25±0.15	0.4±0.1+
3	Physiological saline (n=9)	0.25±0.20	0.50±0.28	1.00±0.25
	Reserpine (n=9)	0.35±0.21	0.35±0.25	0.75±0.22
5	Physiological saline (n=9)	0.25±0.18	0.40±0.25	0.25±0.25
	Reserpine (n=9)	0.50±0.28	0.29±0.21	0.25±0.12
7	Physiological saline (n=9)	0.15±0.05	0.25±0.22	0.75±0.15
	Reserpine (n=9)	0.2±0.1	0.50±0.28	1.25±0.25
5-fluorouracil				
1	Physiological saline (n=9)	0.15±0.12	0.40±0.25	1.55±0.18*
	Reserpine (n=9)	0.25±0.15	0.25±0.15	0.25±0.10+
3	Physiological saline (n=9)	0.12±0.11	0.38±0.22	0.44±0.25
	Reserpine (n=9)	0.23±0.20	0.25±0.18	0.75±0.30
5	Physiological saline (n=9)	2.25±0.25*	0.40±0.25	0.38±0.20
	Reserpine (n=9)	0.25±0.10+	0.25±0.20	0.50±0.28
7	Physiological saline (n=9)	0.25±0.25	0.29±0.15	0.25±0.12
	Reserpine (n=9)	0.38±0.23	0.25±0.12	0.25±0.20

Note. $p<0.05$ compared to: *intact control, +cytostatic without reserpine.

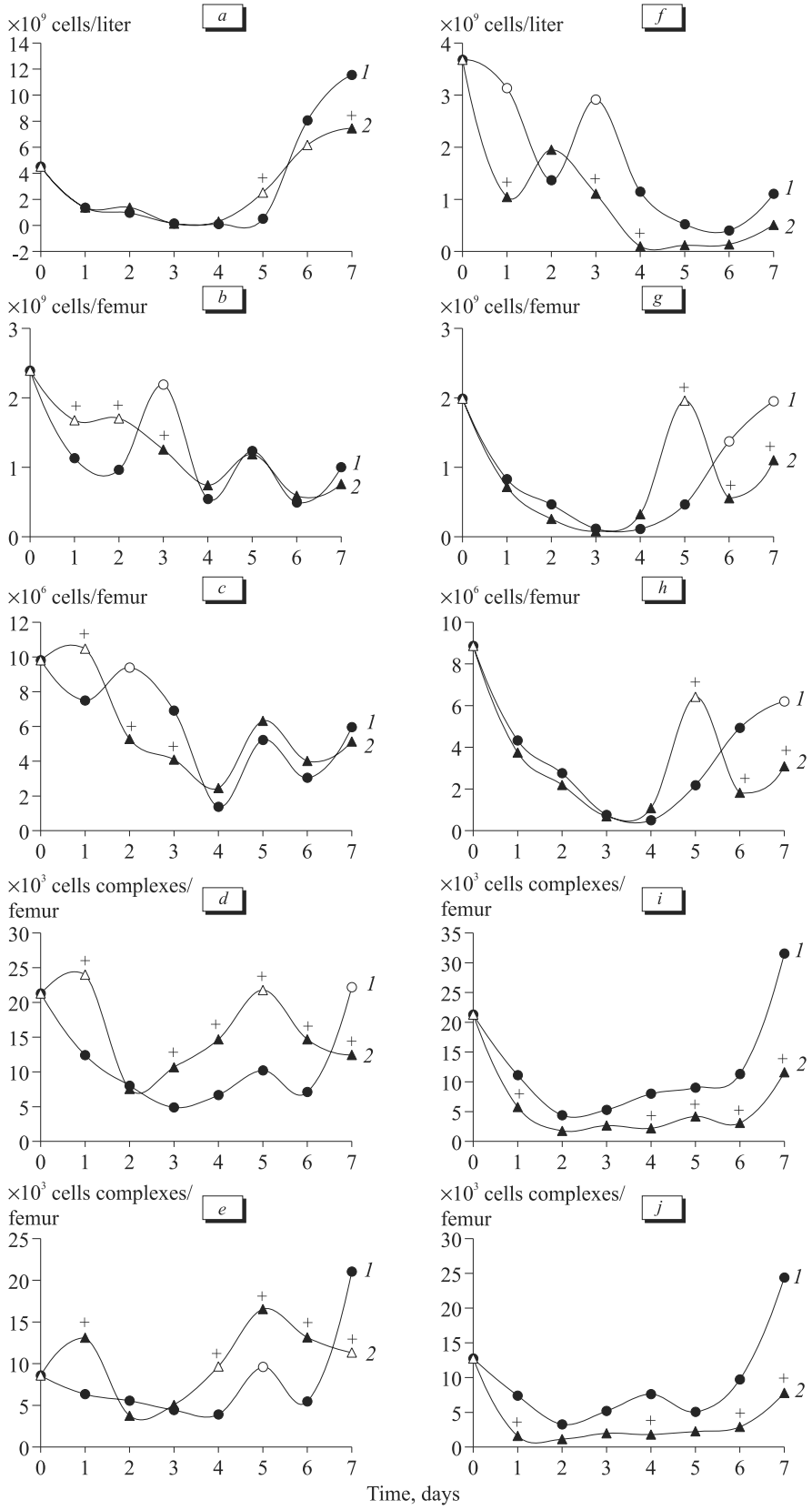

Fig. 1. Dynamics of the content of segmented neutrophils in the peripheral blood (*a*, *f*), immature (*b*, *g*) and mature (*c*, *h*) neutrophils granulocytes, fibroblastoid (*d*, *i*) and granulocytic (*e*, *j*) hemopoietic islets in the bone marrow of CBA/CaLac mice after treatment with cyclophosphamide (*a*-*e*) and 5-fluorouracil (*f*-*g*). *1*) physiological saline; *2*) reserpine. $^{+}p<0.05$ compared to animals receiving physiological saline. Dark symbols marks reliable differences from initial (before treatment) values ($p<0.05$).

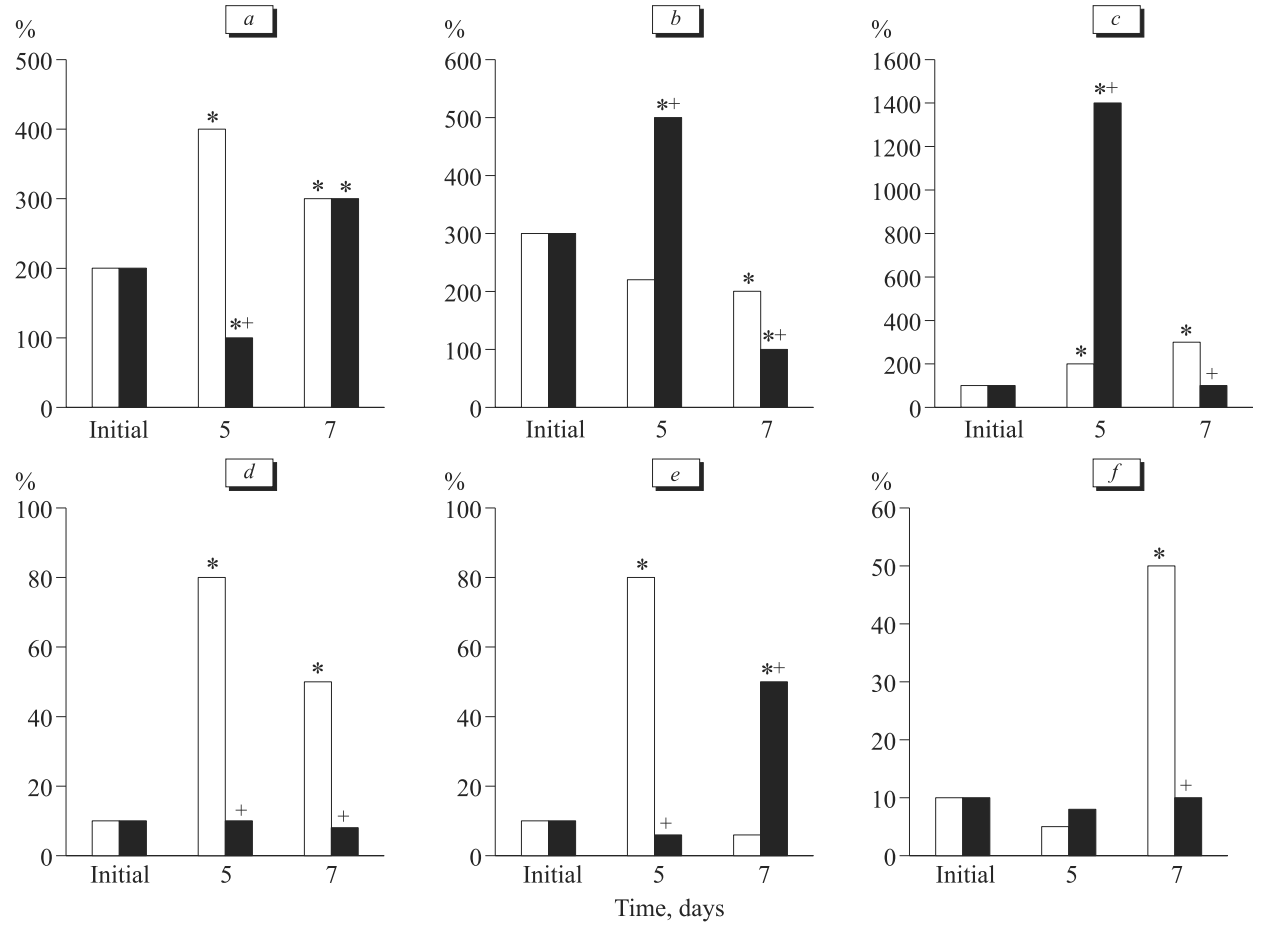

Fig. 2. Intensity of maturation of granulomonocytic precursors (*a-c*) and percent of S-phase CFU-GM (*d-f*) in the bone marrow of CBA/CaLac mice after treatment with cyclophosphamide. Light bars: physiological saline; dark bars: reserpine. Here and on Fig. 3: $p<0.05$ compared to *initial values (before treatment), +animals receiving physiological saline. *In vitro* stimulator G-CSF (*a*, *d*), mezaton (*b*, *e*), or isadrine (*c*, *f*).

while the content of mature forms decreased on days 2-3. Under these conditions, the content of segmented neutrophils in the peripheral blood increased on day 5 of the experiment, but not on days 6-7 (cytostatic control). Under conditions of 5-fluorouracil treatment, changes in the granulocytic hemopoietic stem after depletion of catecholamine depots had other pattern. For instance, normalization of neutrophil content in the hemopoietic tissue was observed earlier (day 5) than in animals receiving no sympatholytic (days 6-7), but this parameter decreased by the end of observation. The number of segmented neutrophils in the peripheral blood on days 1, 3, and 4 of the experiment was 9.0-33.0% from the cytostatic control ($p<0.01$).

Thus, reserpine restored the content of neutrophilic granulocytes in the blood system under conditions of cyclophosphamide treatment and aggravated suppression of granulocytosis in animals receiving 5-fluorouracil. Taking into account the mechanism of sympatholytic action (depletion of catecholamine (primarily, norepinephrine) depots in the central nervous system, we can conclude that cerebral adrenergic structures aggravate disturbances in the hemopoietic tissue caused by the alkylating agent and promote recovery of granulocytopoiesis in animals receiving antimetabolite.

Some regularities were observed in the mechanisms underlying the action of reserpine on granulocytic hemopoietic stem. For instance, decreased activity of central adrenergic structures had no effect on proliferation and differentiation of granulomonocytic precursor cells during the development of hemopoietic tissue hypoplasia in both experimental models. The most pronounced changes in these processes were observed at later terms corresponding to regeneration of the hemopoietic tissue. For instance, depletion of catecholamine stores under conditions of cyclophosphamide treatment and after addition of G-CSF to the bone marrow culture

decreased proliferative activity of precursors and intensity of their differentiation on days 5 and 7 of the experiment (Fig. 2). Treatment with mezaton on day 5 of the experiment decelerated division of CFU-GM compared to the control (cytostatic and adrenoceptor agonist) on the one hand, and accelerated maturation of granulocyte-macrophage cells (Fig. 3). Then (day 7), α-adrenoceptor agonist activated proliferation of CFU-GM, but reduced ClFU-GM/CFU-GM ratio. The effect of isadrine on functional activity of precursors was ambiguous: we observed an increase in the maturation index on day 5 and suppression of processes on day 7.

In experiments with successive administration of the sympatholytic and antimetabolite and addition of G-CSF to the culture of non-adherent myelokaryocytes, the number of DNA-synthesizing CFU-GM increased on day 5, but decreased on day 7 (Fig. 2). At the same time, ClFU-GM/CFU-GM ratio decreased on day 5, but surpassed the level of cytostatic control by the end of observation. Under these conditions, isadrine decreased functional activity of granulomonocytic precursors *in vitro*. Addition of mezaton to the methylcellulose medium accelerated precursor differentiation to the level of intact control (Fig. 3).

Thus, under conditions of cytostatic treatment reserpine suppressed (cyclophosphamide) or modulated (5-fluorouracil) the CSF-mediated mechanisms regulating proliferation and differentiation of granulomonocytic precursors. The suppressor effect of the preparation is related to the peripheral adrenergic structures (this effect is more pronounced after treatment with the alkylating agent). However, on day 5 of the experiment, sympatholytic through α-adrenergic mechanisms considerably intensified maturation of granulomonocytic cells (more markedly under conditions of antimetabolite treatment).

Evaluation of the effect of reserpine on the system of local regulation of granulocytopoiesis showed that this agent promoted recovery of hemopoietic islets in the bone marrow in animals treated

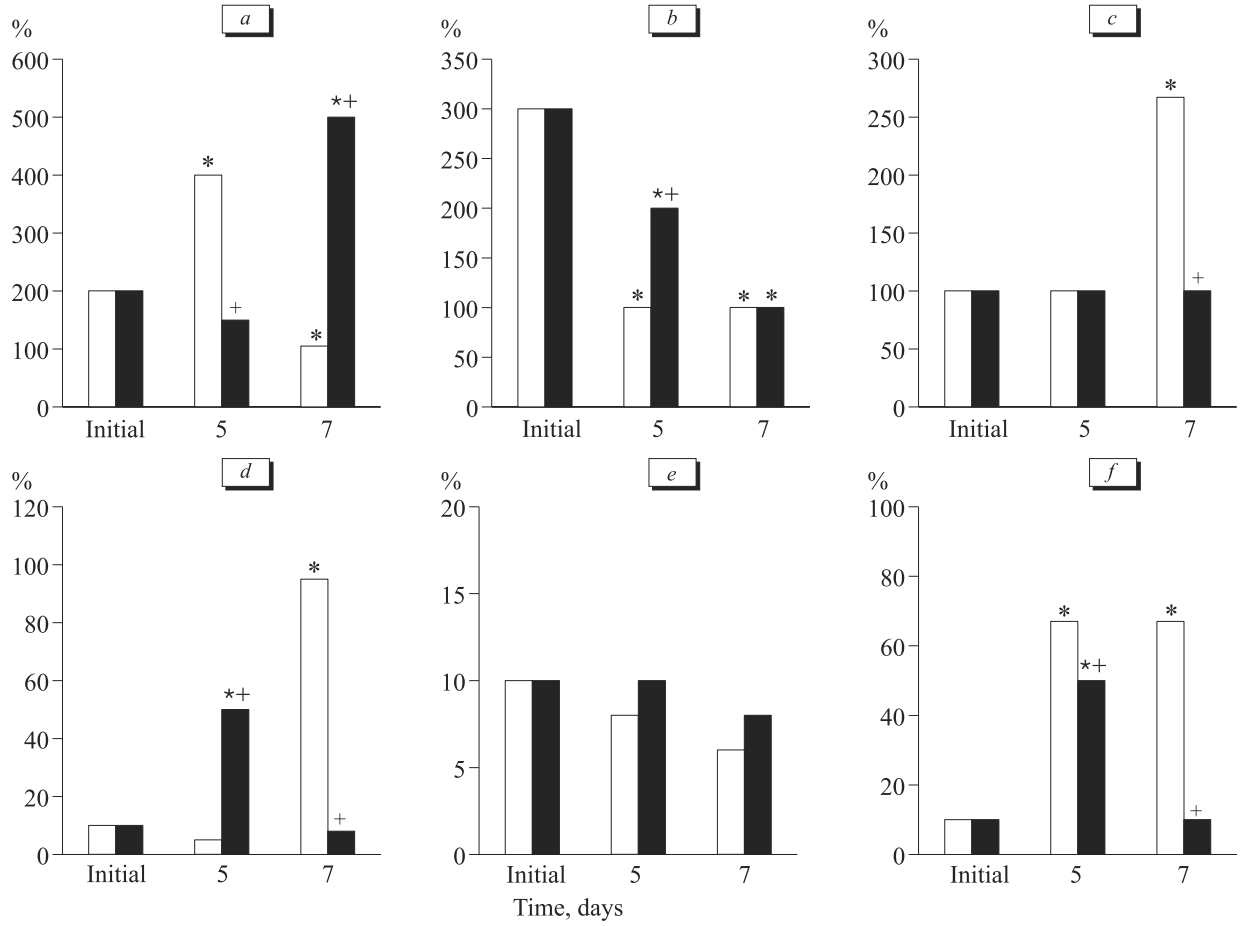

Fig. 3. Intensity of maturation of granulomonocytic precursors (*a-c*) and percent of S-phase CFU-GM (*d-f*) in the bone marrow of CBA/CaLac mice after treatment with 5-fluorouracil.

with cyclophosphamide, which was seem from the increase in the number of fibroblast (days 1 and 3-6) and granulocytic (days 1 and 4-6) cell complexes (Fig. 1). Colony-stimulating activity in myelokaryocyte supernatants in the experimental group was low and practically did not differ from that in cytostatic control and in intact animals (Table 1). Under conditions of 5-fluorouracil treatment, the sympatholytic suppressed the formation of hemopoietic islets (days 1, 4, 6, and 7) and production of colony-stimulating activity by adherent cells of HIM (day 5). In both cases, high serum activity observed in control animals on day 1 of the experiment was abolished by reserpine.

These findings suggest that in animals treated with cyclophosphamide, the recovery of granulocytic stem cellularity under the effect of reserpine is associated with *de novo* formation of hemopoietic islets and production of hemopoietic growth factors by adherent cells of HIM. Disturbed structural and functional integrity of hemopoietic tissue is a mechanism of sympatholytic-induced aggravation of granulocytopoiesis suppression under conditions of 5-fluorouracil treatment.

Thus, under conditions of cytostatic disease, the adrenergic systems realizes its regulatory effects on granulomonocytopoiesis via the corresponding structures on granulomonocytic precursors and the CSF system. Central adrenergic structures are involved in modulation of the secretion of colony-stimulating activity by cell elements of HIM and activity of hemopoietic islet formation, which leads to the corresponding changes in the rate of granulomonocyte division and maturation. Differences in the rate and mechanisms of recovery of the granulocytic hemopoietic stem under conditions of treatment with antitumor drugs are determined by the pecularities of the toxic effects of the antimetabolite and alkylating agent on not only hemopoietic cells, but also HIM elements and peripheral mechanisms of the regulation of functional activity of granulocyte-macrophage precursors by the central nervous system.

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