Effect of Adrenomimetics and Serotonin on Polypotent Stromal and Hemopoietic Precursors in Cytostatic Myelosuppression

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Effects of serotonin and adrenomimetics (phenylephrine and isoprenaline) on bone marrow stromal and polypotent hemopoietic precursors were studied *in vitro* on the model of cyclophosphamide-induced myelosuppression. It was found that under conditions of myelosuppression, adrenomimetics potentiate differentiation of polypotent hemopoietic precursors into mature precursors (granulocyte-macrophage and granulocyte CFU) initiated by granulocytic CSF, while serotonin suppresses these processes. Adrenomimetics (especially, isoprenaline) abolish high rate of division of stromal precursors and suppress the growth of granulocytic CSF induced by fibroblast-like cells. Serotonin does not affect proliferation of stromal precursors, but potentiates the granulocytopoiesis-stimulating effects of fibroblasts.

Key Words: *stromal precursors; polypotent hemopoietic precursors; adrenomimetics; serotonin; cyclophosphamide*

Regulatory properties of stromal cells forming fibroblast colonies (CFU-F) in culture are well studied. Numerous studies demonstrate the capacity of CFU-F to self-renewal and differentiation into mesenchymal and non-mesenchymal elements [7,10-12]. The stromal population of the bone marrow is extremely heterogeneous. Apart from fibroblasts and stromal precursors, it contains undifferentiated cells capable of self-renewal and differentiation. These mesenchymal stem cells (MSC) [7] or mesenchymal precursor cells [10] are precursors for non-hemopoietic cells. It is assumed that MSC similarly to CFU-F can transfer and form the stromal bone marrow microenvironment, mediate adhesion of hemopoietic cells, and supply them with essential cytokines such as IL-6, IL-7, IL-8, IL-11, IL-12, IL-14, IL-15, and growth factors (leukemia inhibiting factor, macrophage CSF, granulocyte-macrophage CSF, and stem cell growth factor) [9,15]. MSC and more mature stromal elements can promote migration of intravenously administered hemopoietic stem cells (HSC) to the bone marrow [9].

Study of nerve terminals in mouse bone marrow revealed adrenergic terminals both around the sinusoids and blood vessels supplying bone marrow tissue and among parenchymal cells [4,8,13,14]. Moreover, published data suggest that norepinephrine-containing fibers directly contact with stromal cells [5]. Hence, substances of neuronal origin participate in the realization of hematological effects of fibroblasts.

Another aspect of this problem is the effect of nervous and humoral stimuli on hemopoietic cells. Monoaminergic regulation of partially determinate and mature hemopoietic precursors in hematological disorders is generally described [2,3], but the effects of monoaminaes on HSC remains unclear.

Here we studied the direct effects of adrenomimetics and serotonin on stromal and polypotent hemopoietic precursors under conditions of cyclophosphamide treatment.

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MATERIALS AND METHODS

Experiments were carried out on 2-2.5-month-old male CBA/CaLac mice (n=120), conventional mouse strain obtained from the nursery of Institute of Pharmacology, Siberian Division of Russian Academy of Medical Sciences.

Cytostatic myelosuppression was induced by single intraperitoneal injections of alkylating cytostatic cyclophosphamide in a dose of 83 mg/kg. Intact animals served as the control. On days 1-7 after cytostatic treatment, the mice were sacrificed by cervical dislocation under ether narcosis and the number of mature and immature forms of neutrophilic granulocytes in the bone marrow was determined. The concentration of bone marrow CFU containing non-differentiated cell elements (CFU-N) was evaluated by the method of limiting dilutions. The cells constituting CFU-N were capable of long-term repopulation (more than 4 passages). Secondary and tertiary colonies had the same size (>1300 blast elements) and morphology as primary cultures: they contained not only non-differentiated cells, but also erythroblasts, granulocytes, and cells of the mononuclear phagocyte system. In our view, these cells were formed from polypotent hemopoietic precursors. We evaluated the intensity of differentiation of polypotent hemopoietic precursors into granulocyte-macrophage CFU (CFU-GM) and granulocyte CFU (CFU-G) under the action of granulocytic CSF (G-CSF, neupogen, Hoffman-La Roche Ltd.; 2 ng/ml) [1].

The yield of granulocytic colonies and clusters in culture of non-adherent myelokaryocytes was determined. Proliferative activity of CFU-G 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) [1]. We also assessed the content of stromal precursor cells forming fibroblast colonies in culture of adherent myelokaryocytes (CFU-F) and the intensity of formation of hemopoietic colonies (CFU-G) on a feeder formed by fibroblast-like cells (10^6 cells per well) [1].

The effect of α-adrenomimetic phenylephrine hydrochloride, β-adrenomimetic isoprenaline hydrochloride, and serotonin (10^{-8} M; all agents were purchased from Sigma) on differentiation of CFU-N, proliferative activity and intensity of differentiation of CFU-G, and on stromal precursors *in vitro* were studied. We also analyzed the effect of adrenomimetics and serotonin on the capacity of adherent bone marrow cells to maintain the growth of CFU-G. To this end, adherent cells were preliminary cultured with the ligands (10^{-8} M) for 2 h and then the medium was replaced with culture medium containing hemopoietic precursors (10^5/ml).

The data were processed by standard methods of variation statistics. Reliability of differences was evaluated using parametric Student's t test, nonparametric Mann–Whitney U test, and Fisher exact test for comparing relative values. The incidence of CFU-N was evaluated using generalized linear model for Poisson distribution.

RESULTS

Using experimental hypoplastic state caused by cyclophosphamide treatment we found that the development of myelosuppression (days 1-4) and inhibition of granulocytic precursors (days 1-5) were accompanied by an increase in the number of polypotent hemopoietic cells in the bone marrow above the level of intact control on days 2 and 4 (by 650 and 200%, respectively, Table 1). The content of non-differentiated cells in experimental CFU-N was ~1800, which surpassed the level of intact control by 50%. Activation of proliferation of polypotent hemopoietic precursors was promoted by their differentiation into mature precursors: CFU-GM on days 2-4 and CFU-G on days 2 (Fig. 1).

After treatment with the alkylating agent, adherent cells actively proliferated on plastic and differentiated

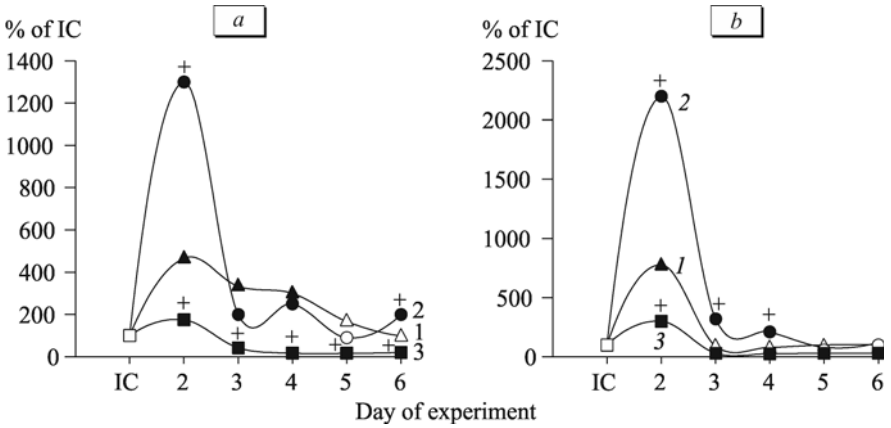

Fig. 1. Effect of adrenomimetics, serotonin, and G-CSF *in vitro* on the growth of CFU-GM (*a*) and CFU-G (*b*) in tertiary cultures of polypotent hemopoietic precursors in CBA/CaLac mice treated with cyclophosphamide. *1*) G-CSF; *2*) adrenomimetic; *3*) serotonin. p<0.05: +compared to G-CSF; dark symbols compared to intact control (IC).

with the formation of discrete colonies consisting of fibroblast-like elements (days 1, 2, and 6; Fig. 2, *a*). These findings indirectly suggest that MSC mobilized by the cytostatic treatment most likely differentiate into stromal precursor cells, *i.e.* hemopoietic microenvironment cells. Under these conditions, the capacity of fibroblast-like cells to maintain the formation of granulocytic colonies increased (days 2 and 4, Fig. 2, *b*).

Thus, cyclophosphamide-induced myelosuppression was accompanied by stimulation of bone marrow stromal and polypotent hemopoietic precursors. It should be noted that initiation of proliferation and differentiation of CFU-N restores the content of granulomonocyte-macrophage and granulomonocyte precursors most sensitive to cytostatic treatment, while mobilization of MSC leads to the formation of stroma essential for the maintenance of homeostasis and functioning of hemopoietic cells.

Then, we studied the effect of aminergic agents on polypotent hemopoietic precursors *in vitro* under conditions of treatment with the alkylating agent. It was found that the intensity of growth of CFU-H in the presence of drugs remained unchanged. In turn, adrenergic stimulation considerably increased the G-CSF-initiated yield of CFU-GM (days 2 and 6) and CFU-G (days 2-4) in tertiary cultures of polypotent hemopoietic precursors (Fig. 1). On the contrary, serotonin inhibited differentiation.

Another aspect of this problem is the specificity of the effect of nerve stimuli on hemopoietic precursor cells of different classes. Experiments with hydroxyurea showed that adrenomimetics reduced the number of mitotically active granulocytic precursors. However, the decrease in proliferative activity of CFU-G was less pronounced than in the group with G-CSF (Fig. 3, *a*). Differentiation of CFU-G was also inhibited by phenylephrine (days 1-5 and 7) and isoprenaline (days 1-6), while growth factor stimulated this process (Fig. 3, *b*). Changes in the parameters of serotonin groups were statistically insignificant.

Similar results were obtained during evaluation of the effects of monoaminergic ligands on stromal precursors. For instance, α- and β-adrenergic stimulation abolished, while serotonin had no effect on the formation of CFU-F observed after cyclophosphamide treatment (days 1, 2, and 6; Fig. 2, *a*). In turn, serotonin considerably improved the capacity of fibroblast-like cells to maintain the formation of granulocytic colonies (days 2 and 3, Fig. 2, *b*). Treatment with phenylephrine and isoprenaline did not change the capacity of fibroblast-like elements to maintain the growth of CFU-G compared to the control.

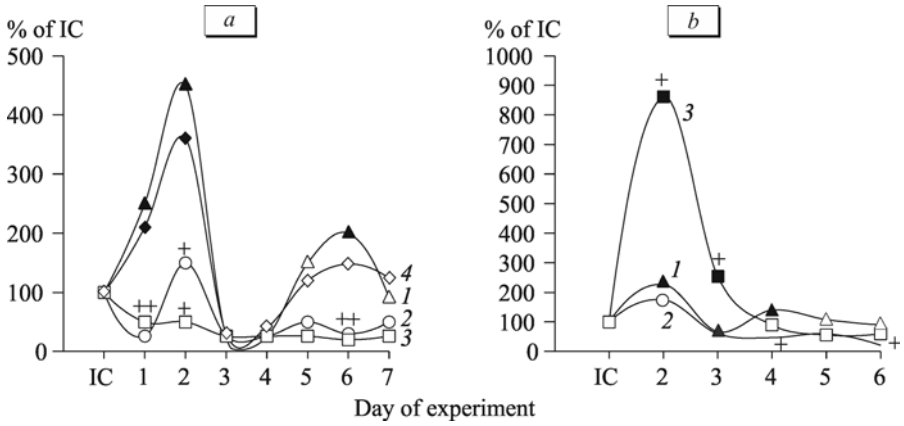

Fig. 2. Effect of adrenomimetics and serotonin *in vitro* on the growth of CFU-F (*a*) and stimulation of the formation of CFU-G mediated by fibroblast-like cells (*b*) in CBA/CaLac mice treated with cyclophosphamide. **p*<0.05 compared to IC (dark symbols). Fragment *a*: *1*) without agents, *2*) phenylephrine, *3*) isoprenaline, *4*) serotonin; +*p*<0.05 compared to the same parameter without treatment with the test agents. Fragment *b*: *1*) G-CSF, *2*) adrenomimetics, *3*) serotonin; +*p*<0.05 compared to G-CSF.

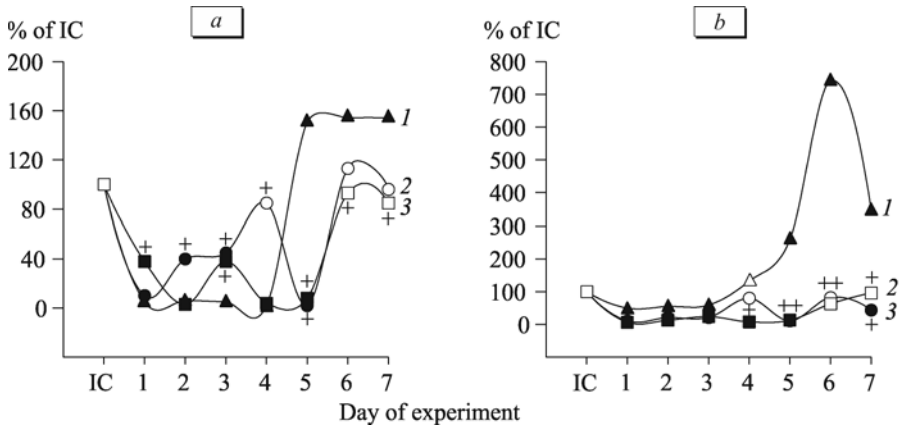

Fig. 3. Effect of adrenomimetics and G-CSF *in vitro* on proliferative activity (*a*) and intensity of differentiation (*b*) of granulocytic precursors in CBA/CaLac mice treated with cyclophosphamide. *1*) G-CSF; *2*) phenylephrie; *3*) isoprenaline. *p*<0.05: +compared to G-CSF; dark symbols compared to intact control (IC).

TABLE 1. Intensity of Growth of CFU-G (10^5 cells) in Culture of Non-Adherent Myelokaryocytes, Content of Morphologically Discernible Cells of Granulocytic Hemopoietic Stem (10^6 cells per femur) in the Bone Marrow of CBA/CaLac Mice Treated with Cyclophosphamide ($X \pm m$)

Time after cytostatic treatment, days		CFU-G	Neutrophilic granulocytes	
			immature	mature
Intact control		10.5±1.5	2.49±0.28	6.35±0.49
Time after treatment, days	day 1	0.25±0.16*	0.60±0.08*	2.23±0.26*
	day 2	0.25±0.16*	0.46±0.03*	1.1±0.08*
	day 3	0.25±0.16*	0.66±0.08*	0.77±0.09*
	day 4	0.25±0.16*	2.84±0.25	3.09±0.28*
	day 5	4.75±0.41*	1.86±0.41	3.09±0.45*
	day 6	11.75±1.05	3.2±0.31	9.10±0.43*
	day 7	12.00±1.36	2.51±0.14	8.64±0.052

Note. *$p<0.05$ compared to intact control.

Thus, adrenomimetics accelerated the rate of G-CSF-induced differentiation of polypotent hemopoietic precursors and suppressed functional activity of granulocytic and stromal CFU under conditions of cyclophosphamide treatment. Serotonin potentiated granulocytopoiesis-inducing activity of fibroblast-loke cells and reduced the rate of CFU-N differentiation.

Our findings suggest that peripheral monoamines are an integral component of the system of local regulation of bone marrow stromal and polypotent hemopoietic precursors. Interestingly, functional activity of CFU-G, but not CFU-N, is suppressed in cytostatic myelosuppression in the presence of phenylephrine, isopleraline, and G-CSF. The growth factor and the α-adrenomimetic produce similar, but more transient inhibitory effect on CFU-GM in mice treated with cyclophosphamide [2]. Moreover, phenylephrine and isoprenaline produce a suppressor effect on stromal precursors. This phenomenon can be explained by high sensitivity of mature mesenchymal (CFU-F) and hemopoietic precursors. In light of this, specific properties of adrenomimetics are expected to manifest at the level of polypotent hemopoietic precursors most resistant to the cytostatic. Undoubtedly, peripheral monoaminergic mechanisms restoring the content of neutrophils in the blood system under conditions of cyclophosphamide treatment are not limited by adrenergic regulation. According to our findings, serotonin promote the recovery of the pool of granulocytic precursors by involving stromal cells of hemopoietic microenvironment. Amine can also counterbalance the effects of catecholamines, because in contrast to adrenergic stimuli in can limit activity of polypotent hemopoietic precursors.

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