Effects of Granulocyte Colony-Stimulating Factor and Hyaluronidase on the Formation of Blood System Reactions

E. D. Goldberg, A. M. Dygai, G. N. Zyuz'kov, and V. V. Zhdanov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 6, pp. 628-633, June, 2008
Original article submitted December 17, 2007.

We studied the possibility of modification of hematotropic effects of granulocytic CSF and hyaluronidase. It was found that hyaluronidase in a dose of 20 U/mouse potentiates the specific effect of granulocytic CSF on granulocytopoiesis, while granulocytic CSF potentiates the stimulating effect of the enzyme on the erythroid stem. Functional activity of hemopoiesis precursors, secretion of humoral factors by adherent myelokaryocytes, and serum content of hemopoietins increased under these conditions. Hyaluronidase (100 U/mouse) against the background of treatment with granulocytic CSF leads to uncoupling of proliferation and differentiation of hemopoietic cells and abolishes the mutually activating hematotropic effect of preparations.

Key Words: *hemopoiesis; granulocytic colony-stimulating factor; hyaluronidase*

Cytokines produced by cells of the hemopoiesis-inducing environment (HIM) play an important role in the regulation of hemopoiesis. One of the best studied bioactive substance is granulocytic CSF (G-CSF) regulating primarily the granulomonocytic stem [1,5]. At the same time, the processes of hemopoiesis are largely determined by the state of extracellular matrix of the hemopoietic tissue, in particular, by hyaluronic acid *in situ* constituting up to 40% of all glycosaminoglycans [9,11,12]. Previous experiments showed [2,3,6] that hyaluronidase, the enzyme cleaving hyaluronic acid to polymers with different molecular weight and various effects on the function of cell elements [12], can modulate the state of the blood system. At the same time, the mechanisms of summary effects of individual cytokines and components of the extracellular matrix to hemopoiesis (*i.e.* mutual modification of specific properties of various factors) are little studied.

Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences, Russia

The aim of the present study is evaluation of the reaction of the blood system and regularities of their formation under conditions of combined treatment with G-CSF and hyaluronidase.

MATERIALS AND METHODS

The experiments were carried out on 2-month-old male and female CBA/CaLac mice (n=258) weighing 18-20 g, conventional mouse strain obtained from the nursery of Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences. The animals received subcutaneous injections of G-CSF (Neupogen, Hoffman-La Roche) dissolved in 0.2 ml RPMI-1640 medium (125 μg/kg/day for 5 days), intraperitoneal injections of hyaluronidase (Lidase, Microgen Research-and-Production Company) dissolved in 0.3 ml physiological saline (20 and 100 arb. units per mouse per day for 2 days), or G-CSF and hyaluronidase simultaneously according to the above schemes. Control mice received vehicle in the corresponding regimens.

0007-4888/08/1456-0682

On days 3, 5, and 8 after the start of treatment, the number of reticulocytes and various forms of leukocytes in the peripheral blood and parameters of the bone marrow hemopoiesis were evaluated [7]. The number of erythroid (CFU-E and granulomonocytic (CFU-GM) precursor cells in the bone marrow, their proliferative activity and intensity of differentiation, and erythropoietic and colony-stimulating activity of conditioned media from adherent and non-adherent elements of HIM and blood serum were studied by cultural methods [4].

The data were processed by methods of variation statistics using Student's *t* test and nonparametric Mann—Whitney test.

RESULTS

Administration of G-CSF to intact animals led to expected changes in hemopoiesis [1,8]. The content of CFU-GM in the bone marrow, their proliferative activity and intensity of maturation increased, which can be explained by the direct effect of the preparation on committed precursors and by stimulation of HIM elements (Fig. 1, 2). These reactions determined the increase in the content of morphologically discernible granulocytic cell elements in the hemopoietic tissue and increase in the count of stab and segmented neutrophils in the peripheral blood (Fig. 3).

In contrast to G-CSF, hyaluronidase in a dose of 20 arb. units/mouse stimulated granulomonocyto- and erythropoiesis. These results coincided with the previously observed hematotropic effects of this enzyme [6]. Treatment with hyaluronidase in the specified dose increased the content of reticulocytes, stab and segmented neutrophils in the peripheral blood and the content of erythrokaryocytes and mature neutrophilic granulocytes in the hemopoietic tissue (Fig. 3). Using the method of cell culture, we studied the relationship between the formation of the described reactions on the content of CFU and CFU-GM in the bone marrow, increase in proliferative activity, and acceleration of their maturation. Functional activation of hemopoietic precursors, in turn, is a result of enhanced production of erythropoietic activity by adherent myelokaryocytes, colony-stimulating activity by non-adherent nuclears of HIM, and increased hemostimulating activity of the serum (Fig. 1, 2).

At the same time, evaluation of possible mutual modification of specific properties of the studied preparations revealed mutual potentiation only for hyaluronidase in a dose of 20 arb. units/mouse. Combined treatment with G-CSF and hyaluronidase (20 arb. units/mouse) more markedly increased the content of reticulocytes in the peripheral blood compared to the corresponding values in animals receiving hyaluronidase alone (by 58.6% on day 8) and more drastically elevated the count of stab neutrophils compared to mice receiving G-CSF alone (by 92.3% on day 8). These changes were paralleled by a sharp decrease in the count of circulating segmented neutrophils, which was probably related to their migration into peripheral tissues [5,6]. This probably resulted from increased permeability of tissue barriers under the effect of hyaluronidase [12] and increased functional activity of neutrophils under the effect of G-CSF [5,8]. The described changes reflected the reactions of the hemopoietic tissue. We also observed an increase in the content of erythrokaryocytes (on day 8) and immature (day 3) and mature (days 3 and 8) neutrophilic granulocytes in the bone marrow compared to the corresponding parameters in mice treated with hyaluronidase and G-CSF, respectively (Fig. 3). The study of the mechanisms of the formation of these hematological shifts revealed significant acceleration of CFU-E proliferation (days 3 and 5) induced by G-CSF under the effect of hyaluronidase and increased content of CFU-GM and their proliferative activity (days 3 and 5) in animals receiving combined treatment compared to mice receiving the cytokine alone (Fig. 1). The reaction of the population of the progenitor cells developed against the background of intensive production of hemopoietically active substances by adherent myelokaryocytes (typical of G-CSF) and secretion of erythropoietic and colony-stimulating activities by adherent nuclears (similar to that observed under conditions of hyaluronidase treatment (Fig. 2).

Thus, combined treatment with G-CSF and hyaluronidase (20 arb. units per mouse) considerably potentiated the hemostimilating properties of each preparation. The observed potentiating effects were determined by enhanced secretory activity of HIM elements. Taking into account the important role of hyaluronic acid as a component of glycocalyx and various cell receptors [10], we can hypothesize that the studied processes are related to modulation of the sensitivity of hemopoietic precursors to cytokines under the effect of the enzyme. *e.g.*, sensitivity of erythroid precursors to administered G-CSF. Moreover, we cannot exclude that the pool of mesenchymal stem cells giving rise to not only HIM elements [1], but also parent hemopoietic cells, participating in the formation of the observed phenomenon. We previously demonstrated the possibility of increasing MSC reserve in the bone marrow by hyaluronidase treatment [2,3].

At the same time, administration of hyaluronidase in a dose of 100 arb. units per mouse led to

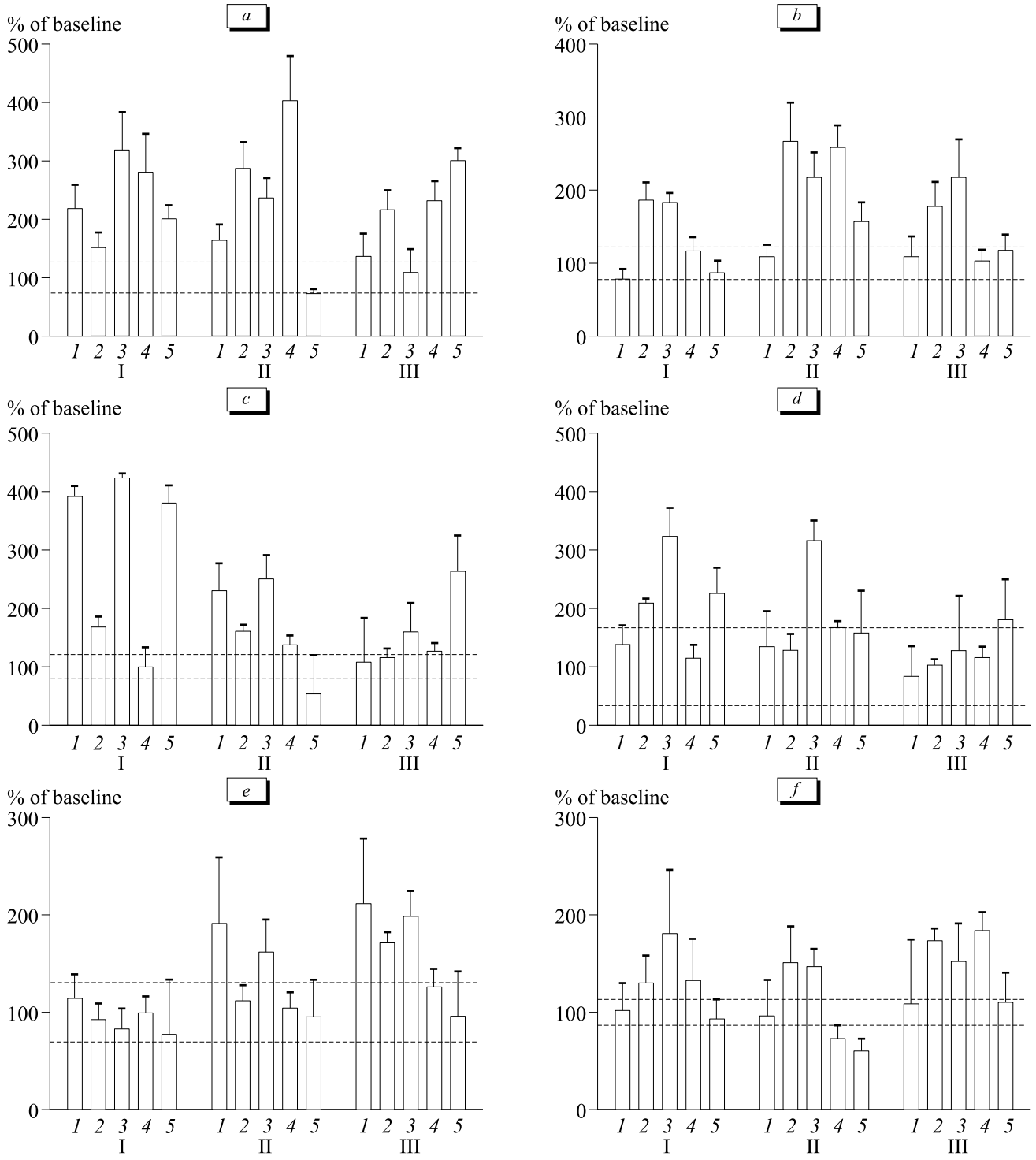

Fig. 1. Dynamics of the content of granulomonocytic (*a, c, e*) and erythroid (*b, d, f*) precursors in the bone marrow of CBA/CaLac mice (*a, b*), number of S-phase precursors (*c, d*), and intensity of maturation (*e, f*) after treatment with G-CSF (*1*), hyaluronidase in a dose of 20 arb. units per mouse (*2*), G-CSF and hyaluronidase in a dose of 20 arb. units per mouse (*3*), hyaluronidase in a dose of 100 arb. units per mouse (*4*), G-CSF and hyaluronidase in a dose of 100 arb. units per mouse (*5*). Here and on Figs. 2 and 3: I: day 3, II: day 5, III: day 8. Area between dotted lines shows confidence interval for the test parameter in intact mice at $p<0.05$.

hemopoiesis disorganization, in particular, to uncoupling of proliferation and differentiation of hemopoietic precursors (Fig. 1), and prevented the formation of blood picture reflecting stimulation of hemopoiesis (Fig. 3). These changes associated with pronounced destruction of hyaluronic acid and chondroitin sulfate of the extracellular matrix [6,12] were observed after administration of hyaluroni-

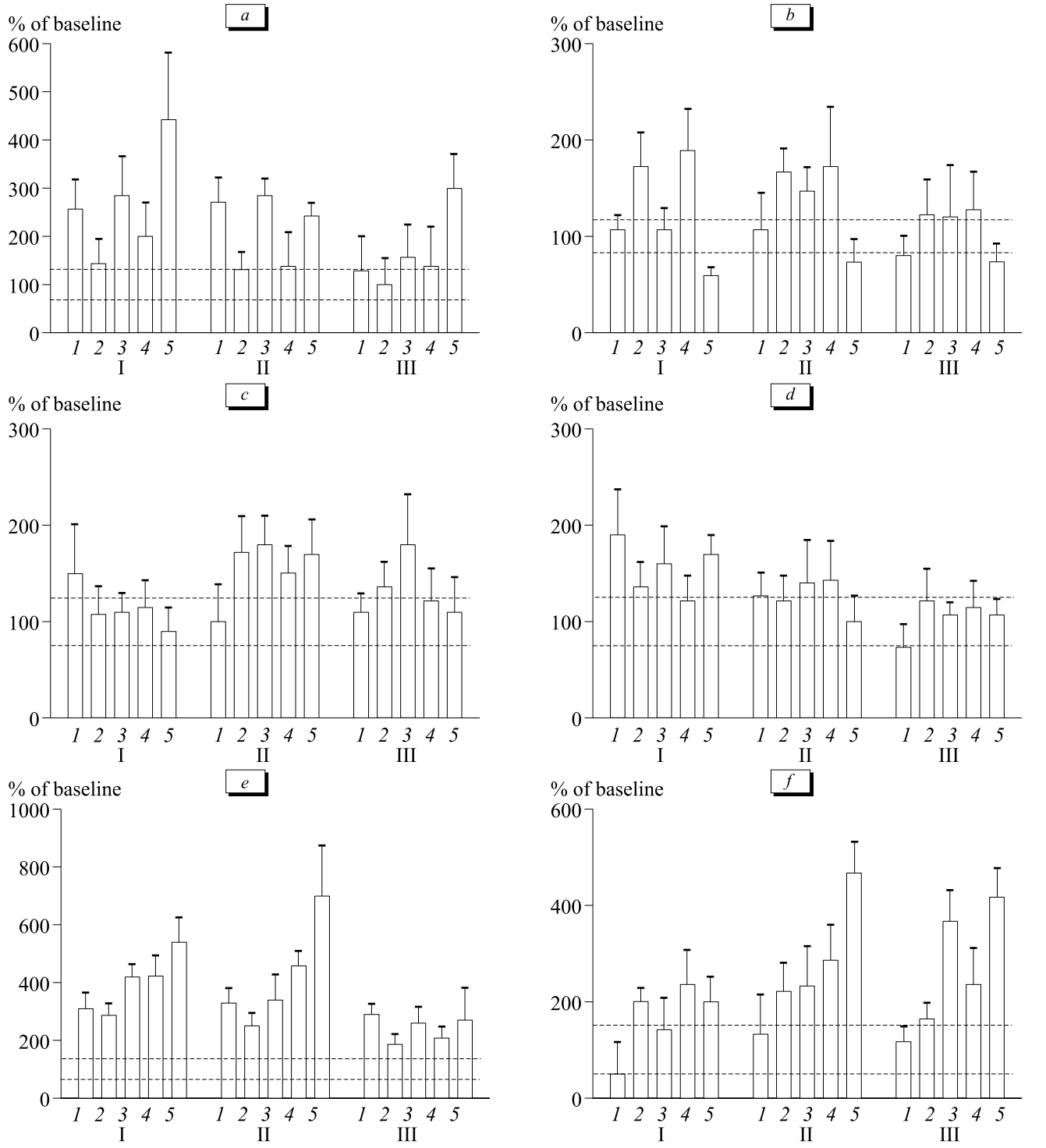

Fig. 2. Dynamics of colony-stimulating (*a, c, e*) and erythropoietic (*b, d, f*) activities in conditioned media from adherent (*a, b*) and non-adherent (*c, d*) myelokaryocytes and blood serum (*e, f*) from CBA/CaLac mice , number of S-phase precursors , and intensity of maturation after treatment with G-CSF (*1*), hyaluronidase in a dose of 20 arb. units per mouse (*2*), G-CSF and hyaluronidase in a dose of 20 arb. units per mouse (*3*), hyaluronidase in a dose of 100 arb. units per mouse (*4*), G-CSF and hyaluronidase in a dose of 100 arb. units per mouse (*5*).

dase alone (which confirmed our previous results [6]) and in combination with G-CSF.

Increasing the dose of hyaluronidase (100 arb. units per mouse) considerably disturbed the reaction of the hemopoietic tissue typically observed after combined treatment with G-CSF and hyaluronidase in a dose of 20 arb. units per mouse. We observed a sharp decrease in the content of various

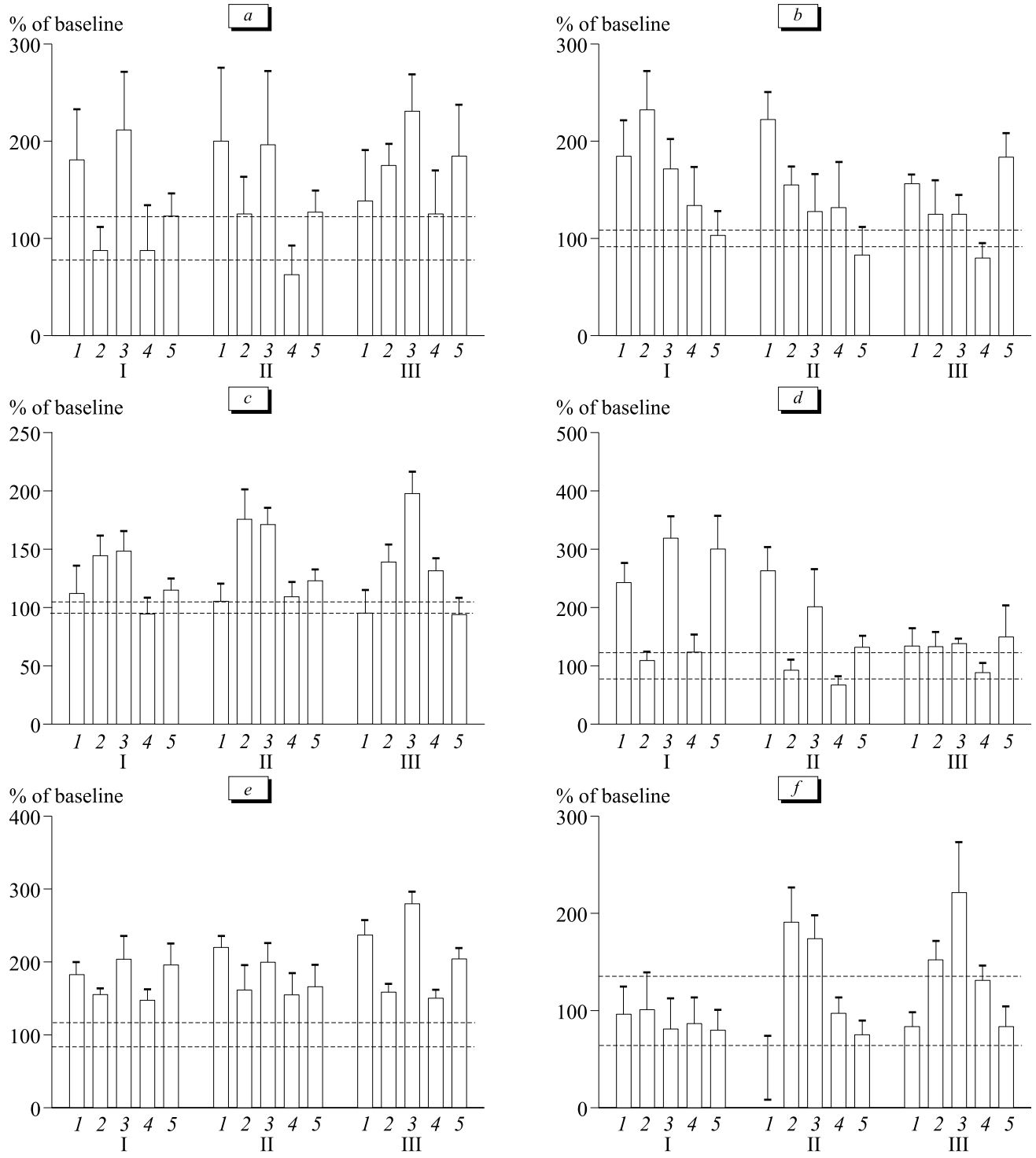

Fig. 3. Dynamics of the content of stab (*a*) and segmented (*b*) neutrophils, and reticulocytes (*c*) in the peripheral blood and immature (*d*), and mature (*e*) neutrophilic granulocytes and erythrokaryocytes (*f*) in the bone marrow of CBA/CaLac mice treated with G-CSF (*1*), hyaluronidase in a dose of 20 arb. units per mouse (*2*), G-CSF and hyaluronidase in a dose of 20 arb. units per mouse (*3*), hyaluronidase in a dose of 100 arb. units per mouse (*4*), G-CSF and hyaluronidase in a dose of 100 arb. units per mouse (*5*).

forms of neutrophils and reticulocytes in the peripheral blood (practically at all stages of the experiments). The number of neutrophils in the circulation was lower than after treatment with G-CSF clone. The content of immature and mature neutrophilic granulocytes and erythrokaryocytes in the bone marrow decreased (Fig. 3). In parallel, we observed a sharp decrease in functional activity of

committed precursors compared to mice receiving the combination of G-CSF and hyaluronidase in a dose of 20 arb. units per mouse (Fig. 1). Nevertheless, the content of G-CSF in the bone marrow and their proliferative activity in these animals considerably surpassed the corresponding parameters in animals receiving G-CSF alone (by 163.9% on day 8). Treatment with hyaluronidase (100 arb. units per mouse) decreased the intensity of CFU-GM maturation induced by the cytokine (by 115.7% on day 8; Fig. 1). The described ambiguous reactions of the pool of hemopoietic precursors developed against the background of enhanced secretion of colony-stimulating activity by primarily adherent myelokaryocytes (on days 3 and 8) and increased serum content of hemopoietins (Fig. 2).

These findings and published data [2,6,9] attest to an important role of hyaluronic acid and hyaluronidase in the regulation of hemopoiesis, in particular under conditions of G-CSSF treatment. The regularities the formation of hemopoietic shifts are largely determined by the degree of hydrolysis of hyaluronic acid and the proportion between its different molecular forms *in situ* [6,11,12]. Thus, our experiments demonstrated modification of specific properties of G-CSF and hyaluronidase under conditions of combined treatment with these agents. The combined treatment with the cytokine and different doses of hyaluronidase can produce various effects of the blood system.

REFERENCES

1. E. D. Goldberg, A. M. Dygai, and G. N. Zyuz'kov, *Hypoxia and Blood System* [in Russian], Tomsk (2006).
2. E. D. Goldberg, A. M. Dygai, G. N. Zyuz'kov, *et al*., *Kletochn. Tekhnol. Biol. Med*., No. 2, 115-119 (2007).
3. E. D. Goldberg, A. M. Dygai, G. N. Zyuz'kov, *et al*., *Byull. Eksp. Biol. Med*., **144**, No. 12, 652-656 (2007).
4. E. D. Goldberg, A. M. Dygai, and V. P. Shakhov, *Methods of Tissue Culture in Hematology* [in Russian], Tomsk (1992).
5. A. M. Dygai and N. A. Klimenko, *Inflammation and Hemopoiesis* [in Russian], Tomsk (1992).
6. G. N. Zyuz'kov, V. V. Zhdanov, A. M. Dygai, and E. D. Goldberg, *Byull. Eksp. Biol. Med*., **144**, No. 12, 690-695 (2007).
7. *Laboratory Methods in Clinical Practice. A Manual*, Ed. V. V. Men'shchikov [in Russian], Moscow (1987).
8. *Cytokine System: Theoretical and Clinical Aspects.* Eds. V. A. Kozlov and S. V. Sennikov [in Russian], Novosibirsk (2004).
9. A. Avigdor, P. Goichberg, S. Shivtiel*, et al., Blood*, **103**, No. 8, 2981-2989 (2004).
10. C. B. Henry and B. R. Duling, *Am. J. Physiol*., No. 277, 508-514 (1999).
11. P. W. Noble, *Matrix Biol*., No. 21, 25-29 (2002).
12. R. Stern, *Glycobiology*, **13**, No. 12, 105-115 (2003).