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Mechanisms of Mobilization of Mesenchymal Precursor Cell under the Effect of Granulocytic Colony-Stimulating Factor and Hyaluronidase

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Binding of mesenchymal precursor cells to intact extracellular matrix and stroma increased under the effect of hyaluronidase and decreased after treatment of adhesion substrates with the enzyme. The stimulation of the release of progenitor elements into the blood by granulocytic CSF depended on the interaction of mesenchymal precursors with the feeder stimulating bone marrow microenvironment. Additional *in vivo* treatment with hyaluronidase potentiated mobilization of precursor cells by granulocytic CSF against the background of increased affinity of precursor to the stroma.

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

The data on functional capacities of mesenchymal stem cells (MSC) gave rise to a new trend based on cell therapy in the treatment of various pathologies. Pharmacological modification of functions of endogenous stem cells is the most physiological and rational method for solving the problems of regeneration medicine. This method is based on imitation of natural regulatory systems of the organism [2,3]. In was found that granulocytic CSF (G-CSF) can mobilize MSC and that hyaluronidase can potentiate this process [3]. However, the mechanisms of recruitment of precursor cells into the blood are little studied. It is assumed that tissue microenvironment (cell elements and components of extracellular matrix) is actively involved into the realization of the mobilization phenomenon and that this phenomenon depends on functional state of precursors [1,2,6].

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Here we studied the effect of G-CSF, hyaluronidase, and their combination on the capacity of mesenchymal precursors to bind with stroma of the hemopoietic tissue and its extracellular matrix.

MATERIALS AND METHODS

The experiments were carried out on 2-month-old male and female CBA/CaLac mice (n=48) 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 G-CSF (Neupogen, Hoffmann—La Roche) dissolved in 0.2 ml RPMI-1640 medium (subcutaneous injections, 125 μg/kg/day for 3 days), hyaluronidase (lidase, Microgen Company) dissolved in 0.2 ml physiological saline (intraperitoneal injections 20 U/mouse/day for 2 days), or both preparations simultaneously according to the above schemes. Cell material for the study was collected on day 3 after the start of treatment (per-

iod of maximum increase in MSC number in the peripheral blood [3]).

The content of fibroblast CFU (CFU-F) in the peripheral blood of experimental animals was evaluated by the method of *in vitro* cloning [4] and the content of MSC was determined by the method of limiting dilutions [6,7]. The capacity of precursor cells to bind the stroma and extracellular matrix was studied under *in vitro* conditions. For obtaining adequate model of the stroma, the whole bone marrow from intact mice (2 mln cells in 1 ml) was cultured for 3 weeks, the resultant monolayer was irradiated in a dose of 20 Gy (Rokus-M γ-apparatus, 1.25 MeV energy), which caused death of actively proliferating elements [5]. For modeling of the extracellular matrix, cell death in 3-week bone marrow culture was induced by concentrated DMSO [9]. Moreover, feeders formed in each case and treated with hyaluronidase (lidase, Microgen Company, 1 U/ml complete nutrient medium) for 24 h were used as additional substrate variants. Then, 500,000 mln unfractionated myelokaryocytes from intact and experimental animals were layered onto the substrates and incubated for 90 min. The medium with nonadherent cells was collected and tested in a test-culture according to the standard method of CFU-F cloning [4]. Initial content of CFU-F in the examined material served as the control. Affinity of precursor cells to various substrates was determined by the difference between the control value and the number of CFU-F not bound to the substrate after incubation.

The data were processed using Student's *t* test and nonparametric Mann—Whitney *U* test. The incidence of MSC in the bone marrow and peripheral blood was evaluated using generalized linear model for Poisson distribution. The correspondence of limiting dilutions to unidimensional Poisson model was evaluated by linear log-log regression. The distribution of theoretic fraction of negative wells μ_i was described by an equation: $\mu_i=\exp(-fx_i)$, where f is the incidence of MSC and x_i is the number of cells seeded to the well [7,8]. Statistica 6.0 software was used.

RESULTS

Treatment with G-CSF increased the number of early progenitor elements and their committed precursors (CFU-F) in the peripheral blood. No mobilization phenomenon was observed in animals receiving hyaluronidase. At the same time, combined administration of preparations led to maximum increase in the pool of circulating MSC and CFU-F. The initial number of committed mesenchymal precursor cells and MSC in the bone marrow was minimum in the group receiving G-CFU, which was probably related to mobilization of progenitor elements into the peripheral blood. The maximum content of CFU-F was found in cell material from animals receiving G-CSF and hyaluronidase simultaneously (Fig. 1). On the whole, the results agree with our previous findings [3].

Evaluation of the capacity of precursors and microenvironment to cooperation revealed differences in the affinity of precursor cells from intact bone marrow to different substrates. The number of bound CFU-F was maximum (85.3%) after incubation on irradiated feeder from 3-week culture and somewhat lower (70.7% from the initial level) after incubation on extracellular matrix. At the same time, enzymatic cleavage of hyaluronic acid, a component of substrates, considerably decreased adhesion of precursors to the stroma and simulated extracellular matrix to 77.1 and 41.3%, respectively (Fig. 2, *a*). Thus, maximum affinity of CFU-F from intact bone marrow to microenvironment requires the presence of stromal cell elements and preserved properties of extracellular matrix of the hemopoietic tissue.

Injection of G-CSF *in vivo* increased the capacity of CFU-F to bind with the sublayer simulating extracellular matrix and weakened their interaction with the stroma on the whole. The numbers of progenitor cells adherent to the matrix and substrate containing viable cell elements were 83.8 and 56.8% of the control, respectively (Fig. 2, *b*). These changes probably resulted from changed adhesion capacity of CFU under the effect of G-CSF. The dynamics of changes in CFU-F binding to the substrates after hyaluronidase treatment did not considerably differ from that in case of intact myelokaryocytes.

Hyaluronidase treatment in contrast to G-CSF increased affinity of CFU-F to not only extracellular matrix, but also the sublayer simulating the bone marrow stroma. In the latter case, the number of adherent precursors was maximum (91.3% of the initial level). Enzyme treatment of the substrate simulating extracellular matrix less markedly modulated adhesion capacity of CFU-F (Fig. 2, *c*). In light of this, it seems logical that systemic administration of the enzyme alone is not accompanied by the release of precursor cells into the peripheral blood.

At the next stage we evaluated the role of the studied mechanisms of mesenchymal precursor recruitment into the peripheral blood after combined application of G-CSF exhibiting independent mobilizing activity and hyaluronidase potentiating this effect of the cytokine [2,3,6]. It was found that G-CSF increased the dependence of CFU-F affinity to

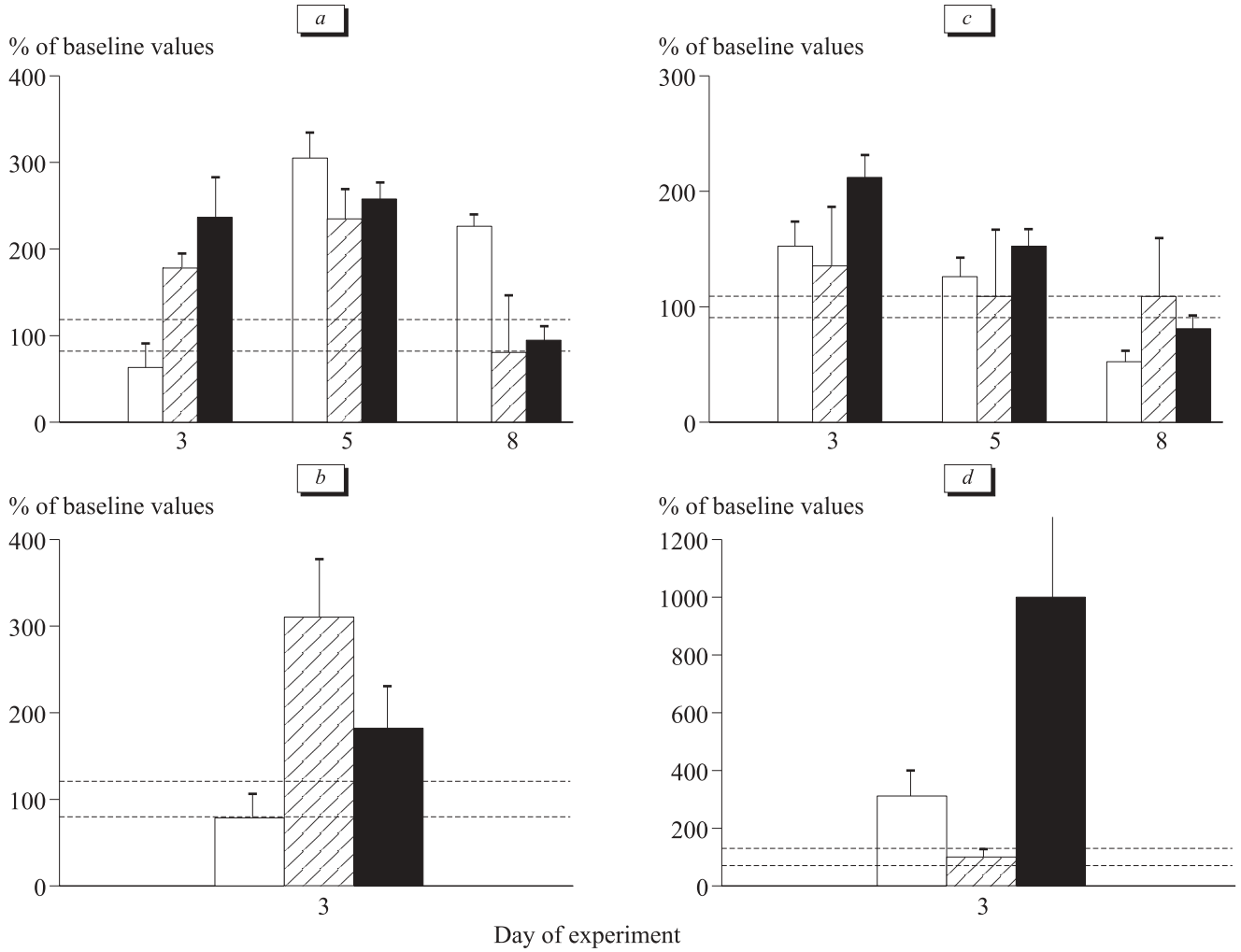

Fig. 1. Content of CFU-F (*a, c*) and MSC (*b, d*) in the bone marrow (*a, b*) and peripheral blood (*c, d*) in CBA/CaLac mice receiving G-CSF (open bars), hyaluronidase (hatched bars), and G-CSF with hyaluronidase (dark bars); area between dotted lines shows confidence interval for the test parameter in intact mice at $p<0.05$.

extracellular matrix on the state of hyaluronic acid in it compared to *in vivo* treatment with hyaluronidase alone. Enzyme treatment of this substrate more markedly disturbed its interaction with CFU-F. At the same time, hyaluronidase injected *in vivo* together with G-CSF diminished disturbances in the cooperation of mesenchymal precursors with the stroma caused by G-CSF. The number of CFU-F adherent to irradiated culture of intact myelokaryocytes increased compared to the corresponding parameter in the bone marrow culture from mice treated with G-CSF alone (Fig. 2, *d*). This fact attests to the involvement of other mechanisms (the most plausible of these is the influence of the enzyme on the microenvironment) into potentiation of mobilization of stem cells, which finally overrides the effect of the enzyme enhancing adhesion capacity of precursor cells [1,6]. It should be emphasized that these changes in the function of progenitor elements under the effect of hyaluronidase can be useful during cell therapy with material obtained from the peripheral blood after combined treatment with the enzyme and G-CSF. Increased adhesion capacity of stem cells is probably associated with their increased engrafting in tissues, *e.g.* in damaged organs in various pathologies.

Our findings attest to important role of hyaluronic acid as a component of extracellular matrix [10] in cooperation of progenitor cells with the microenvironment. Cleavage of this polymer in the feeder simulating the bone marrow stroma weakens their interaction. At the same time, our experiments showed that G-CSF reduces affinity of mesenchymal precursor cells to the stroma against the background of stimulation of their interaction with extracellular matrix. Our findings and published data [1,6] suggest that the effects of the studied preparations consisting in modification of functions of progenitor

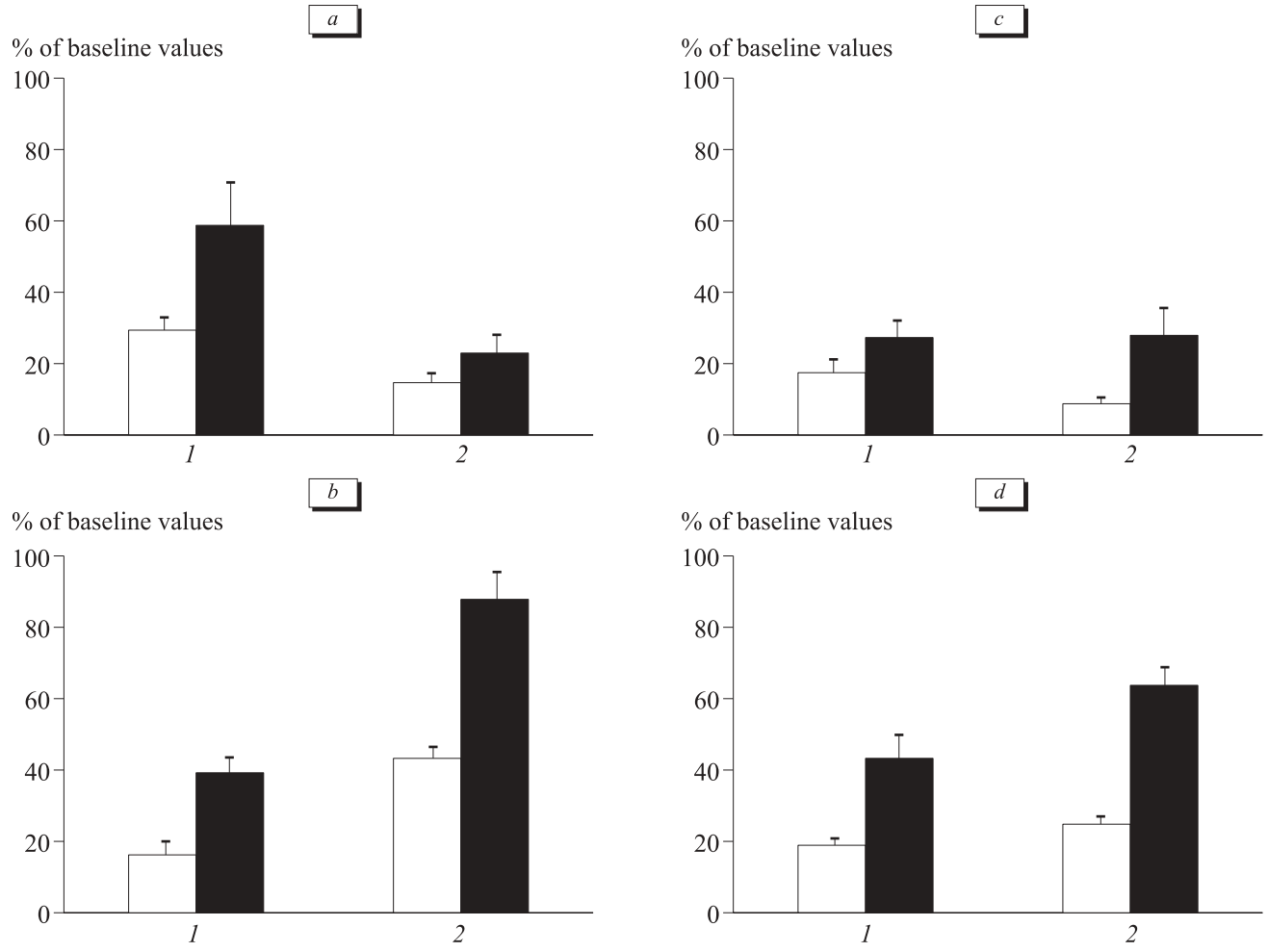

Fig. 2. Percent of bone marrow CFU-F from intact mice (*a*) and mice receiving G-CSF (*b*), hyaluronidase (*c*), and G-CSF+hyaluronidase (*d*) not binding to the substrate. Light bars: intact substrate, dark bars: substrate treated with hyaluronidase. *1*) extracellular matrix, *2*) stroma. Confidence intervals at $p<0.05$.

cells and elements of the microenvironment play an important role in the phenomenon of mobilization of stem cells.

REFERENCES

1. E. B. Vladimirskaya, *Klin. Lab. Diagn*., No. 4, 26-32 (2006).
2. E. D. Goldberg, A. M. Dygai, and G. N. Zyuz'kov, *Hypoxia and Blood System* [in Russian], Tomsk (2006).
3. E. D. Goldberg, A. M. Dygai, G. N. Zyuz'kov, *et al*., *Kletoch. Tekhnol. Biol. Med*., No. 2, 115-119 (2007).
4. E. D. Goldberg, A. M. Dygai, and V. P. Shakhov, *Methods of Tissue Culture in Hematology* [in Russian], Tomsk (1992).
5. E. I. Deryugina, N. I. Drize, and I. L. Chertkov, *Byull. Eksp. Biol. Med*., **104**, No. 7, 96-99 (1987).
6. *Cytokine System. Theoretical and Clinical Aspects*, Eds. V. A. Kozlov and S. V. Sennikov [in Russian], Novosibirsk (2004).
7. *T. Bonnefoix, P. Bonnefoix, M. Callanan, et al.*, *J. Immunol*., **167**, No. 10, 5725-5730 (2001).
8. P. S. in't Anker, W. A. Noort, S. A. Scherjon, *et al., Haematologica*, **88**, No. 8, 845-852 (2003).
9. T. Mosmann, *J. Immunol. Methods*, **65**, Nos. 1-2, 55-63 (1983).
10. R. Stern, *Glycobiology*, **13**, No. 12, 105R-115R (2003).