Mobilization of Progenitor Cells into the Blood by Immobilized Granulocytic Colony-Stimulating Factor

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The effects of granulocytic CSF immobilized on polyethylenoxide by nanotechnology on the bone marrow and circulating pools of mesenchymal and hemopoietic precursors were studied. Immobilized granulocytic CSF caused the release of progenitor cells of different classes into the blood. The effect of injected immobilized granulocytic CSF was superior to that of nonconjugated granulocytic CSF. Specific activity of oral immobilized granulocytic CSF after oral administration was demonstrated.

Key Words: *progenitor cells; mobilization; immobilized granulocytic colony-simulating factor; nanotechnologies*

Recombinant granulocytic CSF (G-CSF) is widely used in practical medicine. The most frequent indications for its use are diseases associated with hemopoiesis disorders [10,11]. In addition, G-CSF is an effective modifier of functions of endogenous stem cells, which determines its high therapeutic activity in some prevalent diseases, which was previously detected in experimental studies [1,5-7]. Clinical use of standard G-CSF preparations is often limited because of its toxicity, immunogenic activity, *etc.* [9,16]. Hence, the development and creation of new drugs or pharmacological compositions with specific activity towards progenitor cells of different classes seems to be an important problem.

We studied the capacity of G-CSF immobilized (IM) by nanotechnology of radiation synthesis on low-molecular-weight polyethylenoxide to induce the release of bone marrow mesenchymal and hemopoietic precursor cells into the blood.

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MATERIALS AND METHODS

Experiments were carried out on 2-month-old CBA/CaLac mice (n=250). Certified first-category animals were obtained from Breeding Center of Institute of Pharmacology. Standard (nonconjugated) nonglycosylated G-CSF (filgrastim analog, Siberian Center of Pharmacology and Biotechnologies) was injected subcutaneously (100 μg/kg) for 5 days. Immobilized G-CSF was also injected subcutaneously (100 μg/kg) for 5 days and administered orally for 10 days. Controls received saline in an equivalent volume (0.2 ml) according to the same protocols.

Immobilized G-CSF is a result of collaborative research carried out at Siberian Center of Pharmacology and Biotechnologies with Institute of Pharmacology, G. I. Budker Institute of Nuclear Physics, Siberian Division of the Russian Academy of Sciences, and Institute of Cytology and Genetics,

Siberian Division of the Russian Academy of Sciences. Molecules of nonglycosylated G-CSF (Siberian Center of Pharmacology and Biotechnologies) were immobilized on low-molecular-weight polyethylenoxide by nanotechnology of radiation synthesis using directed beam of accelerated electrons [15].

The content of granulomonocytic (GM), erythroid (E), and fibroblast (F) CFU in the bone marrow and peripheral blood was evaluated on days 2, 3, 4, 5, 7, and 10 of the experiment by cloning in semisolid culture medium [4]. The counts of MSC in the bone marrow and peripheral blood were evaluated by the method OF limiting dilution on day 3 of THE experiment [12].

The results were statistically processed by methods of variation statistical using Student's *t* test and nonparametric Mann—Whitney *U* test. The incidence of MSC in the bone marrow and peripheral blood was evaluated by the generalized linear model for Poisson's distribution [12].

RESULTS

In controls (a course of saline), the levels of CFU-GM (day 10), CFU-E (days 7 and 10), and CFU-F (day 7) slightly increased in the bone marrow and remained virtually unchanged in the peripheral blood (the only exception was the increase in blood content of CFU-F on day 5; Figs. 1, 2). These reactions presumably resulted from the neurotizing effect of repeated procedures (drug administration via a tube) and were caused by activation of the stress-realizing systems [1,3].

The agents modified significantly the pool of progenitor cells. Treatment with G-CSF (active substance of the studied preparations) led to an increase in the content of granulomonocytic precursors in hemopoietic tissue in all cases. Nonconjugated G-CSF and intragastric immobilized cytokine led to an increase in CFU-GM levels on days 3, 5, 7, and 10 of the study. On the other hand, subcutaneous injection of IMG-CSF led to a longer

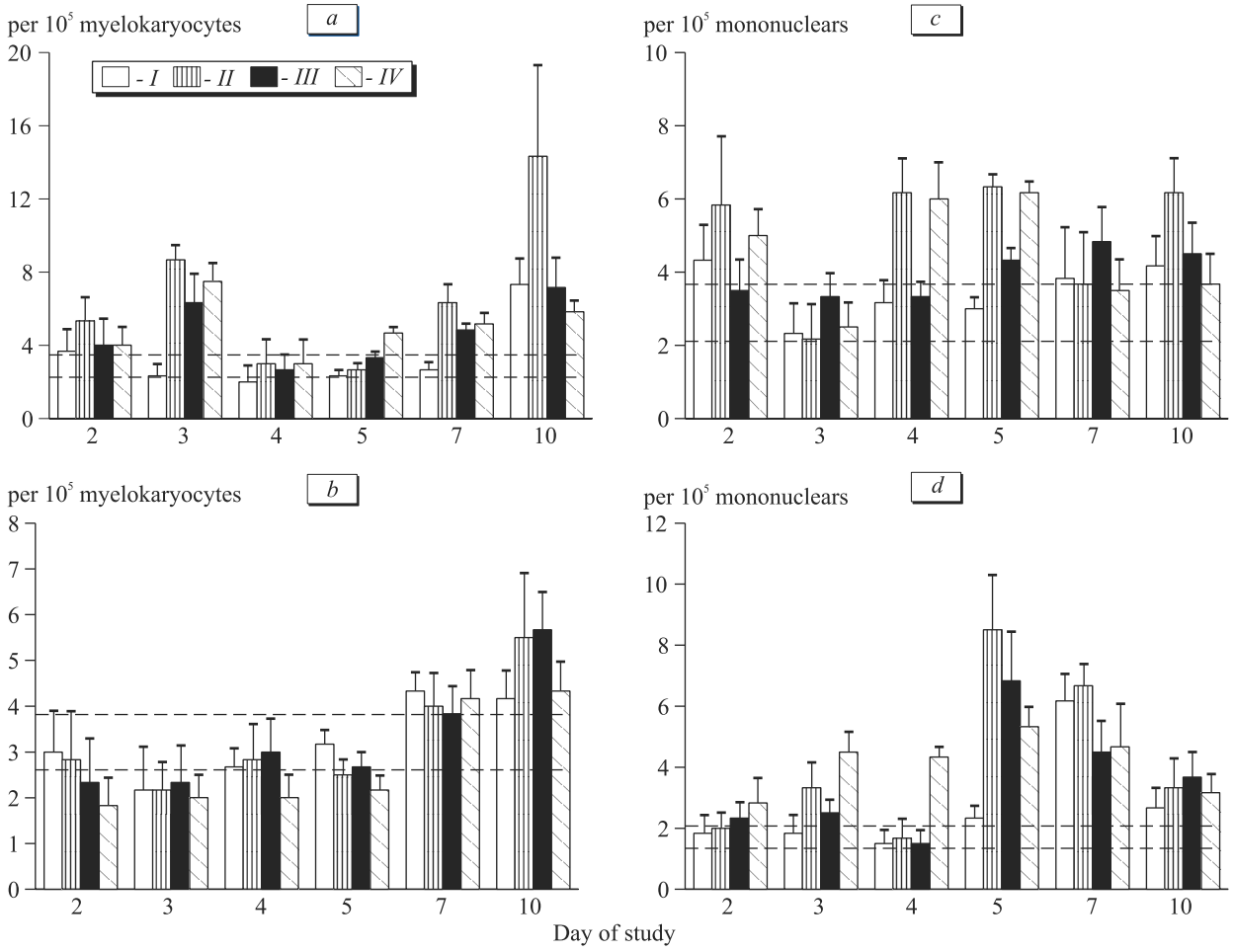

Fig. 1. Content of CFU-GM (*a*, *b*) and CFU-E (*c*, *d*) in the bone marrow (*a*, *c*) and peripheral blood (*b*, *d*) of CBA/CaLac mice receiving saline (*I*), subcutaneous IMG-CSF (*II*), oral IMG-CSF (*III*), and subcutaneous nonconjugated G-CSF (*IV*). Here and in Fig. 2: confidence intervals at $p<0.05$. Area between intermittent lines: confidence interval for the parameter in intact mice at $p<0.05$.

(days 3, 7, 10) and maximally pronounced (on day 3 up to 372.1% compared to controls receiving saline) increment in the bone marrow count of hemopoietic cells (Fig. 1).

On the other hand, mobilization of CFU-GM developed in all experimental groups. The increase in the peripheral blood count of these precursors in response to parenteral G-CSF preparations was noted as early as on day 2 of the experiment. However the increase in their levels, reaching the statistically significant difference in comparison with the control, was recorded on days 4, 5 and on days 4, 5, 10 after treatment with standard and conjugated G-CSF, respectively. On day 10 of the experiment, the blood level of CFU-GM was significantly higher after treatment with IMG-CSF than after nonconjugated G-CSF. In animals receiving oral IMG-CSF, the mobilization of CFU-GM developed later (starting from day 5) and was less pronounced (Fig. 1).

Other effects of G-CSF were observed towards the erythroid precursors. A pronounced reduction (of different degree) in their bone marrow levels was observed in all experimental groups, but only in comparison with the control (animals with sufficiently well-pronounced reaction of stress-realizing systems [1,3]). On the other hand, significant stimulation of CFU-E influx into the blood was observed. Initially (days 2, 3, 4) the highest levels of circulating CFU-E were observed in response to the standard G-CSF, while on day 5 the most intense reaction was induced by IMG-CSF (Fig. 1).

Our results are in complete agreement with published data indicating stimulation of hemopoietic precursors release into peripheral blood under the effect of G-CSF, in parallel with the increase in functional activity of CFU-GM [1,2,8,13].

Similar changes were observed in the CFU-F pool, containing committed stromal elements and MSC [7]. Nonconjugated and immobilized G-CSF, administered according to both protocols, increased CFU-F count in the hemopoietic tissue on days 3, 4, 7 and 4, 7, respectively. These changes in the bone marrow CFU-F functional activity were paralleled by their release into peripheral blood. This reaction was most manifest in animals subcuta-

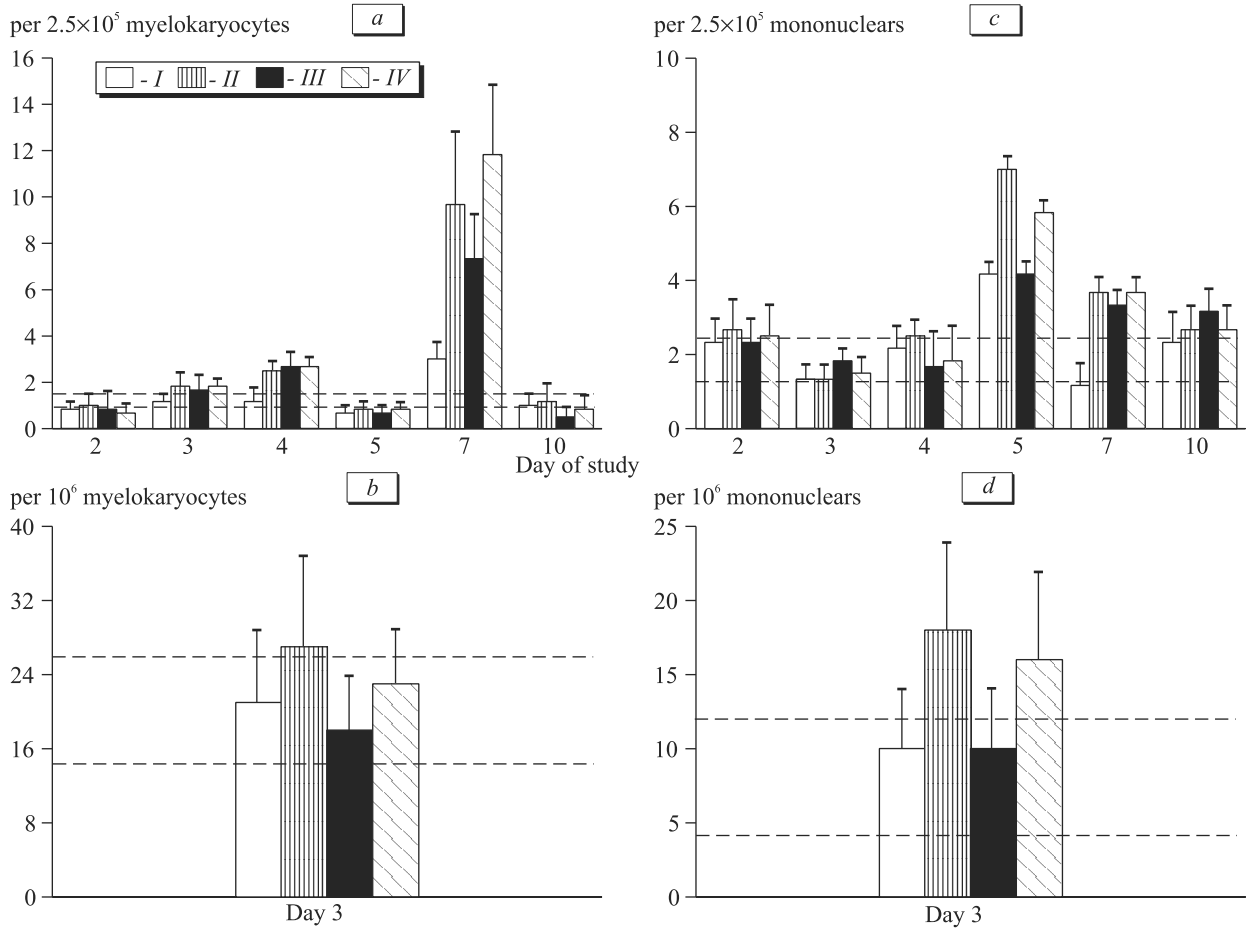

Fig. 2. Content of CFU-F (*a*, *c*) and MSC (*b*, *d*) in the bone marrow (*a*, *b*) and peripheral blood (*c*, *d*) of CBA/CaLac mice receiving saline (*I*), subcutaneous IMG-CSF (*II*), oral IMG-CSF (*III*), and subcutaneous nonconjugated G-CSF (*IV*).

neously injected with IMG-CSF and the least so in response to intragastric immobilized cytokine (Fig. 2).

Study of the content of the true progenitor elements (MSC) in the bone marrow confirmed the data on their poor reaction to humoral factors [1,3]. Treatment with these preparations virtually did not modify the quantitative characteristics of bone marrow MSC population. On the other hand, parenteral treatment with various G-CSF forms led to MSC mobilization into peripheral blood, which was more pronounced (similarly as in all previous cases) in response to the cytokine fixed to a low-molecular-weight polymer molecule and was presumably realized through activation of the microenvironment elements [1,2] (Fig. 2).

Hence, G-CSF immobilized on polyethylenoxide induced the release of progenitor cells of different classes into the blood and an increase in the populations of bone marrow granulomonocytic and stromal precursors. The effect of its parenteral administration was by many parameters (primarily length of effect) superior to the analogous characteristics of nonconjugated G-CSF. These results are in line with published data on modification of the G-CSF modifying effect on stem cells in its pegylation [8,13,14]. On the other hand, we should like to emphasize the possibility of oral use of G-CSF-based preparation, obtained by the radiation synthesis nanotechnology (IMG-CSF), for stimulation of the functional activity and mobilization of progenitor elements of different classes into the blood.

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