Mechanisms of Granulocytopoiesis-Stimulating Activity of Immobilized Granulocytic Colony-Stimulating Factor in Cytostatic Myelosuppression

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The hemostimulatory effects of granulocytic CSF, immobilized on polyethyleneglycol using radiation synthesis nanotechnology, were studied on the model of cyclophosphamide-induced myelosuppression. Immobilization of granulocytic CSF led to stimulation of granulomonocytopoiesis by increasing functional activity of granulomonocytic precursors and secretion of humoral factors by elements of hemopoiesis-inducing microenvironment, and due to more intensive formation of hemopoietic islets. The granulocytopoiesis-stimulating effect of immobilized granulocytic CSF was comparable to the effect of standard nonconjugated granulocytic CSF. Specific activity of immobilized granulocytic CSF in oral treatment was demonstrated.

Key Words: *granulocytopoiesis; immobilized granulocytic colony-stimulating factor; nanotechnologies; cytostatic myelosuppression*

Chemotherapy involves the use of drugs improving tolerance of cytostatics and supporting patient's quality of life. One of the drugs most often used in clinical practice for correction of disorders in the blood system is granulocytic CSF (G-CSF) [1,8]. This drug is characterized by pronounced granulocytopoiesis-stimulating activity, but its use is limited because of high incidence of side effects and complications caused by its toxicity and immunogeneity [8,14]. On the other hand, the characteristics of bioactive substances can be modified by their conjugation with polyethylenglycol. This improves their bioavailability in oral treatment, increases physical stability and solubilization of these preparation, and reduces immunogenic activity and sensitivity to proteolytic enzymes [9,12].

We compared hemostimulatory activity of G-CSF immobilized on polyethylenglycol by radiation synthesis nanotechnology and that of standard recombinant G-CSF on the model of cyclophosphamide-induced myelosuppression.

MATERIALS AND METHODS

Experiments were carried out on 2-month-old male CBA/CaLac mice (n=160; 18-20 g; first-category animals from Breeding Center of Institute of Pharmacology).

Cyclophosphamide (Cyclophosphane-Lance, Veropharm) was injected intraperitoneally in a single MTD (250 mg/kg, maximum tolerated dose). Immobilized G-CSF (IG-CSF; Siberian Center of Pharmacol-

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ogy and Biotechnologies) in a daily dose of 100 μg/kg was applied orally on days 1-10 and subcutaneously on days 1-5 of the study. Nonconjugated recombinant nonglycosylated G-CSF (filgrastim analog; Siberian Center of Pharmacology and Biotechnologies) served as the reference drug and was injected subcutaneously in a daily dose of 100 μg/kg on days 1-5. Controls received saline in an equivalent volume according to the same protocols.

The IG-CSF preparation was created at Siberian Center of Pharmacology and Biotechnologies, Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences, 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. The molecules of G-CSF (Siberian Center of Pharmacology and Biotechnologies) were immobilized on low-molecular polyethylenglycol by the radiation synthesis nanotechnology using directed flow of accelerated electrons [13].

Parameters of peripheral blood and bone marrow hemopoiesis were studied by standard hematological methods on days 1-3, 5, 7, 10, and 13 of the experiment [6]. Bone marrow content of granulocytomacrophagal (CFU-GM) and stromal (CFU-F) precursors, proliferative activity and intensity of CFU-GM differentiation, production of colony-stimulating activity by individual fractions of the hemopoiesis-inducing microenvironment, and structure and functional organization of the bone marrow were studied [5].

The results were processed by variation statistics methods using the Student *t* test and nonparametric Mann—Whitney *U* test.

RESULTS

Cyclophosphamide injection decreased the levels of immature (days 2, 3) and mature (days 2, 3, 5, 7, 13) forms of neutrophilic granulocytes in the bone marrow and segmented neutrophils in the peripheral blood (days 2, 3; Figs. 1, 2). These changes developed in the presence of significant disorders in the structure and functional organization of the hemopoietic tissue, which was seen from low content of macrophage-negative and granulocytic hemopoietic islets (days 1-4; Fig. 2). On the other hand, functional activity of granulomonocytic precursors increased significantly. Bone marrow content of CFU-GM and the rate of their division increased (day 5), their maturation was more rapid (days 5-13; Fig. 3).

Taking into account the important role of cells of the hemopoiesis-inducing microenvironment in hemopoiesis regulation under extreme conditions [3,4], we studied the content of mesenchymal precursors in hemopoietic tissue and secretory function of the bone marrow nuclears. Experiment revealed a significant increase in the content of CFU-F on days 2-5 (with the maximum of up to 417.6% of basal level on day 3), followed by its reduction on days 10, 13, and a higher production of colony-stimulating activity by adherent myelokaryocytes on day 3. These data indicate the development of pronounced compensatory reaction of the microenvironmental stromal elements, including progenitor cells promoting rapid regeneration of the hemopoietic tissue after treatment with alkylating agents [3].

G-CSF preparations stimulated (as expected) the hemopoiesis granulomonocytic stem [7,8,10]. A significant increase in the count of neutrophilic granulocytes

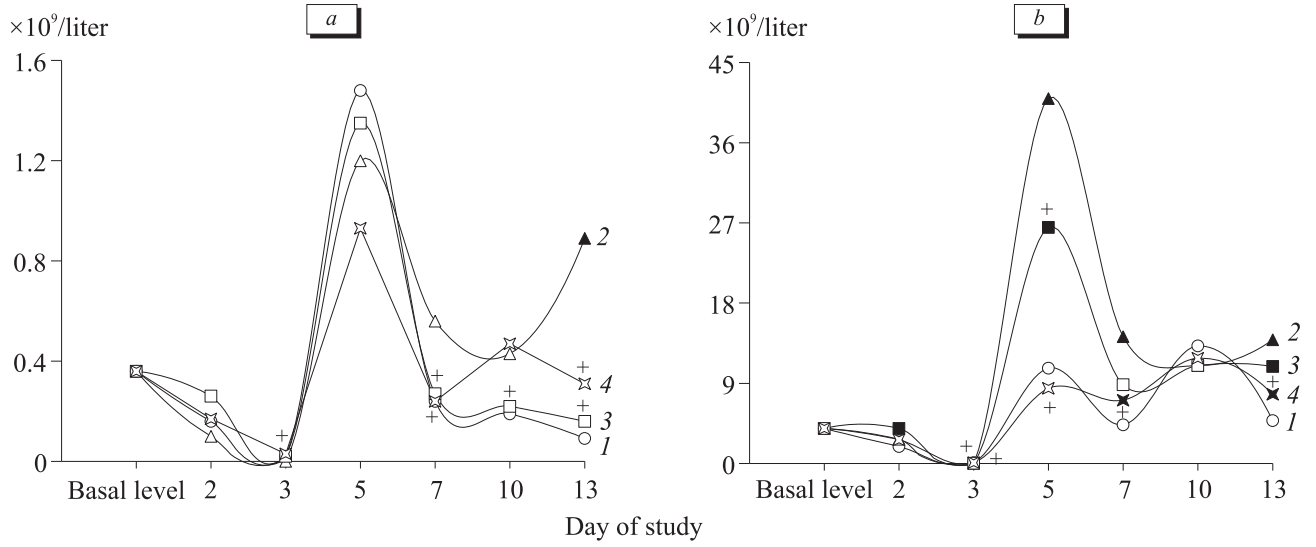

Fig. 1. Counts of stab (*a*) and segmented (*b*) neutrophils in peripheral blood of CBA/CaLac mice after injections of cyclophosphamide (*1*), standard G-CSF (*2*), and IG-CSF subcutaneously (*3*) and orally (*4*). Here and in Figs. 2, 3: dark markers: $p<0.05$ compared to cytostatic control, $^{+}p<0.05$ compared to standard G-CSF preparation.

in hemopoietic tissue was observed in all experimental groups (Fig. 2). The most pronounced changes were observed for the content of immature neutrophilic granulocytes after parenteral treatment with nonconjugated G-CSF and G-CSF pegylated by nanotechnology (with maximum values of up to 238 and 229% of basal level, respectively, on day 7 of the experiment). This was paralleled by a relevant increase in the counts of segmented neutrophils in the peripheral blood, also more pronounced after subcutaneous injections with G-CSF preparations (Fig. 1).

The study of the effects of the preparations on the pool of granulomonocytic precursors revealed similar reactions in all cases. The increase in the content of CFU-GM in the hemopoietic tissue was associated with an increase of their proliferative activity (Fig. 3). On the other hand, more intensive maturation of granulomonocytic precursors was noted on days 5, 7, 13 and on day 15 of the experiment after treatment with standard and immobilized G-CSF, respectively. The least shifts were recorded after enteral treatment with IG-CSF.

Study of the mechanisms of hemostimulatory activity of the preparations modifying the hemopoiesis-inducing microenvironment elements showed that the capacity of stromal cells to form hemopoietic islets increased to a different degree under the effects of nonconjugated and immobilized G-CSF (Fig. 2). Moreover, IG-CSF significantly increased the production of humoral regulators of granulocytopoiesis by adherent myelokaryocytes, reaching 2121.2 and 1112.1% of the basal level on day 2 of the experiment after oral and subcutaneous treatment, respectively.

These changes in feeder activity of the bone marrow adherent fraction were recorded in the presence of a significant increase in the content of mesenchymal precursors in hemopoietic tissue. The increase in the

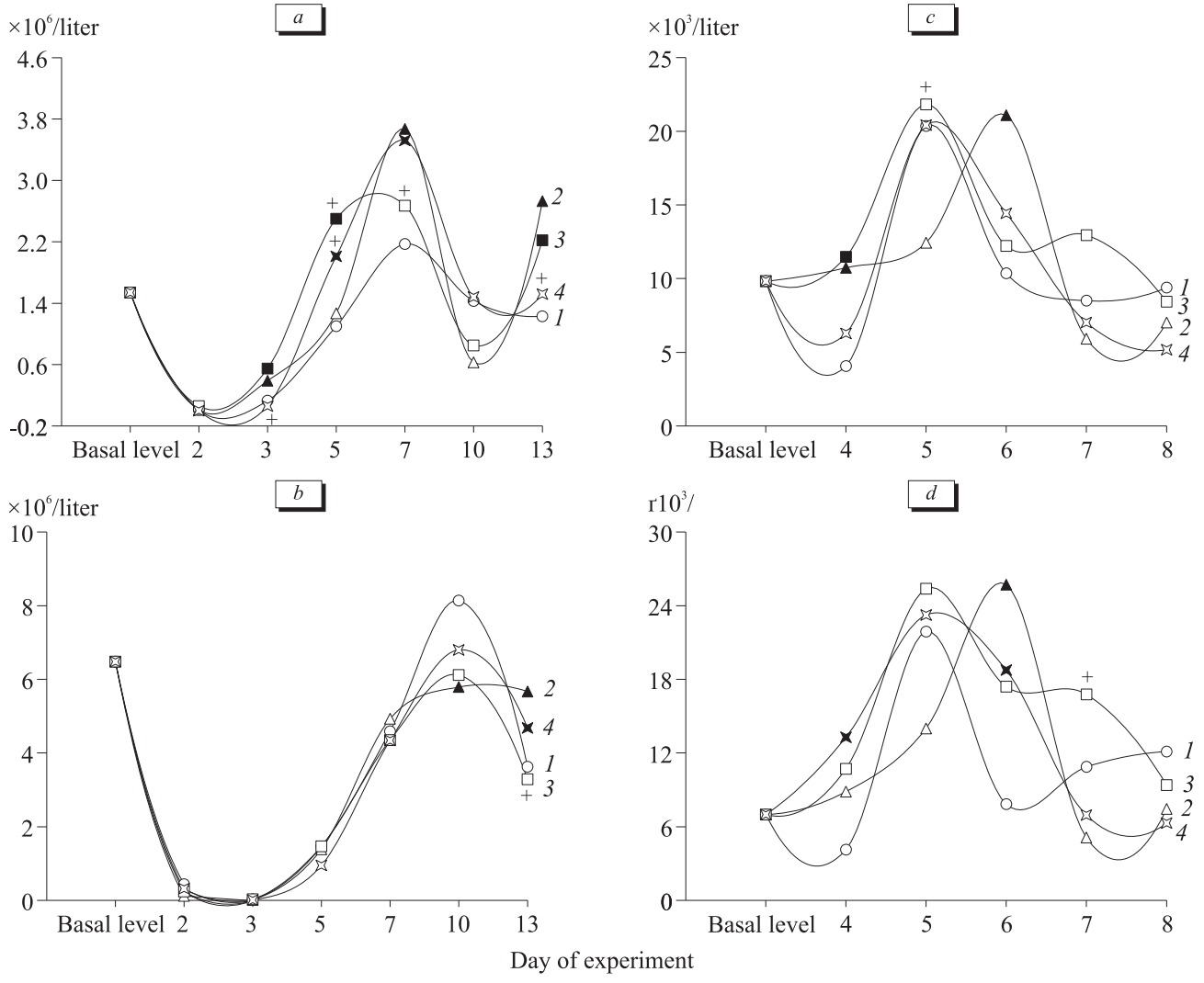

Fig. 2. Bone marrow content of immature (*a*), mature (*b*) neutrophilic granulocytes, macrophage-negative (*c*), granulocytic (*d*) hemopoietic islets in CBA/CaLac mice after injections of cyclophosphamide (*1*), standard G-CSF (*2*), IG-CSF subcutaneously (*3*) and orally (*4*).

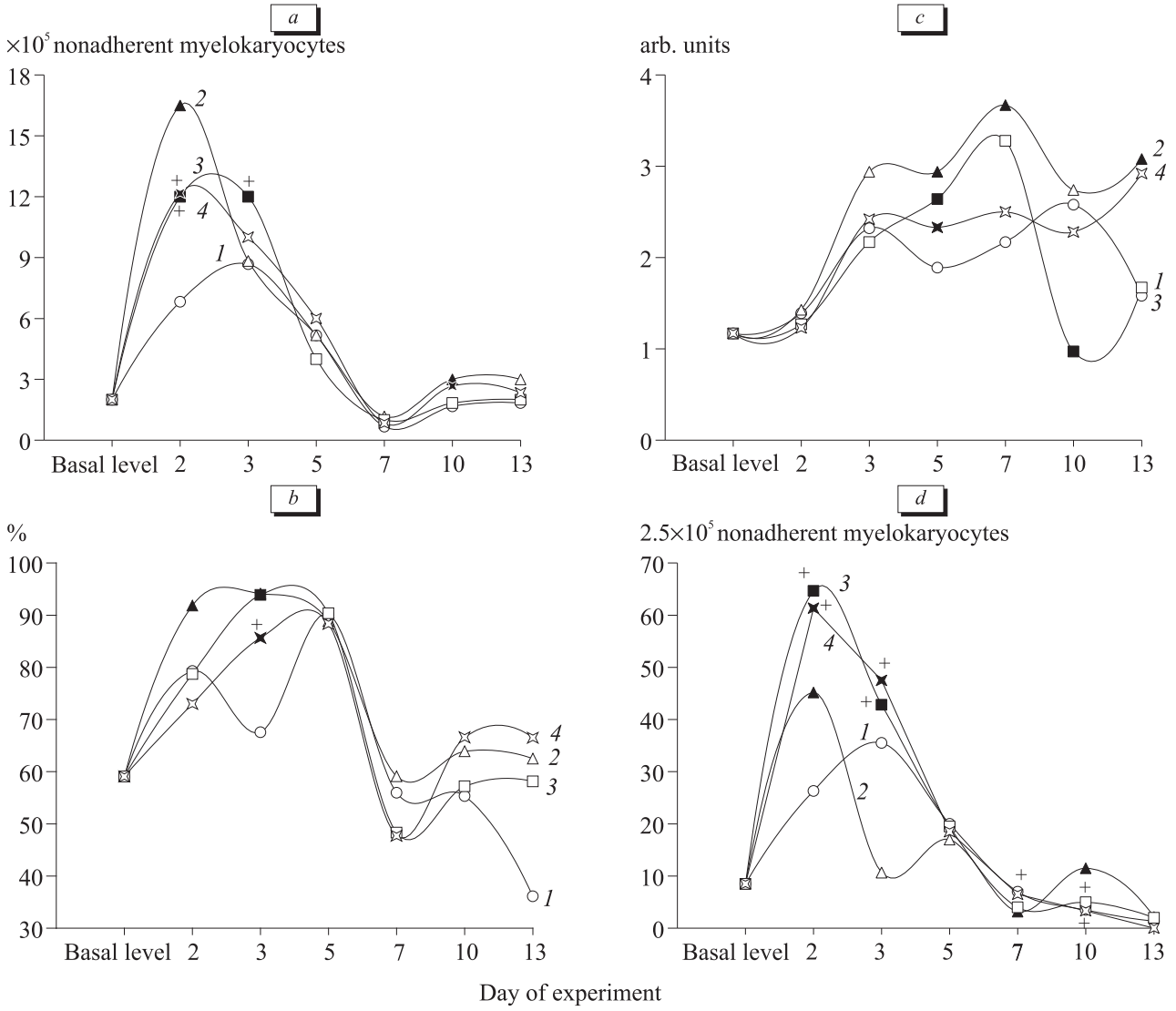

Fig. 3. Content of CFU-GM in the bone marrow (*a*), CFU-GM in the cell cycle S phase (*b*), intensity of granulomonocytic precursor maturation (*c*), and content of CFU-F in the bone marrow (*d*) of CBA/CaLac mice injected subcutaneously with cyclophosphamide (*1*), standard G-CSF (*2*), IG-CSF subcutaneously (*3*), and after oral IG-CSF (*4*).

content of CFU-F in hemopoietic tissue was observed on days 2 and 3 after treatment with IG-CSF, while after treatment with standard preparation the count of stromal precursors was elevated only on day 2 of the experiment. Mesenchymal stem cells with high proliferative and differentiation potential are present among CFU-F [2,3], and hence, it is interesting to investigate the potentialities of IG-CSF as a modifier of the stem cell functions in regenerative medicine.

On the whole, these data indicate the granulocytopoiesis-stimulating effect of G-CSF immobilized by the radiation synthesis nanotechnology. This effect is comparable to that of standard recombinant cytokine. The mechanism of granulomonocytopoiesis stimulation by modified G-CSF consists in stimulation of functional activity of CFU-GM due to direct effect of the cytokine on committed precursor cells (which is characteristic of nonconjugated G-CSF [1,10]) and to stimulation of the secretory function of the stromal elements of hemopoiesis-induced microenvironment. The possibility of enteral use of G-CSF-based preparation is worthy of note.

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