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Effect of Granulocyte Colony-Stimulating Factor on Kinetics of Hemopoietic Precursors in Regenerating Bone Marrow

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We studied processes of proliferation and differentiation of hemopoietic precursor cells in mice treated with granulocyte CSF under conditions of cytostatic myelodepression. It was found that the hemostimulating effect of original granulocyte CSF preparation consists in accelerated maturation of hemopoietic precursors and increased content of DNA-synthesizing CFU-GM in the bone marrow.

Key Words: *granulocyte colony-stimulating factor; cyclophosphamide; granulomonocytopoiesis; differentiation*

It is well known that actively proliferating cell systems are highly sensitive to the toxic effects of cytostatics. Among normal tissues this primarily concerns the hemopoietic tissue. Damage to this tissue leads to toxic aplasia of the bone marrow [1,2]. The search and testing of drugs with hemopoiesis-stimulating activity are an actual problem of oncology and pharmacology. Preparations based on recombinant forms of hemopoietic growth factors are now widely used in clinical practice. These preparations possess very high activity and provide the possibility of targeted and selective modulation of certain hemopoietic lineages [8,10]. Of particular interest for the creation (microbiological synthesis) of these preparation is recombinant granulocyte CSF (G-CSF) [3.6]. *In vitro* studies showed that G-CSF stimulates proliferation and differentiation of immature cells of the neutrophilic lineage. When discussing the mechanisms of *in vivo* hemostimulating effects of this hemopoietin, authors usually note that it activates both these processes. For instance, under conditions of initially balanced hemopoiesis CSF rapidly and dose-dependently increases blood neutrophil count by shortening the time of their maturation from 5 to 1 day, increasing the number of cell divisions, and accelerating the release of mature cells from the bone marrow into the peripheral blood [7-10]. There are no unambiguous data on the effect of G-CSF treatment on the balance between these important factors of cell kinetics under extreme conditions, *e.g.* during myelopoiesis-inhibiting exposures.

Here we evaluated the role of proliferation and differentiation of hemopoietic precursors in the recovery of cytostatic-suppressed hemopoiesis under conditions of G-CSF treatment.

MATERIALS AND METHODS

Experiments were carried out on 2-2.5-month old male CBA/CaLac mice (n=200). The animals (conventional certified inbred mice) were obtained from the nursery of Institute of Pharmacology (Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences). After single intraperitoneal injection of cyclophosphamide (CP) in the maximum tolerated dose (250 mg/kg) the mice received subcutaneously recombinant human G-CSF (rhG-CSF, Vector) in a daily dose of 125 μg/kg for 5 consecutive days (experi-

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mental group) or 0.2 ml distilled water (control). The mice were sacrificed by cervical dislocation on days 4, 5, 6, 7, 8, 10, and 12 after cytostatic treatment. Peripheral blood leukocyte count, total number of myelokaryocytes (TNK) in the bone marrow and their qualitative composition were evaluated using routine hematological methods. The content of committed granulomonocytopoietic precursors (CFU-GM) in the bone marrow was determined by *in vitro* cloning in methylcellulose [4]. The intensity of differentiation of hemopoietic precursors was evaluated by the index of maturation. Proliferative activity of CFU-GM was studied by hydroxyurea-induced cell suicide [4]. The data were processed statistically using Student's *t* test [5].

RESULTS

The total cellularity of the bone marrow did not significantly increase in mice receiving rhG-CSF against the background of CP compared to that in animals receiving the cytostatic alone. However, analysis of myelograms revealed an increased number of immature (on days 5-6) and mature (days 5-8) neutrophils in the hemopoietic tissue compared to the corresponding parameters in the control group. (Fig. 1, *b*, *c*). This difference in the content of mature bone marrow neutrophilic granulocytes (segmented and band forms) between CP+rhG-CSF and CP groups peaked on day 5 of the experiment. However, the number of monocyte-macrophages, lymphoid, and erythroid cells decreased during this period. The absolute count of peripheral blood segmented neutrophilic granulocytes in mice receiving rhG-CSF surpassed that in animals treated with CP alone from day 5 through 8 (Fig 1, *a*). It should be emphasized that administration of rhG-CSF against the background of CP treatment increased monocyte count in the peripheral blood in parallel with the recovery of neutrophilic leukocyte count.

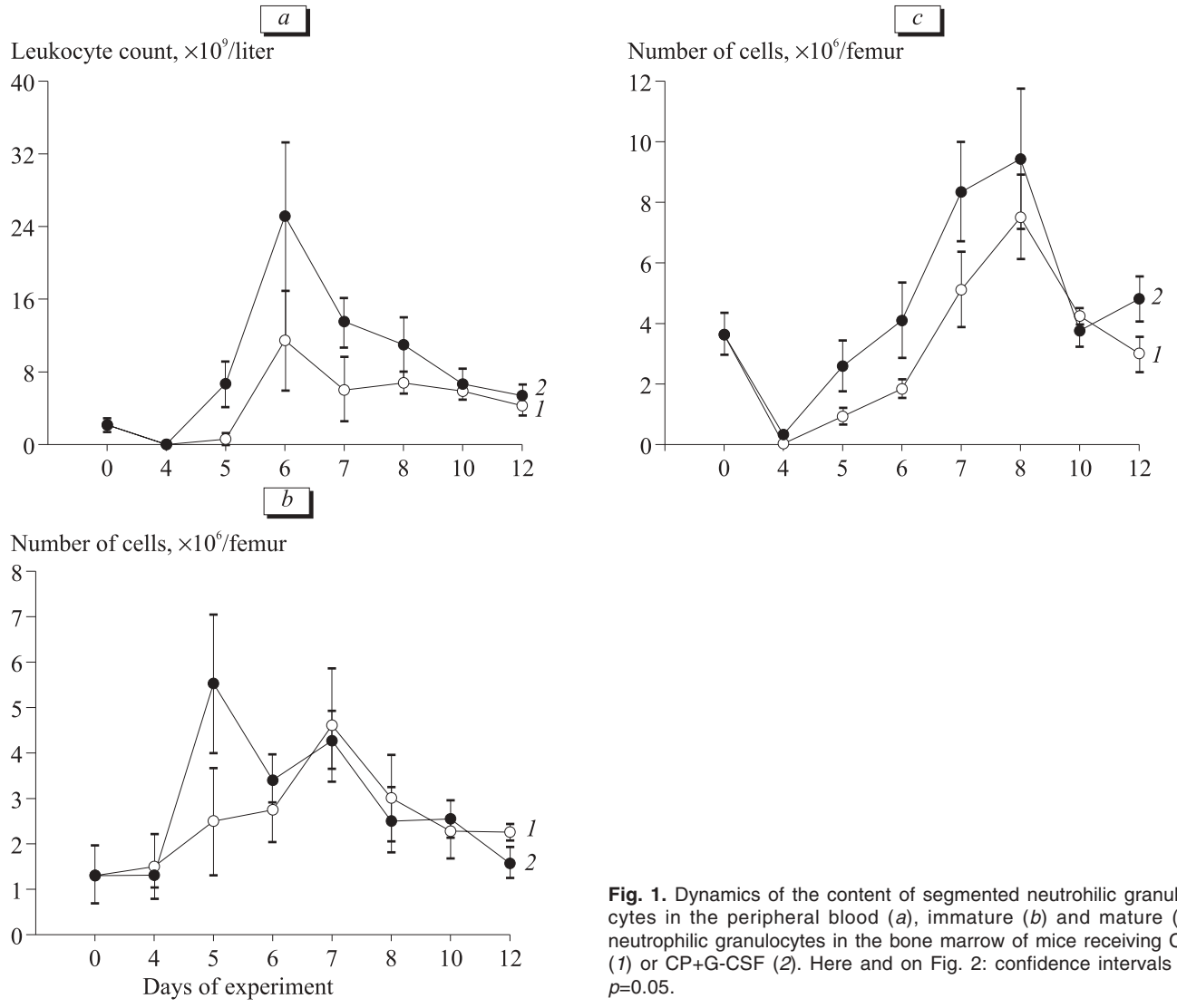

Fig. 1. Dynamics of the content of segmented neutrohilic granulocytes in the peripheral blood (*a*), immature (*b*) and mature (*c*) neutrophilic granulocytes in the bone marrow of mice receiving CP (*1*) or CP+G-CSF (*2*). Here and on Fig. 2: confidence intervals at p=0.05.

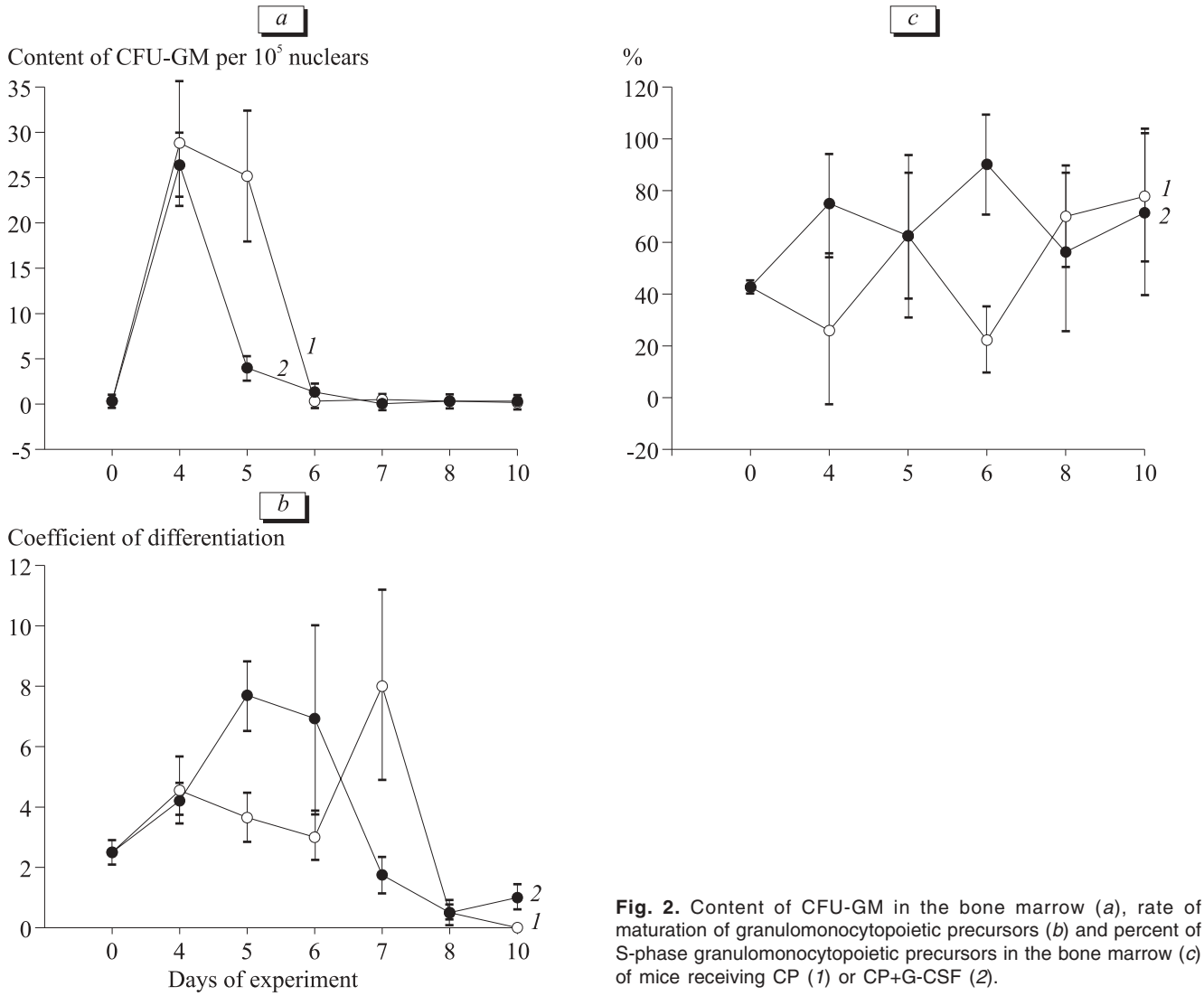

Fig. 2. Content of CFU-GM in the bone marrow (*a*), rate of maturation of granulomonocytopoietic precursors (*b*) and percent of S-phase granulomonocytopoietic precursors in the bone marrow (*c*) of mice receiving CP (*1*) or CP+G-CSF (*2*).

Comparative analysis of the peripheral blood and bone marrow led us to a hypothesis that the drastic increase in peripheral blood neutrophil and monocyte counts in animals receiving rhG-CSF is mediated by redistribution mechanisms.

This assumption is confirmed by the data on colony-forming capacity of the bone marrow in the experimental group. Indeed, rhG-CSF 6-fold reduced colony-forming capacity of the bone marrow in mice receiving CP by the 5th day of the experiment (Fig. 2, *a*). This was determined by considerable intensification of maturation of granulocytomonocytopoietic precursors into morphologically identifiable bone marrow elements (up to 209% on day 5 and 231% on day 6 after cytostatic injection) followed by a drastic decrease in this parameter (to 22%) on day 7 of the experiment, when the release of CFU-GM returned to control values (Fig. 2, *b*). In animals receiving CP alone this activation of maturation of granulocyte-macrophage precursors was delayed to the 7th day and the cellularity of the neutrophilic lineage remained low (Fig. 1, *b*, *c*; Fig. 2, *b*). This also confirms our assumption about mobilization of hemopoietic precursors into the peripheral blood without additional consumption of stem cells. According to published data this is typical of the studied cytokine [11]. Our experiments demonstrated biphasic changes of prolipherative activity in the group treated with rhG-CSF against the background of cytostatic treatment. The number of DNA-synthesizing CFU-GM in the hemopoietic tissue increased 2.9-fold on day 4 and 4-fold on day 6 of the experiment (on day 5 this parameter slightly decreased, Fig. 2, *c*). Administration of CP alone suppressed prolipherative activity on days 4 and 6 by 1.6 and 1.9 times, respectively compared to the initial level (Fig. 2, *c*). At later terms the dynamics of proliferation and differentiation of granulomonocytopoietic precursors in the control and experimental groups were similar.

Thus, changes in the pool of committed hemopoietic precursors play an important role in both postcytostatic regeneration of the granulomonocytopoiesis and its acceleration under the effect of G-CSF. The hemostimulating effect of G-CSF is largely determined by increased proliferative activity of the corresponding committed precursors. However, accelerated maturation of granulocytic elements under the effect of rhG-CSF is an important mechanism of the realization of its hemostimulating effect in the absence of activation of cell proliferation (day 5 of the experiment).

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