Function of Hemopoietic Stem Cells under Conditions of Cytostatic Myelosuppression and Treatment with Hemostimilators

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We compared the function of hemopoietic stem cells under conditions of cytostatic myelosuppression (cyclophosphamide treatment) and during treatment with granulocytopoiesis stimulators. It was found that unipotent hemopoietic precursor cells are most sensitive to cyclophosphamide. Different effects of hemostimulators on stem cells are determined by different proportions between proliferation and differentiation processes.

Key Words: *hemopoietic stem cells; cyclophosphamide, pantohematogen; granulocytic colony-stimulating factor; D-glucuronic acid*

The blood system is very sensitive to extreme influences. Continuous death of blood cells is constantly compensated by the equivalent number of newly formed elements. Tremendous proliferative potential of hemopoietic cells is determined by hemopoietic stem cells (HSC) capable of self-renewal and differentiation towards precursor cells of all myelopoiesis lineages [1].

It is well known that cytostatic drugs often cause deep and long-term toxic aplasia of hemopoiesis due to their toxic effects on proliferating bone marrow cells, which can lead to exhaustion of the pool of precursor cells [3,5-7]. High incidence of myelosuppressions developing after antiblastic treatment prompted the study of HSC functioning in this pathology and during treatment with hemostimulators used in chemotherapy.

Here we compared the function of HSC under conditions of cytostatic myelosuppression (cyclophosphamide) and during treatment with granulocytopoiesis stimulators.

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MATERIALS AND METHODS

Experiments were carried out on 2-2.5-month-old CBA/CaLac male mice (n=200). Certified animals were obtained from the nursery of Institute of Pharmacology, Tomsk Research Center. The animals received an intraperitoneal injection of cyclophosphamide in a maximum tolerable dose (250 mg/kg). After cytostatic treatment experimental animals received pantohematogen (Pantoproek, 50 mg/kg, 7 times *per os*), granulocytic CSF (G-CSF, NIKTI BAV, daily subcutaneous injections in a dose 125 mg/kg for 5 consecutive days), and glycyram (50 mg/kg, *per os* once daily for 5 days) or D-glucuronic acid (Sigma, 50 mg/kg, 3 intravenous injections on days 3, 4, and 5 after cyclophosphamide). Controls received vehicle (distilled water) in an equivalent volume (0.2 ml). The content of granulocyte-erythromacrophage-megakaryocyte (CFU-GEMM) and granulocyte-macrophage (CFU-GM) precursor cells in the bone marrow was determined and their proliferative activity and intensity of differentiation were evaluated on days 4, 5, 6, 8, 10, and 12 after cytostatic treatment [2].

The data were processed statistically using Student's t test.

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RESULTS

Analysis of colony-forming capacity of the bone marrow from CBA/CaLac mice after cyclophosphamide treatment revealed significant increase in the number of CFU-GEMM on day 4 of the experiment (2.3-fold compared to the control, Fig. 1, *a*). This parameter decreased on day 6 (to 33% from the baseline), but then returned to baseline values throughout the experiment (Fig. 1, *a*). Cyclophosphamide produced a more pronounced effect on the content of granulocyte-macrophage precursors in the bone marrow. For instance, the content of committed precursors of granulomonocytopoiesis increased on day 4 (3-fold, Fig. 1, *b*). Then, the content of granulocyte-macrophage precursors returned to the initial level and on day 12 significantly surpassed it (Fig. 1, *b*). The coefficient of CFU-GM maturation increased on days 4-6, but then sharply decreased to the end of the experiment (days 8-12; Fig. 1, *c*). At the same time, the percentage of S-phase granulocyte precursors significantly surpassed the baseline values on days 8, 10, and 12 (Fig. 1, *d*). We can assume that recovery of hemopoiesis at the initial stages is determined by accelerated differentiation of hemopoietic precursors survived after cytostatic treatment, while sharply accelerated division of precursors is a final stage of hemopoietic reparation.

Further experiments were aimed at evaluation of changes in functional activity of hemopoietic precursor cell pool after treatment with granulomonocytopoiesis stimulators under conditions of cytostatic myelosuppression. The yield of granulomonocytopoiesis precursors from the suspension of bone marrow cells of mice receiving pantohematogen considerably (3.7-fold) increased compared to the control group (cyclophosphamide alone). On

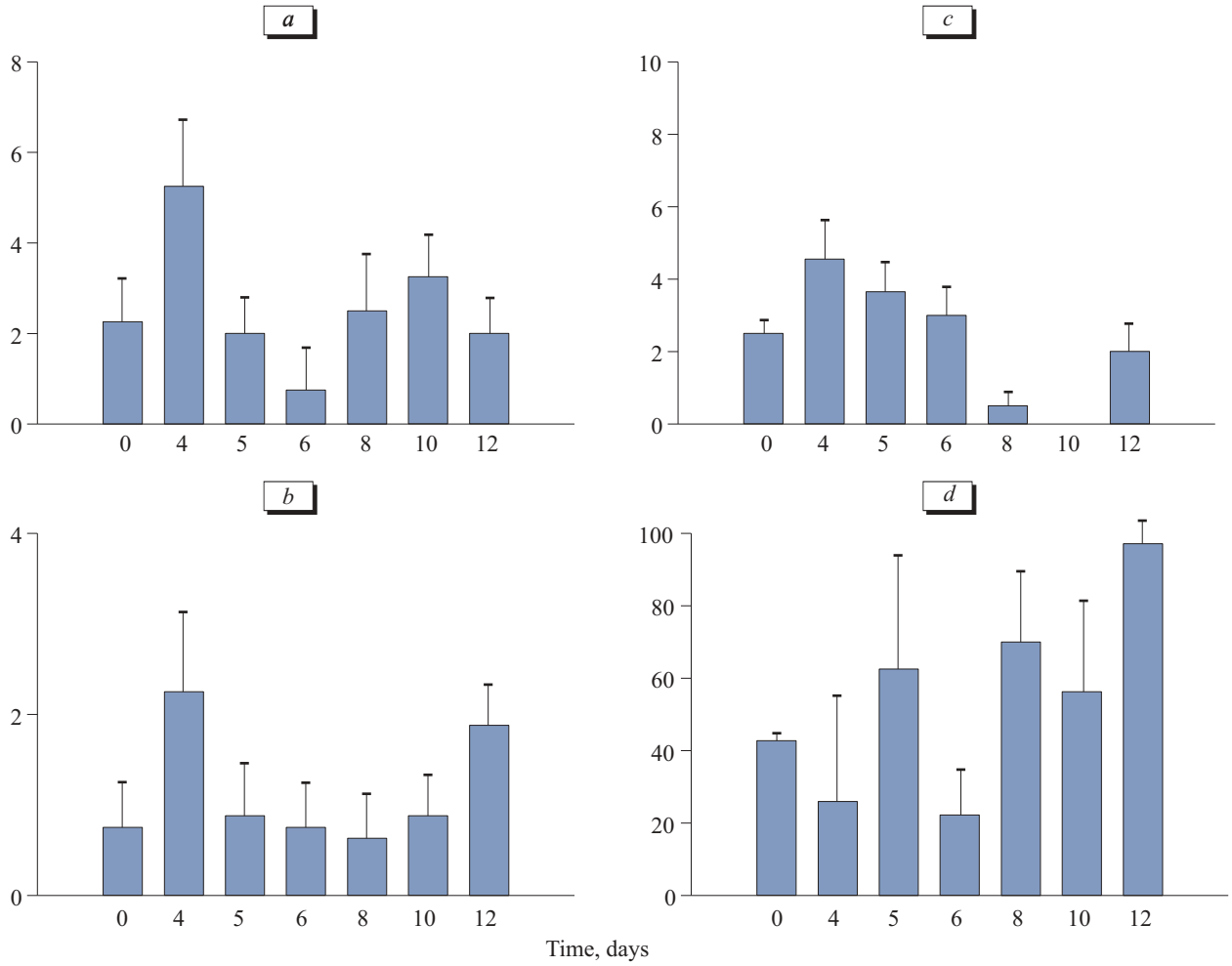

Fig. 1. Content of CFU-GEMM (*a*), CFU-GM (*b*) in the bone marrow, rate of granulomonocytopoiesis precursor maturation (*c*), percent of S-phase granulomonocytopoiesis precursors in the bone marrow (*d*) of mice receiving cyclophosphamide. Ordinate: content of CFU-GEMM per 10^5 nucleated cells (*a*), content of CFU-GM per 10^5 nucleated cells (*b*), coefficient of differentiation (*c*), % (*d*). Confidence intervals at p=0.05.

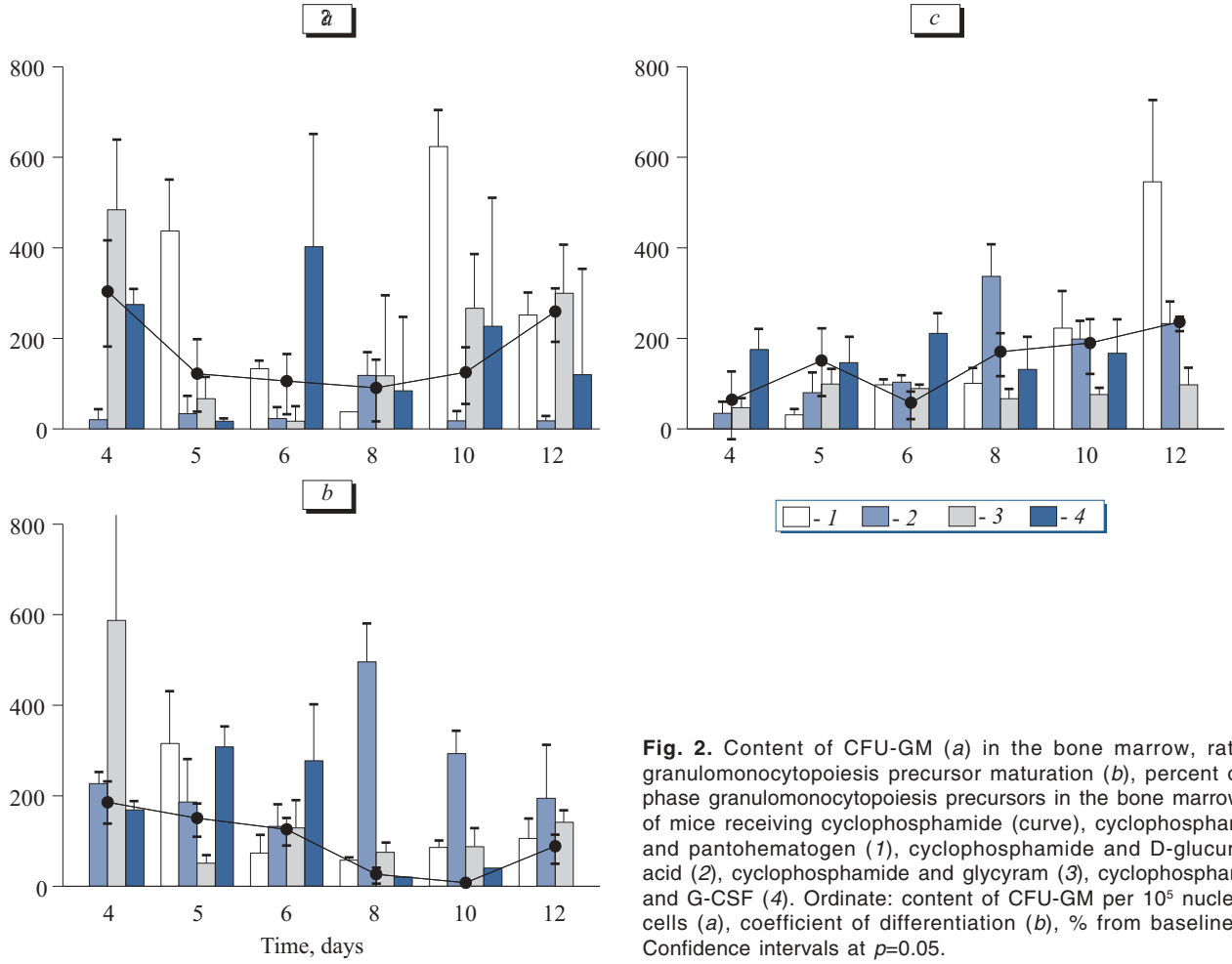

Fig. 2. Content of CFU-GM (*a*) in the bone marrow, rate of granulomonocytopoiesis precursor maturation (*b*), percent of S-phase granulomonocytopoiesis precursors in the bone marrow (*c*) of mice receiving cyclophosphamide (curve), cyclophosphamide and pantohematogen (*1*), cyclophosphamide and D-glucuronic acid (*2*), cyclophosphamide and glycyram (*3*), cyclophosphamide and G-CSF (*4*). Ordinate: content of CFU-GM per 10^5 nucleated cells (*a*), coefficient of differentiation (*b*), % from baseline (*c*). Confidence intervals at p=0.05.

days 10-12, the content of granulomonocytopoiesis precursors in the bone marrow of mice receiving hemostimulator surpassed the corresponding value at these terms in the control group (Fig. 2, *a*). We assumed that these changes were associated with accelerated division of these precursors. Indeed, their proliferative activity increased starting from day 6, while on day 12 the number of DNA-synthesizing granulomonocytopoiesis precursor cells considerably surpassed the control value (Fig. 2, *a*). At the same time, the growth of granulocyte colonies after D-glucuronic acid treatment was suppressed until day 12 with a transient increase in this parameter on day 8 of the experiment (Fig. 2, *a*). This can be explained by variations in the relationship between proliferation and differentiation processes, in particular, by lower rates of proliferation of hemopoietic precursors starting from day 4 after cyclophosphamide treatment and activation of maturation processes on days 8 and 10 of the experiment (Fig. 2, *b, c*).

After glycyram treatment the content of committed hemopoietic precursors in the bone marrow increased and attained the maximum on day 10 (the content of CFU-GM 2.3 times surpassed the corresponding value in mice receiving the cytostatic alone), the intensity of CFU-GM maturation also increased on days 4, and 8-12 of the experiment (Fig. 2, *a-c*).

Administration of G-CSF to mice receiving cyclophosphamide decreased the content of granulocytic precursors at all terms of the experiment, the maximum (6-fold) decrease was observed on day 5, despite increased proliferative activity on days 4 and 6 (Fig. 2, *a, c*). This can be explained by accelerated maturation of precursors (maturation coefficient increased on days 5 and 6 of the experiment) and their mobilization into peripheral blood (Fig. 2, *b*), because stimulation of the release of various stem cells into the circulation is a typical effect of G-CSF [4].

Thus, committed precursor cells, including CFU-GM (bipotent CFU), are most susceptible to the effect of cyclophosphamide, while the reaction of partially determined precursor cells (CFU-GEMM) to cytostatic treatment is less pronounced. This fact suggests that the pool of committed cells plays the

role of a buffer compartment in the hemopoietic tissue. Thus, the use of stimulators of granulocytopoiesis partially compensates the effects of cyclophosphamide on the blood system. Moreover, they produce different effects on stem cells, which is explained by different proportions between proliferation and differentiation of hemopoietic precursors depending on the administered stimulator.

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