Mechanisms of D-Glucuronic Acid Stimulation of Bone Marrow Granulomonocytopoiesis under Conditions of Cytostatic Myelodepression

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The development of pathogenetically-grounded methods for drug correction of hemopoiesis abnormalities based on natural host regulator system simulation is a current topic in hematology. Recombinant forms of bioactive substances such as interleukins and colony-stimulating factor are of late being effectively used in the treatment of hemodepressions [7,8]. Among the products of the vital activity of hemopoiesis-inducing microenvironment cellular elements are acid glycosaminoglycans, high-molecular compounds including D-glucuronic acid (D-GA), which is an effective stimulant of granulomonocytopoiesis [5].

The aim of the present research was to study the mechanisms of the regulatory effect of D-GA on hemopoiesis using a cyclophosphamide-induced myelodepression model.

MATERIALS AND METHODS

Experiments were carried out with 750 CBA mice weighing 18-20 g (Rassvet breeding center, Tomsk) in the fall and winter in the morning hours. Cyclophosphamide (Saransk Drug company) was injected intraperitoneally once in a dose of 250 mg/kg; D-GA (Institute of Organic Synthesis of the Academy of Sciences of Latvia) was injected intravenously in a dose of 50 mg/kg on days 3, 4, and 5 after cyclophosphamide administration, the total dose being 150 mg/kg. The material for the investigation was collected on days 1, 2, 3, 4, 5, 6, 7, 8, 10, and 14 after the administration of the preparations. The total myelokaryocyte count (per femur) was estimated and the myelogram of bone marrow smears assessed.

For determination of the bone marrow content of colony-forming units (CFU) hemopoietic cells were cultured in plasma clots in diffuse microchambers as previously described [1]. The effect of D-GA on the growth of intact murine bone marrow CFU and cluster-forming units (ClFU) was studied *in vitro* in a cultured plasma clot [1]. Interleukin-1 (IL-1) synthesis was assessed by proliferation of murine thymocytes stimulated with a nonmitogenic dose of phytohemagglutinin [8]. The colony-stimulating activity (CSA) of supernatants stimulated with LPS of bone marrow adhesive elements was studied as described previously [6]. Adhesive cells were incubated for 24 h in RPMI-1640 medium with 10% ETS in the presence of *E. coli* LPS (10 μg/ml).

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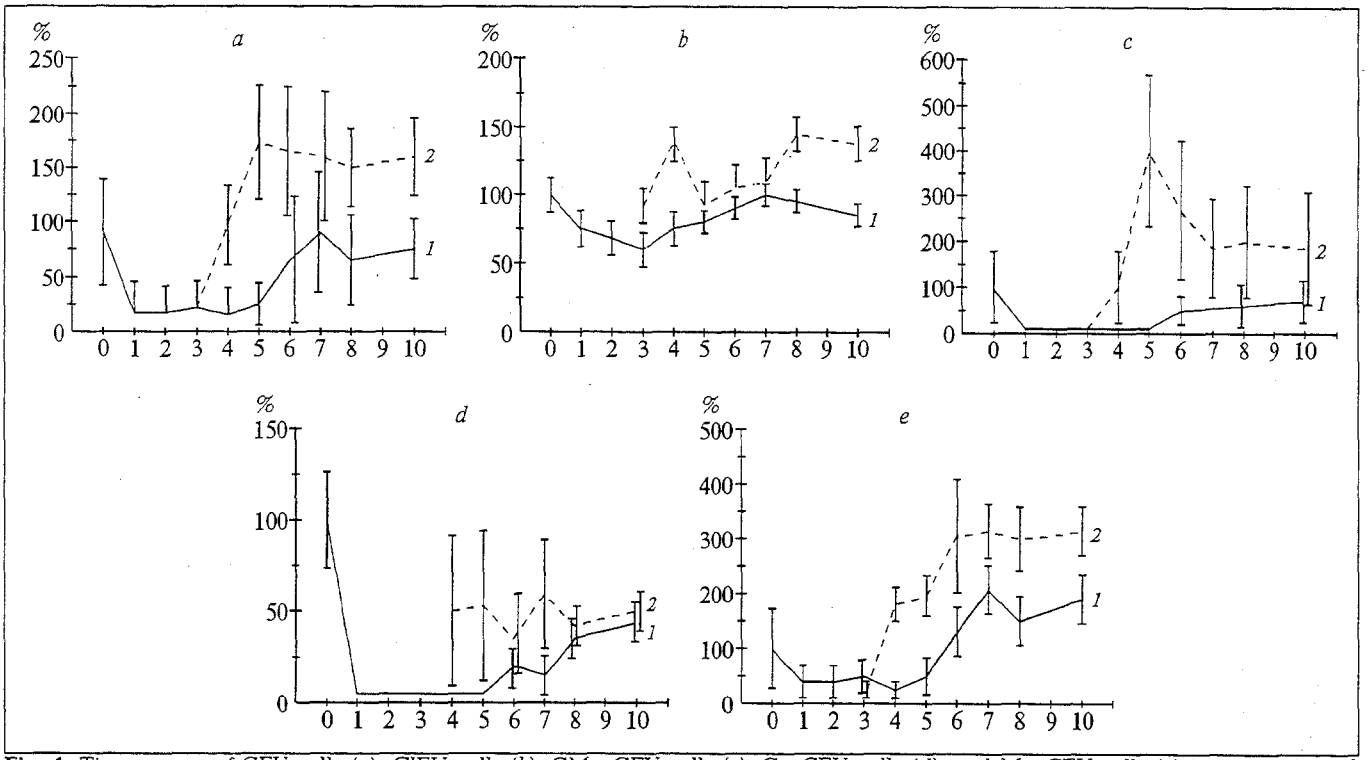

Fig. 1. Time course of CFU cells (*a*), ClFU cells (*b*), GM−CFU cells (*c*), G−CFU cells (*d*), and M−CFU cells (*e*) in mice injected cyclophosphamide (1) or cyclophosphamide + D−GA (2). Abscissa: time elapsed after cyclophosphamide administration; ordinate: colony and cluster content, % of control. Confidence intervals at $p=0.05$.

Hemopoietic islets were studied by the method developed in our laboratory [1,3].

RESULTS

Intravenous injections of D-GA stimulated regeneration of bone marrow granulomonocytopoiesis suppressed by a cytostatic [5]. The bone marrow counts of immature neutrophilic leukocytes in mice administered cyclophosphamide and D-GA or cyclophosphamide alone were, respectively, $0.7\pm2\times10^6$ and $0.01\pm0.003\times10^6$ on day 3, $5.53\pm0.64\times10^6$ and $0.40\pm0.15\times10^6$ on day 4, and $10.64\pm0.93\times10^6$ and $5.2\pm0.46\times10^6$ on day 5 (this value was $2.25\pm0.09\times10^6$ in intact animals).

The study of bone marrow colony- and cluster-forming activities led us to the conclusion that intensive recovery of granulomonocytopoiesis is due to D-GA stimulation of proliferation of type CFU precursor cells: granulocyte macrophagal (GM-CFU), granulocytic (G-CFU), and macrophagal (M-CFU), giving rise, when cultivated in a plasma clot, to GM, G, and M type of colonies. D-GA promoted the recovery of the total CFU cell count on day 4, and on day 5 it was 166% of the initial value, whereas when the cytostatic alone was administered, the CFU cell counts on days 4 and 5 of the experiment were 5.3 and 17.8% of the initial value, respectively (Fig. 1).

According to current notions, the proliferation and differentiation of hemopoietic cells from committed to mature forms takes place in hemopoietic islets, which are structural-functional formations consisting of a centrally located macrophage of fibroblast surrounded by hemopoietic elements [3]. D-GA stimulated active hemopoietic islet production in the bone marrow of mice administered cyclophosphamide.

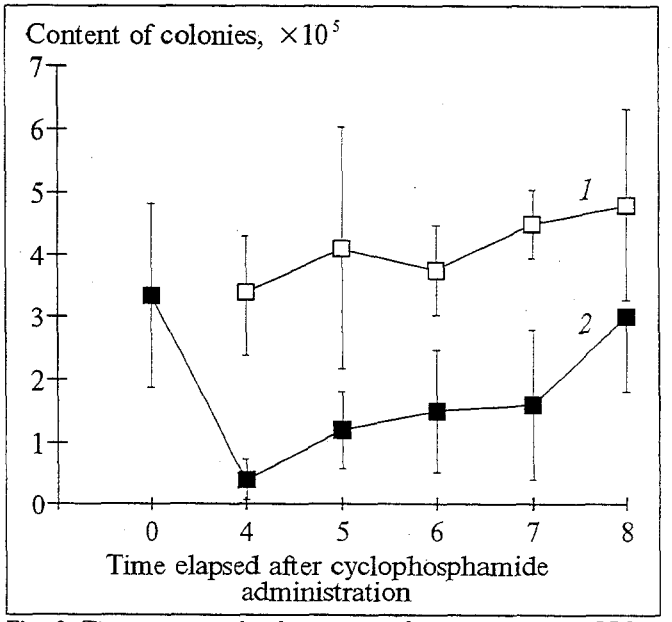

Fig. 2. Time course of colony−stimulating activity of LPS−stimulated bone marrow adhesive cell supernatants in the presence of cyclophosphamide (1) or cyclophosphamide + D−GA (2).

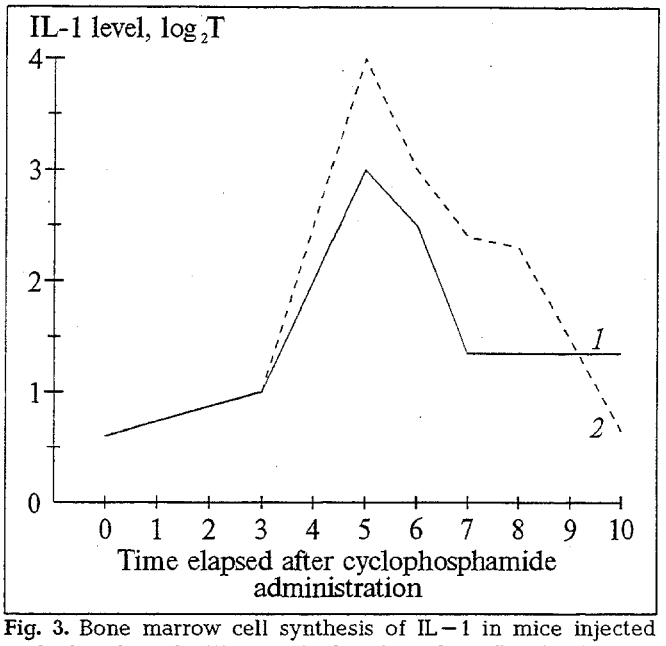

Fig. 3. Bone marrow cell synthesis of IL-1 in mice injected cyclophosphamide (1) or cyclophosphamide + D-GA (2).

Cyclophosphamide induced a significant reduction of the total count of hemopoietic islets from the 1st day to the 5th day of the experiment (down to 30.3% of the initial value on day 2). The counts of these elements in the presence of the cytostatic increased to reach the initial values only on day 6; at the same time, in mice administered both the cytostatic and D-GA the hemopoietic islet counts normalized by day 5. Moreover, on the 6th day the count of hemopoietic islets in animals administered D-GA was twice as high as that in the control animals (administered cyclophosphamide alone). Qualitative analysis of the structural and functional associations forming in the bone marrow showed that D-GA stimulated predominantly the production of granulocytic islets.

Granulomonocytopoiesis activation by D-GA was associated with IL-1 production and CSA stimulation (Figs. 2, 3). IL-1 activity on day 5 of the experiment attained 626% of the initial level (in animals administered cytostatic alone it was 483.3%) (Fig. 3), colony-stimulating activity in animals injected cyclophosphamide and D-GA normalized on day 4, whereas after cyclophosphamide alone this parameter recovered only by the end of the follow-up (on days 7-8) (Fig. 2). When interpreting these data, one should bear in mind that IL-1 may induce active proliferation of hemopoietic stem cells [4] and stimulate colony-stimulating factor production by hemopoiesis-inducing microenvironment cells [3,9].

Noteworthy is the fact that addition of D-GA to the tissue culture *in vitro* in a concentration of 10^{-12} mole/liter was associated with marked stimulation of the proliferative activity of GM-CFU cells, this being indicated by the increased efficacy of granulocytomacrophagal precursor cloning (by 204% as against the initial level). In discussing the possible mechanisms of the effect of D-GA on hemopoietic cells, we would emphasize that incubation of a bone marrow myelokaryocyte culture with D-GA or its potassium salt increased the levels of Ca^{2+} and cAMP and enhanced ^{3}H-thymidine incorporation in DNA. Similar results were obtained after intraperitoneal injections of these agents to mice [2].

Hence, the data indicate a high efficacy of D-GA as a hemostimulant in cytostatic myelodepressions. Increased production of colony-stimulating and IL-1 activity by hemopoiesis-inducing microenvironment cellular elements, and stimulation of the formation of hemopoietic islets and of various types of committed granulomonocytopoiesis precursor cells underlie the activating effect of D-GA on bone marrow myelopoiesis. When speaking about the mechanisms of the effect of D-GA on hemopoiesis, we should remember that a single intravenous injection of D-GA in a dose of 150 mg/kg to intact mice is associated with a rapid increase of serum acid glycosaminoglycans (within 15 min), followed by an increase of their bone marrow content (within 24 h) [5].

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