Effect of Granulocytic Colony-Stimulating Factor on Cytostatic-Suppressed Granulocytopoiesis under Conditions of Exhausted Catecholamine Depot

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Exhaustion of catecholamine depot in the CNS before modeling of cytostatic myelosuppression potentiates the stimulatory effect of granulocytic CSF on regeneration of the granulocytic hemopoiesis stem. The increase in granulocyte count in the blood system under these conditions is caused by recovery of the structural and functional organization of the hemopoietic tissue (at the expense of reduction of the suppressive effect of catecholamines on the formation of granulocytic hemopoietic islets) and simultaneous stimulation of division and maturation of granulomonocytic precursors.

Key Words: *granulocytopoiesis; granulocytic colony stimulating factor; catecholamines; hemopoietic islets; cyclophosphamide*

Preparations of granulocytic CSF (G-CSF) are used for prevention and treatment of neutropenias (and prevention of low resistance to infectious complications) and for normalization of hemopoiesis in cancer patients treated with cytostatics [2,6]. The use of G-CSF is limited because of its numerous side effects [1,6,9].

Hemopoiesis suppressed by cytostatics is highly sensitive to neuropharmacological agents (sympatholytics, adrenoblockers, neuroleptics, antiserotonin drugs) [3,5,7,8]. Their effects on hemopoietic cells are largely mediated via modulation (recovery) of the hemopoiesis-inducing microenvironment. It can be hypothesized that functional recovery of cells of the hemopoiesis-inducing microenvironment will potentiate the effect of G-CSF.

Here we studied the stimulatory effect of G-CSF on cytostatic-suppressed granulocytopoiesis under conditions of exhaustion of the catecholamine depot by sympatholytic treatment.

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MATERIALS AND METHODS

Experiments were carried out on 2-2.5-month-old female CBA/CaLac mice (n=550). First-category conventional mice were obtained from Breeding Center of Institute of Pharmacology.

Cytostatic myelosuppression was induced by a single intraperitoneal injection of $^1/_3$ MPD of alkylating agent cyclophosphamide (83 mg/kg). Thirty minutes before the cytostatic, the experimental animals received a single intraperitoneal dose of sympatholytic reserpine (Polfa; 2 mg/kg) dissolved in sterile saline directly before the injection. The animals of the experimental groups received subcutaneous injections of recombinant human G-CSF (100 μg/kg, in sterile phosphate buffer (pH 7.2), Institute of Cell Cultures, Vector Center) for 5 days starting from the next day after the cytostatic injection. Controls were injected with an equivalent vo-

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lume (0.2 ml) of saline. Intact animals served as the reference group.

On days 1-7 after cytostatic injection, the counts of peripheral blood neutrophils was determined. The animals were sacrificed by cervical dislocation under ether narcosis and the counts of mature and immature neutrophilic granulocytes in the bone marrow were evaluated [4]. The structural and functional organization of the bone marrow was studied by enzymatic isolation of hemopoietic islets (HI) and subsequent evaluation of their quantitative and qualitative composition. The content of granulomonocytic colony- and cluster-forming units (CFU-GM and ClFU-GM) in the bone marrow was studied *in vitro* by cloning of nonadherent myelokaryocytes in methylcellulose culture medium [4]. Proliferative activity of granulomonocytopoiesis precursors was evaluated by the method of hydroxyurea cell suicide. The intensity of their differentiation was evaluated by the maturation index (clusters/colonies ratio in the same well) [4].

The significance of differences between the results was evaluated using parametric Student's *t* test or nonparametric Mann—Whitney *U* test.

RESULTS

Cyclophosphamide significantly reduced the count of immature (days 1-3) and mature (days 1-5) neutrophilic granulocytes in the bone marrow and the counts of stab (days 1-3) and segmented (days 1-5) neutrophils in the peripheral blood (Fig. 1). Suppression of the granulocytic hemopoiesis stem was paralleled by inhibition of the formation of macrophage-negative (days 1-5) and granulocytic HI (days 1-3, 5, and 7) in the hemopoietic tissue. The production of hemopoietic growth factors by microenvironment cells was disordered for a long period [7,8]. Cyclophosphamide stimulated proliferative activity of granulocytomonopoiesis precursors (days 1-7), which stimulated the production of CFU-GM in hemopoietic tissue culture (days 1, 5, 7; Fig. 2). The intensity of maturation of granulomonocytic precursor cells was inhibited on days 3 and 7 and increased on day 5.

It is obvious that disorders in functional activity of local regulation system were primarily responsible for myelosuppression caused by injection of the alkylating agent in $^1/_3$ MPD.

G-CSF stimulated regeneration of granulocytopoiesis after cyclophosphamide injection, which was seen from accumulation of immature (days 2, 3) and mature (day 5) neutrophilic granulocytes in the hemopoietic tissue (Fig. 1). An appreciable increment in the peripheral blood counts of segmented neutrophils was observed on days 2, 4, 5 (up to the development of moderate neutrophilic leukocytosis on days 4, 5). It is known that the hemostimulatory effect of G-CSF is due to direct stimulation of proliferation of committed precursors and maturation of neutrophilic granulocytes [2]. In our study, the cytokine mainly increased the count of HI containing stromal cells (days 2, 3) and granulocytes (days 2, 3, 5, 7).

Reserpine also stimulated granulocytopoiesis regeneration after injection of the alkylating agent, which was seen from accumulation of neutrophilic granulocytes in the bone marrow (days 3, 5; Fig. 1). The count of segmented neutrophils in the peripheral blood also increased (days 2, 4), but was 1.8-5.9 times lower than in experiments with G-CSF. Exhaustion of catecholamine depot increased the number of cell complexes with the central stromal element and the number of granulocytic associations (days 1-4). It can be hypothesized that the effect of reserpine (in contrast to G-CSF) was related to *de novo* formation HI. Moderate reduction of CFU-GM growth by reserpine in methylcellulose medium also supports this hypothesis (Fig. 2).

The recovery of the granulocytic stem cellularity under conditions of steady exhaustion of catecholamine depot and G-CSF treatment was more intensive than in experiments with G-CSF and reserpine. As early as on days 3 and 4, the counts of immature and mature neutrophils in the bone marrow virtually did not differ from the initial levels, and on days 6-7 granulocytopoiesis hyperplasia was detected. More active release of segmented neutrophils into peripheral blood under these conditions led to the development of pronounced leukocytosis on days 4, 5, 7.

Treatment with reserpine and G-CSF led to active recovery of the structural and functional organization of the hemopoietic tissue. Macrophage-negative (days 1-3, 7) and granulocytic (days 1-3, 5-7) cell complexes were accumulating (Fig. 1). Cells of granulocytic HI were presented mainly by morphologically recognizable granulocytes (for example, segmented neutrophils). The counts of nucleated cells in HI were significantly lower in groups treated with reserpine or G-CSF than after successive treatment with both preparations. After successive treatment with reserpine and G-CSF, the formation of additional foci of granulocytic hemopoiesis preceded the development of bone marrow hyperplasia and neutrophilic leukocytosis in the peripheral blood.

Hence, exhaustion of catecholamine depot before simulation of cytostatic myelosuppression potentiated the stimulatory effect of G-CSF on regeneration of the hemopoietic granulocytic stem with

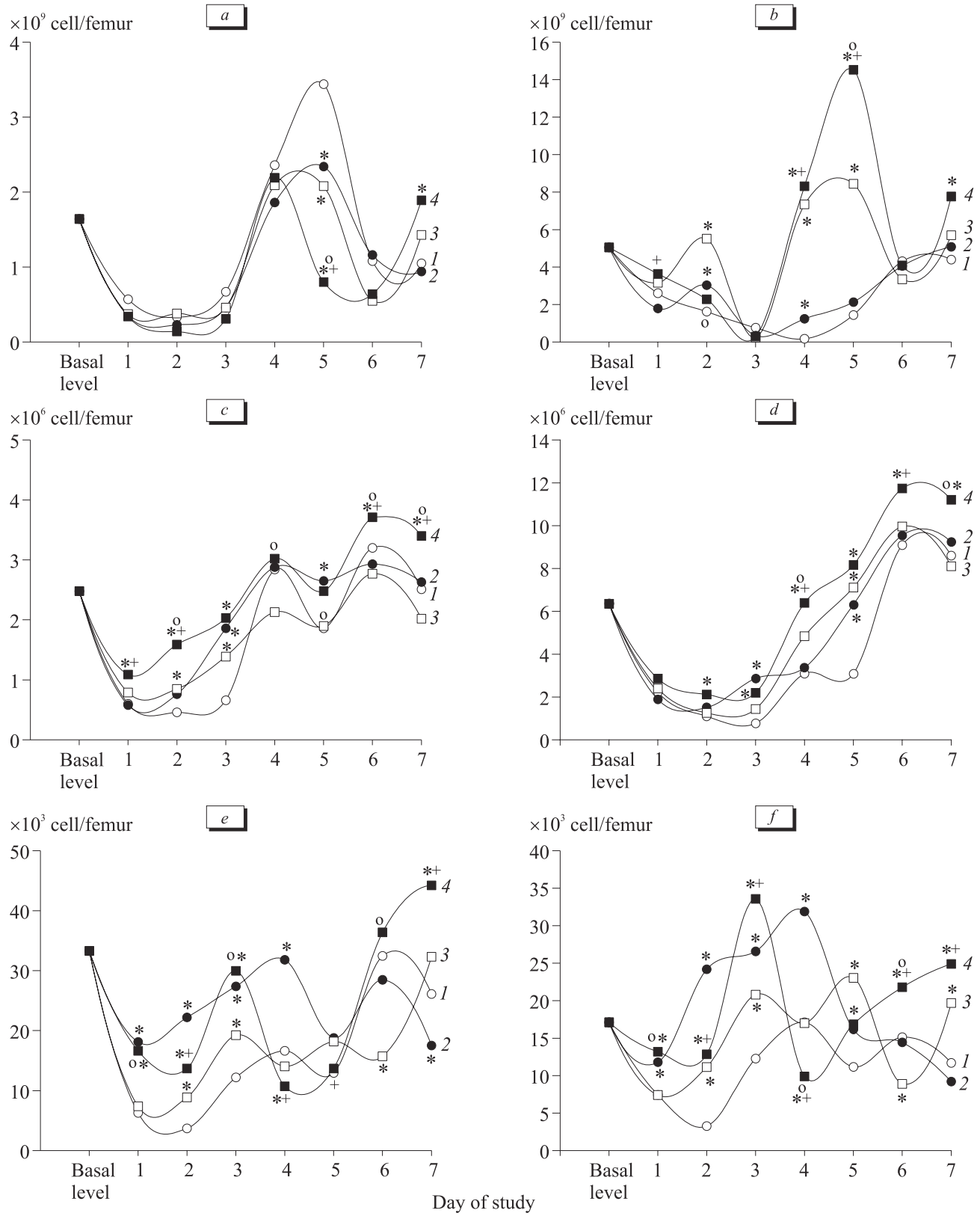

Fig. 1. Effects of reserpine, G-CSF, and their combination on the time course of peripheral blood counts of stab (*a*) and segmented (*b*) neutrophils and bone marrow counts of immature (*c*) and mature neutrophils (*d*), macrophage-negative (*e*) and granulocytic (*f*) HI in CBA-CaLac mice injected with cyclophosphamide. Treatment: *1*) saline and cyclophosphamide; *2*) reserpine and cyclophosphamide; *3*) cyclophosphamide and G-CSF; *4*) reserpine, cyclophosphamide, and G-CSF. $p<0.05$ compared to: **1*, $^{+}$*2*, °*3*.

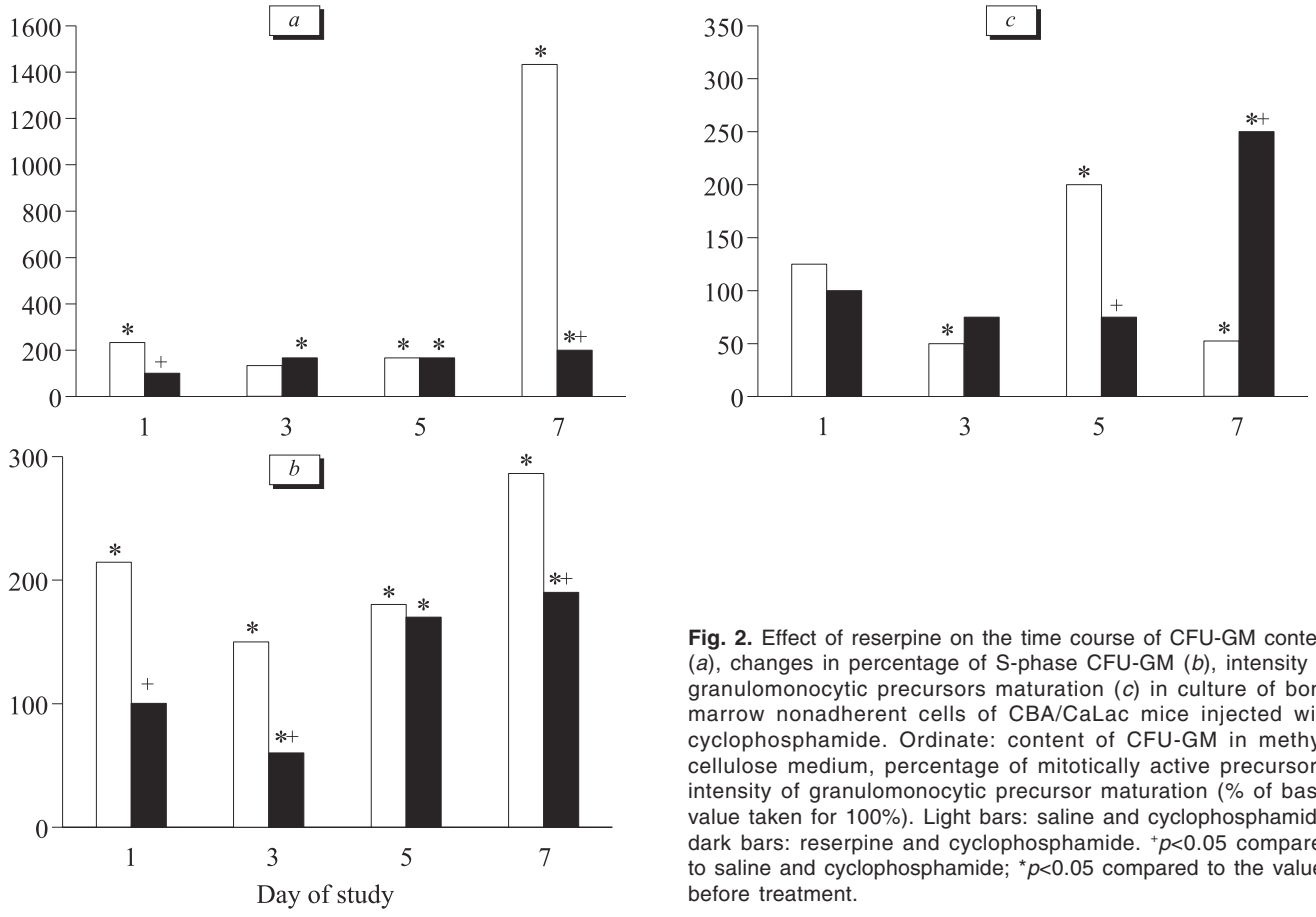

Fig. 2. Effect of reserpine on the time course of CFU-GM content (*a*), changes in percentage of S-phase CFU-GM (*b*), intensity of granulomonocytic precursors maturation (*c*) in culture of bone marrow nonadherent cells of CBA/CaLac mice injected with cyclophosphamide. Ordinate: content of CFU-GM in methylcellulose medium, percentage of mitotically active precursors, intensity of granulomonocytic precursor maturation (% of basal value taken for 100%). Light bars: saline and cyclophosphamide; dark bars: reserpine and cyclophosphamide. $^{+}p<0.05$ compared to saline and cyclophosphamide; $^{*}p<0.05$ compared to the values before treatment.

the development of bone marrow hyperplasia and blood neutrophilosis. It seems that under conditions of myelosuppression caused by cytostatics, catecholamines of the CNS produce a destructive effect on structural and functional organization of the bone marrow (HI). These data suggest that G-CSF therapy for cytostatic leukopenia should be supplemented with drugs reducing activities of the adrenergic and dopaminergic structures of the brain [7].

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