Hemostimualting Properties of Preparation Containing Ultralow Doses of Antibodies to Stem Cell Factor in Cytostatic Myelosuppression

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The preparation containing ultralow doses of antibodies to stem cell factor considerably activates bone marrow myelopoiesis suppressed by cyclophosphamide. This effect of the preparation is based on stimulation of proliferation of committed hemopoietic precursors and increase in functional activity of adherent elements of the hemopoiesis-inducing microenvironment.

Key Words: *hemopoiesis; myelosuppression; stem cell factor; antibodies; ultralow doses*

A great part in the regulation of blood cell production is now allocated to stem cell factor (SCF), an early acting hemopoietin affecting the earliest progenitor cells for not only hemopoietic, but also other tissues [5]. The mechanism of the action of SCF includes immediate influence on bone marrow stem cells and on mobilization of precursor of different maturity from depots into circulation [8]. The effect of preparations primarily targeted to the blood system largely depends on their effects on structures locally regulating the hemopoiesis processes [1], in particular, on stromal elements of the hemopoietic microenvironment. The state of fibroblast population, stromal mechanocytes, in turn, depends on the function of their precursors, mesenchymal stem cells [6].

SCF belongs to a class of substances, which, despite their high *in vitro* activity, are practically not used *in vivo* due to the absence of appropriate medicinal forms [4]. At the same time, culturing of progenitor cells *in vitro*, even during 3-4 passages, can lead to their tumor transformation [9,10]. Moreover, the preparations of most growth factors produce side effects considerably limiting their clinical use [11]. This problem can be solved by using the preparation of ultralow doses of antibodies to SCF, because it was proven that potentiated preparations of antibodies in ULD can stimulate production of the substance they are directed [7].

Here we studied myeloprotective activity of a preparation containing ULD of antibodies to SCF modulating the functions of stem cells and the mechanisms underlying.

MATERIALS AND METHODS

Experiments were carried out on 2-month old male CBA/CaLac mice (n=114) obtained from the nursery of Institute of Pharmacology, Tomsk Research Center.

Myelosuppression in experimental mice was modeled by single intraperitoneal injection of cyclophosphamide in MTD (250 mg/kg). The preparation containing ULD of antibodies to SCF (C12+C30+C200) was administered *per os* to experimental animals in a volume of 0.2 ml once a day for 10 days starting from

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day 1 after cytostatic injection. The controls injected with cyclophosphamide received distilled water according to the same scheme. Ten mice maintained under the same conditions served as the reference group (baseline values).

The animals were decapitated under ether narcosis on days 3, 5, 7, and 10 after cyclophosphamide injection. Parameters of the peripheral blood (total number of reticulocytes, leukocytes, differential leukocyte count) were determined by routine methods, the state of the peripheral components of the erythrone (hemoglobin content, erythrocyte count, hematocrit, and mean corpuscular concentration of hemoglobin) was evaluated using a hematological analyzer ABACUS (Diatron) operated in veterinary mode.

The content of committed erythro- and granulomonocytic precursors in the bone marrow was measured by culturing the non-adherent fraction of myelokaryocytes in semisolid medium on the basis of RPMI-1640 supplemented with 0.5 U/ml recombinant erythropoietin or 0.4×10^{-5} M 2-mercaptoethanol and 5 ng/ml recombinant granulocytic colony-stimulating factor.

For culturing of fibroblast CFU containing stromal precursors and true (mesenchymal) stem cells, the concentration of bone marrow cells was brought to 5×10^5 per 1 ml semisolid nutrient medium based on DMEM with 20% FCS. Colonies were counted under an inverted microscope after 7-day incubation.

We also evaluated the intensity of cell proliferation by the method of cell suicide with hydroxyurea and the intensity of differentiation (by index of maturation) of erythro- and granulomonocytic precursors. Functional activity of the hemopoiesis-inducing microenvironment (GIM) was evaluated by the intensity of secretion of factors constituting colony-stimulating and erythropoietic activities by HIM elements [2].

RESULTS

A pronounced depression of reticulocytes in the peripheral blood was observed in mice of control and experimental groups. Dynamics of this parameter was typical of cyclophosphamide treatment. However, the number of circulating reticulocytes in experimental mice was higher than in controls throughout the observation period and attained the baseline values by the end of the experiment (Fig. 1).

The content of erythrocytes in the peripheral blood was less sensitive to the cytostatic treatment [3]. In the control group, this parameter decreased on day 7 after cyclophosphamide injection; on the contrary, in the experimental group the number of erythrocytes on day 5 surpassed the initial level. Cytostatic treatment significantly decreased hematocrit in control animals on days 7-10 of the experiment and only on day 10 in the experimental group. Moreover, the mean corpuscular concentration of hemoglobin in experimental mice considerably increased by the end of the observation period, which confirms the positive effect of ULD of antibodies to SCF on saturation of erythrocytes with hemoglobin under conditions of cytostatic myelosuppression (Fig. 1).

In the control group, blood leukocyte count after cytostatic leukopenia returned to normal on day 7 of the experiment and then surpassed it. In mice of the experimental group, this parameter significantly surpassed the control value (1.82×10^9/liter and 4.4×10^9/liter, respectively) at the expense of more than 2-fold increase in the number of segmented neutrophils (Fig. 2).

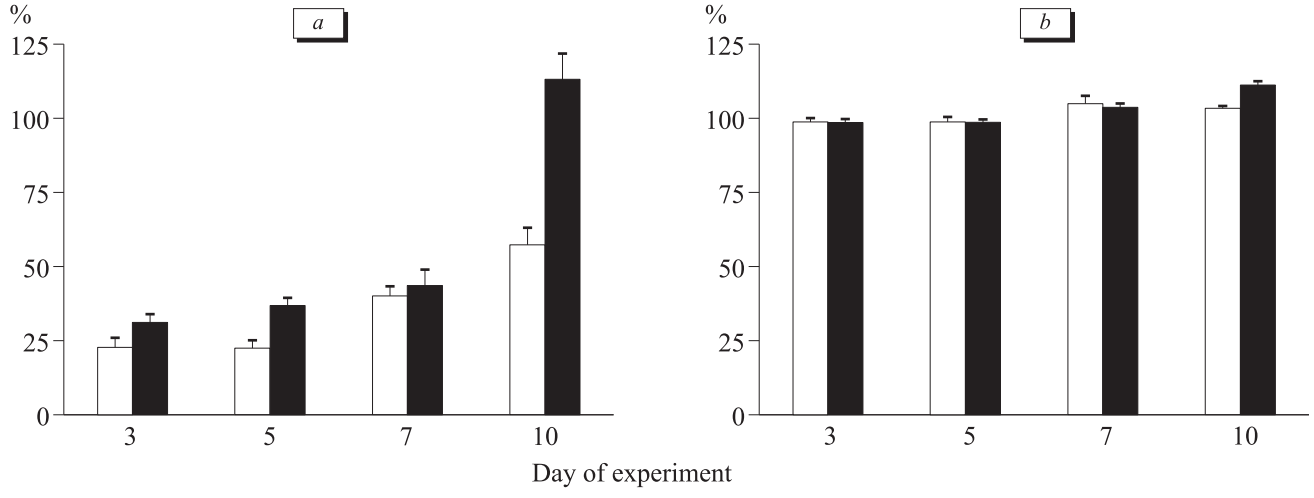

Fig. 1. Dynamics of the number of reticulocytes (*a*) and the degree of erythrocyte saturation with hemoglobin (*b*) in mice with cytostatic myelosuppression induced by cyclophosphamide injection in MTD under the effect of ULD of antibodies to SCF. Here and on Figs. 2 and 3: open bars: control; dark bars: experiment.

Changes in the total number of myelokaryocytes in mice of the compared groups were similarly directed, corresponded to the dynamics of changes of peripheral blood parameters, and were characterized by a decrease in cellularity of the bone marrow, which attained a minimum on day 5 of the experiment and then gradually increased. However, this parameter returned to normal on day 10 of the experiment only in the experimental group, which resulted from higher content of mature neutrophils, eosinophils, and erythroid cells in the bone marrow. The content of lymphoid cells remained below the initial level in all animals.

These findings suggest that the preparation containing ULD of antibodies to SCF can stimulate myelopoiesis under conditions of suppression of hemopoiesis caused by cytostatic treatment. Taking into account the general property of preparations containing ULD of antibodies, stimulation of the production of molecules to that they are directed, we can assume that ULD of antibodies to SCF can stimulate production of SCF by HIM cells [7]. The latter, being an early acting growth factor, can stimulate the production of several hemopoietic stems, which agrees with our observation.

The study of the effect of ULD of antibodies to SCF on the state of erythro- and granulomonocytic precursors showed that colony-forming capacity of the bone marrow in all animals decreased by more than 2-fold on day 3 of the experiment. Abortive rise of this parameter on day 5 of the experiment changed by repeated suppression of colony formation, but the

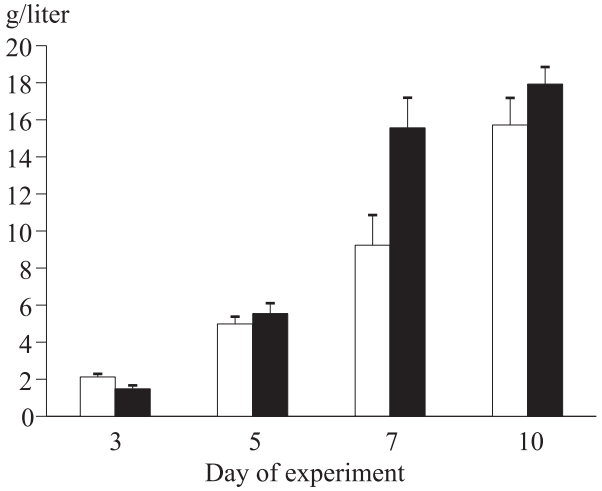

Fig. 2. Dynamics of leukocyte count in mice with cytostatic myelosuppression induced by cyclophosphamide injection in MTD under the effect of ULD of antibodies to SCF.

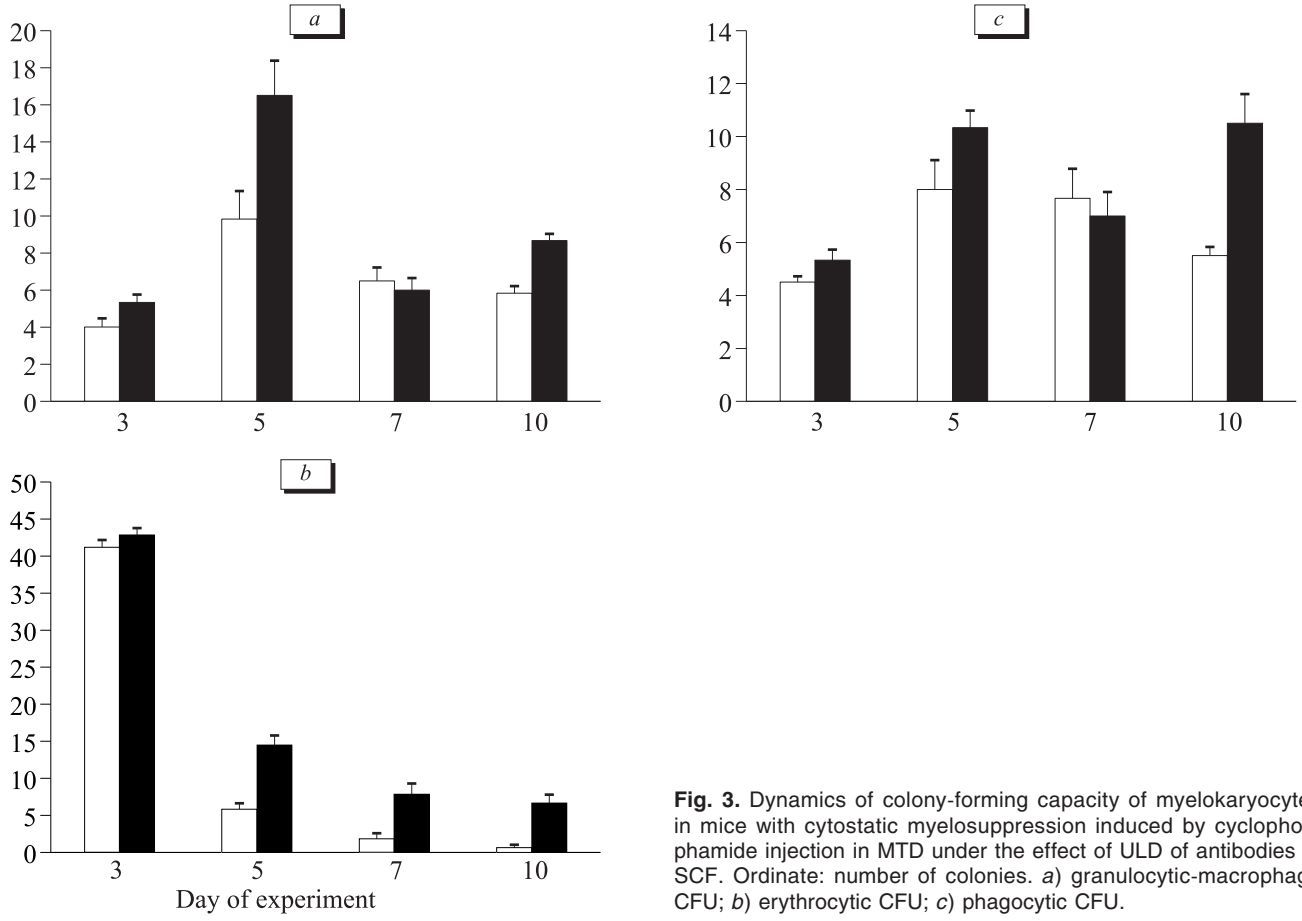

Fig. 3. Dynamics of colony-forming capacity of myelokaryocytes in mice with cytostatic myelosuppression induced by cyclophosphamide injection in MTD under the effect of ULD of antibodies to SCF. Ordinate: number of colonies. *a*) granulocytic-macrophage CFU; *b*) erythrocytic CFU; *c*) phagocytic CFU.

number of hemopoietic precursors of both types considerably surpassed the control values at all terms of the experiment and on day 10 practically returned no the baseline values (Fig. 3, *a*, *b*)

Evaluation of the state of bone marrow cell pool giving rise to stromal elements forming HIM showed that their content in the bone marrow was considerably reduced at all terms after cytostatic treatment, except day 3 when the number of fibroblast CFU considerably surpassed the initial values (Fig. 3, *c*). At later terms this parameter in animals receiving antibodies to SCF was higher than in the control group. Thus, the preparation containing ULD of antibodies to SCF induces accumulation of stromal precursors in the bone marrow.

Analysis of mechanisms underlying these changes in the state of the pool of hemopoietic precursors revealed a sharp increase in the content of DNA-synthesizing precursors of erythro- and granulomonocytopoiesis under the effect of ULD of antibodies to SCF on day 5 of the experiment (after pronounced depression of their content). Then, the intensity of proliferation of clonogenic myeloid elements considerably decreased, while that of erythroid elements surpassed the corresponding parameter in intact animals. No significant differences in the intensity of maturation of hemopoietic precursors were noted.

Changes in secretory activity of HIM elements on the whole were characterized by increased colony-stimulating activity of both adherent and non-adherent cells on day 3 after cyclophosphamide treatment, which reflects unspecific reaction of HIM to the cytostatic treatment [1]. ULD of antibodies to SCF increased the production of colony-stimulating activity by adherent cells (to 921% of the initial level) on day 3 of the experiment, but had little effect on activity of non-adherent myelokaryocytes.

Erythropoietic activity of adherent cells of HIM also increased by day 3 of the experiment and did not depend on administration of antibodies to SCF. In the control group we observed an increase in this parameter for non-adherent bone marrow cells on day 3 of the experiment; administration of ULD of antibodies to SCF abolished this effect probably due to modulation of the production of endogenous SCF by this preparation [7].

Thus, our findings suggest that the preparation containing ULD of antibodies to SCF considerably activates bone marrow erythropoiesis and granulomonocytopoiesis suppressed by cyclophosphamide. The hemostimulating properties of the preparation are determined by stimulation of proliferation of committed hemopoietic precursors. Accelerated production of hemopoietic precursors, in turn, is related to an increase in functional activity of adherent elements of HIM.

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