Participation of Humoral Factors in the Regulation of Hemopoiesis in Cytostatic Myelodepression

A. M. Dygai, V. V. Zhdanov, M. Yu. Minakova, and E. D. Gol'dberg

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 8, pp. 161-165, August, 1997
Original article submitted June 3, 1996

Production of hemopoietic factors responsible for colony-stimulating and erythropoietic activities by hemopoietic microenvironment is measured and the serum level of these activities in CBA mice treated with various cytostatics is determined. It is shown that the key role in direct stimulation of suppressed hemopoiesis is played by hemopoietic growth factors produced by adherent and nonadherent cells of hemopoietic microenvironment.

Key Words: *hemopoietic microenvironment; colony-stimulating activity; erythropoietic activity; cytostatics*

Hormones and hormone-like substances produced by various organs and tissues play an important role in the regulation of hemopoiesis in health and under the action of hemopoiesis-disturbing factors. The most potent hemopoietic regulators are hemopoietins and other cytokines produced by cells of the hemopoiesis-inducing microenvironment (HIM) and involved in local regulation of hemopoiesis [9]. Organism also produces other endogenous humoral factors, for instance, hormones of the adrenal cortex, sex hormones, eicosanoids, biogenic amines, etc., which modulate proliferation and differentiation of hemopoietic cells [1,7,11].

We have previously demonstrated that various extreme factors which have no direct myelodepressive effect (immobilization, blood loss, and inflammation) induce universal and nonspecific changes in the blood system, typical for the general adaptation syndrome [3,5,6].

All these reaction are based on enhanced secretion of stimulators of hemopoiesis by HIM cells and increased serum level of colony-stimulating (CSA) and erythropoietic (EPA) activities.

Institute of Pharmacology, Tomsk Research Center, Russian Academy of Medical Sciences

The aim of the present study was to elucidate the role of local (bone marrow) and remote components of the humoral hemopoiesis regulation in regeneration of hemopoiesis in cytostatic myelodepression.

MATERIALS AND METHODS

Experiments were carried out on 225 male CBA mice weighing 18-20 g (*Rassvet* nursery, Tomsk). The animals were intraperitoneally injected with 5-fluorouracil (*Darnitsa* Chemopharmaceutic Association), cyclophosphane (*Biokhimik* Complex, Saransk), or adriamycin (Farmitalia Carlo Erba) in the maximum tolerated doses: 228, 250, and 6 mg/kg, respectively, judging from the data of probit-analysis. Control group consisted of intact mice. The mice were sacrificed by cervical dislocation under ether anesthesia at different times postinjection. The total number and subpopulation content of karyocytes were studied on smears stained by the Nocht—Maksimov method [8].

Suspension of bone marrow nuclear cells was fractionated to the adhesive and nonadhesive fractions by adhesion to plastic dishes. Live cells of each fraction were adjusted to a concentration of 2×10^6 cells/ml with a medium containing 90% RPMI-1640

0007-4888/97/0008-0764$18.00

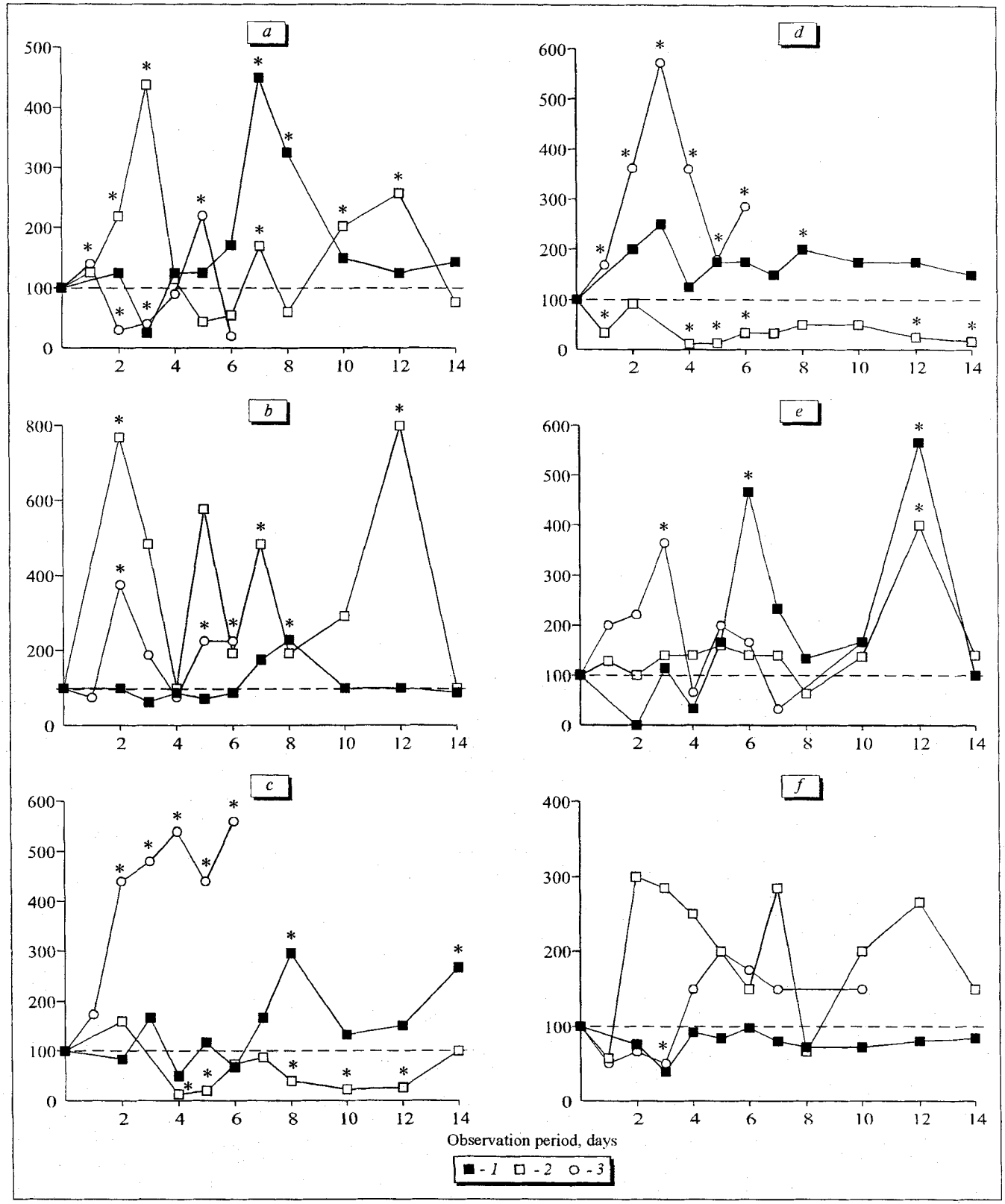

Fig. 1. Erythropoietic and colony-stimulating activities in supernatants of adherent (*a*, *b*) and nonadherent (*c*, *d*) myelokaryocytes and in serum (*e*, *f*) of CBA mice treated with 5-fluorouracil (*1*), cyclophosphane (*2*), and adriamycin (*3*) in maximum tolerated doses. Ordinate: humoral activity (% of initial level). *$p<0.05$ compared with the initial level (dashed line).

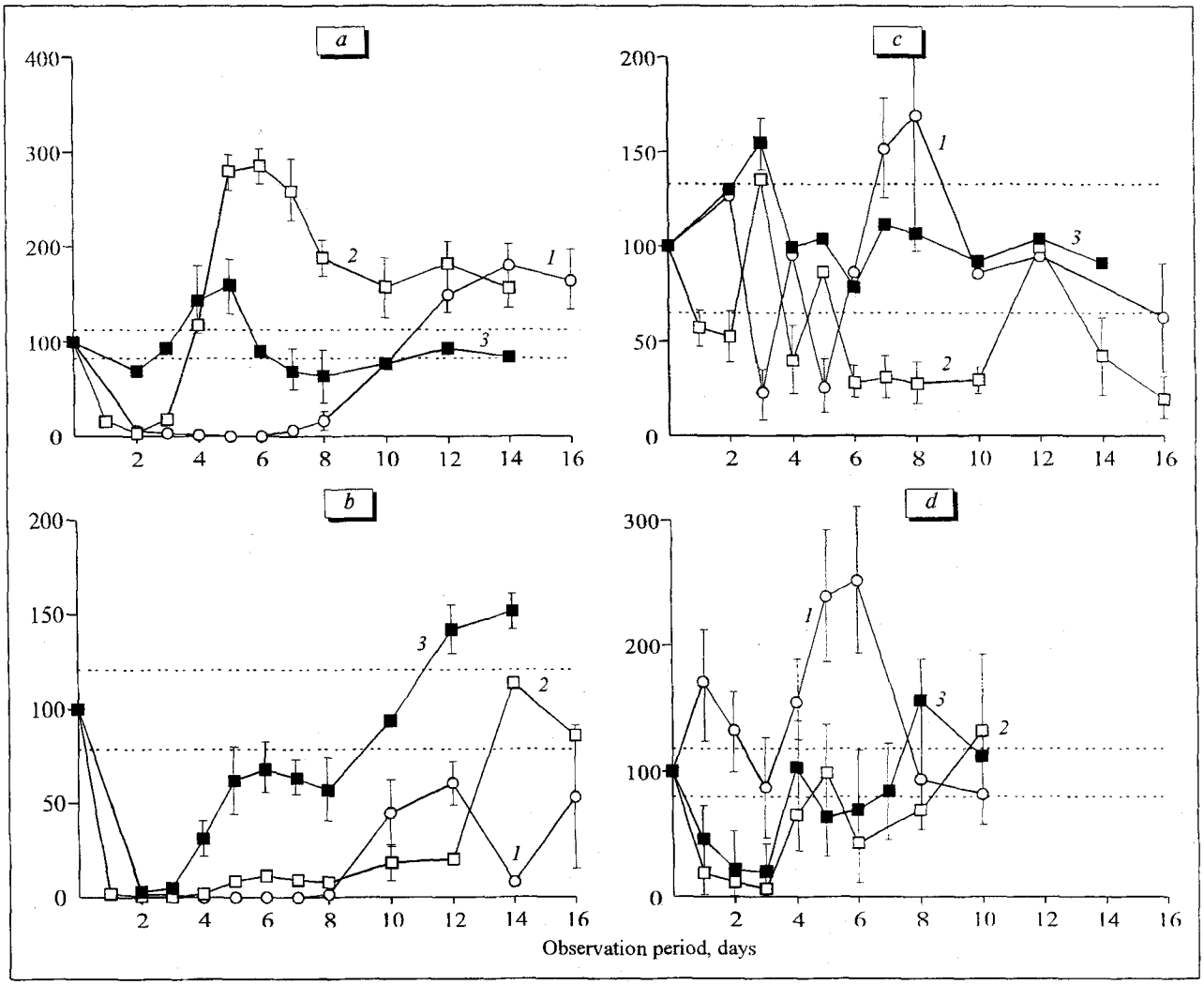

Fig. 2. Content of immature neutrophil granulocytes (*a*), erythroid cells (*b*), monocytes and macrophages (*c*), and Thy-1$^+$ cells (*d*) in the bone marrow of CBA mice treated with 5-fluorouracil (*1*), cyclophosphane (*2*), and adriamycin (*3*) in maximum tolerated doses. Ordinate: cell content in bone marrow (% of the initial level). Confidence intervals at p=0.05.

(Vektor), 10% embryonal calf serum (Vektor), 280 mg/liter L-glutamine (Sigma), 50 mg/liter gentamicin (Bosnalijek), and 5×10^{-5} M 2-mercaptoethanol and incubated for 24 h at 37°C, 3.5% CO_2 and 100% humidity. The supernatants were collected and stored at -20°C for no more that one month.

CSA and EPA of the conditioned media from the adherent and nonadherent myelokaryocytes and in sera from the control and experimental mice were assayed in methyl cellulose cultures as described previously [4]. The results were expressed as the number of colonies formed by 10^5 intact syngeneic nonadherent myelokaryocytes in the presence of the test samples. The content of T regulators in the bone marrow was determined by the complement-dependent cytotoxic test using monoclonal anti-Thy-1 antibodies (Diagnotekh).

The data were processed by analysis of variances using the Student's t test or nonparametric Mann—Whitney U test (when variances did not fit to normal distribution).

RESULTS

Cytostatics considerably changed the production of various cytokines by bone marrow nuclear cells. In our experiments, the total hemopoiesis-stimulating activity of these cytokines was judged from CSA and EPA. Cyclophosphane enhanced the production of these factors by adhesive myelokaryocytes (among these, monocyte-macrophage precursors exhibited the maximum hemopoiesis-stimulating activity) by 500-800% from the initial level soon postinjection, and this parameter remained above the baseline throughout the experiment (Fig. 1, *a*, *b*). Adriamycin also stimulated the production of CSA and in some experimental points EPA by adherent HIM cells, but the latter parameter decreased on days 2-3 post-

injection. On the other hand, in the supernatants of adhesive cells from 5-fluorouracil-treated mice, the rise of EPA and CSA was noted only on days 7-8 of the experiment (Fig. 1, *a*, *b*), which points to a toxic effect of this antimetabolite on the functional activity of monocytes and macrophages. It should be noted that the content of monocyte-macrophage precursors in the bone marrow of cytostatic-treated mice tended to decrease (Fig. 2, *c*), while the rise of specific CSA and EPA of the adherent fractions coincided with the periods when the number of these cells returned to or even surpassed the normal. This fact suggests that cyclophosphane and adriamycin induce an early (days 2-3) increase in the total CSA and EPA produced by stromal bone marrow cells, while the effect of 5-fluorouracil is noted only on days 7-8 postinjection.

Cytotoxic test revealed different dynamics of Thy-1$^+$ cells in the hemopoietic tissue of mice treated with different cytostatics. These cells belong to the T lymphocyte population [10] and are characterized by maximum cytokine production (among adhesive myelokaryocytes) [10,13]. Cyclophosphane sharply reduced the content of Thy-1$^+$ cells in the bone marrow; this suppression persisted to day 10 of experiment (Fig. 2, *d*). Simultaneous decrease in the secretory activity of nonadherent fractions from the cyclophosphane-treated mice (Fig. 1, *c*, *d*) attests to markedly reduced CSA and EPA of total bone marrow T cells population (cyclophosphane is a potent immunodepressant [12]). In mice injected with adriamycin, the content of Thy-1$^+$ cells in the bone marrow was reduced during the first 3 days (20% of the initial level of day 3 postinjection), nevertheless specific CSA and EPA in the supernatants of nonadhesive myelokaryocytes were considerably elevated starting from day 1 of the experiment. The pool of regulatory cells restored starting from day 4 (Fig. 2, *d*) against the background of intense production of EPA and CSA. Injection of 5-fluorouracil induced accumulation of T cells in the bone marrow on days 4-6 followed by augmentation of their secretory activity, CSA and EPA in the conditioned media of nonadherent cells peaked on day 8 of the experiment (Fig. 1, *c*, *d*). These findings clearly demonstrate the important role of humoral factors produced by HIM cells in the stimulation of cytostatic-suppressed hemopoiesis.

Treatment with cytostatics is by a number of parameters a stress factor for the organism. In particular, the development of general adaptive syndrome manifests itself in activation of the neurotransmitter systems of the hypothalamus, adrenal cortex, and the sympathoadrenal system as soon as one day after injection of 5-fluorouracil and cyclophosphane in doses close to those used in our experiments [2]. However, none of the administered antitumor drugs increased serum CSA (Fig. 1, *f*), representing an integral index of the total effect produced by all bioactive serum components. This fact, however, does not contradict the above-mentioned published data, since stress-realizing hormones, in particular, glucocorticoids, have an indirect effect on proliferation and differentiation of hemopoietic cells and not only stimulate but also inhibit these processes [1,3,6]. Dynamics of serum EPA in mice injected with cytostatics also did not correspond to the dynamics of activation of the stress-realizing systems (the absence of an EPA peak on day 1 postinjection). Serum EPA is primarily determined by renal erythropoietin [14], and therefore the rise of this parameter at the later stages of experiment is most probably related to anemia due to considerable hypoplasia of the erythron (Fig. 2, *b*).

Our findings suggest that in cytostatic myelodepressions, hemopoietic growth factors produced by HIM cells are more important for restoration of hemopoiesis than humoral factors of peripheral blood. Secretory activity of HIM strongly determines the intensity of recovery of granulo- and erythropoiesis after administration of different cytostatics (Fig. 1 and 2)

REFERENCES

1. Yu. M. Bala, B. I. Sidel'nikova, and V. M. Lifshits, *Pat. Fiziol.*, No. 4, 60-61 (1991).
2. K. P. Balitskii and Yu. P. Shmal'ko, *Stress and Metastatic Spreading of Malignant Tumors* [in Russian], Kiev (1987).
3. E. D. Gol'dberg, A. M. Dygai, Yu. M. Zakharov, *et al.*, *Pat. Fiziol.*, No. 3, 7-10 (1991).
4. E. D. Gol'dberg, A. M. Dygai, and V. P. Shakhov, *Tissue Culture in Hematology* [in Russian], Tomsk (1992).
5. A. M. Dygai, N. A. Klimenko, E. V. Abramova, *et al.*, *Pat. Fiziol.*, No. 6, 28-31 (1991).
6. A. M. Dygai, V. P. Shakhov, E. V. Kirienkova, *et al.*, *Biol. Nauki*, No. 12, 71-76 (1990).
7. K. P. Zak, *Endocrinology Today* [in Russian], Kiev (1982), pp. 173-191.
8. V. V. Men'shikov (Ed.), *Laboratory Tests in the Clinical Practice. A Manual* [in Russian], Moscow (1987).
9. D. G. Natan and K. A. Ziff, *Hematol. Transfuziol.*, **39**, No. 2, 3-10 (1994).
10. I. G. Khrushchev, V. I. Starostin, E. I. Domaretskaya, *et al.*, *Advances in Science and Technology. Ser. Human and Animal Morphology* [in Russian], Vol. 13, Moscow (1988), pp. 73-81.
11. A. P. Yastrebov, B. G. Yushkov, and V. N. Bol'shakov, *Regulation of Hemopoiesis under the Action of Extreme Factors on the Organism* [in Russian], Sverdlovsk (1988).
12. B. Glass, L. Uharek, T. Gaska, *et al.*, *Bone Marrow Transplant*, **12**, No. 1, 41-47 (1993).
13. D. Metcalf, *Lancet*, No. 8642, 825-827 (1989).
14. S. Sakata, Y. Enoki, and M. Ueda, *Zool. Sci.*, **9**, No. 6, 1251 (1992).