Role of Cell-Cell Interactions in the Regulation of Hemopoiesis during Cytostatic-Induced Myelosuppression

V. V. Zhdanov, E. V. Udut, L. A. Gur'yantseva, M. G. Danilets, A. M. Dygai, and E. D. Gol'dberg

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 132, No. 8, pp. 156-160, August, 2001
Original article submitted April 4, 2001

The content of hemopoietic precursors in the bone marrow, its morphological composition, structural and functional organization, binding of hemopoietic precursors by adherent cells, and expression of receptors for erythroblasts and sialoadhesin on bone marrow macrophages were studied in CBA/CaLac mice with hypoplasia of hemopoiesis caused by etoposide in a maximum permissible dose. It was found that recovery of hemopoiesis after cytostatic treatment was related to accelerated maturation of hemopoietic precursors and increased ability of microenvironmental cells to bind hemopoietic cells.

Key Words: *etoposide; hemopoietic microenvironment; hemopoietic islet; erythroblast receptors; sialoadhesin receptors*

Bone marrow hemopoiesis depends on secretion of humoral regulators by cells of the hemopoiesis-inducing microenvironment (HIM) and presence of cell-cell contacts [3,12,13]. Mature bone marrow macrophages playing an important role in the maturation of hemopoietic cells are the main components of HIM [1]. These macrophages express various receptors and adhesion molecules, including receptors for erythroblasts and sialoadhesin, that bind immature erythroid and granulocytic cells through specific ligands [13]. The cooperative interaction of macrophages, fibroblasts, or endotheliocytes with hemopoietic cells contributes to the formation of specific structural and functional elements named hemopoietic islets (HI). Proliferation and maturation of hemopoietic cells occur in HI [4,6,12]. Previous studies of the effects of various cytostatics (cyclophosphamide, 5-fluorouracil, and adriamycin) on HIM revealed pronounced structural disturbances in the bone marrow. It was shown that the ability of HIM cells to bind hemopoietic precursors plays an important role in the formation of HI and recovery of hemopoiesis. The antitumor plant preparation etoposide widely used in anticancer therapy markedly suppresses hemopoiesis [7,10]. Here we studied the mechanisms underlying changes in structural and functional characteristics of the bone marrow and their role in the recovery of hemopoiesis during etoposide-induced myelosuppression.

Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences, Tomsk

MATERIALS AND METHODS

Experiments were performed on 170 male CBA/CaLac mice aging 2.0-2.5 months and weighing 18-22 g (nursery of the Institute of Pharmacology, Tomsk Research Center). The animals received single intraperitoneal injection of etoposide (Bristol-Myers) in a maximum permissible dose (MPD 10 mg/kg, probit analysis) [10]. The animals were euthanized by cervical dislocation under ether anesthesia on days 1-8, 10, 12, 14, and 16 after cytostatic administration. The total count of bone marrow myelokaryocytes was estimated. The qualitative composition of the bone marrow was assayed in smears stained by the method of Nokht—Maksimov. Cloning of erythropoietic (CFU-E) and granulomonocytopoietic precursors (CFU-GM) from nonadherent myelokaryocytes was performed in a methylcellulose culture [2]. We counted colonies of bone

0007-4888/01/1322-0744$25.00

marrow cells from intact mice before and after 2-h incubation with adherent bone marrow cells from cytostatic-treated animals. The ability of adherent bone marrow cells from etoposide-treated mice to bind intact CFU-GM and CFU-E was estimated by the difference between the counts of colonies. To evaluate structural and functional organization of the bone marrow, HI were enzymatically isolated, stained with neutral red, and counted in the Goryaev chamber [2]. Expression of sialoadhesin receptors on bone marrow macrophages was determined using the suspension of sheep erythrocytes in phosphate-buffered saline containing fetal bovine serum [12]. Expression of erythroblast receptors on bone marrow macrophages was determined using a suspension of embryonic liver (nonadherent fraction) in phosphate-buffered saline containing fetal bovine serum [14]. The results were analyzed by Student's *t* test [8].

RESULTS

Etoposide decreased the cellularity of granulocytic and erythroid bone marrow stems. Normalization of the contents of immature neutrophilic granulocytes, mature neutrophilic leukocytes, and erythroid cells in the bone marrow started 3 days after etoposide administration (Fig. 1, *a*, *b*).

The counts of CFU-GM and CFU-E in the hemopoietic tissue increased 1-7 and 1-8 days after treatment, respectively, returned to normal on day 10, and remained unchanged to the end of observations (Fig. 1, *c*, *d*).

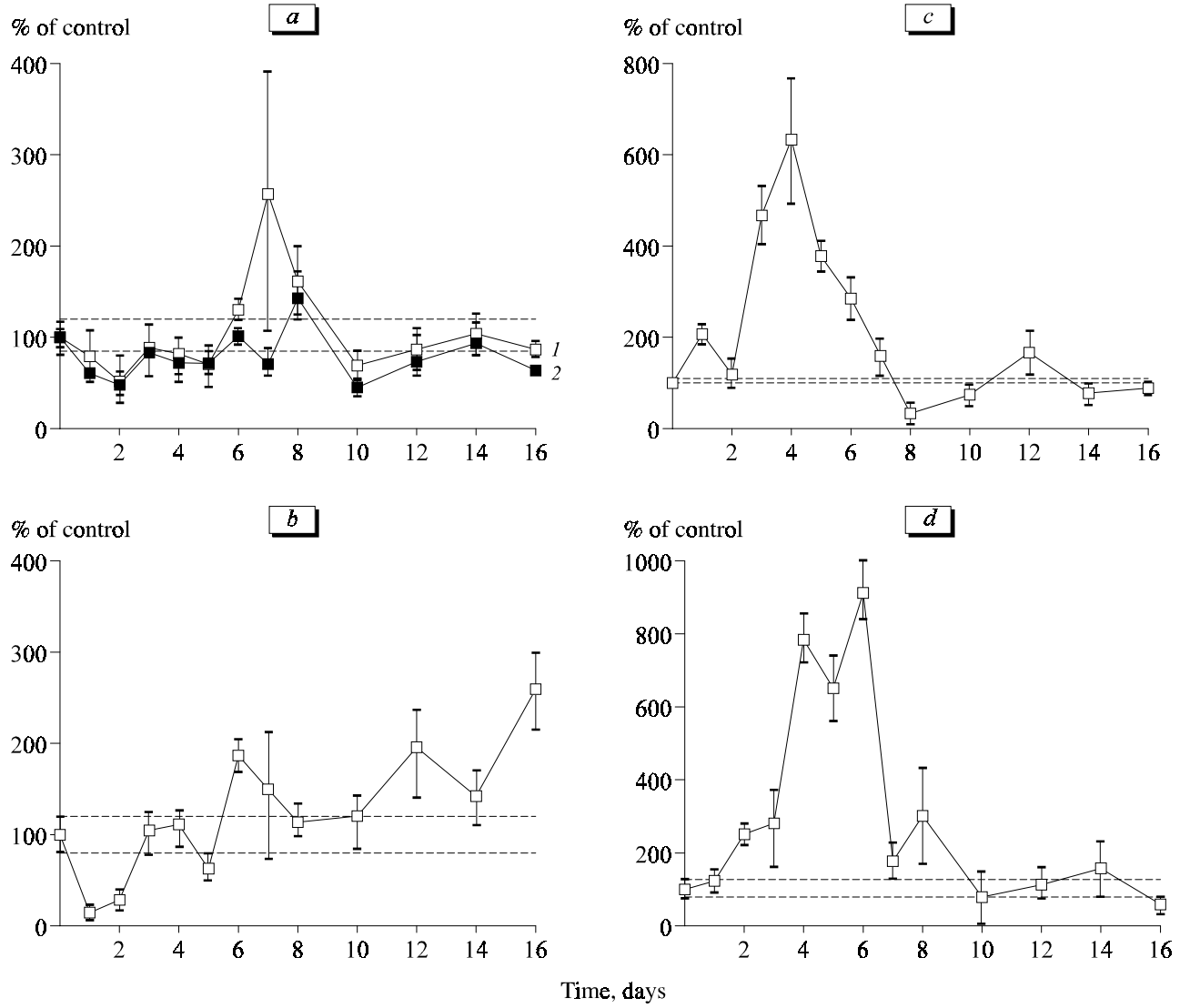

Fig. 1. Contents of immature (*1*) and mature (*2*) neutrophilic granulocytes (*a*), erythrokaryocytes (*b*), granulomonocytopoietic (*c*) and erythropoietic CFU (*d*) in the bone marrow of CBA/CaLac mice receiving etoposide in MPD. Here and in Figs. 2 and 3: confidence intervals at p=0.05 are shown by dotted lines.

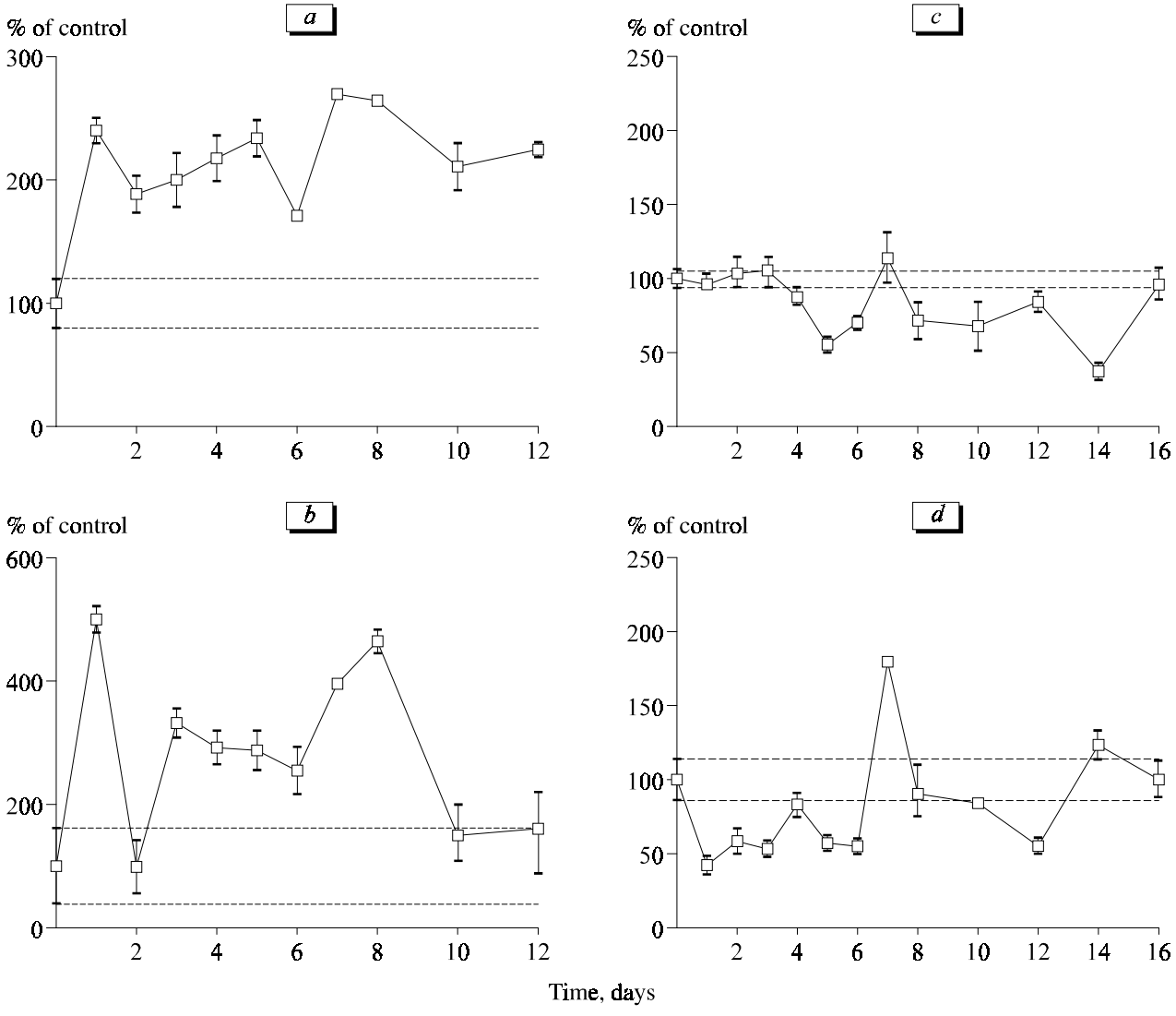

Fig. 2. Binding of intact granulomonocytopoietic (*a*) and erythropoietic CFU (*b*) by adherent bone marrow cells and contents of granulocytic (*c*) and erythroid hemopoietic islets (*d*) in the bone marrow of CBA/CaLac mice treated with etoposide.

This process was accompanied by structural and functional in changes the bone marrow. The total number of HI islets decreased, but then increased to 124% of the initial level (day 7). This was primarily related to an increase in the count of erythroid HI (Fig. 2, *d*). Migration of granulocytic cells from the bone marrow was suppressed after cytostatic treatment (Fig. 2, *c*).

Expression of membrane-bound erythroblast receptors on macrophages in cytostatic-treated mice tended to decrease by 12 and 17% on days 2 and 10 after treatment, respectively (insignificantly). These changes were followed by a decrease in the count of erythroid HI by 53 and 55% on days 3 and 12 after cytostatic administration, respectively (Fig. 3, *a*). The increase in the expression of sialoadhesin receptors on bone marrow macrophages by 125% on days 7, 8, and 10 probably contributed to normalization of the number of granulocytic HI (Fig. 3, *b*). Since these changes occur later than reparative processes, they do not underlie the recovery of erythroid and granulocytic hemopoietic stems.

The binding of CFU-GM and CFU-E by adherent bone marrow cells increased to 240 and 500%, respectively, on the next day after treatment (Fig. 2, *a*, *b*). The count of intact precursors bound to adherent bone marrow cells surpassed the control in all periods after cytostatic administration. This increase in the affinity of adherent HIM cells to hemopoietic precursors against the background of severe structural and functional disorganization of the bone marrow probably potentiates precursor binding to fibroblasts and macrophages not involved in the formation of HI. These changes lead to the formation of class III HI with high cellularity, which is related to the binding of several precursors to one central cell [5].

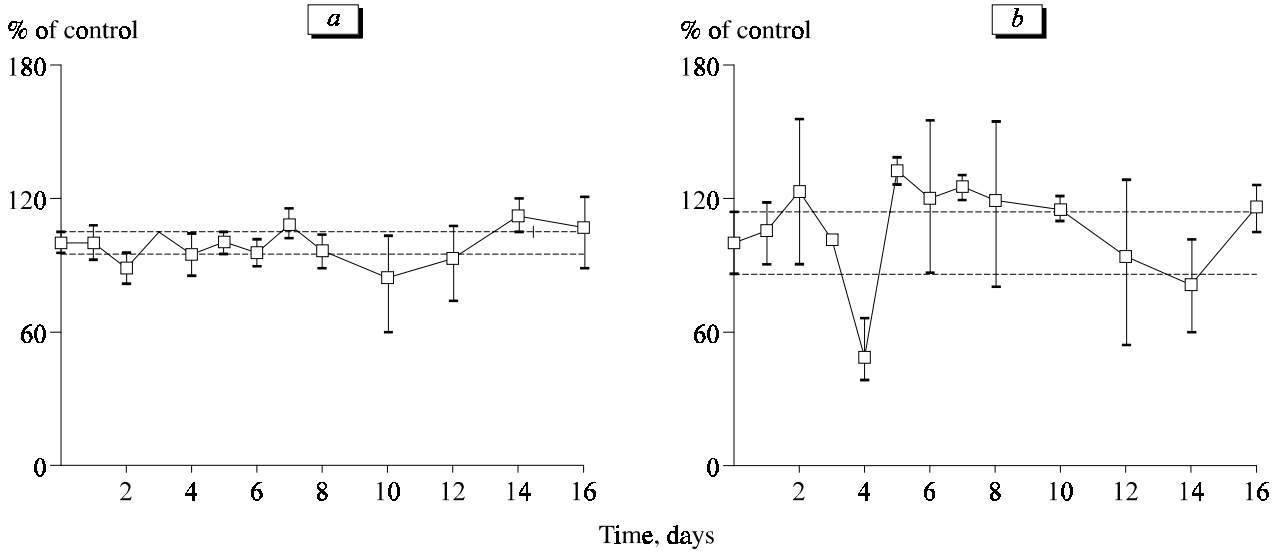

Fig. 3. Expression of erythroblasts (*a*) and sialoadhesin (*b*) receptors on bone marrow macrophages from CBA/CaLac mice receiving etoposide in MPD.

Our results and previous data on changes in secretion of hemopoietic growth factors indicate that adhesion of hemopoietic cells to HIM elements contributes to the regulation of functional activity of hemopoietic precursors [11]. The increase in the ability of stromal cells to interact with CFU-E probably plays the major role in the regeneration of the erythroid hemopoietic stem at the early stages after cytostatic treatment. The recovery of erythropoiesis at the late stages after etoposide administration is associated with intensive secretion of hemopoietic growth factors by adherent bone marrow cells (erythropoietic activity) and increase in plasma erythropoietic activity. These processes are simultaneously involved in the normalization of bone marrow granulocytopoiesis.

REFERENCES

1. E. D. Gol'dberg, A. M. Dygai, and V. V. Zhdanov, *Role of Hemopoietic Microenvironment in the Regulation of Hemopoiesis during Cytostatic Myelosuppressions* [in Russian], Tomsk (1999).
2. E. D. Gol'dberg, A. M. Dygai, and V. P. Shakhov, *Tissue Cultures in Hematology* [in Russian], Tomsk (1992).
3. E. D. Gol'dberg, A. M. Dygai, and V. P. Shakhov, *Role of Cell-Cell Interactions in the Regulation of Hemopoiesis* [in Russian], Tomsk (1989).
4. A. M. Dygai, N. A. Klimenko, B. Yu. Gumilevskii, and E. D. Gol'dberg, *Byull. Eksp. Biol. Med.*, **113**, No. 5, 514-516 (1992).
5. Yu. M. Zakharov and L. P. Varypaeva, *Gematol. Transfuziol.*, No. 10, 21-23 (1992).
6. Yu. M. Zakharov and I. Yu. Mel'nikov, *Ibid.*, No. 10, 51-56 (1984).
7. G. V. Karpova, T. I. Fomina, E. A. Timina, *et al.*, *Eksp. Klin. Farmakol.*, **61**, No. 2, 51-53 (1998).
8. G. F. Lakin, *Biometry* [in Russian], Moscow (1973).
9. O. I. Naumenko, *Eksp. Onkol.*, **14**, No. 1, 11-20 (1992).
10. E. A. Timina, *Myelotoxicity of Vepeside*, Abstract of Cand. Med. Sci. Dissertation, Tomsk (1998).
11. E. V. Udut, *Problems of Experimental and Clinical Pharmacology* [in Russian], Tomsk (2000), pp. 48-50.
12. I. Blazsek, X. H. Liu, and A. Anjo, *Exp. Hematol.*, **23**, No. 4, 309-319 (1995).
13. P. R. Crocker and S. Gordon, *J. Exp. Med.*, **162**, No. 3, 993-1014 (1985).
14. P. R. Croker, L. Moris, and S. Gordon, *J. Cell Sci.*, **9**, Suppl. P, 185-206 (1988).
15. N. Soni, N. J. Meropol, L. Pendyala, *et al.*, *J. Clin. Oncol.*, **15**, No. 2, 766-772 (1997).