Role of Hemopoietic Growth Factors in Regeneration of Hemopoiesis during Etoposide-Induced Myelosuppression

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We studied structural and functional organization of the bone marrow, production of hemopoietic growth factors by hemopoietic cells, and plasma colony-stimulating and erythropoietic activities in CBA/CaLac mice treated with etoposide. The effects of etoposide on cultured hemopoietic and microenvironmental cells were also evaluated. Our results indicate that hemopoietic growth factors secreted by adherent bone marrow cells play the major role in the normalization of hemopoiesis during etoposide-induced myelosuppression.

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

Hemopoietic microenvironment (HIM) plays an important role in the regulation of hemopoiesis under physiological and pathological conditions. Local regulation of proliferation and differentiation of hemopoietic elements by HIM cells is realized through secretion of humoral substances and signal transduction during cell-cell interactions [1,3,8]. Alteration of HIM cells caused by extreme factors, including cytostatics, modulate suppression or recovery of hemopoiesis [1].

The podophyllotoxin derivative etoposide and other cytostatics widely used in anticancer therapy markedly suppress hemopoiesis [4,6,7,9]. The mechanisms of changes caused by etoposide and other preparations should be taken into account during prevention and correction of their toxic effects on the hemopoietic tissue. Although the effects of etoposide are extensively studied, the state of HIM and peculiarities of local regulation of postcytostatic regeneration of hemopoiesis after treatment with this substance are still poorly understood. Here we studied the role of HIM and its elements in the regulation of hemopoiesis during etoposide-induced myelosuppression.

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MATERIALS AND METHODS

Experiments were performed on 170 male CBA/CaLac mice aging 2-2.5 months and weighing 18-22 g (Institute of Pharmacology, Tomsk Research Center). The animals received single intraperitoneal injection of etoposide (Bristol-Myers) in a maximum tolerated dose (MTD 10 mg/kg, probit analysis) [7]. The animals were euthanized by cervical dislocation under ether anesthesia on days 1-8, 10, 12, 14, and 16 after cytostatic treatment. The total count of bone marrow myelokaryocytes was estimated and qualitative analysis of the bone marrow was performed on smears stained by the method of Nocht—Maksimov. The contents of committed erythropoietic (CFU-E) and granulomonocytopoietic precursors (CFU-GM) in the bone marrow were determined by *in vitro* cloning [2]. For evaluation of the structural and functional organization of the bone marrow, the number of hemopoietic islets (HI) was estimated after collagenase dissociation and neutral red staining [2]. Colony-stimulating (CSA) and erythropoietic activities (EPA) of conditioned media of adherent and nonadherent HIM cells and blood plasma were tested on intact mouse myelokaryocytes cultured in semisolid medium [2]. *In vitro* effects of 10^{-6} M etoposide on HIM elements were evaluated by the

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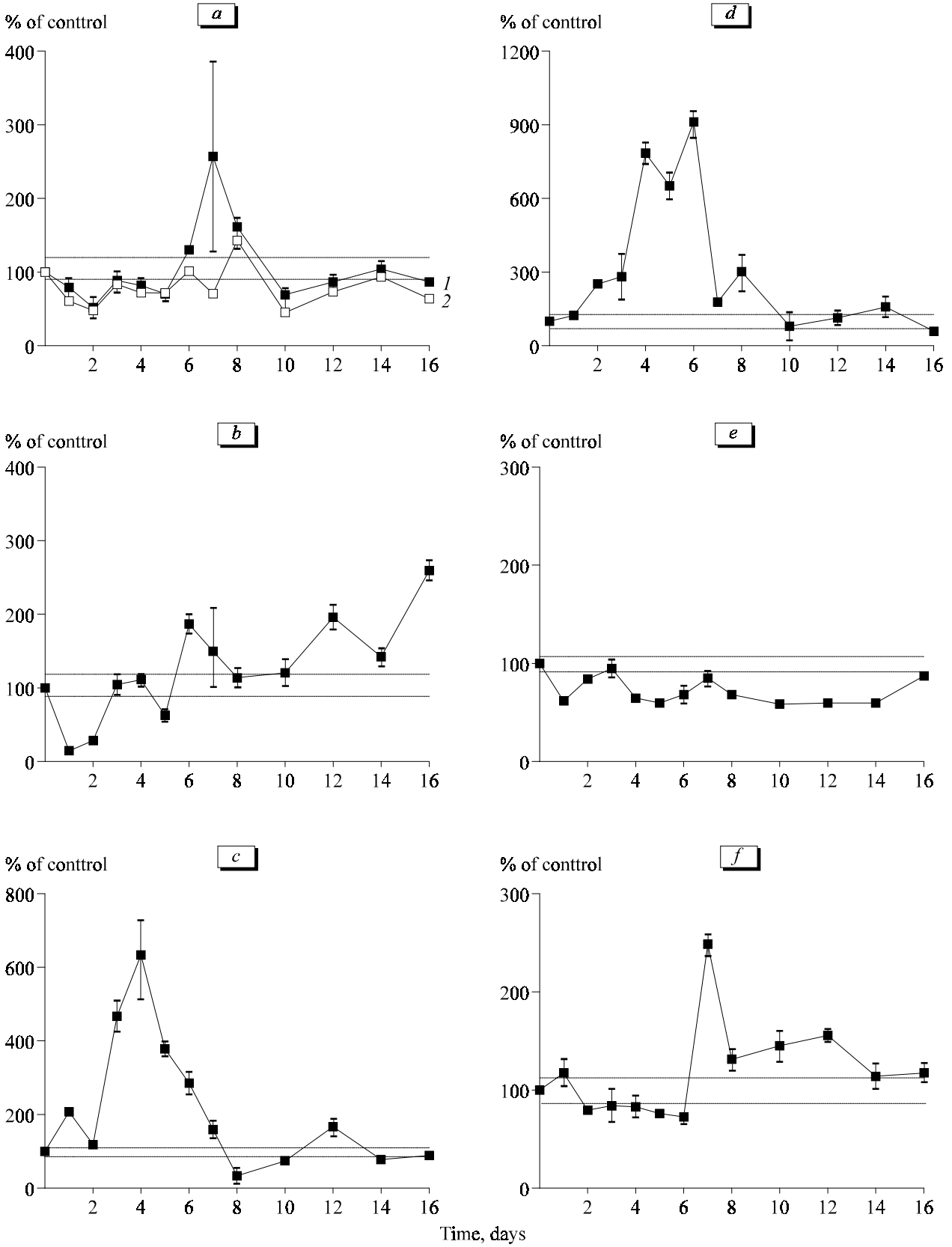

Fig. 1. Contents of immature (*1*) and mature (*2*) neutrophilic granulocytes (*a*), erythrokaryocytes (*b*), CFU-GM (*c*), CFU-E (*d*), and macrophage-negative (*e*) and macrophage-positive hemopoietic islets (*f*) in the bone marrow of CBA/CaLac mice treated with etoposide in MTD. Here and in Figs. 2 and 3: confidence intervals at p=0.05 are shown by dotted lines.

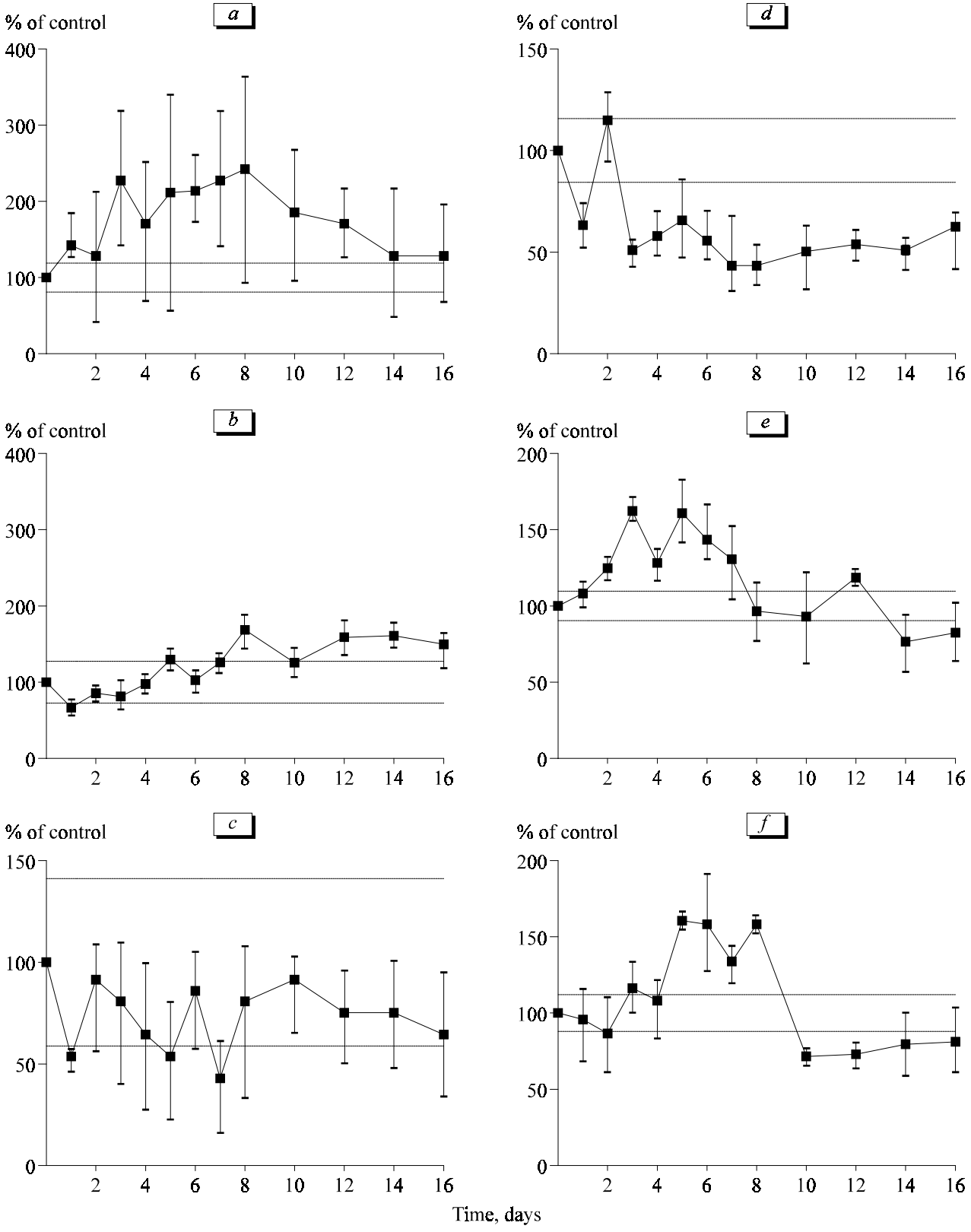

Fig. 2. Colony-stimulating and erythropoietic activities in supernatants of adherent (*a*, *b*) and nonadherent myelokaryocytes (*c*, *d*) and plasma (*e*, *f*) in CBA/CaLac mice treated with etoposide in MTD.

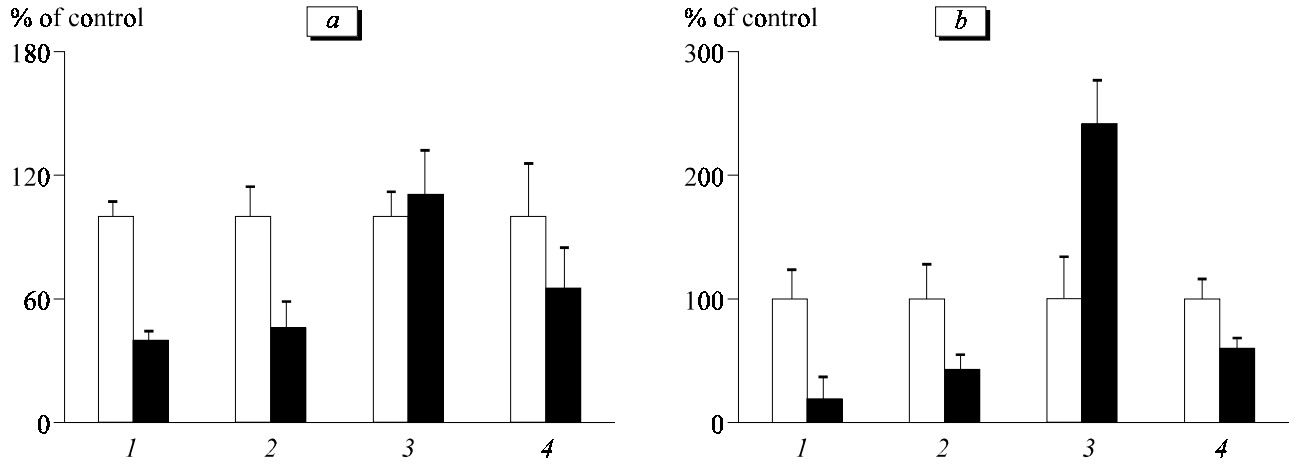

Fig. 3. Effects of 10^{-6} M etoposide on the growth of erythroid (*a*) and granulocyte-macrophage (*b*) colonies from the bone marrow of intact mice: unfractionated bone marrow cells (*1*), nonadherent (*2*) and adherent bone marrow cells (*3*), and nonadherent bone marrow cells preincubated (dark bars) or not preincubated with etoposide (light bars). Ordinate: bone marrow colony-forming ability.

ability of cytostatic-treated cultured cells to maintain the growth of granulocyte-macrophage and erythroid colonies from intact nonadherent myelokaryocytes. The results were analyzed by Student's *t* test [5].

RESULTS

Single injection of etoposide in MTD decreased the total cellularity of the bone marrow due to a decrease in the content of immature and mature neutrophilic granulocytes, nucleated erythroid cells, lymphoid elements, and monocytes-macrophages. Normalization of the neutrophilic stem started 3 days after etoposide administration. The contents of immature and mature granulocytes surpassed the control on days 6-8 and 8, respectively (Fig. 1, *a*). The content of bone marrow erythroid cells returned to normal 3 days after etoposide treatment, and after 6 days this parameter reached 187% of the control (Fig. 1, *b*).

These changes in the myelogram were preceded by an increase in the bone marrow CFU-GM content (days 1-7), which was maximum 4 days after treatment (633% of the control). The count of CFU-GM decreased to 33% of the control on day 8, but returned to the control 10 days after etoposide treatment (Fig. 1, *c*). The count of CFU-E increased 1-8 days after treatment, reached maximum on days 4 and 6 (784 and 912% of the control, respectively), and did not differ from the initial level from day 10 to the end of observations (Fig. 1, *d*).

Changes in structural and functional organization of the bone marrow were characterized by a decrease in the total number of HI. This parameter increased only on day 7 after treatment (124% of the control), which was associated with an increase in the number of macrophage-positive HI (Fig. 1, *f*). Migration of stromal cell-containing structural and functional elements from the bone marrow was suppressed throughout the experiment (Fig. 1).

Production of humoral hemopoietic regulators is the major parameter characterizing functional activity of HIM cells. The maximum increase in CSA in conditioned media of adherent mouse myelokaryocytes was observed on days 3, 6, and 7 after etoposide administration (Fig. 2, *a*). It can be hypothesized that the increased CSA in supernatants of adherent cells determines the increased content of committed and morphologically visualized granulocytic cells at the early stages after cytostatic treatment (despite impaired structural and functional organization of the bone marrow). EPA peaked on days 8-16 after cytostatic administration (Fig. 2, *b*).

Production of CSA by bone marrow nonadherent cells remained practically unchanged, while secretion of EPA by these cells decreased and dropped to a minimum on days 7-8 (Fig. 2, *c*, *d*).

To exclude the effects of systemic reactions to etoposide, we studied functional activity of HIM cells treated with cytostatic in culture. Treatment of adherent hemopoietic cells with etoposide enhanced their ability to maintain the growth of granulocytic and erythroid precursors by 142 and 11%, respectively (Fig. 3), but decreased feeder activity of nonadherent cells with respect to both CFU-GM and CFU-E (Fig. 3).

We also studied hemopoiesis-stimulating properties of peripheral blood plasma from mice treated with etoposide. Plasma CSA increased on days 2-7, reached maximum on days 3 and 5 (Fig. 2, *e*), and then returned to normal. Plasma EPA increased on day 5, but decreased 10 days after treatment (Fig. 2, *f*).

Therefore, etoposide stimulates secretory and feeder activities of adherent cells (monocytes, macrophages, and fibroblasts), but impairs functional properties of nonadherent cells (lymphocytes).

Our findings indicate that the recovery of bone-marrow granulocytopoiesis in animals receiving etoposide in MPD is related to intensive secretion of hemopoietic growth factors by adherent bone marrow cells and high plasma CSA in the early stage after cytostatic treatment. Reparation of the erythron does not depend on stimulation of secretory activity of HIM cells and plasma erythropoietin concentration, since these processes occur at the late stages after cytostatic treatment.

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