Role of Humoral Factors in the Regulation of Hemopoiesis during Cytostatic-Induced Myelosuppression

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The content of hemopoietic precursors in the bone marrow, its morphological composition, production of hemopoietic growth factors by cells of the hemopoietic microenvironment, erythropoietic activity, and plasma content of endogenous erythropoietin were studied in CBA/CaLac mice with hypoplasia of hemopoiesis induced by adriamycin in a maximum permissible dose. Cytostatic treatment affected production of substances determining erythropoietic activity by adherent and nonadherent cells of the bone marrow. Erythropoietic activity of peripheral blood plasma considerably increased. Recovery of erythroid hemopoiesis was determined by the effects of endogenous erythropoietin and other substances secreted by cells of the hemopoietic microenvironment and present in the peripheral blood.

Key Words: *adriamycin; erythropoietic activity; erythropoietin*

Study of the mechanisms underlying regulation of hemopoiesis under normal and pathological conditions is an important problem of experimental hematology. Cytostatic-induced myelosuppression is a suitable model for evaluation of functional activity in the regulatory apparatus of the blood system under extreme conditions. These myelosuppressions are induced by antitumor preparations with various mechanisms of action producing different effects on hemopoiesis-regulating structures [2]. Previous experiments showed that the anthracycline antibiotic adriamycin in high doses suppresses bone marrow hemopoiesis (primarily erythropoiesis) and produces opposite changes in structures responsible for local and distant regulation of hemopoiesis. These changes are manifested in the formation of hemopoietic islets by mature macrophages, increase in secretory activity of cells in the hemopoietic microenvironment, and rise in erythropoietic activity (EPA) of the plasma [1]. The role of individual humoral factors regulating erythropoiesis in its recovery should be evaluated to understand the mechanisms underlying regulation of hemopoiesis. According to current concepts, erythropoietin secreted into the blood by peritubular cells of the kidneys, hepatocytes, and mononuclear phagocytes is the major regulator of proliferation and maturation of erythroid cells [6,13,14].

Here we studied the role of erythropoietin in the regulation of erythroid hemopoiesis during adriamycin-induced myelosuppression.

MATERIALS AND METHODS

Experiments were performed on 350 CBA/CaLac mice (class I conventional mouse strain) aging 2 months and obtained from the nursery of the Laboratory of Experimental Biomedical Modeling (Institute of Pharmacology, Tomsk Research Center). The animals received single intraperitoneal injection of adriamycin in a maximum permissible dose (MPD, 6 mg/kg). The

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mice were euthanized by cervical dislocation on days 1-12 after cytostatic treatment. The count of peripheral blood erythrocytes, reticulocytes, and leukocytes was determined by routine blood tests. The total number of bone marrow myelokaryocytes was estimated. The qualitative composition of the bone marrow was assayed in smears stained by the method of Nokht—Maksimov [7]. The content of committed erythroid precursors (CFU-E) in the bone marrow was estimated by *in vitro* cloning in a methylcellulose culture [3]. EPA in conditioned media of adherent and nonadherent hemopoietic cells and blood plasma was measured using a semisolid medium with myelokaryocytes from intact mice [3]. The role of endogenous erythropoietin in the formation of EPA was evaluated by suppression of erythroid colony growth after treatment of bone marrow supernatants and peripheral blood plasma with monoclonal antibodies against mouse erythropoietin (PharMingen). Erythropoietin content in the plasma from experimental animals was measured by enzyme immunoassay with Sangui Bio Tech Inc. kits according to methodical recommendations. The results were analyzed by Student's *t* test.

RESULTS

Adriamycin produced considerable changes in the blood system of animals. Suppression of bone marrow hemopoiesis (primarily erythropoiesis) was observed 1 day after single intraperitoneal injection of the preparation. On days 3-5 after treatment the total number of peripheral blood erythrocytes and count of reticulocytes decreased by 20 and 94%, respectively. The test parameters progressively returned to normal starting from day 6. Pronounced reticulocytosis was revealed 9 days after cytostatic treatment (166% of the baseline level, Fig. 1, *a*). The total number of myelokaryocytes decreased most significantly on day 3, which was related to suppression of erythroid and lymphoid hemopoiesis. Erythrokaryocyte count in the femur decrea-

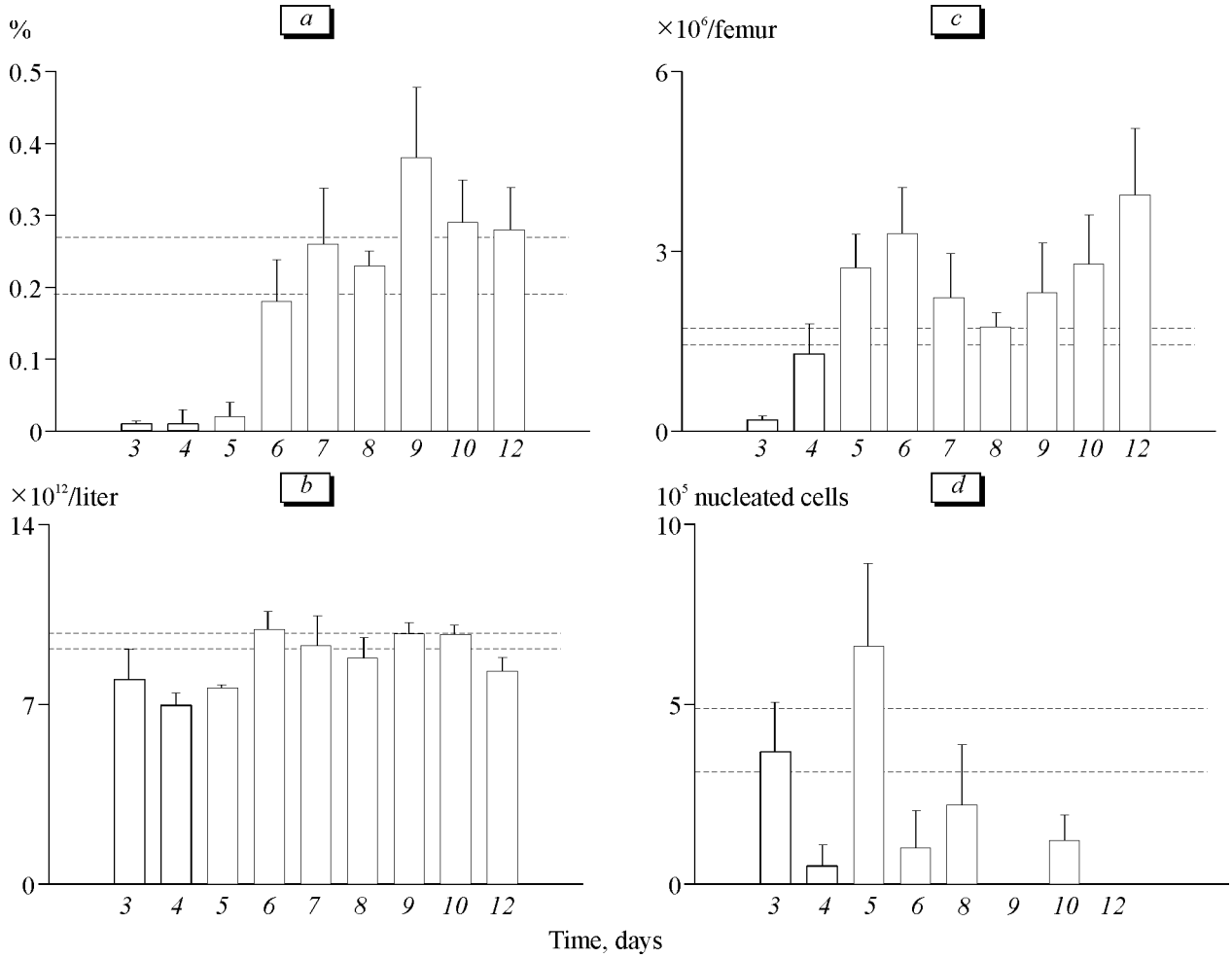

Fig. 1. Contents of reticulocytes (*a*) and erythrocytes in the peripheral blood (*b*) and erythrokaryocytes (*c*) and committed erythroid precursors in the bone marrow (*d*) of CBA/CaLac mice receiving adriamycin in MPD. Here and in Fig. 2: upper and lower confidence limits (p=0.05) for baseline levels are shown by dotted lines.

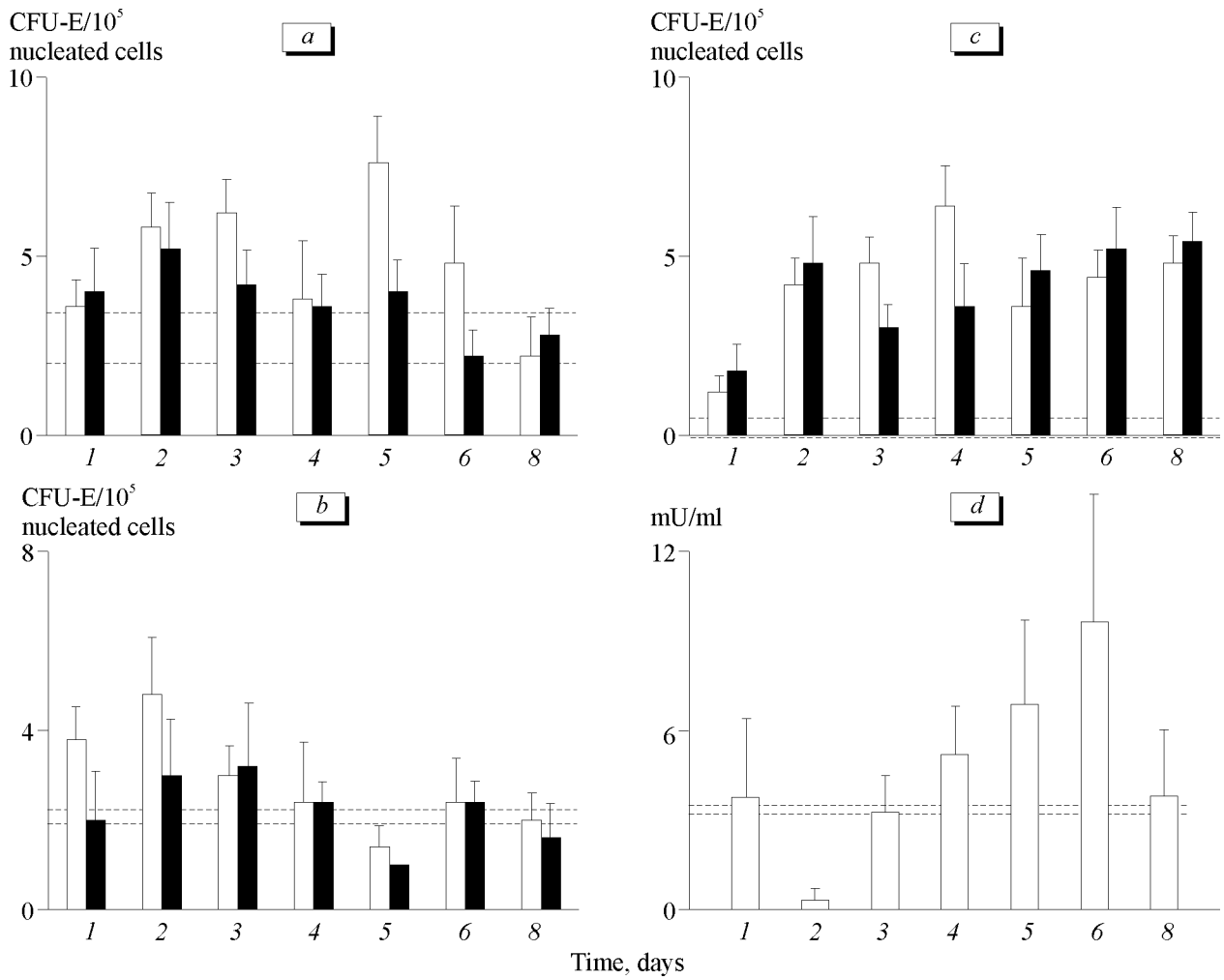

Fig. 2. Erythropoietic activity in supernatants of nonadherent (*a*) and adherent bone marrow cells (*b*) and peripheral blood plasma (*c*) and plasma erythropoietin content (enzyme immunoassay, *d*) in CBA/CaLac mice receiving adriamycin in MPD. Dark bars, additional treatment of samples with monoclonal antibodies against mouse erythropoietin; light bars: untreated samples (*a*, *b*, *c*).

sed from $(1.58\pm0.07)\times10^6$ to $(0.19\pm0.03)\times10^6$, but then rapidly returned to normal and even surpassed it on day 5 (Fig. 1, *c*). Intensive regeneration of the erythroid stem in the bone marrow was accompanied by a transient decrease in its cellularity to the baseline level (day 7), which is consistent with published data [1,4].

Anthracycline antibiotics produce damage to proliferating morphologically identified hemopoietic cells and their precursors [1]. It was observed in studying the population of committed erythroid precursors. The number of erythroid colonies in 3-day-old cultures decreased most significantly on day 4 after adriamycin administration (0.05×10^5 *vs.* 4.0×10^5 nonadherent myelokaryocytes in the control, Fig. 1, *d*). The count of erythroid colonies increased on day 5 (165% of the initial level), but then decreased and remained below the control.

A comparative analysis of myelograms and colony-forming ability of the bone marrow indirectly reflects the relationship between proliferation and differentiation of hemopoietic precursors [4]. The ratio between the counts of morphologically differentiated erythrokaryocytes and CFU-E showed that recovery of the cellularity in the erythroid stem of the bone marrow was accompanied by suppression of colony formation (Fig. 1, *c*, *d*). These results indicate that maturation of erythroid precursors plays a key role in the recovery of erythropoiesis after adriamycin administration [1].

Humoral factors present in peripheral blood plasma and secreted by cells of the hemopoietic microenvironment are involved in proliferation and differentiation of hemopoietic cells [5,10,12,15]. We studied the effects of adriamycin on the content of factors determining EPA of peripheral blood plasma and supernatants of bone marrow cells. Cytostatic treatment was followed by an increase in EPA of the plasma (days 1-8), which reached maximum on day 4 (Fig. 2, *c*). Experiments with neutralization of EPA in bone marrow supernatants and peripheral blood plasma with

monoclonal antibodies against mouse erythropoietin (MABE) showed that these antibodies attenuated the increase in EPA of blood plasma on days 3-4 (Fig. 2, *c*). However, MABE did not abolish the increase in EPA of blood plasma at other terms. It was related to the involvement of other factors differing from erythropoietin (*e.g.*, hormones, interleukins, and leukotrienes) in the formation of EPA at these terms [2,11].

EPA in supernatants of nonadherent bone marrow cells increased 2-3 and 5-6 days after adriamycin administration. MABE abolished the increase in EPA on days 5 and 6 (53 and 46%, respectively, Fig. 2, *a*).

Cytostatic treatment was followed by a short-term increase in EPA of adherent bone marrow cells (days 1-2). These changes were related to the influence of erythropoietin. MABE 2-fold decreased EPA in supernatants of adherent bone marrow cells in these periods (Fig. 2, *b*).

Plasma erythropoietin content was measured by enzyme immunoassay. Erythropoietin content in the plasma decreased 2 days after adriamycin administration, but then progressively returned to normal, increased, surpassed the initial level on day 6, and did not differ from the control on day 8 (Fig. 2, *d*).

Intensive production of EPA-determining substances by bone marrow cells in the early period after cytostatic administration probably reflects the reaction of the hemopoietic microenvironment to this treatment [1,5]. Adherent cells primarily secret erythropoietin, which is related to properties of monocytes and macrophages. Nonadherent secretory active nuclear cells (T lymphocytes) produce other substances capable of stimulating the growth of erythroid precursors (interleukin-3 and granulocyte/macrophage colony-stimulating factor) [6,8].

Humoral factors of the peripheral blood underwent similar nonspecific changes in the early period after treatment. Erythropoietin produced by the kidneys was involved in the formation of EPA starting from day 3 after adriamycin administration. It was probably associated with hypoxia, since in this period we observed anemia in the peripheral blood. These changes contributed to the increase in plasma erythropoietin content up to the 6th day after treatment. It should be emphasized that the concentration of other substances stimulating erythropoiesis and partially abolishing the effect of erythropoietin in neutralization remained high in the blood [11].

The synergetic influence of erythropoietin and other humoral factors promotes recovery of erythroid hemopoiesis suppressed by adriamycin.

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