Effect of Cytostatics on Bone Marrow Stem Cells

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Morphological composition of the bone marrow, content of hemopoietic precursors, intensity of their proliferation and differentiation, and the size of mesenchymal precursor pool were studied in experiments on CBA/CaLac mice with myelosuppression induced by adriamycin, cyclophosphamide, or 5-fluorouracil in the maximum tolerated doses. The dynamics of changes in the content of granulomonocyte precursors in the bone marrow after cytostatic treatment was similar to that of erythroid precursors. Changes in the content of stromal mechanocytes were specific for each cytostatic. Different character of the reaction of the hemopoietic and mesenchymal stem cells to the test cytostatics was demonstrated.

Key Words: *cytostatics; hemopoietic precursors; mesenchymal precursors; stem cells*

Changes developing in the organism under the effect of pathogenic factors are largely determined by the state of parent tissue cells. Among them are hemopoietic and mesenchymal stem cells giving rise to hemopoietic and connective tissue cells. The important role of hemopoietic and connective tissue cells (macrophages, lymphocytes, fibroblasts, endotheliocytes, adipocytes and some other cells) in the formation of organism's reactivity [9] is determined by the fact that apart from specialized functions they are components of different systems of local regulation of physiological functions [2,4].

There are ample published data on the sensitivity of hemopoietic cells at different stages of maturation to antineoplastic drugs [1,2,5,8]. However, the effect of cytostatic treatment on mesenchymal stem cells and its progeny is little studied. The importance of this problem is obvious and is related to important regulatory function of fibroblasts and adipocytes. This function considerably increases under conditions of regeneration of cytostatic-damaged tissues, which was clearly demonstrated using various models on myeloinhibiting exposures [2,6].

Here we compared the effects of some cytostatic drugs with different mechanisms of action on the content of hemopoietic and mesenchymal stem cells in the bone marrow.

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MATERIALS AND METHODS

Experiments were carried out on 2-month-old male CBA/CaLac mice (n=160). The animals (conventional certified inbreed mice) obtained from the nursery of Institute of Pharmacology (Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences) were divided into 3 groups. Group 1 mice received fluoropyrimidine antimetabolite 5-fluorouracil (5-FU, Darnitsa Chemical and Pharmaceutical Plant), group 2 animals were treated with alkylating cytostatic cyclophosphamide (CP, Biokhimic Plant, Saransk), and group 3 mice were given anthracycline antibiotic adriamycin (Adr, Walter Buchnell). All antitumor drugs were injected intraperitoneally in the maximum tolerated doses of 228, 250, and 6 mg/kg, respectively (probit-analysis). The animals were sacrificed by cervical dislocation under ether narcosis. The total number of myelokaryocytes (TNK) in the bone marrow was determined by routine hematological methods and their qualitative composition was evaluated on smears stained by the method of Nocht—Maksimov [7].

The suspension of bone marrow nuclears was separated into adherent and nonadherent fractions. Nonadherent karyocytes were cultured in methylcellulose

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for evaluation of the content of colony- and cluster-forming granulomonocytopoietic [3] and erythropoietic [3] precursors; the content of mesenchymal precursors was determined by the number of fibroblast colony-forming units (CFU-F) [2,10,14].

The intensity of differentiation of hemopoietic precursors was determined by the index of maturation (clusters to colonies ratio). Proliferative activity of granulocyte-macrophagic and erythroid precursors was measured using the method of hydroxyurea-induced cell suicide [3].

The data were processed statistically using Student's *t* test.

RESULTS

The most pronounced and long-lasting decrease in the total content of myelokaryocytes and cellularity of individual hemopoietic stems was observed after injection of 5-FU (Fig. 1). Active recovery of bone marrow neutrophol count was noted in animals receiving Adr or CP. In the latter case regeneration of the granulocytic stem was most pronounced. The erythroid stem more rapidly recovered in mice receiving Adr compared to other groups.

The content of granulocyte-macrophage precursors in the bone marrow of mice receiving Adr or CP and the content of erythroid precursors after Adr treatment recovered later than the content of morphologically identifiable cells of the corresponding stems (Fig. 2). This phenomenon was determined by different ratio between proliferation and differentiation of hemopoietic precursors (Fig. 3). For instance, the rate of CFU division for the granulocytic stem decreased on days 4-6 after injection of Adr and, especially, CP. However, the intensity of maturation of these precursors markedly increased starting from day 2 after administration of the anthracycline antibiotic and from

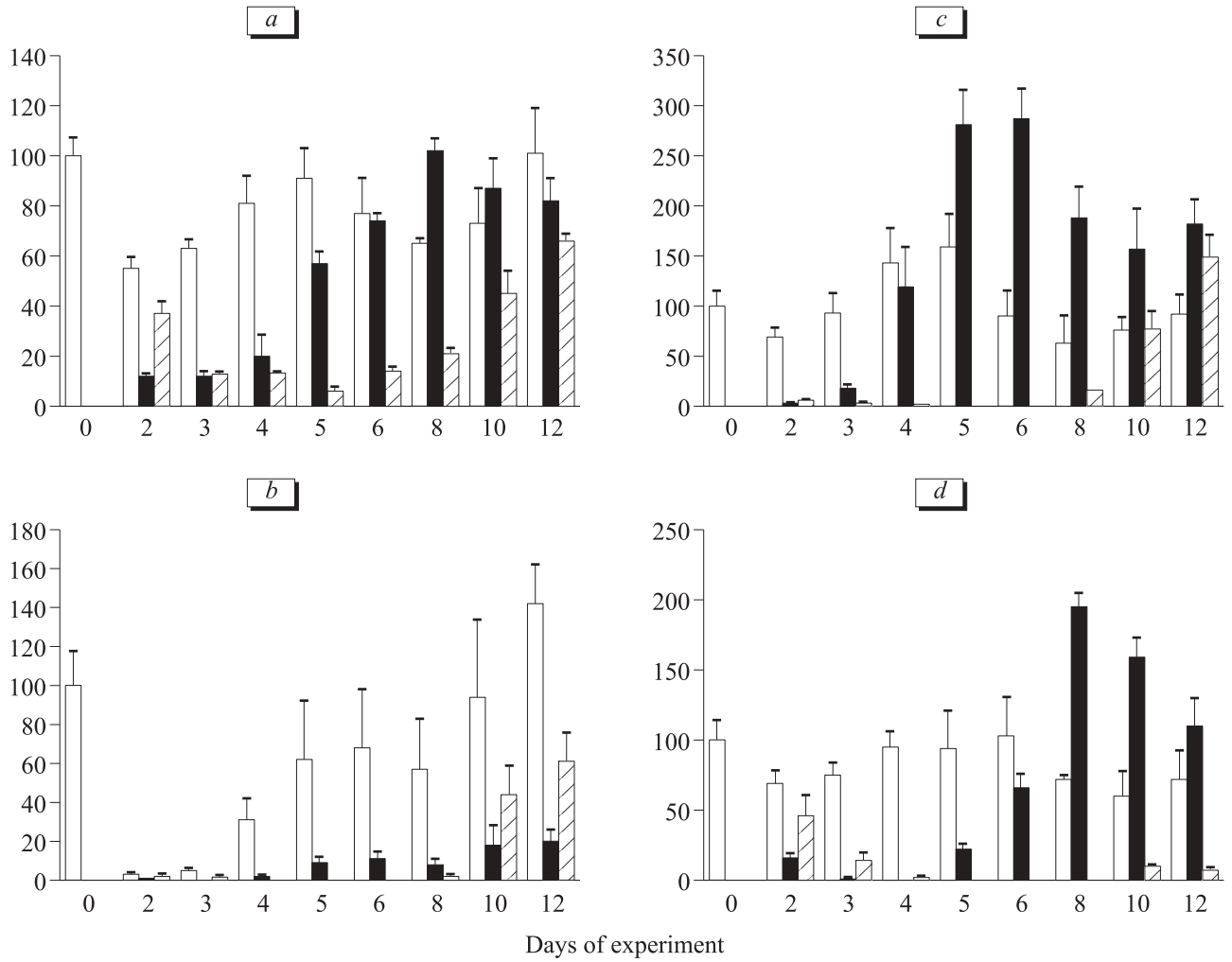

Fig. 1. Dynamics of the total content of myelokaryocytes (*a*), erythroid cells (*b*), immature (*c*), and mature (*d*) forms of neutrophilic granulocytes in the bone marrow of CBA/CaLac mice treated with adriamycin (open bars), cyclophosphamide (dark bars) and 5-fluorouracil (hatched bars). Ordinate: content of cells in the bone marrow, % of background values. Here and on Figs. 2 and 3: confidence intervals at p=0.05.

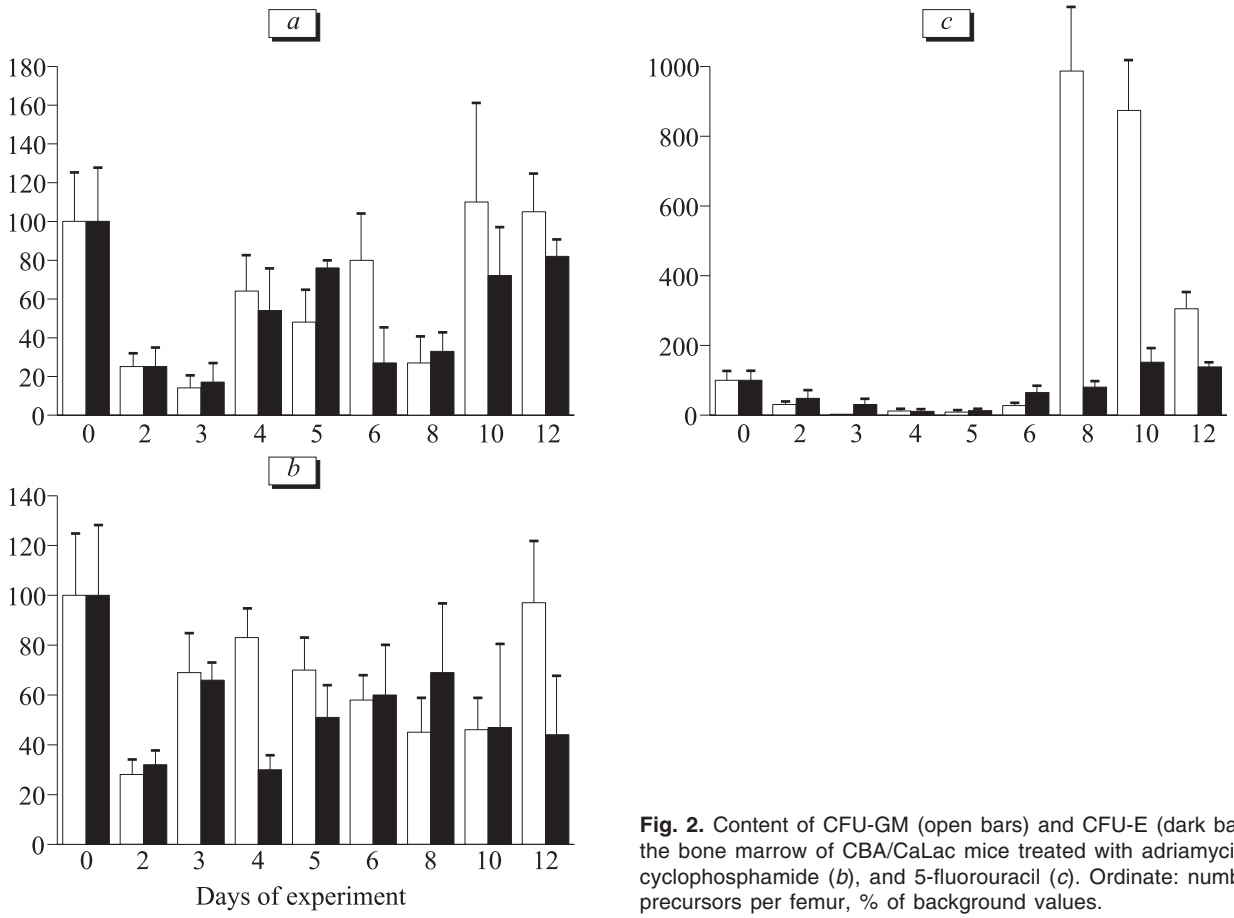

Fig. 2. Content of CFU-GM (open bars) and CFU-E (dark bars) in the bone marrow of CBA/CaLac mice treated with adriamycin (*a*), cyclophosphamide (*b*), and 5-fluorouracil (*c*). Ordinate: number of precursors per femur, % of background values.

day 4 after injection of the alkylating agent. This suggest that accelerated differentiation of hemopoietic precursors is the leading mechanism of the recovery of bone marrow parameters after administration of the test cytostatic drugs.

Injection of 5-FU led to a gradual accumulation of both granulocyte-macrophage and erythroid precursors in the bone marrow (Fig. 2) without increasing their proliferative activity. Accumulation of committed precursors occurred against the background of depletion of more mature granulo- and erythrokaryocytes and was determined by sharp inhibition of differentiation of hemopoietic precursors after administration of the fluoropyrimidine antimetabolite (Fig. 3).

Thus, the dynamics of the content of granulomonocytopoietic precursors in the bone marrow after injection of all cytostatics was similar to that of erythroid precursors.

In further experiments we studied changes in the pool of mesenchymal precursors during the development of cytostatic disease. It is well established that the bone marrow contains a special population of stem cells maintaining hemopoiesis and capable of differentiating into cells of different lineages. These cells were first described by A. Ya. Fridenshtein *et al*. in 1974 as fibroblast stem cells capable of forming colonies (CFU-F) [10]. Later these cells were named mesenchymal stem cells [13-16]. These cells are morphologically similar to fibroblasts and are characterized by high replication capacity; after appropriate stimulation they can differentiate into at least eleven cell types: osteocytes, chondrocytes, adipocytes, tenocytes, myocytes (cardiomyocytes, smooth muscle cells, and striated muscle fibers), astrocytes, olygodendrocytes, neurons, and stromal cells capable of maintaining hemopoiesis [14,16,17].

Administration of Adr considerably increased the efficiency of colony formation by precursors of stromal mechanocytes throughout the experiment. The most pronounced stimulation of the growth of fibroblastoid colonies (by 3.5 times) was observed after transplantation of the bone marrow on day 4 of the experiment (Table 1). CP reduced the number of clonogenic fibroblast precursors in the bone marrow at early terms of the experiment. The yield of CFU-F gradually decreased and was completely ceased on day 6 after administration of the alkylating agent. At later terms we observed sharp stimulation of the growth of colonies formed by stromal mechanocyte precursors (to 8 CFU-F per 100,000 explanted nonadherent mye-

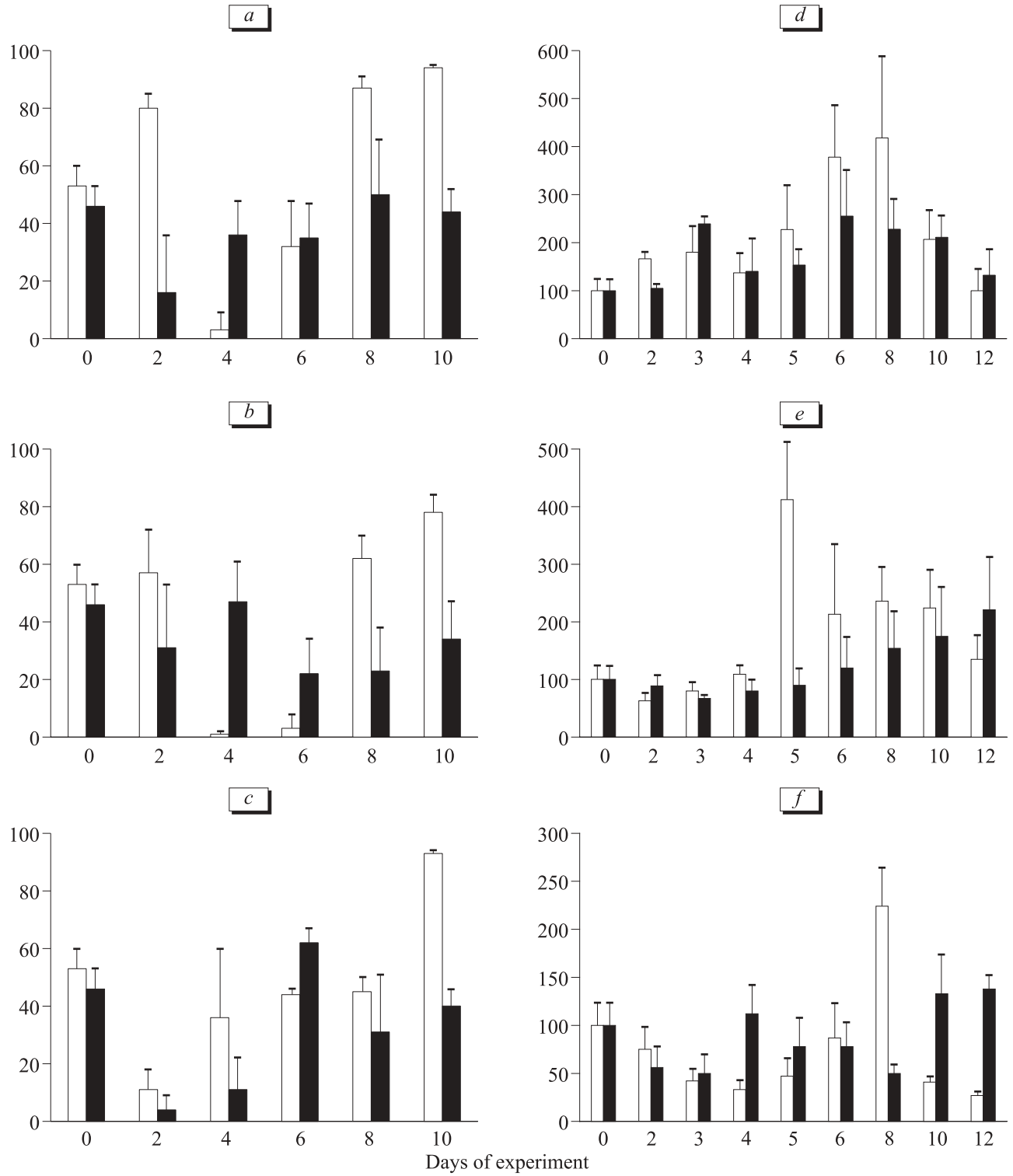

Fig. 3. Percentage of CFU-GM (open bars) and CFU-E (dark bars) in S phase of the cell cycle (*a-c*); intensity of maturation (*d-f*) of CFU-GM (open bars) and CFU-E (dark bars) in the bone marrow of CBA/CaLac mice treated with adriamycin (*a*, *d*), cyclophosphamide (*b*, *e*) and 5-fluorouracil (*c*, *f*). Ordinate: percentage of precursors in S phase (*a-c*); index of maturation, % of background (*d-f*).

lokaryocytes by the 10th day *vs.* 2.38 in intact animals, Table 1). 5-FU produced a pronounced toxic effect not only on hemopoietic cells, but also on mesenchymal stem cell progeny starting from the earliest terms of their development. Indeed, neither fibroblasts, nor fibroblast colonies were found in the cultures from the start of the experiment. Solitary CFU-F appeared in bone marrow samples only on day 8 after administration of

TABLE 1. Dynamics of CFU-F Content (per 10^5 Myelokaryocytes) in the Bone Marrow of Mice Treated with Adr, CP, and 5-FU in MTD ($X \pm m$)

Group	Before treatment	Day 2	Day 4	Day 6	Day 8	Day 10
Adr	2.38±0.32	4.50±0.43 $p<0.01$	8.38±0.42 $p<0.001$	3.67±0.42 $p<0.05$	3.86±0.26 $p<0.01$	4.38±0.46 $p<0.01$
CP	2.38±0.32	2.33±0.88	0.71±0.29 $p<0.01$	0	5.50±0.43 $p<0.001$	8.00±0.31 $p<0.001$
5-FU	2.38±0.32	0	0	0	0.29±0.18 $p<0.001$	1.14±0.26 $p<0.02$

Note. *p*: compared to the corresponding parameter before treatment.

antimetabolite in MPD, but their content did not return to the initial level (in contrast to that in mice receiving Adr or CP, Table 1).

Thus, mesenchymal precursors similarly to hemopoietic ones are very sensitive to the cytostatic treatment. However, the dynamics of changes in their bone marrow content induced by different cytostatics (Adr, 5-FU) considerably differ from that of hemopoietic clonogenic cells. This fact can be explained by different sensitivity of histogenetically different precursors to direct effects of cytostatic agents and plasticity of mesenchymal stem cell. This property allows mesenchymal stem cell to transform into cells of other differentiation lineages starting from the early stages of its development [11-13].

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