Role of Proliferation and Differentiation of Hemopoietic Precursors in Regeneration of Hemopoiesis in Cytostatic Myelodepressions

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

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Quantitative and qualitative composition of hemopoietic tissue and the intensity of proliferation and differentiation of committed hemopoietic precursors are studied in mice treated with adriamycin, cyclophosphane, or 5-fluorouracil. It is shown that intense maturation of colony-forming units results in rapid regeneration of hemopoiesis even under conditions of reduced proliferation of these cells, whereas inhibition of their differentiation suppresses restoration of bone marrow cellularity despite intense proliferation of hemopoietic precursors.

Key Words: *precursors cells; hemopoiesis; myelodepression*

The balance between proliferation and differentiation of hemopoietic precursors determines the state of precursor population and quantitative composition of differentiated pools of the hemopoietic system [6,9, 10,12,13]. We have previously suggested [4,5] that cellularity of hemopoietic tissue in a cytostatic disease is restored mainly due to accelerated differentiation of precursor cells under conditions of suppressed cell proliferation. However, there are only few and contradictory reports on the role of proliferation and differentiation of these cells in reparation of hemopoiesis in cytostatic myelodepression [1,3].

The aim of the present study was to evaluate the contribution of differentiation and proliferation of hemopoietic precursors to the regeneration of hemopoiesis suppressed by antitumor drugs with different mechanisms of action.

MATERIALS AND METHODS

Experiments were carried out on 160 CBA male mice weighing 18-22 g (*Rassvet* nursery, Tomsk). The animals were divided into 3 groups. Group 1 mice received the fluoropyrimidine antimetabolite 5-fluorouracil (*Darnitsa* Chemicopharmaceutical Association), group 2 mice were treated with the alkylating cytostatic cyclophosphane (CP, *Biokhimik* Company, Saransk), and group 3 animals were injected with the anthracycline antibiotic adriamycin (AD, Wolter Buchnell). All preparation were injected intraperitoneally in the maximum tolerated doses: 228, 250, and 6 mg/kg, respectively (data of probit-analysis). The animals were sacrificed by cervical dislocation under ether narcosis at different times postinjection. The total number of myelokaryocytes in the bone marrow was determined using standard hematological methods; quantitative shifts were studied on smears stained by the Nocht—Maksimov method [7].

Suspension of bone marrow mononuclear cells was divided to adhesive and nonadhesive fractions. The content of colony- and cluster-forming granulomonocytopoietic (CFU-GM and ClFU-GM) and erythropoietic (CFU-E and ClFU-E) precursors in the hemopoietic tissue of experimental and control animals was counted using *in vitro* cloning of nonadhesive karyocytes in methyl cellulose [2].

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

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The intensity of differentiation of hemopoietic precursors was assessed by the index of maturation (clusters to colonies ratio) [8]. Proliferative activity of CFU-GM and CFU-E was determined by the method of cell suicide using hydroxyurea [2,11].

The data were processed by ANOVA using the Student's *t* test.

RESULTS

The most pronounced hemodepression was observed in 5-fluorouracil-treated mice. It was accompanied by significant decrease in the number of immature (days 2-10 postinjection) and mature (days 2-14 postinjection) neutrophilic granulocytes. On day 2 postinjection, the number of bone marrow erythroid cells decreased to 2.02% of the initial value (Fig. 1).

In CP-treated mice granylocyto- and erythropoiesis were considerably suppressed on days 2-4 followed by their gradual recovery. The content of immature neutrophilic granulocytes on day 2 after CP injection constituted only 3.5% of the initial level; however, on day 5 it increased to 280.9% and remained at this level throughout the observation period. The number of mature neutrophils peaked on day 8, and returned to normal on day 12 postinjection. The content of erythroid cells sharply decreased to 0.4% of the baseline level on day 3 and returned to normal only on day 14.

In AD-treated mice we observed two-phase changes in the studied hemodynamic parameters. The first (transient) rise of immature and mature neutrophilic granulocytes to the baseline values was noted on days 3-6 and 4-7, respectively, while final restoration of bone marrow cellularity was attained

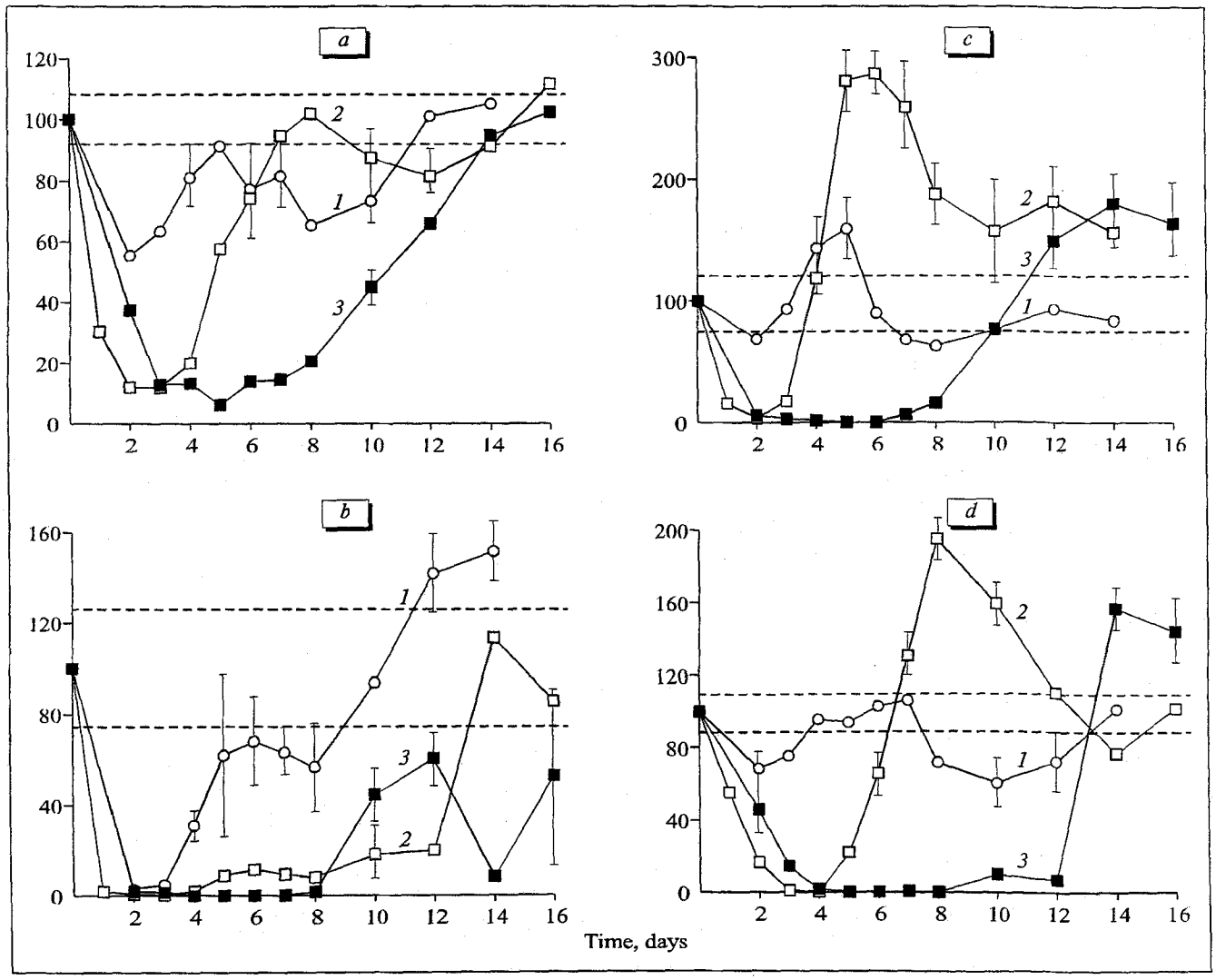

Fig. 1. Dynamics of total myelokaryocyte population (*a*), erythroid cells (*b*), immature (*c*), and mature (*d*) neutrophilic granulocytes in the bone marrow of CBA mice treated with adriamycin (*1*), cyclophosphane (*2*), or 5-fluorouracil (*3*). Ordinate: number of cells, % of background level.

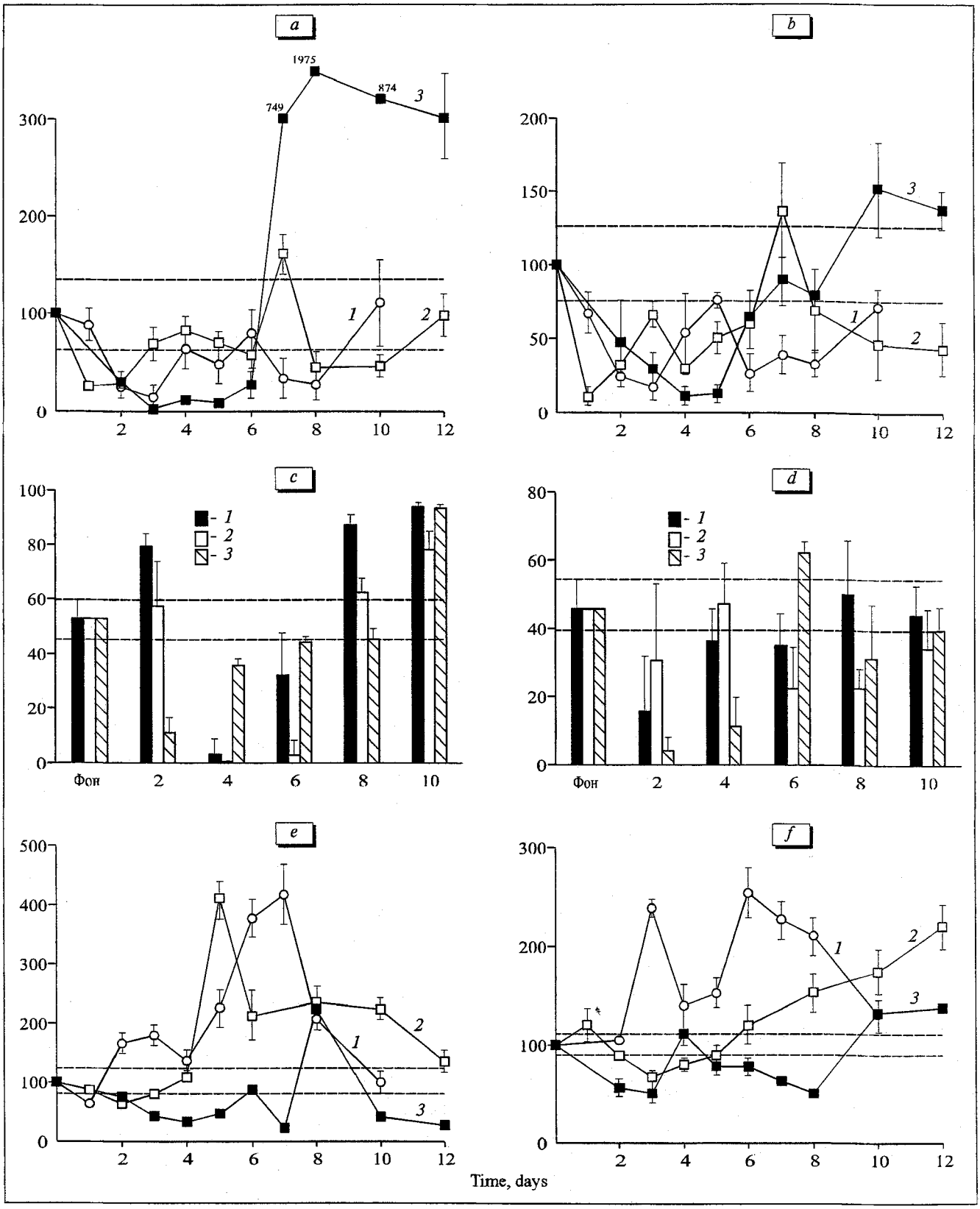

Fig. 2. Number of CFU-GM (*a*) and CFU-E (*b*), percentage of CFU-GM (*c*) and CFU-E (*d*) in the S phase, intensity of differentiation of CFU-GM (*e*) and CFU-E (*f*) in the bone marrow of CBA mice injected with adriamycin (*1*), cyclophosphane (2), or 5-fluorouracil (*3*). Ordinate, number of precursors in the femur, % of baseline value (*a*, *b*); portion of precursors in the S phase, % (*c*, *d*); index of maturation, % of baseline value (*e*, *f*).

only on day 12 of the experiment. Adriamycin produced the strongest effect on suppressing erythroid cells: the content of erythrokaryocytes on day 2 constituted 2.97% of the initial value. Abortive rise of this parameter on days 5-6 was induced another suppression followed by gradual recovery (starting from day 10, Fig. 1).

When studying the mechanisms of hemopoietic reparation we noted gradual accumulation of committed hemopoietic precursors after their transient decrease in mice injected with 5-fluorouracil. The number of CFU-E returned to normal on day 6 of the experiment (Fig. 2); the number of CFU-GM markedly rose starting from day 7 and on day 9 attained 1947.5% of the control level. While measuring the proliferative activity of hemopoietic precursors we found that in mice treated with 5-fluorouracil the number of CFU-GM and CFU-E in S phase rapidly returned to normal on days 4 and 6, respectively, while their differentiation was considerably suppressed to days 12 and 8, respectively (Fig. 2, *e*, *f*). We think that this phenomenon is responsible for accumulation of CFU-GM and long-lasting suppression of mature karyocytes in the bone marrow of animals treated with this metabolite.

Both AD and CP decreased the number of CFU-GM in the bone marrow (on days 10 and 12, respectively). Interestingly, both drugs induced a transient rise of this parameter on days 4-6 and on days 3-5, respectively. This coincided with a considerable intensification of CFU-GM differentiation: to 412% (on day 5 after injection of CP) and to 377.5% (on day 6 after injection of AD). In animals treated with AD or CP, the number of DNA-synthesizing CFU-GM in hemopoietic tissue decreased on days 4-6 (Fig. 2, *c*). These findings suggest that the primary transient rise of granulocytopoiesis in AD- and CP-injected mice results from accelerated differentiation of survived hemopoietic precursors. Active differentiation of hemopoietic precursors against the background of reduced proliferation rate is an imperfect mechanism of post-cytostatic regeneration and rapidly depletes the pool of hemopoietic precursors. This leads to a second decrease in the bone marrow cellularity in AD-treated mice. Complete regeneration of granulocytopoiesis was attained through marked acceleration of CFU-GM proliferation (days 8-10) against the background of intense differentiation of granulocyte precursors, implying an important role of both proliferative processes and enhanced differentiation in regeneration of hemopoiesis suppressed by AD and CP treatment.

The number of erythrokaryocytes was most rapidly restored in the bone marrow of AD-treated animals despite the fact that CFU-E proliferation was not accelerated throughout the experiment (Fig. 2, *d*). In the AD-treated mice, the rate of differentiation of erythroid precursors from day 3 through 8 was considerably higher than in animals injected with other cytostatics.

These findings confirm our hypothesis that accelerated differentiation of hemopoietic precursors is an important mechanism of hemopoietic regeneration in post-cytostatic hemodepression; it promotes regeneration of some hemopoietic clones even without enhanced proliferation of the corresponding committed precursors [4].

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