ROLE OF HEMATOPOIETIC PRECURSOR CELLS IN THE RESTORATION OF HEMATOPOIESIS AFTER TREATMENT WITH CYTOSTATICS

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The development of methods of correcting hemodepressive states is directly linked with the study of depression and regeneration of hematopoiesis. A convenient model with which to study the mechanisms of restoration of hematopoiesis is hypoplasia of the bone marrow created by a single injection of a cytostatic [1]. Data showing that regeneration of the hematopoietic system after exposure to extremal factors takes place through the proliferation of stem cells and committed cells, have been obtained chiefly on models of postradiation restoration of hematopoiesis. Meanwhile, high-dosage chemotherapy of malignant neoplasms, with the use of specific stimulators of hematopoiesis (colony stimulating factor, interleukin-1, interleukin-3), requires more detailed study, to reveal the role of different types of precursor cells in the restoration of hematopoiesis, for each type of hematopoietic precursor responds in its own way to an increase in concentration of a given hematopoietin. The aim of the present investigation was to study the content of splenic colony-forming units (CFUS) and of colony-forming units in diffusion chambers (CFU_d) after injection of phosphamide.

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice (from the "Rassvet" Nursery, Tomsk). A standard preparation of cyclophosphamide (USSR) was dissolved in physiological saline immediately before use and injected once intraperitoneally in the maximal tolerated dose (MTD), namely 250 mg/kg. At different times after injection of the cytostatic, the animals were killed by cervical dislocation. The total number of myelokaryocytes (TNM) was counted and the myelogram calculated. The number of precursor cells committed to granulomonocytopoiesis in the hematopoietic tissue was determined by cloning bone-marrow nucleated cells in a plasma clot in diffusion chambers [2]. The final concentration of cells in the culture was $0.5 \cdot 10^5$. The cells were cultured for 7 days. The term colonies was applied to foci of hematopoiesis containing more than 50 cells. To study the morphological composition of the colonies monolayer preparations were obtained and stained with azure-II-eosin or by the cytochemical test for hemoglobin [4]. The number of cells forming splenic colonies on the 5th, 8th, and 12th days was estimated as in [7]. Bone marrow karyocytes in a concentration of $0.6 \cdot 10^5$ cells per mouse were transplanted intravenously into animals lethally irradiated in a dose of 8 Gy.

EXPERIMENTAL RESULTS

Injection of the cytostatic led to the development of marked hypoplasia of medullary hematopoiesis from the 1st through the 4th days of the experiment. The maximal reduction in the number of nucleated cells was observed in these experiments on the 3rd day (to 15% of the initial level; Fig. 1a). Analysis of the myelograms showed that at this time inhibition of the granulocytic and erythroid branches of hematopoiesis was observed. Later, in mice receiving cyclophosphamide, restoration of the hematopoietic foci took place. For instance, the number of immature forms of neutrophilic

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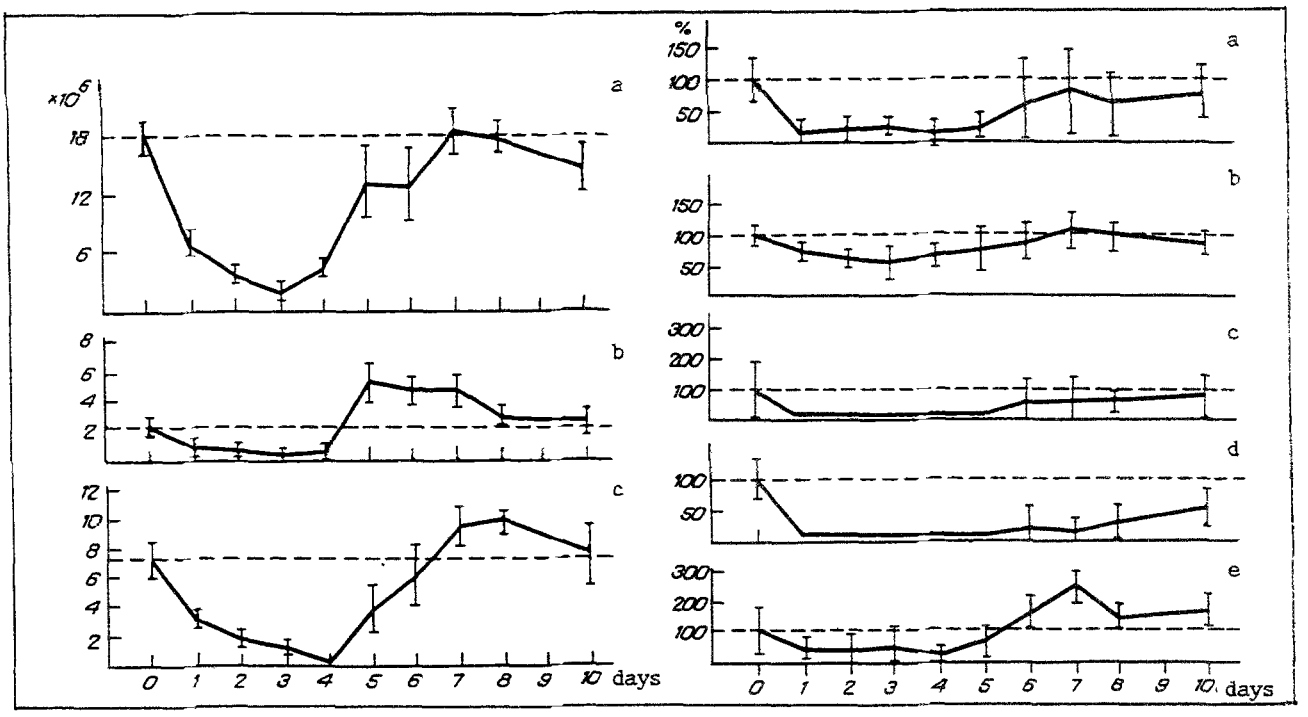

Fig. 1 Fig. 2

Fig. 1. Dynamics of TNM (a) and immature (b) and mature neutrophilic granulocytes (c) in bone marrow of CBA mice after injection of cyclophosphamide in MTD. Abscissa, time after injection of cytostatic (days); ordinate, number of cells ($\cdot 10^6$/femur). Confidence limits at $p = 0.05$.

Fig. 2. Time course of number of CFU_d (a), $ClFU_d$ (b), CFU_d-GM (c), CFU_d-G (d), and CFU_d-M (e) in bone marrow of CBA mice after injection of cyclophosphamide in MTD. Abscissa, time after injection of cytostatic (days); ordinate, number of colonies and clusters (% of control). Confidence limits at $p = 0.05$.

TABLE 1. CFU_s Content in CBA Mouse Bone Marrow (5th, 8th, and 12th days) at Various Times after Injection of Cyclophosphamide in MTD ($\bar{X} \pm m$)

Time of investigation	CFU_s content		
	CFU_s-5	CFU_s-8	CFU_s-12
Control	5.0±1.75	22.6±5.52	20.8±1.35
1st day	3.25±0.87	5.0±4.4**	5.6±1.8**
4th day	3.25±1.0	25.2±8.67	15.0±4.0
7th day	1.5±0.5**	2.8±2.22**	11.6±2.45*

Legend: * — $p < 0.05$, ** — $p < 0.01$.

leukocytes increased tenfold from the 4th through the 5th days (Fig. 1b). The number of erythrokaryocytes, however, was not restored until the 10th day of the experiment. The increase in the number of granulocytes took place against the background of stimulation of mitotic activity of immature forms of neutrophilic leukocytes (Fig. 2c). The number of CFU_s forming colonies on the 8th and 12th days after transplantation of syngeneic myelokaryocytes from mice exposed to the action of the cytostatic was reduced as early as 24 h after injection of the cyclophosphamide. By the beginning of intensive regeneration of medullary hematopoiesis (4 days) the number of CFU_s and CFU_s-12 was restored to initial values (Table 1). The number of CFU_s-5 at this period did not differ from that in animals of the control group. Later (7th day after

injection of the cytostatic) a significant decrease was observed in the numbers of CFU_s-5, CFU_s-8, and CFU_s-12 compared with the initial values (Table 1).

The study of colony and cluster forming ability of the bone marrow by the diffusion chamber method showed that CFU_d are more sensitive to the action of cyclophosphamide than cluster-forming units ($ClFU_d$) (Fig. 2a, b). Moreover, restoration of the number of $ClFU_d$ and the number of morphologically identifiable granulocytic cells took place at the same times and coincided with the period of active proliferation of myelokaryocytes (Fig. 1a-c; Fig. 2b). Meanwhile the number of CFU_d began to increase later (after the 5th day). Morphological analysis of cell aggregates formed under these circumstances revealed inhibition by cyclophosphamide of proliferation and differentiation of precursor cells of granulomonocytopoiesis (CFU_d-GM) and granulocytopoiesis (CFUd-G) (Fig. 2c, d). Colonies of these types were virtually not found during the period of intensive proliferation of cells of the myeloid series (from the 1st through the 5th day of the experiment).

The results are evidence that the role of CFU_d in regeneration of granulocytopoiesis is unimportant under the conditions described. After exposure to the cytostatic the mechanisms of "shunt" hematopoiesis are activated, when more rapid differentiation takes place of polypotent precursor cells (CFU_s-8 and CFU_s-12), which have preserved their viability, with an increase in the proliferative activity of the more mature cell populations. This is shown by the time course of restoration of the parameters examined above (the increase in proliferative activity of the myeloid cells and in the number of cells of the granulocytic branch of hematopoiesis is preceded by an increase in the number of CFU_s-8 and CFU_s-12, whereas CFU_d-GM and CFU_d-G do not appear in the bone marrow until the 6th day of observation. The mechanisms of more rapid differentiation of stem cells are activated under the influence of extremal factors (cytostatics with different mechanisms of action, ionizing radiation) [3, 5, 6]. The fact that the cell pool forming splenic colonies is exhausted by the 7th day of the experiment will be noted, for this may give rise to late disturbances in the blood-forming system after exposure to cytostatics.

LITERATURE CITED

1. E. D. Gol'dberg and V. V. Novitskii, Antitumor Antibiotics of the Anthracycline Series and the Blood System [in Russian], Tomsk (1986).
2. A. M. Dygai and V. P. Shakhov, The Role of Intercellular Interactions in the Regulation of Hematopoiesis [in Russian], Tomsk (1989).
3. E. A. Zherbin and A. B. Chukhlovin, Radiation Hematology [in Russian], Moscow (1989).
4. F. G. J. Hayhoe and D. Quaglino, Hematological Cytochemistry [Russian translation], Moscow (1983).
5. R. Lerza, G. Bogliolo, et al., Chemotherapy, **34**, No. 4, 354 (1988).
6. A. Razek, F. Valeriotte and T. Vietti, Cancer Res., **32**, 1496 (1972).
7. J. Till and E. McCulloch, Radiat. Res., **14**, 213 (1961).