GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Significance of the Binding Ability of Hemopoietic Microenvironment Cells for Restoration of Individual Hemopoietic Stem Precursors Suppressed by Cytostatic Drugs

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Restoration of granulomonocytopoiesis in CBA mice treated with maximum tolerated doses of adriamycin or cyclophosphane is strongly dependent on the maturation of precursor cells. Accelerated maturation is provided by the binding of the microenvironment cells to stem precursor cells. Restoration of erythroid precursors damaged by cytostatics is slower in the absence of such a binding.

Key Words: *hemopoietic microenvironment; cell-to-cell contacts; hemopoietic precursors; cytostatics*

Hemopoiesis-inducing microenvironment (HIM) plays an important role in the regulation of hemopoiesis both under normal and extreme conditions. There is a selective association between the microenvironment elements and various hemopoietic precursors [2,3] provided by at least two mechanisms. The first involves receptor binding and membrane-bound hemopoietic growth factors or adhesion molecules (integrin). The second consists in nonspecific binding realized by the extracellular matrix components [4-6,8-10]. It is likely that changes in adhesive properties of interacting cells contribute to the adaptation of the blood to extreme situations.

In the present study we attempted to evaluate the role of the binding between stromal components and precursor cells in the restoration of individual hemopoietic stem cells damaged by cytostatics with different mechanisms of action.

MATERIALS AND METHODS

Experiments were performed on 120 male CBA mice weighing 18-20 g. Adriamycin (Wolter Buchnell) and cyclophosphane (Biokhimik, Saransk) were dissolved in normal saline immediately before use and injected intraperitoneally in the maximum tolerated dose (6 and 250 mg/kg, respectively, according to preliminary analysis). The mice were killed by cervical dislocation under ether anesthesia 1, 2, 3, 4, 5, 6, 7, 8, 10, and 12 days after administration of the cytostatics. The total myelokaryocyte count per femur was determined, and the red marrow was analyzed qualitatively using smears stained by the method of Nocht—Maksimov.

In order to isolate adherent and nonadherent cell fractions, suspension of the bone marrow cells

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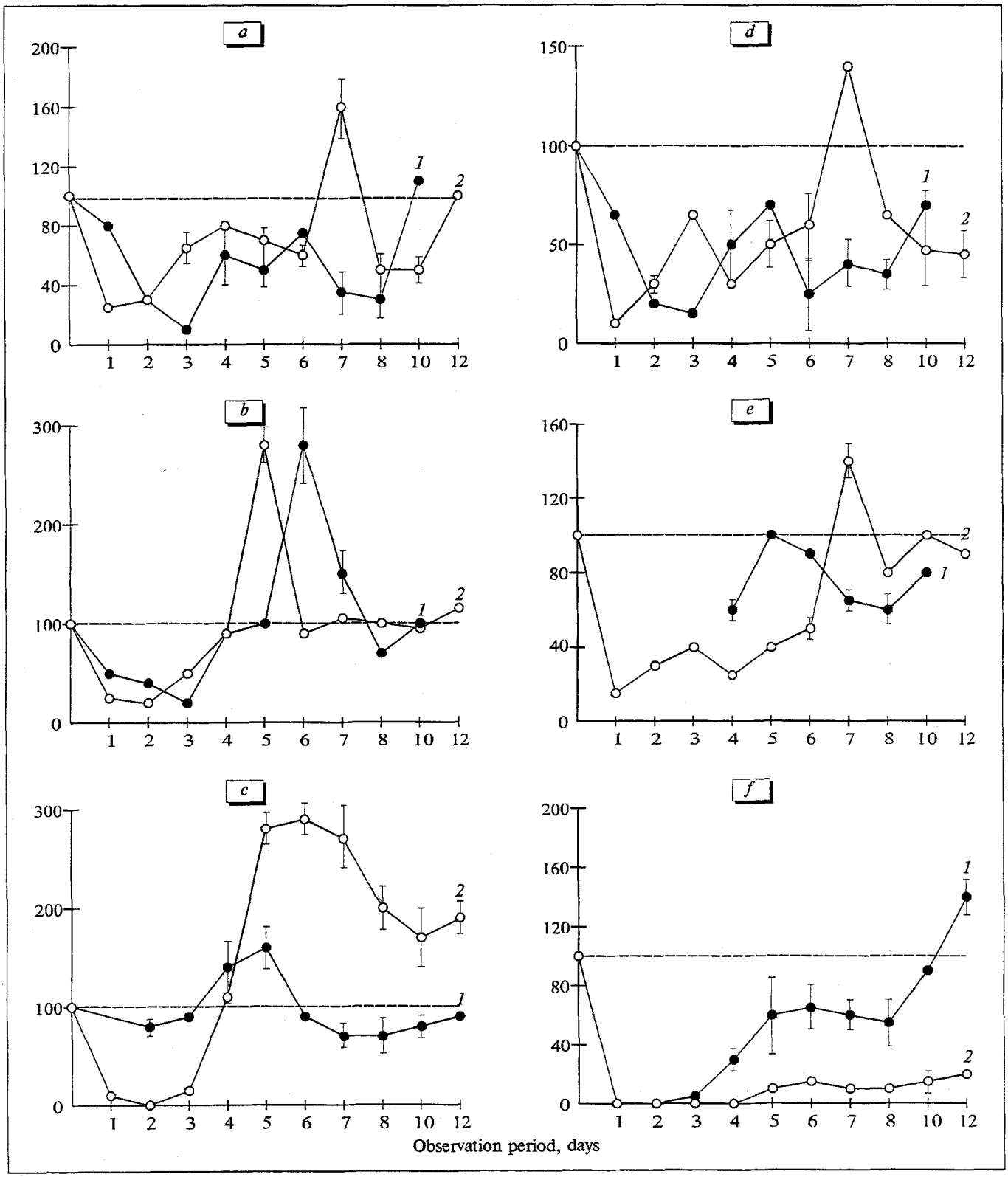

Fig. 1. The contents of CFU-GM (*a*), CIFU-GM (*b*), immature neutrophilic granulocytes (*c*), CFU-E (*d*), CIFU-E (*e*), and erythronormoblasts (*f*) in the bone marrow of CBA mice treated with adriamycin (*1*) or cyclophosphane (2). Ordinate: number of precursor cells per femur (% of the baseline level).

from the second femur (5×10^6 cells/ml) was incubated in plastic Petri dishes for 45 min at 37°C in RPMI-1640 medium (Vektor) containing 5% fetal calf serum (Serva) in a humidified atmosphere (100%)

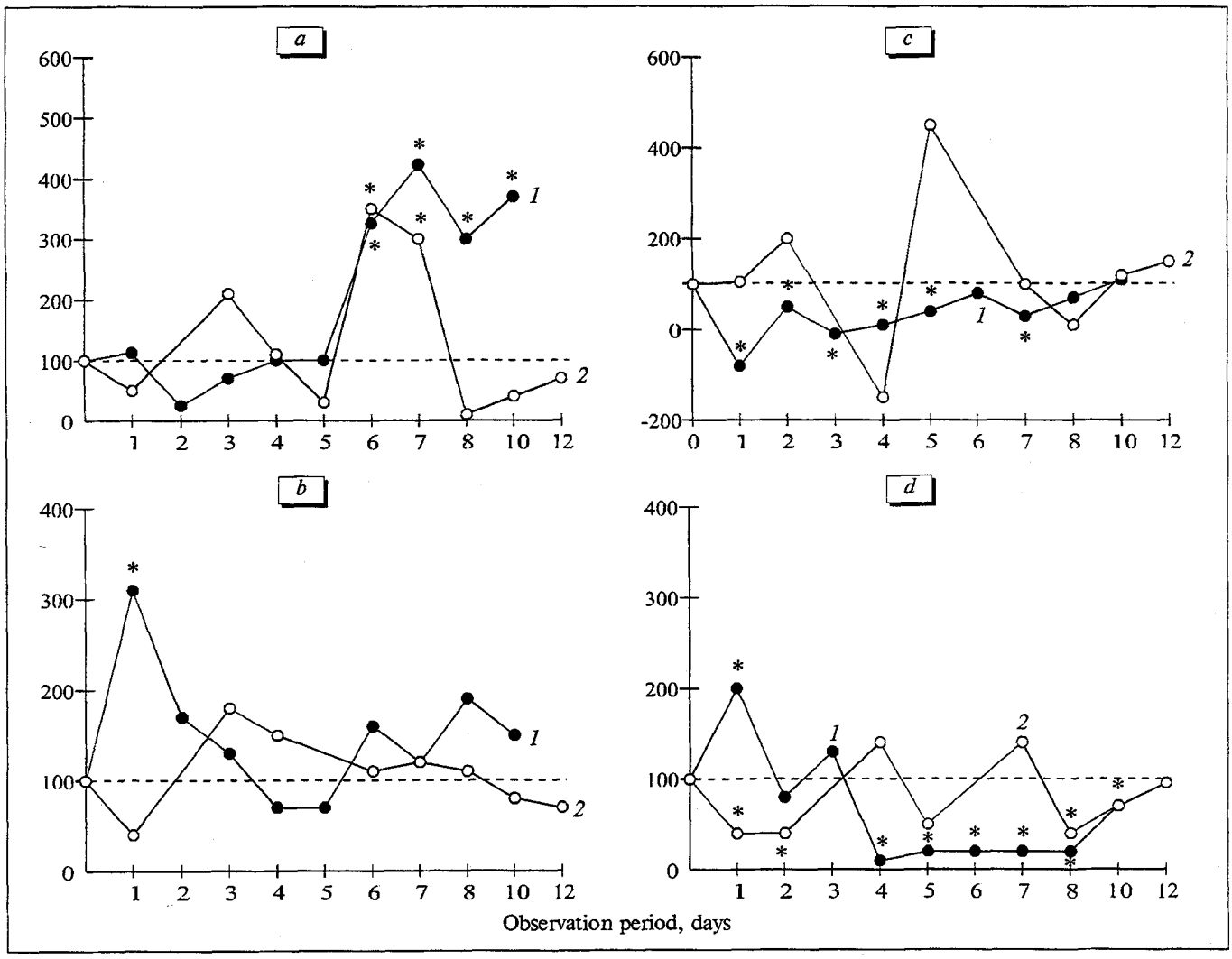

Fig. 2. The ability of adherent cells isolated from the bone marrow of CBA mice treated with adriamycin (*1*) or cyclophosphane (*2*) to bind intact CFU-GM (*a*) and CFU-E (*c*). The contents of CFU-GM (*b*) and CFU-E (*d*) bound to stroma. Ordinate: the number of precursor cells per 10^5 adherent cells (% of the baseline level). *$p<0.05$ compared with the original level.

with 5% CO_2. The number of colony- and cluster-forming units of granulocyte-macrophage (CFU-GM and ClFU-GM, respectively) as well as that of CFU-erythropoietic (CFU-E) and ClFU-E was determined by cloning nonadherent myelokaryocytes on methylcellulose *in vitro* [1,7].

Adherent cells from mice treated with the cytostatics were harvested with a siliconized policeman, washed, and transferred to 24-well plates (5×10^5 cells/well). Equal amounts of nonadherent myelokaryocytes from intact mice were added to some wells and incubated for 2 h in RPMI-1640 containing 10% fetal calf serum, 280 mg/ml L-glutamine (Sigma), and 5×10^{-5} M 2-mercaptoethanol (Sigma). Incubation medium containing nonadherent cells was removed, adherent cells were washed two times with RPMI-1640, and covered with a semisolid medium containing the stimulators for cloning CFU-GM or CFU-E [1,7]. The colonies formed in the medium in the adherent layer were counted. The ability of adherent cells to bind intact CFU-GM and CFU-E was expressed as the difference between CFU counts in the medium and adherent layer.

The results were analyzed using the Student's *t* test and Wilcoxon—Mann—Whitney *U* test.

RESULTS

Adriamycin and cyclophosphane reduced the number of CFU-GM in mouse bone marrow and induced a transitory increase in the number of CFU-GM on day 4-6 (adriamycin) or 4-7 (cyclophosphane), which was followed by a decrease. The CFU-GM count was normalized only on day 10-12 (Fig. 1, *a*). Interestingly, after transitory suppression, the number of ClFU-GM increased to 284% of the original level

on day 6 (adriamycin) and to 280% on day 5 (cyclophosphane) (Fig. 1, *b*). As the count of CFU-GM in the bone marrow of mice treated with the cytostatics decreased, the count of immature neutrophilic granulocyte increased, the increase being particularly pronounced (almost 3-fold on day 5-6) in cyclophosphane-treated mice (Fig. 1, *c*). Based on this finding and on the fact that in a cell culture clusters are formed by cells more mature than CFU-GM [7], we have hypothesized that accelerated maturation of CFU-GM to ClFU-GM and neutrophils is the main mechanism providing restoration of the bone marrow granulomonocytopoiesis under our experimental conditions.

The content of CFU-E in the bone marrow of mice treated with the cytostatics was decreased during a prolonged time period. The number of CFU-E was not restored faster than that of ClFU-E, which was normalized by day 7 after administration of cyclophosphane and did not vary considerably by the end of the observation period (Fig. 1, *d-f*). From these findings it cannot be concluded that accelerated maturation of hemopoietic precursors is important for restoration of the erythron damaged by either adriamycin or cyclophosphane.

The number of granulocyte-macrophage colonies grown from the adherent cells from mice treated with the cytostatics did not change considerably relative to the number of adherent cells. However, upon culturing with intact myelokaryocytes the number of these colonies increased substantially on day 6-10 (adriamycin) and day 6-7 (cyclophosphane). We believe that this is due to a higher ability of adherent cells to bind CFU-GM (Fig. 2, *a*, *b*). The CFU-E content in the layer of adherent HIM cells remained decreased. The ability of these cells to bind exogenous CFU-E was not increased. By contrast, in adriamycin-treated mice the binding activity of adherent cells decreased, which led to "dissociation" of erythroid precursors from HIM cells (Fig. 2, *c*, *d*).

Presumably, the ability of the stroma to bind intact precursor cells depends predominantly on the number of free binding sites [10] on adherent cells. An increase in the number of these sites at a practically constant number of bound precursors may be caused by a more active expression of the homing receptors and an increase in the number of nonspecific binding sites for CFU-GM on the surface of adherent HIM cells. Restoration of the erythron in adriamycin- or cyclophosphane-treated mice is much slower compared with that of granulomonocytopoiesis due to decreased ability of the adherent HIM cells to bind CFU-E.

Our results show that the binding of hemopoietic precursor cells by adherent HIM cells is important for the restoration of hemopoiesis after administration of cytostatics with various mechanisms of action.

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