GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Role of Thy 1.2$^+$ Cells in Hemopoietic Regulation in Cytostatic-Induced Hemosuppression

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Effect of Thy 1.2$^+$ cells (direct and mediated by stromal elements) on the growth of granulomonocyte and erythroid colonies in the bone marrow is studied on CBA mice with cytostatic disease induced by single injection of adriamycin, cyclophosphamide, and 5-fluorouracil in maximum permissible doses. It is shown that Thy 1.2$^+$ cells stimulate colony formation in regenerating bone marrow, the effect depending on functional activity of hemopoiesis-inducing microenvironment.

Key Words: *cytostatics; hemopoiesis; microenvironment; precursor cells; Thy 1.2$^+$ cells*

Immunocompetent cells, in particular, T cells play an important role in the regulation of hemopoiesis under normal and extreme conditions [3,4,11]. These cells act in cooperation with other nuclear cells, which are the components of hemopoiesis-inducing microenvironment (HIM) [1,4,7,15]. We have previously demonstrated [3,10] that T cells with phenotype Thy 1.2$^+$, Lyt 1$^+$, Lyt 2$^+$, L3T4$^-$ directly stimulate the growth of granulocyte-macrophage (CFU-GM) and erythroid (CFU-E) colony-forming units and activate macrophage-dependent mechanisms of hemopoietic regulation in acute inflammation and immobilization stress.

The aim of the present study was to investigate the interaction between Thy 1.2$^+$ cells with other elements of HIM and hemopoietic precursors in the course of postcytostatic recovery of hemopoiesis.

MATERIALS AND METHODS

Experiments were carried out on 100 male CBA mice weighing 18-22 g (Rassvet nursery, Tomsk). The animals received the fluoropyrimidine antimetabolite 5-fluorouracil (Darnitsa Chemicopharmaceutical Association), alkylating cytostatic cyclophosphamide (Biokhimik Company, Saransk), or anthracycline antibiotic adriamycin (Wolter Buchnell). All preparation were injected intraperitoneally in the maximum permissible doses: 228, 250, and 6 mg/kg, respectively (data of probit-analysis). The animals were sacrificed by cervical dislocation under ether narcosis 2, 4, 6, 8, and 10 days postinjection; 10 intact mice served as the control.

The content of regulatory T cells in the bone marrow was determined by complement-dependent cytotoxic test with monoclonal antibodies. The titer of monoclonal anti-Thy 1.2 antibodies (clone 5A-8 CL 8600A, Cedarlane) and complement cytotoxicity were evaluated in preliminary experiments on thymocytes from intact mice. The titer was 1:100. To evaluate the role of Thy 1.2 cells in the regulation of colony formation, suspension of bone marrow cells was purified from erythrocytes and fractionated to plastic adherent and nonadherent fractions. The concentration of nonadherent cells was adjusted to 2×10^6 cells/ml with RPMI-1640 medium containing 10 mM HEPES and incubated at 4°C for 45 min with or without monoclonal anti-Thy 1.2 antibodies (10 μl/ml cell suspension). The samples were then fixed with

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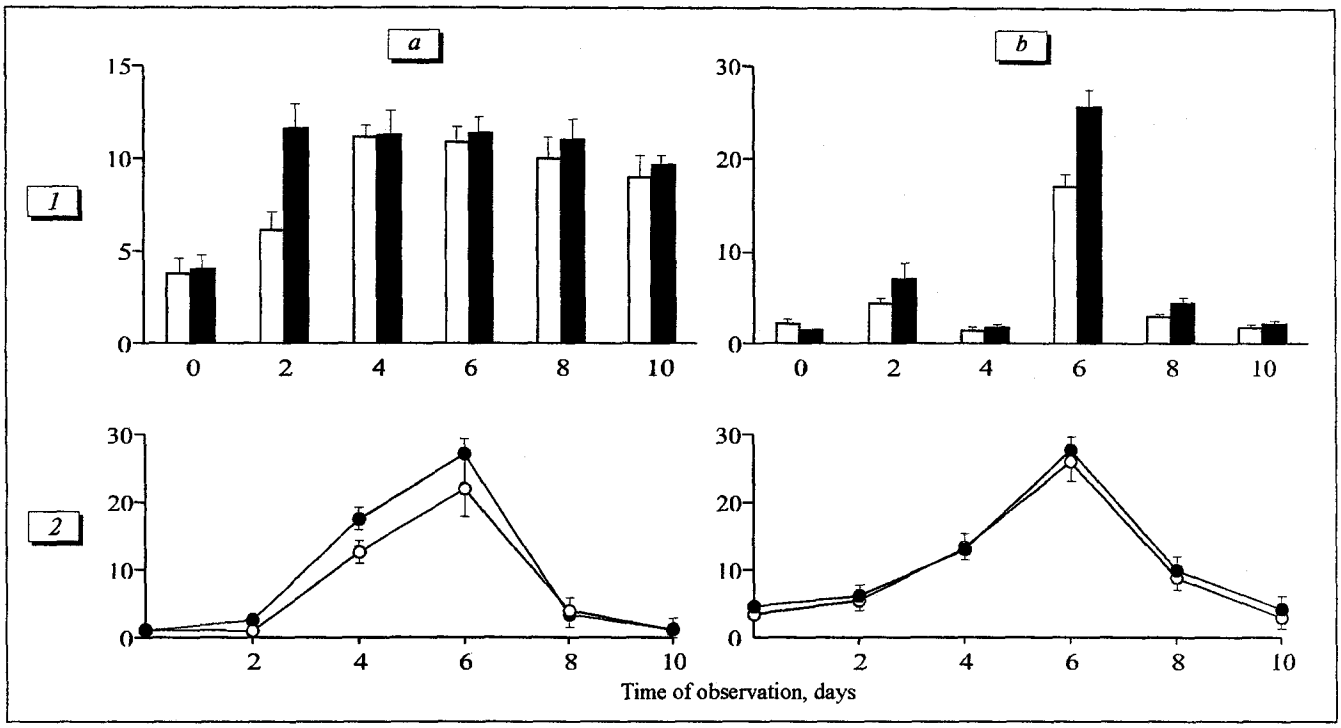

Fig. 1. Number of granulocyte-macrophage (*a*) and erythroid (*b*) colonies formed by adherent (*1*) or nonadherent (*2*) bone marrow cells from adriamycin-treated CBA mice. Here and in Figs. 2 and 3: suspension of nonadherent myelokaryocytes is free from Thy 1.2^+ cells (light bars and markers) or treated with complement only (shaded bars and markers). Ordinate: colony-forming capacity of bone marrow cells (per 10^5 nonadherent myelokaryocytes). Confidence intervals at p=0.05.

rabbit serum (source of complement, 1:10 v/v ratio) and incubated for 45 min at 37°C. Experimental and control cells were twice washed by centrifugation and adjusted to a concentration of 2×10^5 nuclears per 1 ml cultural medium consisting of 79% RPMI-1640, 1% methyl cellulose, 20% fetal calf serum and sup-

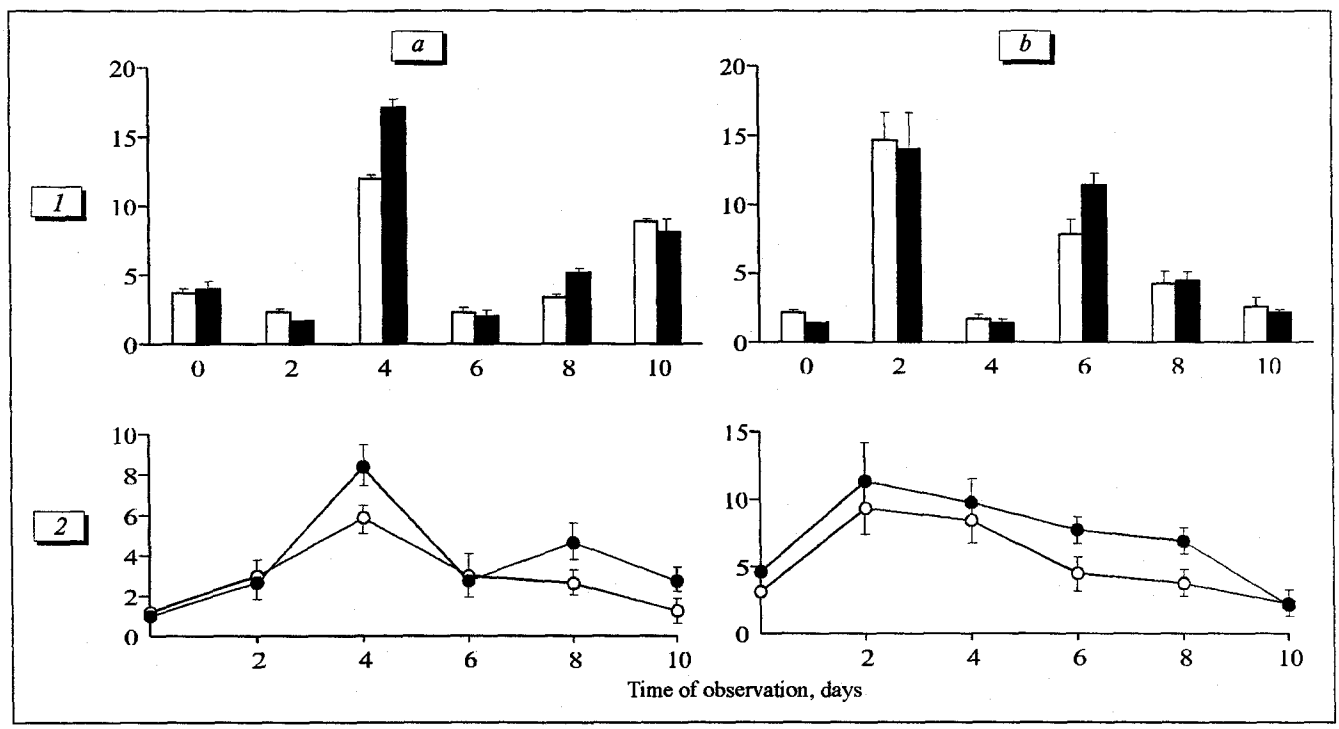

Fig. 2. Number of granulocyte-macrophage (*a*) and erythroid (*b*) colonies formed by adherent (*1*) or nonadherent (*2*) bone marrow cells from cyclophosphamide-treated CBA mice.

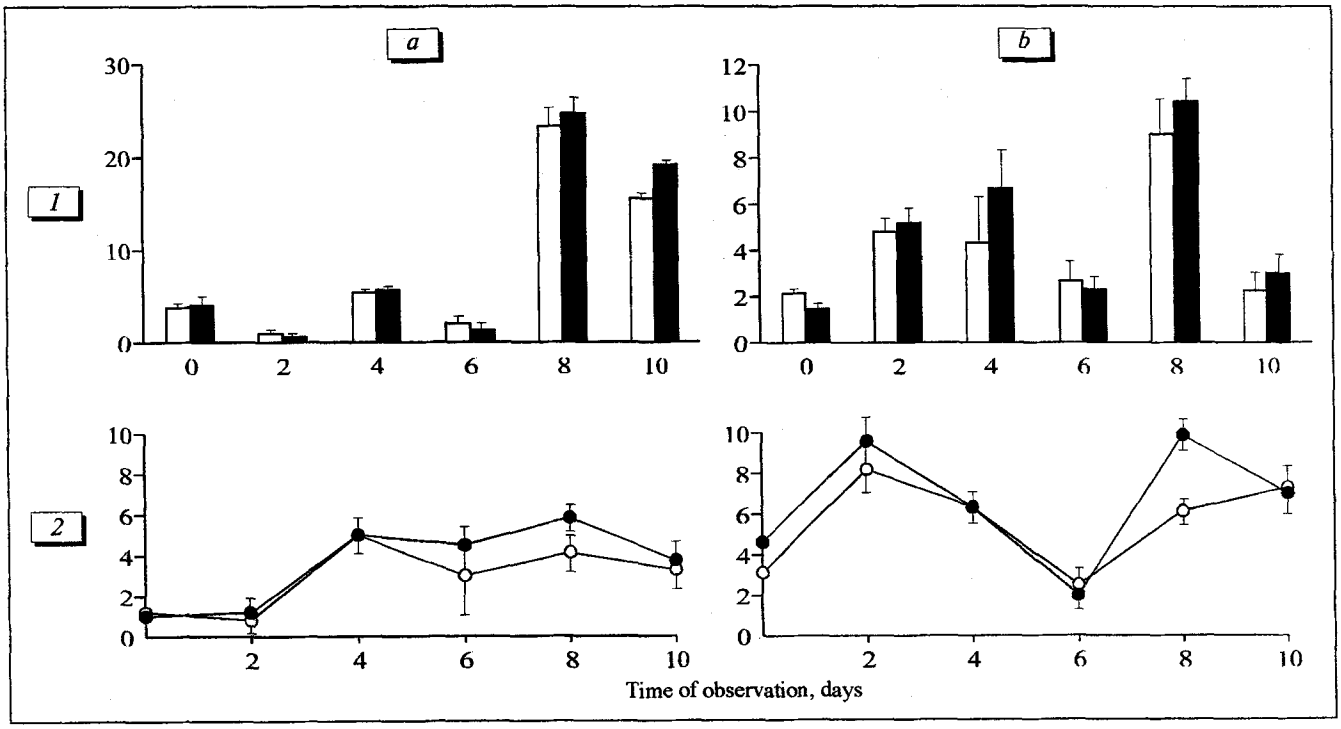

Fig. 3. Number of granulocyte-macrophage (*a*) and erythroid (*b*) colonies formed by adherent (*1*) or nonadherent (*2*) bone marrow cells from 5-fluorouracil-treated CBA mice.

lemented with 280 mg/ml L-glutamine, 4 μM 2-mercaptoethanol, 50 mg/liter gentamicin (all reagents from Sigma). Part of semivicious cell suspension was transferred to 24-well plates (0.5 ml per well) containing 5×10^5 washed adherent myelokaryocytes isolated from mice of the same group. The remainder volume was mixed with 1 ng/ml granulocyte-macrophage colony-stimulating factor (Sigma) or 2 U/ml recombinant erythropoietin (Sigma) and transfered to 24-well plates (0.5 ml/well). All plates were incubated in a CO_2 incubator at 37°C, 5% CO_2 and 100% humidity, and the num-ber of granulocyte-macrophage and erythroid colonies was counted 7 or 3 days later, respectively.

The data were processed by analysis of variances using the Student *t* test.

RESULTS

Function of CFU-GM and CFU-E under normal conditions does not depend on the presence of Thy 1.2^+ cells, since the removal of these cells had practically no effect on colony formation from intact bone marrow cells (Figs. 1-3).

Adriamycin caused a transient decrease in the number of Thy 1.2^+ cells in mouse bone marrow to 22% of the initial level (Table 1). However, their colony-stimulating capacity considerably increased as soon as on day 2 postinjection. In samples containing Thy 1.2^+ cells, the feeder activity of adherent bone marrow cells with respect to CFU-GM was moderately increased (by 24% on day 6 postinjection, Fig. 1, *a*).

Table 1. Content of Thy 1.2^+ Cells in Bone Marrow of CBA Mice Injected with Adriamycin, Cyclophosphamide, or 5-Fluorouracil in Maximal Permissible Doses (% of Initial Level, *X*±*m*)

Days postinjection	Adriamycin	Cyclophosphamide	5-Fluorouracil
Before treatment	100.00±7.61	100.00±8.88	100.00±8.88
2	21.45±21.40 (0.002)	11.99±8.61 (0.000)	132.55±14.95 (0.071)
4	103.09±9.84 (0.805)	65.12±20.36 (0.110)	154.67±18.09 (0.012)
6	69.16±25.66 (0.217)	42.72±9.81 (0.001)	251.75±44.38 (0.002)
8	156.26±21.70 (0.018)	68.80±9.74 (0.037)	93.51±30.50 (0.821)
10	111.99±24.65 (0.609)	132.15±21.09 (0.148)	81.54±13.22 (0.252)

Note. Significance of differences between given and baseline values are shown in arentheses.

Cyclophosphamide induced a considerable and long-term decrease in the number of T helpers in the bone marrow (days 2-8 postinjection, Table 1), but the presence of even minor quantities of these cells in cultures had a substantial effect on proliferation and differentiation of hemopoietic precursors. Maximum feeder activity of Thy 1.2^+ cells with respect to CFU-GM (days 4-10) and CFU-E (days 6-8) was observed in the presence of adherent bone marrow fraction (Fig. 2) despite pronounced and sustained depletion of this fraction induced by cyclophosphamide [2].

Injection of 5-fluorouracil induced accumulation of Thy 1.2^+ cells in the bone marrow to 252% of the initial level (Table 1). However, these cells only slightly stimulated proliferation of hemopoietic precursors (on days 8-10) and primarily in cooperation with adherent cells of HIM (Fig. 3)

An important role in the interaction between HIM different elements and between them and hemopoietic precursors is played by both secreted cytokines and cell-to-cell transmembrane contacts [6-9,12-14]. On the other hand, cytostatics strongly modulate production of humoral factors by HIM cells and their ability to bind hemopoietic precursors [2,5]. The observed discrepancy between the number of HIM cells and stimulation of hemopoietic precursors growth after injection of cyclophosphamide and 5-fluorouracil is probably due to their more potent suppressive effect on nonadherent and adherent HIM cells, respectively, in comparison with adriamycin [2,5].

Thus, bone marrow cells with Thy 1.2^+ phenotype play an important role in regulation of hemopoietic reparation after myelosuppression induced by different cytostatics. The ability of T cells to stimulate proliferation of hemopoietic precursors (both direct and mediated via adherent elements) depends rather on functional state than on the number of HIM cells in the bone marrow of experimental animals.

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