Regulation of Cytokine Production in the Bone Marrow during Postcytostatic Regeneration by SC-1$^+$ and Thy-1$^+$ Cells

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The role of SC-1$^+$ and Thy-1$^+$ cells in the regulation of interleukin-3 production and erythropoietic and colony-stimulating activity of bone marrow cells is studied in cyclophosphamide-treated mice. It is shown that elimination of SC-1$^+$ and Thy-1$^+$ cells has no effect on the production of these cytokines during the early postcytostatic period and upregulates production of some factors stimulating proliferation and colony-formation in the spleen.

Key Words: *T cell precursors; cytokines; cytostatic treatment*

Under physiological conditions, when organism needs no enhanced generation of blood cells, stem cells are exposed to a variety of negative and positive regulatory molecules (signals) produced by their microenvironment consisting of endothelial and fibroblastoid cells and macrophages [6]. Under pathological conditions there often arises a need for additional signals promoting proliferation and differentiation of hemopoietic precursors [2]. This role is played by interleukins (IL) produced by activated immune cells [7]. In particular, bone marrow precursors of T cells produce regulatory peptides stimulating and inhibiting hemopoiesis [4]. The aim of the present study was to evaluate the role of SC-1$^+$ and Thy-1$^+$ cells in the regulation of IL-3 production and in erythropoietic (EPA) and colony-stimulating (CSA) activities of bone marrow cells during postcytostatic regeneration.

MATERIALS AND METHODS

Experiments were performed on 200 male (CBA× C57Bl/6)F_1 mice weighing 20 g. Experimental mice received single intraperitoneal injection of cyclophosphamide (Saransk Drug Plant) in the maximum permissible dose of 250 mg/kg. Bone marrow cells were fractionated by panning as described elsewhere [10]. For elucidation of the role of SC-1$^+$ and Thy-1$^+$ cells in the regulation of cytokine synthesis, bone marrow cells were treated with anti-SC-1 rabbit antiserum and anti-Thy-1 monoclonal antibodies and nontoxic rabbit complement [3]. The supernatant was collected after 18-h incubation in RPMI-1640 medium supplemented with 5% embryonic calf serum. The effect of the supernatant on colony formation in the spleen was assessed by incubating 10^6/ml Ig$^-$ SC-1$^-$Thy-1$^-$ bone marrow cells for 2 h in the presence of 2-50% supernatants. CSA and EPA of the conditioned media were evaluated as described previously [1]. Synthesis of IL-3 by nonadherent bone marrow cells was estimated by the increase in ^{3}H-thymidine incorporation into IL-3-dependent FDCP-2 cell strain and expressed in arbitrary units. The dilution of supernatant causing 2-fold increase in ^{3}H-thymidine incorporation was taken as a unit of activity.

For more comprehensive characteristics of the test supernatants we used *in vitro* cell proliferation reactions. Proliferative response of thymic and splenic

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cells to the same supernatants and in comitogenic tests with 1 μg/ml concanavalin A and proliferative response of Ig^+ and Thy-1^+ splenocytes obtained after panning were evaluated. Target cells in 96-well plates were incubated for 4 days in a complete medium. ^{3}H-thymidine (0.5 μCi) was added 18 h before the end of incubation. Radioactivity was measured in a Mark-III counter.

The data were processed by ANOVA (Student *t* test).

RESULTS

Nonadherent bone marrow cells exhibited a high level of IL-3 production 3 days after cyclophosphamide injection. Treatment with anti-SC-1 and anti-Thy-1 antibodies and complement had no effect on IL-3 production during the first 4 days postinjection, increased it on the 5th day and inhibited on the 6th day postinjection (Fig. 1).

Effect of cell supernatants on splenic colony formation of $Ig^-SC\text{-}1^-Thy\text{-}1^-$ cells from intact bone marrow was assessed on the 1 and 3 days after cytostatic treatment (Fig. 2). Supernatants obtained 1 day postinjection markedly stimulated splenic colony formation, and this effect did not depend linearly on their concentration. For instance, in concentrations 50 and 2%, supernatants equally stimulated colony formation, while incubation with 10% supernatant was less effective. Elimination of anti-SC-1^+ and anti-Thy-1^+ cells had no effect on the production of factor(s) stimulating colony formation. Supernatants collected on postcytostatic day 3 also stimulated colony formation, but their effect depended on the dose of supernatant and decreased after treatment with SC-1 and Thy-1 antibodies (at supernatant concentration 50%).

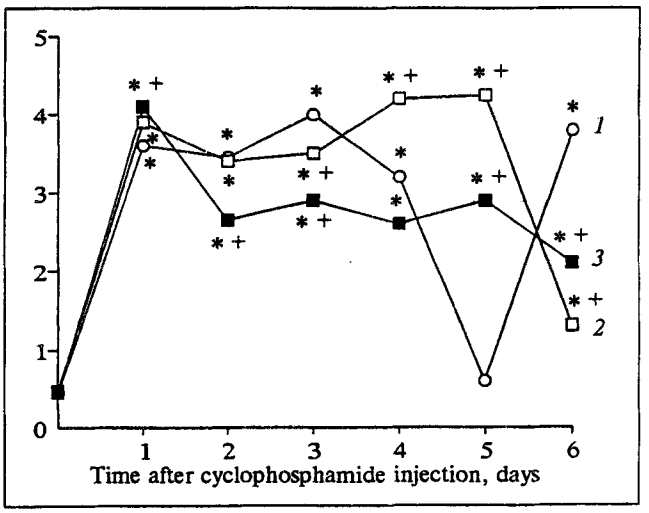

Fig. 1. Dynamics of interleukin-3 production by nonadherent bone marrow cells of (CBA×C57Bl/6)F_1 mice treated with maximally permissible dose of cyclophosphamide. Ordinate: synthesis of interleukin-3, arb. units. Here and in Fig. 2-3: *1*) control, treatment with anti-SC-1 (*2*) and anti Thy-1 (*3*) antibodies. $p<0.05$: *compared with the control, +compared with *1*.

Figure 3 shows the effect of supernatants on proliferation of committed precursors from intact bone marrow. Their ability to stimulate *in vitro* growth of erythroid colonies decreased on postcytostatic days 2 and 7, while CSA with respect to proliferation of granulocyte/macrophage precursors remained unchanged. Anti-Thy-1 monoclonal antibodies had no effect on CSA and EPA production in regenerating bone marrow. On the other hand, treatment of bone marrow nuclear cells with anti-SC-1 antiserum enhanced EPA on days 2 and 7 and CSA on days 3 and 7 postinjection.

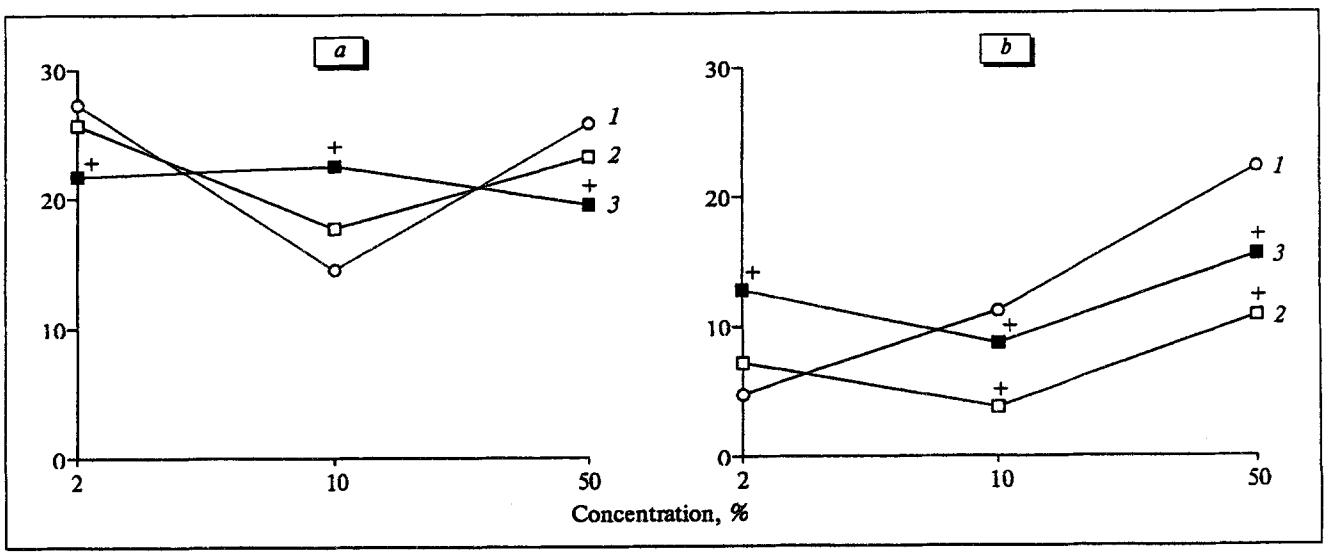

Fig. 2. Splenic colony formation of bone marrow cells from intact mice in the presence of supernatants of bone marrow cells from (CBA×C57Bl/6)F_1 mice obtained 1 (*a*) and 3 (*b*) days after injection of cyclophosphamide. Ordinate: number of colonies.

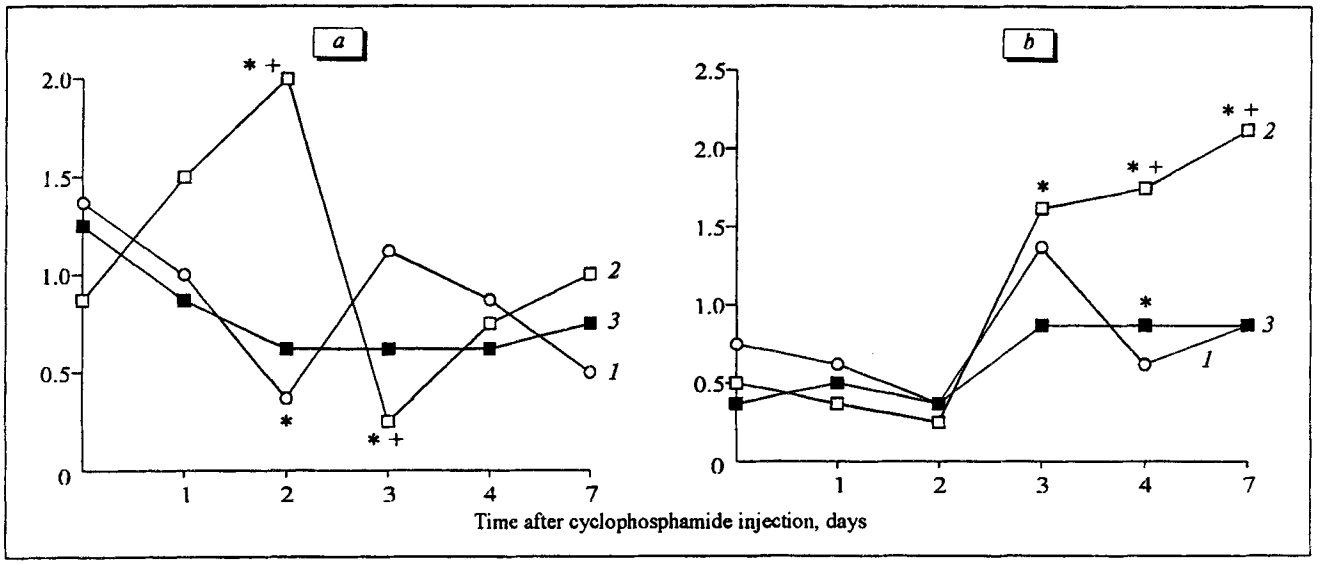

Fig. 3. Effect of supernatants of bone marrow cells from (CBA×C57Bl/6)F_1 mice obtained different times after cyclophosphamide treatment on the growth of erythroid (*a*) and granulocyte/macrophage precursor cells (*b*) from bone marrow of intact mice. Ordinate: number of colonies, $\times 10^5$ cells.

Conditioned media of myelokaryocytes isolated after cytostatic treatment stimulated ^{3}H-thymidine incorporation into thymocytes and splenocytes under conditions of the comitogenic test, and into splenocytes and T cells in the absence of mitogen. This stimulation was most pronounced one day after cytostatic treatment and persisted until day 7.

Of particular importance is the fact that supernatants of intact bone marrow cells treated with anti-SC-1 and anti-Thy-1 antibodies and complement became able to stimulate proliferation of thymocytes and splenocytes in the comitogenic test and spontaneous proliferation of splenocytes and T cells. Conditioned media of regenerating bone marrow cells obtained on day 1 after cytostatic treatment and treated with the same antibodies also stimulated splenocyte proliferation. Treatment of bone marrow cells obtained later than 1 day postinjection with antibodies and complement had no effect on stimulating activity of their supernatants.

It can be hypothesized that cytostatic treatment activates mechanisms of bypass hemopoiesis triggered by IL-3 and IL-6 synergistically modulating hemopoiesis [9]. The presence of IL-6 in the supernatants is indirectly confirmed by stimulation of thymocyte and splenocyte proliferation in the comitogenic test and spontaneous proliferation of splenocytes and T cells [8]. Moreover, supernatants of adherent cells obtained within 5 days after cytostatic treatment contain IL-1, which can act as factor of stem cell differentiation, shorten the cell cycle, and stimulate IL-3 receptors [5,11].

Unfortunately, we failed to demonstrate the role of SC-1$^+$ and Thy-1$^+$ cells in cytokine production by regenerating bone marrow cells. Moreover, elimination of these cells stimulated production of factors enhancing splenocyte proliferation and colony formation in the spleen, which agrees with experiments demonstrating production of hemopoiesis-inhibiting factors by SC-1$^+$ cells [4].

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