Growth-Inhibitory Activity of Bone Marrow Cells during CCl_4-Induced Liver Cirrhosis

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During CCl_4-induced liver cirrhosis, cells of the hemopoiesis-inducing microenvironment in the bone marrow of BALB/c mice produced activity inhibiting the growth of erythropoiesis and granulomonocytopoiesis precursors. Stimulation with yeast polysaccharide zymosan increased the inhibitory activity (especially in relation to granulomonocytic precursors). The highest growth-inhibitory activity was produced by the bone marrow adherent fraction (residual bone marrow macrophages). Tumor necrosis factor-α is probably responsible for the inhibition of the growth of myeloid precursors in mice with CCl_4-induced liver cirrhosis.

Key Words: *liver; CCl_4; cirrhosis; bone marrow; hemopoiesis; macrophages; tumor necrosis factor-α*

In animals with CCl_4-induced liver cirrhosis, the ability of the blood system to respond to additional stimulating influences sharply decreases [3,8]. The number of progenitor cells, especially granulomonocytic precursors in the bone marrow decreased considerably. Functional activity of the blood system is determined by the type of specific hemopoiesis-inducing microenvironment formed by stromal cell elements of hemopoietic organs: fibroblasts, adipocytes, and macrophages [11]. This regulation is effected via production of factors stimulating and inhibiting hemopoiesis [4]. Insufficiency of the blood system in cirrhotic animals and its paradoxical reaction to stimulatory factors are probably due to both low production of factors maintaining proliferation and maturation of myeloid precursors and the production of growth-inhibitory activity (or activities) by hemopoietic microenvironment in the bone marrow. Here we determined the presence (or absence) of the growth-inhibitory activity in supernatants of various fractions of bone marrow cells (BMC) in mice with CCl_4-induced liver cirrhosis.

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MATERIALS AND METHODS

Experiments were performed on 40 male and female BALB/c mice weighing 20-25 g. Liver cirrhosis was induced by administration of CCl_4 (oil solution) for 16 weeks. A suspension of zymosan granules (100 mg/kg body weight) was administered 24 h before euthanasia to stimulate hemopoiesis. Total, adherent, and nonadherent fractions of BMC were isolated from intact and cirrhotic mice before and after stimulation with zymosan [13]. In animals with liver cirrhosis, fractionation of BMC was performed 3 days after the last CCl_4 injection.

Erythropoietin-like and colony-stimulating activities of isolated fractions were determined [2] by adding one-day-old supernatants to a culture of normal syngeneic bone marrow cells with standard inductors of erythroid (CFU-E) or granulomonocytic (CFU-GM) precursors. The serum of syngeneic mice obtained 24 h after acute blood loss (2.5% of body weight) was used as the standard inductor of CFU-E. The serum of syngeneic mice obtained 48 h after administration of zymosan served as the standard inductor of CFU-GM [3]. In experimental series, test supernatants (0.1 ml) were added to cell cultures simultaneously with CFU-E and CFU-GM inductors. In control series,

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equivalent volumes of supernatants of unfractionated BMC from normal syngeneic mice were added to the cell culture. Recombinant tumor necrosis factor-α (TNF-α, Sigma) in concentrations of 1, 10, 100 ng/ml was added to BMC culture to study its effects on the growth of hemopoietic precursors. All experiments were performed in three (or two) replicate.

The results were analyzed by Student's t test.

RESULTS

Supernatants of unfractionated BMC from normal BALB/c mice added to cultures with standard CFU-E inductor did not increase the number of CFU-E, while supernatants obtained from stimulated animals increased the growth of CFU-E by 1.8 times (Figure 1, *a*). By contrast, supernatants of the total and adherent fractions obtained from unstimulated cirrhotic mice inhibited the growth of CFU by 1.4 and 2.7 times, respectively. Thus, the adherent fraction displayed more pronounced inhibitory activity, while the nonadherent fraction did not display such activity in relation to erythroid precursors. The inhibitory activity in BMC supernatants increased 24 h after administration of zymosan. Supernatants of the total and adherent fractions added to cultured erythroid cells decreased the number of CFU-E by 5 and 8.4 times, respectively. Zymosan induced the appearance of the inhibitory activity in nonadherent cells.

The addition of these supernatants to CFU-GM induced similar but more pronounced changes (Fig. 1, *b*). Supernatants of total BMC fraction obtained from normal and stimulated mice increased the growth of CFU-GM by 1.3 and 2 times, respectively ($p<0.001$). However, supernatants of the total and adherent fractions from cirrhotic mice decreased the growth of CFU-GM by 3.7 and 10.2 times, respectively, compared with this index in the culture of cells of intact animals in the presence standard CFU-GM inductor. Supernatants of nonadherent cells possessed no CFU-GM growth-inhibitory activity. Zymosan sharply increased the growth-inhibitory activity. Supernatants of the total and adherent fractions inhibited the growth of CFU-GM by 9.2 and 17.9 times, respectively. Similar activity that was not observed in unstimulated animals was found in the fraction of nonadherent cells.

Thus, our findings indicate that BMC of animals with CCl_4-induced liver cirrhosis produce activity inhibiting the growth of CFU-E and CFU-GM. This production sharply increases in stimulated animals. The highest activity is found in supernatants of adherent BMC fraction containing macrophages. The appearance of similar activity in supernatants of the nonadherent fraction from zymosan-treated animals is probably associated with activation of the admixture of adherent cells or with transformation of lymphoid cells (T lymphocytes) into hemopoiesis-inhibiting suppressor cells [10].

The question arises: what is the nature of this activity? Permanent systemic endotoxemia accompanying chronic inflammatory destructive processes (including liver fibrosis) [1] maintains long-term activation of macrophages releasing a variety of anti-inflammatory cytokines. High concentrations of cytokines are known to inhibit myelopoiesis. Macrophage cytokines, in particular transforming growth factor-α, TNF-α, and MIF-1-α, regulate proliferation of myeloid precursors [5]. The main effector of pathogenic effects of endotoxins is TNF-α [9]. Therefore, we

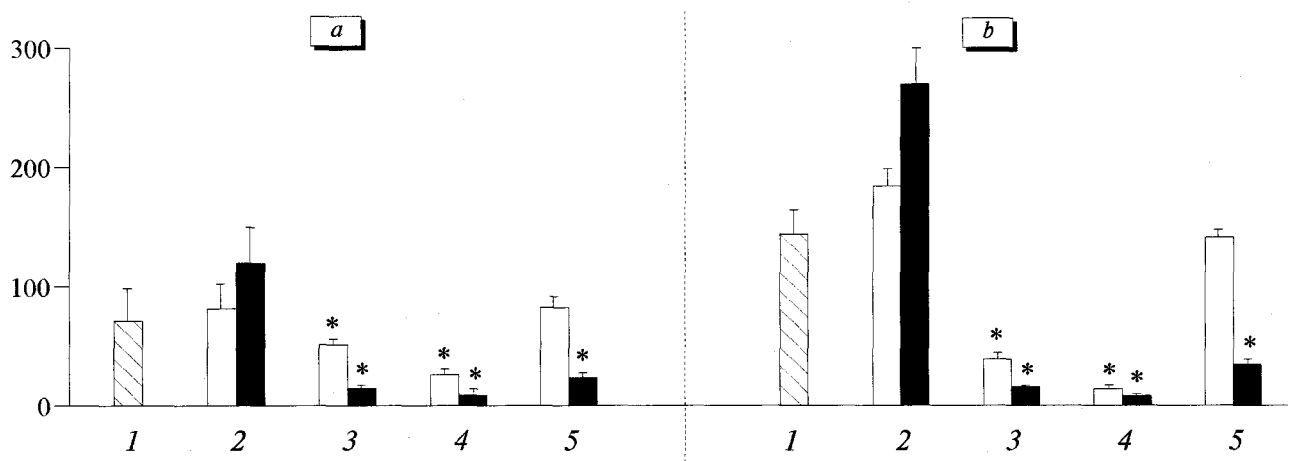

Fig. 1. Effects of supernatants of various bone marrow fractions from mice with CCl_4-induced liver cirrhosis on the growth of erythroid (CFU-E) (*a*) and granulomonocytic (CFU-GM) precursors (*b*). Ordinate: number of precursors per 10^5 bone marrow cells. *1*) control culture of normal bone marrow cells with standard growth inductor of CFU-E; *2*) supernatants of the total bone marrow fraction from normal BALB/c mice; *3*) total fraction; *4*) adherent bone marrow cells; *5*) nonadherent bone marrow cells from mice with CCl_4-induced liver cirrhosis. *$p<0.001$ compared with the control. Light and dark bars show unstimulated and zymosan-stimulated mice, respectively.

TABLE 1. Effects of Various Concentrations of TNF-α on Growth of CFU-E and CFU-GM in BMC Culture (*M±m*)

TNF-α concentration, ng/ml	CFU-E	CFU-GM
	×10⁵ BMC	
Control	63.7±5.24	132.0±11.11
1	64.4±1.16	15.1±1.54
10	37.0±1.36	10.8±0.78
100	29.3±1.95	9.9±2.11

studied the effects of recombinant TNF-α on the growth of hemopoietic precursors *in vitro*.

TNF-α in a concentration of 1 ng/ml did not affect the growth of CFU-E (Table 1), while in concentrations of 10 ng/ml and 100 ng/ml it decreased the number of CFU-E in the culture by 1.7 and 2.2. times, respectively. On the other hand, the minimum concentration of TNF-α (1 ng/ml) sharply decreased the number of CFU-GM (by 8.7 times), but increasing the concentration by 10 and 100 times did not potentiate this effect. Thus, TNF-α displayed a dose-dependent inhibitory effect on the growth of CFU-E and to a greater extent CFU-GM. This effect of TNF was similar to the growth-inhibitory activity of BMC supernatants of cirrhotic mice.

TNF-α (one of the most important macrophagic cytokines) can inhibit hemopoiesis [7,12]. Our findings suggest that TNF-α produced by the adherent BMC fraction is responsible for the inhibition of the growth of myeloid precursors. This primarily refers to bone marrow macrophages producing TNF-α [6]. However, it can not be excluded that the growth-inhibitory effect can be produced by a combination of antiinflammatory cytokines secreted by activated macrophages [4,5,12].

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