Activated Lymphocytes Produce Mediators Potentiating the Antitumor Cytostatic Activity of Bone Marrow Cells

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Mitogen-activated murine T and B lymphocytes produce soluble mediators that potentiate the inhibitory effect of bone marrow cells on the growth of leukemic cells *in vitro*. γ-Interferon is a T-cell product increasing the cytostatic activity of bone marrow cells. An increase in the cytostatic activity under the effect of T-cell soluble mediators was characteristic of bone marrow cells from *nude* mice.

Key Words: *T and B lymphocytes; bone marrow cell; tumor growth inhibition*

Bone marrow cells (BMC) phenotypically similar to bone marrow natural suppressor cells inhibit the growth of leukemic cells *in vitro* [1,2,8-10]. Tumor growth suppression by BMC is not associated with tumor cell destruction and is mediated, at least partially, by soluble cytostatic products [9].

We investigated the ability of activated T and B lymphocytes to regulate the cytostatic activity (CSA) of BMC by producing soluble factors.

MATERIALS AND METHODS

(C57BL/6×DBA) F_1 (BDF1, H-2^b/H-2^d) mice aged 3-6 months from the Breeding Center of Siberian Division of the Russian Academy of Medical Sciences were used. BALB nu^+/nu^+ mice were obtained from the Department of Experimental Biomedical Modeling of Tomsk Research Center. The animals received sterile fodder and acidified (pH 2.8) boiled water.

Mastocytoma P815 (H-2^d) and lymphocytic leukemia L1210 (H-2^d) cells were obtained from N. N. Blokhin Cancer Research Center (Moscow) and maintained by *in vitro* culturing.

Cells were cultured in RPMI-1640 with 10 mM HEPES, 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, antibiotics, and 7% fetal calf serum (all reagents from Sigma) in a humidified atmosphere with 5% CO_2.

Splenocytes from normal BDF1 mice were activated with concanavalin A (Pharmacia, 5 μg/ml) or lipopolysaccharide (*E. coli* 055:B5:, Sigma, 20 μg/ml) in 25 cm^2-plastic flasks (Linbro) (10 ml) for 24 h. T and B lymphocytes activated with concanavalin A and lipopolysaccharide, respectively, were isolated by the positive penning method [3]. Immunofluorescence analysis of cells [3] showed that the purity of isolated T and B lymphocytes was close to 100%.

For preparing culture supernatants, activated lymphocytes ($3\text{-}4\times10^6$ cells/ml) were cultured in a 24-well plate (Linbro) for 24 h. The supernatant was obtained by centrifugation and stored at -20°C until use.

In the cytostatic test, BMC (3×10^5/well) were cultured with (25%) or without the supernatant from activated T or B lymphocytes in round-bottom 96-well plates (BDSL) for 20 h. After the medium replacement, P815 or L1210 cells were added to BMC into wells (10^4 cells/well) and cultured for 24 h. In the control tests, tumor cells were cultured without adding any cells or with thymocytes incapable of suppressing leukemic cell growth *in vitro* [1,8]. The level of proliferative activity was measured routinely by incorporation of ^{3}H-thymidin (Izotop) added in a dose of 0.75 μCi to all wells 5 h before the end of culturing.

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The percentage of proliferation suppression was calculated from the formula:

$$\% \text{ suppression} = (1 - \frac{\text{in experiment}}{\text{pulse in control}}) \times 100.$$

Each test was represented by 3-4 parallel cultures. The data were reproduced in three experiments. The results were statistically processed using Student's t test. All the differences were significant at $p<0.05$.

RESULTS

T lymphocytes activated in an allogenic mixed culture produce mediator(s) stimulating the cytostatic activity of BMC, which is tested by suppression of P815 and L1310 cell growth *in vitro* [9]. Both T and B lymphocytes produce soluble factors potentiating the antitumor activity of BMC (Fig. 1). Lymphokine-mediated stimulation of CSA was not associated with generation of killer cells in the cytolytic test with ^{3}H-thymidin-labeled L1210 cells as the target (data not presented).

The cytostatic BMC effectors are similar to natural suppressor cells in many aspects [8,10]. Immunosuppressive activity of the latter is induced and maintained mainly by γ-interferon (IFN-γ) [4,6]. In our experiments the neutralization of IFN-γ activity by specific antibodies (Genzyme) decreased but did not completely suppress the ability of T-cell supernatant to enhance antitumor activity of BMC. These antibodies did not affect the CSA increase under the effect of the B-cell supernatant. T and B lymphocytes activated during immunogenesis may increase the BMC-mediated suppression of tumor growth through the production of IFN-γ and other soluble mediators. This is in line with our data [9] demonstrating the stimulating effect of recombinant human interleukin-2 on antitumor cytostatic activity of BMC.

The entire antitumor activity of freshly isolated normal BMC is probably determined by the immunogenesis processes normally proceeding in the body under the effect of environmental microflora. However, BMC of *nude* mice actively suppressed leukemic cell growth (Fig. 2). Moreover, their CSA markedly increased under the effect of mediators in the culture medium from activated T lymphocytes. Natural CSA of *nude* mice BMC at least partially does not depend on the constant thymus-regulated immune processes in the organism.

The relationship between BMC and T lymphocytes may play an important role in the formation of an antiproliferative barrier preventing the development of leukemia. The L5178 lymphoma quiescent cells may be present in the bone marrow of a syngeneic DBA mouse for a long time, ensuring threshold antigenic stimulation needed for the formation of stable antitumor immunity mediated by T cells [7].

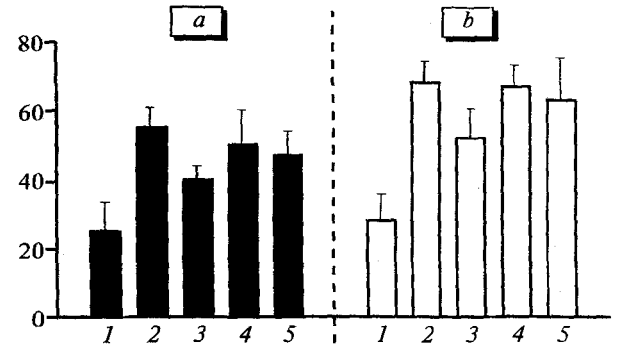

Fig. 1. Increase in the bone marrow cell cytostatic activity by lymphokines. Bone marrow cells of BDF1 mice were cultured for 20 h in medium (*1*) with 25% T-cell supernatant without (*2*) or with 4 mg/ml antibodies to γ-interferon (*3*) and with B-cell supernatant without (*4*) or with antibodies (*5*). The duration of subsequent culturing of bone marrow cells with tumor cells was 24 h. Control incorporation of ^{3}H-thymidine in P815 and L1210 cells was 172,000 and 182,000 cpm, respectively. Here and in Fig. 2: ordinate: % of P815 (*a*) and L1210 (*b*) cell growth suppression.

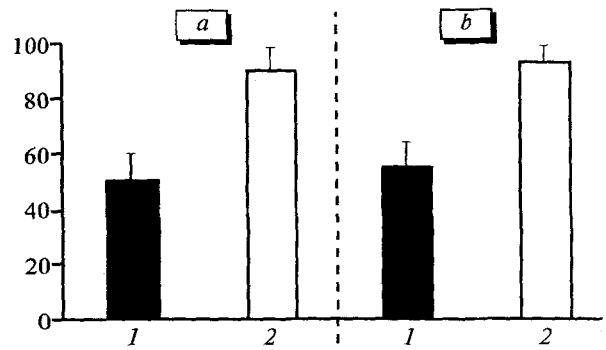

Fig. 2. Cytostatic activity of bone marrow cells isolated from nude mice. Bone marrow cells of nude mice were cultured without (dark bars) or with 25% T-cell supernatant (light bars) for 20 h. The duration of subsequent culturing of bone marrow cells with tumor cells was 24 h. Control proliferation of P815 and L1210 cells was 152,000 and 165,000 cpm, respectively.

Together with our previous findings [9], these results suggest that T cells involved in immunogenesis increase (through the production of IFN-γ, interleukin-2, and other mediators) the cytostatic activity of BMC and thus prevent the development of leukemia at the sites of intense hemopoiesis. If so, the efficacy of BMC-mediated antitumor cytostatic mechanisms might increase during prolonged immune processes associated with the development of, for example, chronic graft-versus-host reaction. This reaction in allogenic bone marrow recipients is associated with a low probability of leukemic relapse [5].

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