Production of Transmitters Stimulating Antitumor Cytostatic Activity of Bone Marrow Cells by Activated Lymphocytes

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Murine lymphocytes preactivated with mitogen (both T and B) produce soluble transmitters which stimulate cytostatic activity of normal mouse bone marrow cells, tested by *in vitro* inhibition of P815 mastocytoma and L1210 lymphoma cell growth. γ-Interferon is one of T-cell products stimulating antitumor activity of bone marrow cells. Cytostatic activity stimulated by T-cellular soluble transmitters is another characteristic of bone marrow cells isolated from nude mice.

Key Words: *T, B lymphocyte; bone marrow cell; tumor growth inhibition*

Bone marrow cells (BMC) similar by many phenotypical signs to bone marrow natural suppressor cells suppress the growth of leukemic cells *in vitro* [1,2,8-10]. Tumor growth suppression by BMC does not involve tumor cells destruction and is mediated, at least partially, by soluble cytostatic products [9]. We studied the capacity of activated T and B lymphocytes to regulate cytostatic activity of BMC by producing soluble products.

MATERIALS AND METHODS

Three-six-month-old (C57BL/6×DBA) F_1 (BDF_1, H-2^b/H-2^d) mice from Breeding Center of Siberian Division of Russian Academy of Medical Sciences, and nude BALB/c nu^+/nu^+ mice from the Department of Experimental Biomedical Simulation, Tomsk Research Center, Russian Academy of Medical Sciences, were used. The animals received sterilized fodder and acidified (pH 2.8) boiled water. P815 mastocytoma (H-2^d) and L1210 lymphoma (H-2^d) cells were obtained from the Oncology Research Center, Russian Academy of Medical Sciences and preserved by *in vitro* culturing.

Cells were cultured in RPMI-1640 with 2 g/liter $NaHCO_3$, 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 BDF_1 mice were activated by concanavalin A (5 μg/ml, Pharmacia) or lipopolysaccharide (*E. coli* 055:B5, 20 μg/ml, Sigma) in plastic 25 cm^2 Linbro flasks (10 ml) for 24 h. T and B lymphocytes preactivated with concanavalin A and lipopolysaccharide, respectively, were isolated by positive penning [3]. Immunofluorescent staining of [3] showed the purity of isolated T and B lymphocytes to be almost 100%.

For preparing culture supernatants, preactivated lymphocytes ($3\text{-}4\times10^6$/ml) were cultured in 24-well plates (Linbro) for 24 h. Then the supernatant was purified from cells by centrifugation and stored at -20°C.

In the cytostatic test, BMC (3×10^5/well) were cultured with or without the supernatant (25%) from preactivated T or B lymphocytes in round-bottom 96-well plates (BDSL) for 20 h. After the medium

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had been replaced, 10^4 P815 or L1210 cells were added in wells with BMC. Cell mixtures were cultured in medium without the supernatant for 24 h. Control cultures consisted of tumor cells alone or with thymocytes not suppressing leukemic cell growth *in vitro* [1,8]. The level of cellular proliferative activity was evaluated routinely by incorporation of ^{3}H-thymidine (Izotop) added to all wells in a dose of 0.75 μCi 5 h before the end of culturing. The percentage of cell proliferation suppression was calculated from the formula:

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

Each test was represented by 3 parallel cultures. The data were reproduced in 3 similar experiments. Results were statistically processed using Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

T lymphocytes activated in an allogenic mixed culture produce mediator(s) stimulating cytostatic activity of BMC tested by P815 or L1210 cell growth suppression *in vitro* [9]. Figure 1 demonstrates that both T and B lymphocytes produce soluble compounds stimulating antitumor activity of BMC. The cytolytic test with ^{3}H-thymidine-labeled L1210 cells as the target showed that lymphokine-mediated stimulation of BMC cytostatic activity was not associated with generation of killer cells.

According to previous reports [8,10], cytostatic BMC effectors are similar to natural suppressor cells in many aspects. γ-Interferon (γ-IF) is considered to be the main agent inducing and maintaining the immunosuppressor activity of natural suppressor BMC [4,6]. In our experiments, neutralization of γ-IF activity by specific antibodies (Genzyme) significantly decreased but does not completely block the capacity of T cellular supernatant to stimulate the antitumor activity of BMC. On the other hand, antibodies to γ-IF did not notably affect the process of BMC cytostatic activity stimulation by B cellular supernatant. Therefore, T and B lymphocytes, activated during immunogenesis, may stimulate through the production of γ-IF and other soluble transmitters the BMC-mediated suppression of tumor growth. Such a hypothesis is in line with our previous report [9] about the stimulating effect of recombinant human interleukin-2 on antitumor cytostatic activity of BMC.

These data suggest that the entire antitumor activity of fresh-isolated normal BMC directly depends

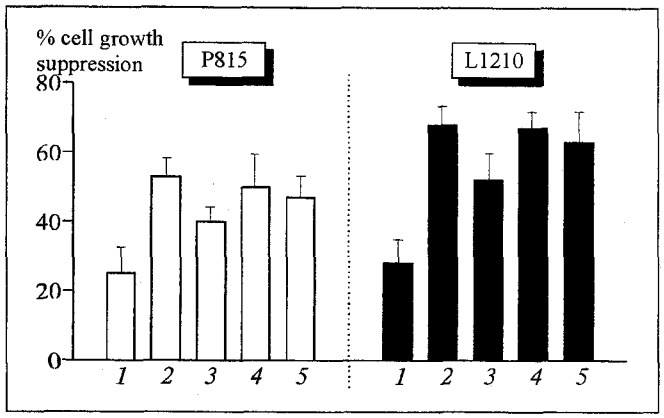

Fig. 1. Stimulation of bone marrow cell cytostatic activity by lymphokines. Bone marrow cells of BDF_1 mice were precultured for 20 h in medium alone (*1*), with 25% T cell supernatant without (*2*) or with 4 μg/ml antibodies to γ-interferon (*3*), and with B cell supernatant without (*4*) or with antibodies to γ-interferon (*5*). Control incorporation of ^{3}H-thymidine in P815 and L1210 cells was 172,000 and 182,000 pulses/min, respectively.

on immunogenesis processes permanently occuring in an organism under the effect of environmental microflora. However, BMC of nude mice rather actively suppress the growth of leukemic cells (Fig. 2). Moreover, their cytostatic activity significantly increases under the effect of mediators of culture supernatant from preactivated T lymphocytes. The overwhelming majority of immune reactions are based on T-cell reactivity. Natural cytostatic activity of nude mice BMC seems to indicate that it does not depend on the immune processes, at least partially.

Interaction between BMC and T lymphocytes might play an important role in the formation of antiproliferative barrier preventing the development of leukemia. Recent studies demonstrated L5178 lymphoma dormant cells to persist in bone mar-

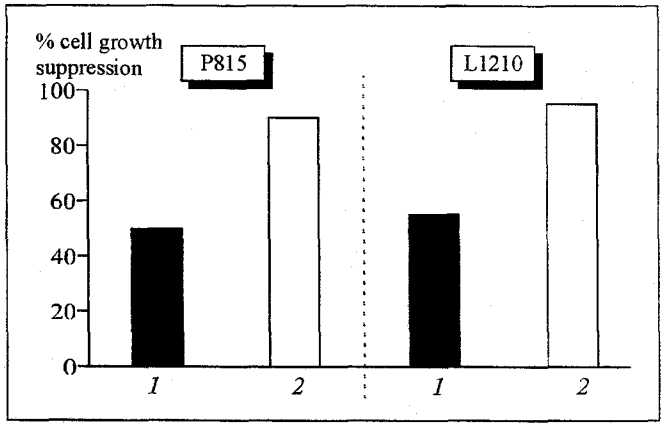

Fig. 2. Cytostatic activity of nude mice bone marrow cells. Bone marrow cells of nude mice were preincubated without (*1*) or with 25% T cell supernatant (*2*) for 20 h. Control proliferation of P815 and L1210 cells was 152,000 and 165,000 pulses/min. Mean arithmetic deviations were no more than 10% of the respective means.

row of a syngeneic DBA mouse for a long time and ensure a threshold antigenic stimulation needed for the formation of a stable antitumor immunity mediated by T cells [7]. These data together with our findings [9] suggest that T cells involved in immunogenesis might stimulate cytostatic activity of BMC through the production of γ-IF, interleukin-2, and probably other transmitters, and thus prevent the development of leukemia at sites of intense hemopoiesis. If so, the efficacy of BMC-mediated antitumor cytostatic mechanisms can notably increase during long-lasting immune processes occuring during the development of chronic "graft-versus-host" reaction. In bone marrow allograft recipients this chronic reaction is associated with a much lower probability of leukemic relapse [5].

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