ONCOLOGY

Decrease of Antitumor and Suppressor Activities of Nonadherent Bone Marrow Cells by Culturing in Medium Conditioned with Ehrlich Adenocarcinoma Cells

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It was demonstrated that proliferation of bone marrow cells cultured in medium conditioned with tumor cells is somewhat increased. A much more potent stimulation of bone marrow cell proliferation is observed after the removal of cells carrying the erythroblast antigen.

Key Words: *suppressor cells; tumor cells; suppressor factor*

It is known that, along with spleen and thymus cells, bone marrow cells (BMC) exhibit a cytostatic activity toward some types of tumor cells *in vitro*. It was demonstrated that splenocytes and thymic cells of T-cell origin capable of cytostatic interaction elicit their effect on tumor cells upon direct contact [1], while the natural suppressor cells (NSC) located in the bone marrow may inhibit the proliferation of immunocompetent and tumor cells *in vitro* by releasing a soluble suppressor factor [4,5,7,12,13] and *in vivo* [2,5]. NSC have no markers of mature immunocompetent cells and are confined to the fraction of BMC which do not adhere to plastic [14]. On the other hand, it is known that during growth, tumor cells secrete various compounds modulating the function of immunocompetent cells [11].

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Our goal was to study the influences of the factors released by Ehrlich adenocarcinoma cells *in vitro* on antitumor, suppressor, and proliferative activity of nonadherent BMC and their production of the suppressor factor.

MATERIALS AND METHODS

BALB/c, DBA/2, and C57Bl/6 mice aged 2-3 months were used. Ehrlich adenocarcinoma (BALB/c mice) and mastocytoma P815 (DBA/2 ice) were inoculated by intraperitoneal transplantation tumor (10^6 cells per animal).

The cells were cultured in RPMI 1640 medium containing 2 mM HEPES, 2 mM L-glutamine, 10% fetal calf serum (Flow), 5×10^{-3} M 2-mercaptoethanol (Fluka), 100 U/ml penicillin, and 100 μg/ml streptomycin.

Ehrlich adenocarcinoma cells (EAC) were isolated from the abdominal cavity on day 7-9 after inoculation and twice washed with medium 199 (Vektor, Novosibirsk). The growth medium was conditioned with EAC as follows: 2×10^4 tumor

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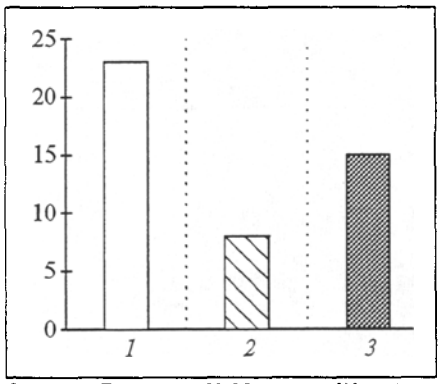

Fig. 1. Effect of BMC on proliferation of P815 mastocytoma cells. Ordinate: ^3H-thymidine incorporation in P815 cells (cpm×10): *1*) in the absence of other cells; *2*) in the presence of normal BMC; *3*) in the presence of BMC preincubated in culture medium conditioned with Ehrlich adenocarcinoma cells (final dilution 1:2).

cells were cultured in 24-well plates (Costar) at 37°C for 48 h (5% CO_2) and centrifuged at 3000g for 5 min, after which the supernatant diluted 2-fold was added to normal BMC (4×10^6 cells/ml) and cultured at 37°C for 72 h. The cells were then washed 3 times, and their cytostatic activity was determined.

The fraction of nonadherent BMC was used in all experiments, since it is known that NSC are confined to this fraction [14]. For removal of adherent cells 4-6×10⁶ BMC were incubated at 37°C for 90 min with the culturing medium in Petri dishes or in 6-well plates.

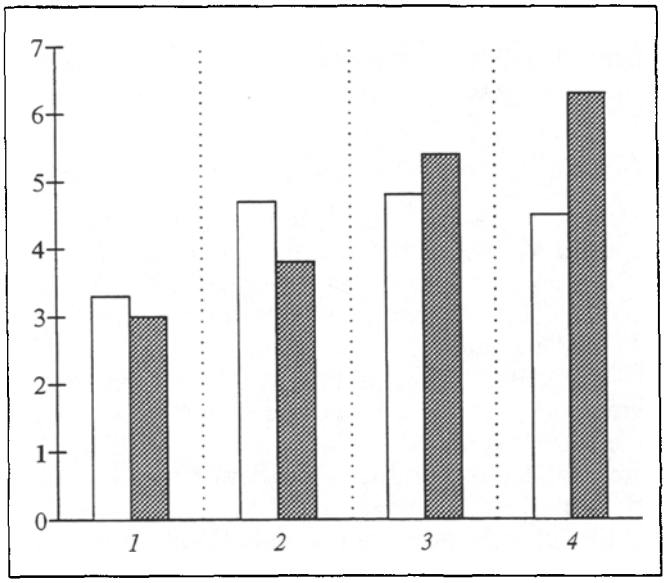

Fig. 2. Effect of culture medium conditioned with Ehrlich adenocarcinoma cells on proliferation of BMC. Ordinate: ^3H-thymidine incorporation in nonadherent BMC (cpm×10) before (black bars) and after (shaded bars) removal of AG-SE-positive cells in the absence of culture medium conditioned with Ehrlich adenocarcinoma cells (*1*) and after the addition of conditioned medium at a final dilution of 1/16 (*2*), 1/8 (*3*), and 1/2 (*4*).

Cytostatic activity of BMC toward P815 mastocytoma cells was evaluated. For this purpose 2×10^4 of P815 cells/well and 2×10^5 BMC/well were cultured in 96-well plates for 16 h under the above-mentioned conditions. Four hours before the end of the culturing 1 μCi ^3H-thymidine was added in each well, and its incorporation was determined by the standard method. Radioactivity was measured in a Mark-III β-counter. The cytostatic index (CI) was determined from the following formula:

$$CI = \left(1 - \frac{\text{cpm per well (effector cells + target cells)}}{\text{cpm in the well with target cells}}\right) \times 100.$$

In order to obtain the suppressor factor (SF) of BMC, 4×10^5 cells/ml were incubated in the culture medium in 24-well plates (Costar) at 37°C for 48 h and then centrifuged at 1000 g for 5 min. The pellet was removed and the supernatant was tested for antitumor and suppressor activities. For evaluation of the antitumor activity different dilutions of SF were added to 2×10^4 EAC or P815 cells per well and incubated under the above-mentioned conditions for 16 h. Tritiated thymidine (1 μCi/ml) was added 4 h before the end of culturing, and radioactivity was measured in a β-counter. For the determination of the suppressor effect of the culture medium conditioned with BMC the medium was added to normal splenocytes (3×10^5 cells/well) with presence of 2.5 μg/ml concanavalin A (ConA) and the cells were cultured in it for 72 h. ^3H-Thymidine was added 18 h before the end of culturing. Antitumor and suppressor activities were evaluated by the label incorporation in cultured tumor cells and splenocytes. The inhibition index (II) was calculated from the following formula:

$$II = \frac{\text{cpm per well (target cells without the factor)}}{\text{cpm per well (target cells in the presence of the factor)}}.$$

The effect of factors produced by Ehrlich adenocarcinoma cells on the proliferation of normal BMC was evaluated by the standard method [8].

The removal of BMC carrying the erythroblast antigen was performed by panning [10]. Monoclonal antibodies to sheep erythrocyte antigens (AG-SE) were isolated with the use of MAE-15 hybridoma cells [3].

The data were statistically analyzed using Student's *t* test.

RESULTS

Cells from normal bone marrow incubated for 3 days in conventional culture medium without tu-

TABLE 1. Effect of Suppressor Factor Produced by BMC Preincubated with Culture Medium Conditioned with Ehrlich Adenocarcinoma Cells ($M \pm m$)

Proliferation of target cells without additives	SF added to BMC at a final dilution	Proliferation of target cells after preincubation in the presence of culture medium conditioned with Ehrlich adenocarcinoma cells in dilution:			
		0	1/16	1/8	1/2
Mastocytoma P815 41,733±2,644	1/4	19 347±4201	31 457±2300	31 354±3719	38 977±3635
	1/8	34 127±4302	35 509±4439	42 054±7671	46 532±2421
	1/16	33 476±3546	43 016±3132	41 843±4336	35 302±4249
	1/32	37 946±2616	39 946±6174	35 742±5350	35 088±4116
Ehrlich adenocarcinoma cells 46,766±4,532	1/4	13 431±2337	23 333±1985	21 990±2296	27 873±2815
	1/8	34 440±1197	37 342±656	40 763±3742	44 232±3319
	1/16	36 630±5301			23 691±4837
Splenocytes stimulated with ConA 14,591±684	1/2	6385±1646	–	–	14 496±1243
	1/8	15 682±1195	–	–	19 048±1597
	1/16	14 958±4250	–	–	18 694±2225
	1/32	17 342±2087	–	–	16 499±2166

Note. Absolute values of ^3H-thymidine incorporation (cpm) are presented.

mor-produced factors induced a pronounced cytostatic effect *in vitro* on P815 mastocytoma cells (Fig. 1). Coculturing of BMC with medium conditioned with EAC resulted in an almost 2-fold (up 37.2%) decrease in the ability of BMC to inhibit proliferation of target cells.

In order to assess the effect of the factors produced by Ehrlich adenocarcinoma on the production of SF by BMC the medium conditioned with nonadherent BMC was added to P815 cells and Ehrlich adenocarcinoma cells (Table 1). The culture medium conditioned with BMC (4-fold dilution) produced an inhibitory effect on the proliferation of both EAC (II was 71%) and P815 mastocytoma cells (53%). When the conditioned medium was further diluted, a dose-dependent reduction in inhibition occurred, and the effect of the suppressor factor was minimal at a dilution of 1/16. The culture medium conditioned with BMC preincubated with Ehrlich adenocarcinoma cells produced a much lesser inhibitory effect. At 1/4 SF the inhibitory index was equal to 67.6% for P815 cells and 40% for EAC; when the medium was diluted 1/8 the bone marrow SF was practically without effect on both types of tumor cells. Thus, incubation of normal nonadherent BMC with medium conditioned with EAC leads to a decrease in the production of bone marrow SF.

Similar results were obtained in the experiments studying the suppressor activity of culturing medium conditioned with BMC with the use of normal syngeneic mitogen-stimulated splenocytes as the control.

Figure 2 illustrates the data characterizing the effect of factors secreted by EAC on the proliferative activity of BMC upon coculturing. These data indicate that proliferation of BMC under the influence of EAC-conditioned culture medium is somewhat increased. Bearing in mind the observations of [9,11], one could assume the increase in proliferation in this case to be related to tumor cell production of factors with colony-stimulating activity. However, recently it was found that for a direct influence of various types of recombinant and highly purified colony-stimulating factors (CSF) on BMC, induction of suppressor cells of myeloid origin is possible [15]. Despite the apparent contradiction, our results agree with the data of [15], since, first, we studied only the nonadherent fraction of BMC containing natural suppressor cells and, second, the culture medium conditioned with EAC in addition to CSF contains numerous factors which may modulate the influence of CSF.

It is interesting to note that preincubation BMC with culture medium conditioned with EAC slightly potentiated the proliferative activity of BMC confined to the nonadherent fraction. However, the stimulatory effect of culture medium conditioned with EAC on the proliferation of BMC becomes much more pronounced after the removal of BMC carrying the erythroblast antigen (Fig. 2). This is consistent with the concept that the natural suppressor activity of BMC is associated with a definite cell population bearing the surface erythroblast antigen and sensitive to MAE-15 monoclonal antibodies [6].

It can be concluded that nonadherent normal BMC reduce their antitumor and suppressor activi-

ties after being treated with substances secreted by EAC, probably due to diminished production of the suppressor factor.

REFERENCES

1. I. V. Bogdashin, *Byull. Eksp. Biol. Med.*, **101**, № 6, 744-746 (1986).
2. S. A. Kusmartsev and V. I. Ogreba, *Eksp. Onkol.*, № 5, 23-25 (1989).
3. E. B. Mechetner, E. N. Rozinova, E. Ievleva, *et al.*, *Dokl. Akad. Nauk SSSR*, **282**, 282-285 (1985).
4. I. G. Sidorovich, V. V. Lyakhov, A. A. Vlasov, *et al.*, *Immunologiya*, № 4, 67-69 (1987).
5. R. M. Khaitov, T- an B-Suppressors and Contrasuppressors in Tumor Growth, *VINITI. Ser. Onkologiya*, **13** (1984).
6. V. V. Cheglyakova, I. G. Tsyrlova, and V. A. Kozlov, *Immunologiya*, № 3, 52-55 (1989).
7. A. K. Duwe and S. K. Singhal, *Cell Immunol.*, **39**, 79-86 (1978).
8. C. L. Hardy and L. Balducci, *J. Nat. Cancer Inst.*, **76**, 535-540 (1985).
9. S. Hosoe, T. Ogura, R. Komuta, *et al.*, *Cancer Immunol. Immunother.*, **28**, 116-122 (1989).
10. M. G. Mage, I. McHugh, and T. L. Rothstein, *J. Immunol. Meth.*, **15**, 47-53 (1977).
11. Y. Tsuchiya, M. Igarashi, R. Suzuki, *et al.*, *J. Immunology*, **141**, 699-708 (1988).
12. J. Subiza *et al.*, *Int. J. Cancer*, **44**, 307-314 (1989).
13. K. Sugiura, M. Inaba, H. Ogata, *et al.*, *Cancer Res.*, **50**, 2582-2586 (1990).
14. M. R. Young, M. Newby, and H. T. Wepsic, *Ibid.*, **47**, 100-105 (1987).
15. M. Young, M. Wright, and M. Young, *Cancer Immunol. Immunother.*, **33**, 146-153 (1991).

Androstenedione Conversion in Human Peripheral Blood Lymphocytes

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The ability of human peripheral blood lymphocytes to convert the androgenic steroid metabolite androstenedione diminishes in women over 45 years old or during menopause. In patients with breast cancer in menopause the ability of lymphocytes to convert androstenedione is enhanced compared with the control. The intensity of conversion in circulating lymphocytes correlates with the blood concentration of sex steroids.

Key Words: *androstenedione; lymphocytes; peripheral blood*

Lymphocytes have surface and cytosol receptors for various hormones [9]. However, these cells have recently been regarded not only as sites of hormonal action but also as sites of hormone synthesis and metabolism. In fact, lymphocytes can produce a number of peptide hormones or hormone-like factors [4] and metabolize one of the most active human glucocorticoids - cortisol [8]. Previously [1] we speculated that lymphocytes may be a site of extragonadal synthesis of estrogens: a process consisting of aromatization of androgenic precursors (notably androstenedione (A) and/or testosterone) and being an intrinsic property primarily of fat and muscle tissues, i.e., tissues which are peripheral in relation to the gonads and adrenals [12]. This assumption was based on observations of $^3H_20$ formation during incubation of ^{3}H-1β-A with human lymphocytes [1]. Due to its stoichiometric peculiarities (theoretically, 1 M estrogens should be formed per M $^3H_20$), this reaction has been used for the evaluation of aromatous activity since the mid-1970s [13]. However, later

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