ONCOLOGY

Mechanism of Antitumor Activity of Bone Marrow Natural Suppressor Cells

Yu. P. Bel'skii, V. K. Patrushev, N. V. Bel'skaya, M. G. Danilets, E. S. Trofimova, and V. I. Agafonov

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Co-culturing of P-815 tumor cell strain and intact mouse bone marrow cells nonadherent to plastic resulted in the appearance of soluble mediators with antitumor activity. Bone marrow cells start releasing these antiproliferative factors only after signal exchange with the target tumor cells. The cell—cell contact is an important factor for the induction of antitumor activity. Antitumor activity of bone marrow cells (similarly as immunosuppressive activity) is realized through suppressor factors; the appearance of these factors is induced by target tumor cells.

Key Words: *natural suppressor cells; antitumor activity*

The capacity of hemopoietic cells of different degree of maturity and belonging to different stems to suppress proliferation of T and B cells in response to antigens and mitogens *in vitro* and *in vivo* without preliminary contact with the targets and restriction for the histocompatibility complex antigens was called natural suppressor activity [2,3,5,6,12]. One more manifestation of natural suppressor activity of immature hemopoietic cells (natural suppressor cells — NSC) is their capacity to suppress proliferation of some tumor cells *in vitro* [8,10]. NSC factors causing immunosuppressive effect are well known (*e. g.*, transforming growth factor-β, NO, prostaglandins, *etc.* [7,8,13]), but little is known about mediators of antitumor activity. There are only few reports that this activity does no depend on NO [1,11]. The mechanism of induction of immunosuppressor activity in bone marrow NSC is well described, but there are no data about approaches to activation of the antitumor effects. As for immunosuppressor activity, the target (activated lymphocyte) induces NO production by intact bone marrow NSC [4]; NO is the only NSC factor. Antitumor effect is based on an NO-independent mechanism not working for lymphocytes. In cases when tumor cell serves the target, NSC is activated by this target. If this activity exists before the contact with the target, we can admit that lymphocytes are insensitive to the antitumor factor, which is less probable. Here we studied the mechanisms of antitumor activity of bone marrow cells (BMC).

MATERIALS AND METHODS

The study was carried out on certified C57Bl/6 (n=10) and DBA/2 (n=12) mice aged 8-12 weeks from Institute of Pharmacology, Tomsk Research Center. The animals were kept under conditions of incomplete barrier system with free access to water (boiled water acidified with hydrochloric acid, pH 4-4.5) and food (sterilized granulated fodder). Tumor strains were maintained in ascitic form in DBA/2 (P-815 mastocytoma) and C57Bl/6 (Ehrlich carcinoma) mice.

In order to prepare cell suspensions, the animals were sacrificed by cervical dislocation under light

Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences. ***Address for correspondence:*** belsky@pharm.tsu.ru. Yu. P. Bel'skii

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ether narcosis, myelokaryocytes were obtained by perfusion of the femoral and tibial bones. The cells were cultured under conditions of 5% CO_2 and absolute humidity in a medium of the following composition: RPMI-1640 (Sigma) with 10% FCS (ICN), 20 mM Hepes, 0.05 mM 2-mercaptoethanol (both from Sigma), 50 μg/ml gentamicin, and 2 mM L-glutamine (both from Flow Lab.). Experiments were carried out on suspensions containing at least 95% viable cells (according to the Trypan Blue test), with only BMC nonadhesive to plastic, obtained after 1-h culturing of $3\text{-}5\times10^6$ cells/ml in plastic Petri dishes (Costar).

P-815 cells (0.15 ml) were cultured for 40 h in 96-well plates (2×10^4 cells/well); ^{3}H-thymidine (0.5 μCi/well) was added 16 h before the end of culturing. After culturing the cells were transferred onto filters and radioactivity was measured on a β-counter.

Cell supernatants were obtained after 24-h culturing in 90-mm Petri dishes (10^8 BMC/10 ml) or in 96-well plates (in 0.15 ml; 4×10^5 BMC, 2×10^4 P-815 cells).

The role of cell—cell contacts was studied by cell co-culturing in wells divided by a semipermeable membrane (Transwells, Costar) allowing interactions between the cells through soluble factors, but excluding direct cell—cell contacts. The studied cells (10^5 tumor cells or 2×10^6 BMC) were placed into 6.5-mm wells and plunged into other wells (15 mm in diameter) of a 24-well plate with syngeneic BMC (5×10^6), P-815 tumor cells, or Ehrlich carcinoma cells (3×10^5), or both. The cells were cultured for 20-22 h in a total volume of 2 ml, after which BMC from inserted wells were transferred into fresh medium and used for preparing the supernatant. Proliferative activity of tumor cells cultured in inserted wells was evaluated by the radioisotope method or colorimetry [9]. In this case 0.05 ml cell suspension from inserted wells was placed into 96-well plate and 0.1 ml fresh medium was added into each well. The cells were cultured for 4 h with 0.5 μCi/well ^{3}H-thymidine or 200 μg/ml 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Serva). The cells with radioactive label were transferred onto filters and radioactivity was counted on a scintillation β-counter. If MTT was used, the supernatant was discarded from the wells after the end of culturing, the precipitate was dissolved in DMSO, and absorption was measured at λ=550 nm. The proliferative activity was expressed in cpm or as a logarithm of light absorption percent.

The results were statistically processed using Student's *t* test. The differences were considered significant at $p<0.05$.

TABLE 1. Proliferation of P-815 Mastocytoma Cells in the Presence of Different Supernatants ($X\pm m$)

Culture	Proliferation, cpm
No supernatant	115 876±19 393
Medium (control)	84 506±1912
BMC	87 348±4652
P-815 cells	80 657±3275
BMC+P-815 cells	69 696±1911*
BMC precultured with medium	103 213±7104
BMC precultured with P-815	106 317±116 80

Note. *$p<0.05$ compared to the control.

RESULTS

In order to find out whether proliferation of tumor cells was suppressed by factor(s) or by another way, we studied the effects of supernatants of cell cultures of different composition on tumor cell proliferation. Supernatant of BMC monoculture did not modify proliferation of the target cells, while supernatant of a mixed culture containing BMC and P-815 mastocytoma cells inhibited it (Table 1). Presumably, similarly as during induction of immunosuppressive activity, the targets (tumor cells) produced inductor of the antitumor factor.

In order to verify this hypothesis, we studied the effect of BMC supernatant on proliferative activity of P-815 cells after preculturing of myelokaryocytes with P-815 tumor cell supernatant. No suppressor factor appeared (Table 1). Presumably, a contact with target tumor cells is required for inducing its production by BMC. It is also possible that tumor cells can produce the antiproliferative factor and BMC can serve as the source of its inductor. Moreover, the appearance of the suppressor factor can result from a succession of events and be a result of several signal exchanges between cell populations.

The following experiments were carried out in order to clear out which of these variants was true. Tumor cells or BMC were cultured for 24 h in the presence of other cells separated by a semipermeable membrane, after which changes in proliferative activities of mastocytoma and Ehrlich carcinoma cells were evaluated; in another group of experiments BMC capacity to produce antitumor factors was evaluated.

BMC cultured with tumor cells separated by a membrane did not produce the suppressor factor (Table 2). Only the supernatant of myelokaryocytes present in the mixed culture of BMC+tumor cells suppressed proliferation of P-815 cells (by 48%). Proliferation of tumor cells cultured with BMC without direct contact with these cells decreased significantly by 22% (P-815) and by 11% (carcinoma; Table 2).

Hence, antitumor activity of nonadhesive BMC (similarly as immunosuppressant activity) is realized

TABLE 2. Proliferative Activity of P-815 and Ehrlich Carcinoma Cells Precultured with BMC Separated by a Semipermeable Membrane ($X \pm m$)

Culture	Proliferation of P-815 cells, cpm		Proliferation of Ehrlich carcinoma cells, lg%
	no supernatant	with BMC after culturing	
Medium (control)	63 684±4093	103 376±5956	0.840±0.020
BMC	49 414±2985*	108 817±9351	0.745±0.021*
P-815 cells	71 173±2002	106 325±7490	0.849±0.092
BMC+P-815 cells	36 265±2758*+	53 568±8896*	0.575±0.055*+

Note. $p<0.05$ *compared to the control, +compared to proliferation of tumor cells precultured with BMC.

through suppressor factors produced as a result of signal exchange between BMC and tumor cell; cell—cell contacts are an important stage in this process. These results are in line with previous data [10] and indicate that the contact between NSC and tumor cell is essential for induction of antitumor activity.

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