ONCOLOGY

Antitumor Activity of Normal Bone Marrow Cells

I. V. Avdeev, V. I. Seledtsov, I. V. Prokopenko, G. V. Seledtsova, and V. A. Kozlov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 120, № 8, pp. 181-183, August, 1995
Original article submitted September 7, 1994

After separation of normal murine bone marrow cells in a Percoll density gradient cellular fractions with densities of 1.076 and 1.060 g/ml are capable of suppressing the *in vitro* growth of leukemia cells. The cytostatic activity of these fractions, however, does not surpass the level of antitumor antiproliferative activity intrinsic to intact bone marrow cells. These cells were found to be capable of joining the splenocytes, thymocytes, and lymph node cells in effector cytostatic cooperation and thus enhance the final antitumor effect.

Key Words: *bone marrow cells; tumor growth suppression; intercellular cooperation*

Deficiency of antitumor cytolytic activity is known to be a typical feature of bone marrow [4,5]. This feature is due, among other things, to the presence of natural suppressor cells in bone marrow, which are characterized by an ability to directly and indirectly suppress the generation and functional activity of both nonspecific and specific cytolytic effector cells [6,7,9]. On the other hand, previous studies [1,2] demonstrated that bone marrow cells (BMC) possessing no cytolytic activity are characterized by a relatively high capacity for suppressing the *in vitro* growth of leukemia cells.

The aim of this research was to analyze the antitumor activity of BMC fractionated in a Percoll density gradient and to investigate the possibility of cytostatic effector cooperation of BMC with the cells of various hemoimmunopoietic organs.

MATERIALS AND METHODS

(C57Bl/6×DBA/2) F_1, (BDF_1, H-2^b/H-2^d) mice aged 3 to 6 months bred at the Stolbovaya breeding center (Moscow Region) were used in the experiments.

Institute of Clinical Immunology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk

BMC were isolated by washing the femurs and tibias in RPMI-1640 medium cooled to 4°C [4,5]. Cell suspensions of lymphoid organs (spleen, thymus, lymph nodes) were prepared by squeezing out cells from fragments of organs into cold RPMI-1640 medium with 1% fetal calf serum. The resultant cells were washed three times and placed in complete culture medium. The viability of cells was assessed by the trypan blue exclusion method.

For fractionation, BMC were layered onto the stepwise Percoll gradient (Pharmacia) prepared as described previously [10] and centrifuged at 400 g for 30 min. Cells of each interphase were collected with pasteur pipette, washed three times, and placed in culture medium.

Cell suspensions were cultured in RPMI-1640 medium with 10% fetal calf serum, 2 mM L-glutamine, 2 g/l $NaHCO_3$ (all the reagents were from the Vektor Research and Manufacturing Conglomerate, Russia), and 30 μg/ml gentamicin (Pharmachim) at 37°C in a humid atmosphere with 5% CO_2.

Culture supernatants were prepared by placing cells in a concentration of 5×10^6/ml in a 24-well Linbro plate, 2 ml per well, and culturing for 48

0007-4888/95/0008-0826$12.50

h. Supernatants were obtained by 6-min centrifugation of the cell suspensions at 250 g.

For assessment of the cytostatic activity, mastocytoma P815 (H-2^d, DBA/2) or lymphoma L1210 (H-2^d, DBA/2) cells in a dose of 10^4/well were cultured with effector cells or in the presence of supernatants from them (with the medium in the control) in a 96-well round-bottom BDL plate for 24 or 40 h. ^{3}H-thymidine in doses of 0.75 and 0.5 mCi/well was added 6 and 16 h, respectively, before the end of culturing. For assessment of the level of ^{3}H-thymidine incorporation, the cells were transferred from the wells onto a fiber glass filter. Radioactivity was measured using a Delta 300 b-counter. The cytostatic index (CI) expressed in percent was calculated using the formula:

$$CI=(1-A-B/C)\times 100\% \text{ or } (1-A/C)\times 100\%$$

in tests with effector cells and cell supernatants, respectively, where A is cpm for tests when tumor targets were cultured with effectors or supernatants, B is cpm for tests where effectors were without tumor targets, and C is cpm for tests with tumor cells alone. The value of C varied from 150,000 to 300,000 counts in different tests, whereas B usually did not surpass 20,000 counts. Each test was represented by 3 parallel cultures.

The results were statistically processed using Student's t test, differences being considered reliable at $p<0.05$.

RESULTS

In order to obtain a BMC subpopulation rich in cytostatic effectors, BMC were divided into 4 fractions in a stepwise Percoll gradient. Figure 1 shows

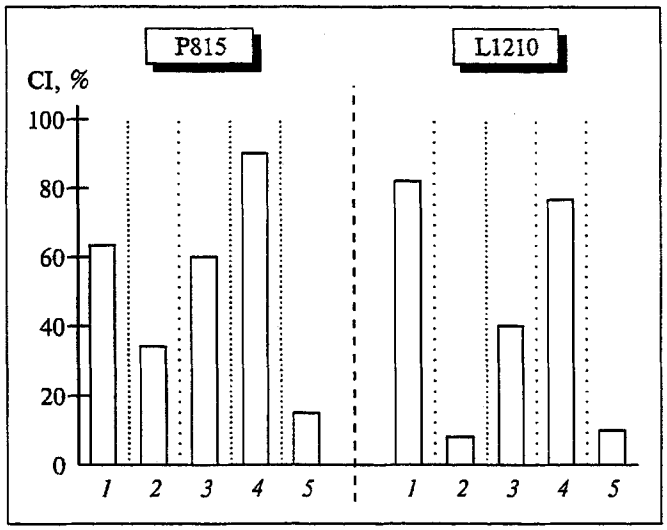

Fig. 1. Antitumor activity of BMC fractionated in a Percoll density gradient. CI characterizes the antitumor cytostatic activity of nonfractionated BMC (3.5×10^5 per well, *1*) and of cell fractions of 1.090 (*2*), 1.076 (*3*), 1.060 (*4*), and 1.046 (*5*) g/ml. Duration of culturing 48 h. Typical results of one out of 6 experiments are presented. Arithmetic mean deviations did not exceed 10%.

that fractions with 1.060 and 1.076 g/ml densities are characterized by the most pronounced antiproliferative properties toward tumor targets. Attempts at appreciably enhancing the antileukemic effect in any of the fractions in comparison with intact BMC, however, failed in these experiments. It should be noted that the BMC population reconstructed from the Percoll cell fractions possessed the same antitumor activity as nonfractionated BMC (data not shown). Hence, we may assume that the population of bone marrow cytostatic effectors is heterogeneous and that intercellular cooperation may play an important role in limiting tumor growth.

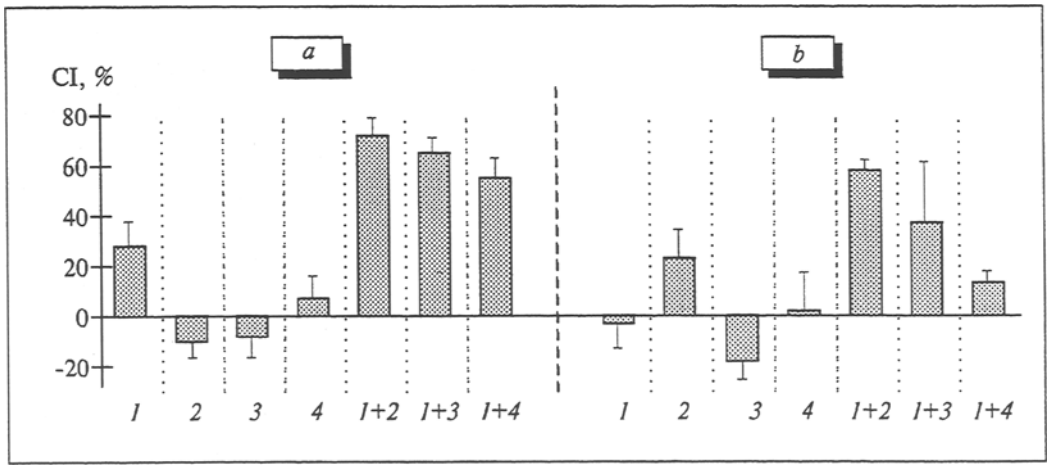

Fig. 2. Intercellular cooperation in tumor growth suppression. CI characterizes the cytostatic activity of BMC (*1*) in a well in a concentration of 1.75×10^5 (*a*) or 0.87×10^5 (*b*), as well as of splenocytes (*2*), thymocytes (*3*), and lymph node cells (*4*) in wells in concentrations of 1.75×10^5 (*a*) or 2.63×10^5 (*b*), which were cultured either separately or in combinations with BMC in a 1:1 (*a*) or 0.5:1.5 (*b*) ratio of BMC to other effector cells together with P815 cells (10^4/well) for 24 h. Mean values of 3 experiments are presented.

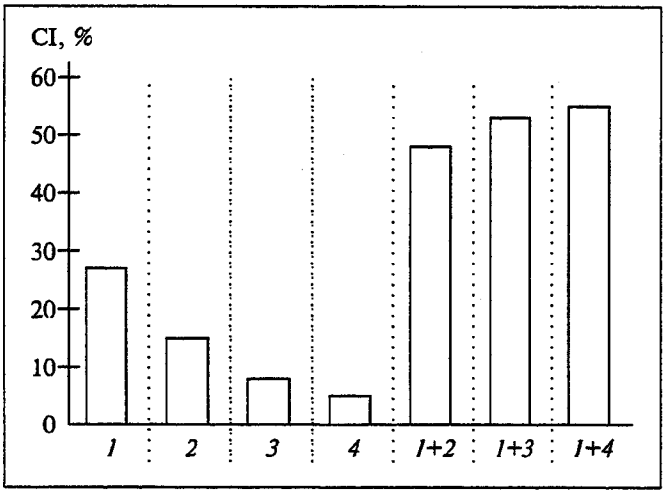

Fig. 3. Tumor growth suppression by soluble cellular factors. CI characterizes the cytostatic activity of 25% culture supernatant from BMC (*1*), splenocytes (*2*), thymocytes (*3*), and lymph node cells (*4*), as well as of combinations of supernatants. P815 cells were cultured in the presence of supernatants for 24 h. Results of one of two experiments performed are presented; the arithmetic mean deviations did not exceed 10%.

Moreover, it is possible that cell-to-cell cooperation aimed at suppressing tumor growth is not confined to the bone marrow (Fig. 2). A synergic intensification of the antiproliferative effect was observed when tumor cells were cultured with BMC combined in a 1:1 ratio with splenocytes, thymocytes, or lymph node cells (Fig. 2, *a*). At a ratio of 0.5:1.5 (Fig. 2, *b*) the BMC combination with lymph node cells was an exception, where cytostasis was only negligibly increased. Parallel experiments carried out to investigate the lysis of tumor cells prelabeled with ^{3}H-thymidine demonstrated that cytostasis of P815 cells enhanced by cellular combinations was not associated with their cytolysis (data not presented).

Studies of the cytostatic characteristics of supernatants from cells of the above organs showed (Fig. 3) that the formation of a synergistic antitumor effect of BMC with splenocytes, thymocytes, and lymph node cells may be due, at least in part, to products released by the cells into the medium.

Results similar to the above were obtained in tests with lymphoma L1210 cells used as targets (data not presented).

Analysis of the data demonstrated a phenotypic similarity of the bone marrow cytostatic effectors with natural suppressor cells capable of inhibiting both cellular and humoral immune reactions [6,8]. Both are present in normal bone marrow, being detected in relatively low-density fractions upon separation in Percoll, neither possess features typical of mature immunocompetent cells, and both are characterized by nonspecific effects unrestricted for major histocompatibility antigens [6-9]. There may well be a universal BMC-mediated mechanism which is capable of nonspecifically suppressing the high proliferative and functional activity of different types of cells. Just how the natural immunosuppressive and cytostatic antitumor cellular mechanisms interact and how much they overlap remain to be investigated.

The data indicate that cellular cooperation within the hemopoietic system may play an important role in the formation of a mechanism capable of tumor growth suppression. It is possible that disorders in the components of this cytostatic mechanism are observed in hemoimmunopoiesis disturbances characterized by various types of aplasia and cell imbalance. Such disorders are frequently noted in the early postleukemic period of hemoblastosis development [3]. Probably, at least part of the cytostatic cooperation can be attributed to the effects exerted on tumor cells by cytostatic molecules secreted by different cells. It is important to note that BMC may in fact be the principal element in the cooperative cytostatic mechanism, because in our experiments the cell combinations which did not include BMC were far less active in suppressing tumor growth than BMC-containing combinations (data not shown).

REFERENCES

1. Yu. P. Bel'skii, I. M. Agranovich, and S. A. Kusmartsev, *Byull. Eksp. Biol. Med.*, **117**, № 5, 514 (1994).
2. N. N. Belyaev, G. K. Zakir'yanova, and A. B. Beklemishev, *Immunologiya*, № 3, 27 (1991).
3. A. Butturini and R. P. Gale, *Leuk. Res.*, **15**, 1 (1991).
4. G. C. Koo, J. R. Peppard, and W. H. Mark, *J. Immunol.*, **132**, 2300 (1984).
5. G. C. Koo and C. L. Manyak, *Ibid.*, **137**, 1751 (1986).
6. T. Maier, J. N. Holda, and H. N. Claman, *Immunol. Today*, **7**, 312 (1986).
7. S. C. Moore, M. A. Shaw, and L. S. F. Soderberg, *J. Leukoc. Biol.*, **52**, 596 (1992).
8. D. C. Saffron and Sh. K. Singal, *Cell. Immunol.*, **128**, 301 (1990).
9. D. C. Saffron and Sh. K. Singal, *Transplantation*, **52**, 685 (1991).
10. M. L. Wood, A. P. Monaco, and R. Gottschalk, *Ibid.*, **51**, 208 (1991).