Soluble Factor and Cell-Cell Interaction in Cytostasis Induced by Bone Marrow Cells

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Cell-cell interaction and soluble low-molecular-weight products are probably involved in *in vitro* inhibition of leukemic cell growth by bone marrow cells.

Key Words: *bone marrow; leukemia; cell-cell contact; cytostasis*

Normal bone marrow cells (BMC) inhibit *in vitro* proliferation of cultured tumor cells [1,2,4,7-9,11]. Antiproliferative activity of bone marrow cytostatic effectors is not restricted by major histocompatibility complex antigens and is realized without cell lysis [1,2, 7-11]. These effectors do not express surface markers of immunocompetent cells, but possess some properties typical of natural immunosuppressor cells [4,10]. Soluble suppressor factors play the major role in the inhibition of tumor growth by BMC. It is believed that direct contact between BMC and target cells is not necessary for cytostasis [11]. However, this notion is not absolutely true. Here we studied the role of cell-cell interactions in the cytostasis.

MATERIALS AND METHODS

Experiments were performed on (C57Bl/6×DBA/2)F1 (BDF_1, H-2b/H-2k) mice aging 4-6 months and obtained from Rassvet nursery (Tomsk).

Lymphocytic leukemia L1210 (H-2d) and mastocytoma P815 (H-2k) cells were cultured in RPMI-1640 medium containing 2 mM L-glutamine (Sigma) and 10% fetal bovine serum (Institute of Clinical Immunology).

BMC were routinely washed out from the tibia with cold (4°C) RPMI-1640 medium [3].

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Conditioned media were obtained after culturing of BMC (5×10^6/ml) in Iscove's serum-free medium (Sigma) containing 5 mM L-glutamine in 24-well plate (Linbro). Molecules with a molecular weight below 5 kD were isolated by ultrafiltration using special Millipore tube filters.

Tumor cells were cultured with BMC or their soluble factors in RPMI-1640 medium containing 2 mM L-glutamine, antibiotics (Sigma), and 7.5% fetal bovine serum at 37°C and 5% CO_2.

To evaluate the role of cell-cell interactions in the inhibition of tumor growth, BMC were cultured with tumor cells in 96-well plates with V-shaped, U-shaped, or flat bottom for 24 (series I) or 7 h (to minimize the contribution of bone marrow soluble suppressor factors in cytostasis, series II). Before culturing, the samples were precipitated by centrifugation at 1000 rpm for 3 min. In control samples BMC were replaced with thymocytes, which do not suppress tumor growth [2,7,8].

Four hours before the end of culturing, ^{3}H-thymidine was added into wells (0.75 μCi/well), and its incorporation was evaluated routinely (the samples were placed on a fiberglass filter, and radioactivity was measured on a β-counter). Inhibition of tumor growth was calculated by the formula:

$$(1-(I_T-I_C)/I_C)\times100\%,$$

where I_T and I_C are ^{3}H-thymidine incorporation in the test and control samples, respectively.

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Experiments were performed in triplicates. The results were analyzed using Student's *t* test. Only statistically significant data ($p<0.05$) are presented.

RESULTS

Proliferation of P815 and L1210 cells in control samples was 18.9×10^4 and 25×10^4 cpm, respectively. Soluble products present in whole supernatants after 24-h culturing of BMC inhibited growth of L1210 and P815 cells *in vitro* (Fig. 1). Most (if not all) bone marrow cytostatic products had relatively low molecular weight (below 5 kD), because after separation of large molecules by ultrafiltration the supernatant retained its cytostatic activity. However, the effect of BMC can be explained not only by production of soluble cytostatic molecules. Inhibition of tumor growth was much more pronounced after 24-h culturing of 2×10^5 or 10^5 BMC with 10^4 leukemia cells in wells with V-shaped bottom than in wells with flat bottom (Fig. 2); cell culturing in wells with U-shaped bottom produced an intermediate effect. It should be emphasized that BMC in maximum concentration (4×10^5/well) produced the same inhibitory effect on cell growth irrespective on

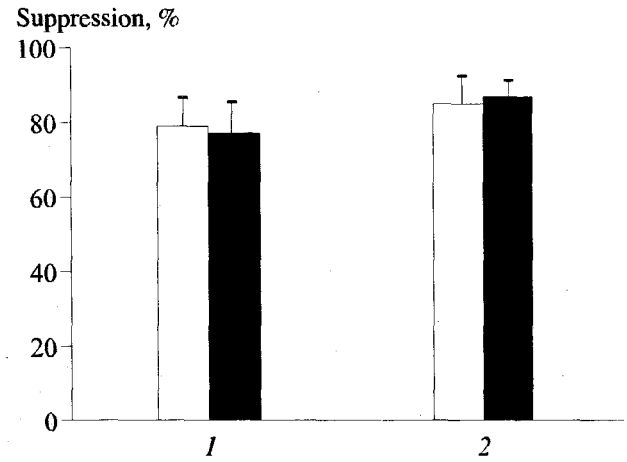

Fig. 1. Proliferative activity of P815 (*1*) and L1210 (*2*) cells after culturing with bone marrow supernatant (light bars) or its ultrafiltrate (dark bars).

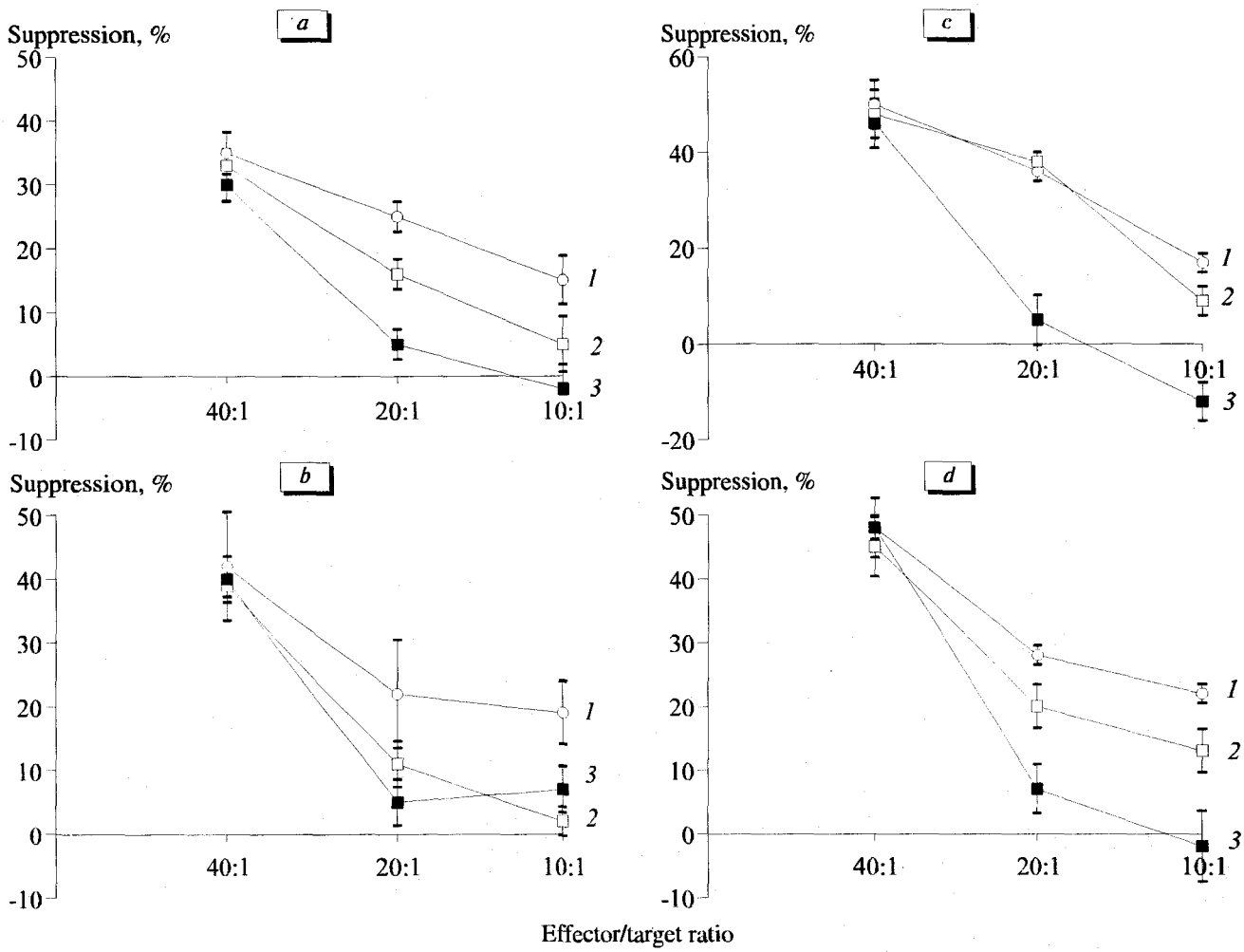

Fig. 2. Cytostasis as a function of cell density in co-cultures of P815 (*a*, *b*) and L1210 cells (*c*, *d*) with bone marrow cells in 96-well plates with V-shaped (*1*), U-shaped (*2*), or flat bottom (*3*) for 24 (*a*, *c*) and 7 h (*b*, *d*).

the bottom shape. These data imply that suppression of tumor growth depended on cell-cell interaction and cell density. Culturing of precipitated BMC and tumor cells for 7 h confirmed our assumption. Under these conditions, cell-cell interactions played the major role in the formation of antiproliferative effects, while the contribution of soluble cytostatic molecules was minimized. Even after short-term culturing, cytostasis depended on cell-cell interactions (Fig. 2).

Our findings are consistent with published data on cell production of nonspecific soluble cytostatic factor(s) [1,2,4,7-9,11]. The role of bone marrow low-molecular-weight products in the regulation of cell proliferation attracts considerable attention. One of these products, reptimed with a molecular weight of 1.8 kD, suppresses proliferative activity of myeloid tumor cells. The interaction between this factor and soluble product responsible for cytostatic effect in our experiments is now extensively studied.

It is known that malignant transformation is associated with the loss of cell sensitivity to contact inhibition. Our results indicate that not only soluble products, but also cell-cell interactions are involved in the inhibition of tumor growth by BMC. Leukemic cells are sensitive to contact inhibition of normal, but not tumor cells. Hence, close contacts between tumor cells protecting them from the contacts with normal cells probably plays the major role in oncogenesis.

Membrane molecules involved in BMC-induced cytostasis are not yet identified. Previous studies showed that leukemic cells express VLA molecules, which interact with stromal cells by recognizing the corresponding ligands [5,6,12]. However, the effects of this interaction on tumor growth are still elusive. Further studies of the role of cell adhesion molecules in the regulation of tumor growth are important for the understanding of cytostasis and its contribution in antitumor defense.

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