Comparative Study of Antitumor Cytostatic and Natural Suppressor Activity of Bone Marrow Cells

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Cells remaining after removal from bone marrow population of cells aggregated by wheat rudiment agglutinin retained the capacity to inhibit the growth of P815 mastocytoma and L1210 lymphoma cells *in vitro*, while their capacity to suppress concanavalin-induced lymphoblastogenesis dropped. Contrary to antiproliferative activity of bone marrow natural suppressor cells, their natural antitumor cytostatic activity did not depend on the presence of interferon-γ in the medium and was not mediated by nitric oxide. Thus, bone marrow antitumor cytostatic effectors are probably not equivalent to bone marrow natural suppresser cells by cell composition and by the mechanism of antiproliferative effect.

Key Words: *bone marrow cells; tumor growth inhibition; natural suppressor activity*

Bone marrow cells of normal adult mice nonspecifically suppress the growth of various leukemic cells *in vitro* [1,2,8-10]. Suppression of tumor growth by bone marrow cells is not associated with tumor cell destruction and is mediated, at least partially, by soluble cytostatic products [1,9]. Bone marrow antitumor cytostatic effectors (BACE) differ from mature T and B lymphocytes and macrophages, and after fractionation in Percoll density gradient are detected mainly in relatively low-density (1.060-1.075 g/liter) fractions [9,10]. Published reports note a similarity between BACE and bone marrow natural suppressor cells (BNSC) with the so-called "zero" phenotype; these cells are believed to play an important role in the maintenance of immune homeostasis [3,6,7].

Our purpose was to compare bone marrow cells potentially capable of suppressing cell proliferation.

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MATERIALS AND METHODS

(C57Bl/6×DBA) F_1 (BDF_1, H-2^b/H-2^d) mice aged 4-7 months were used. The animals were fed sterile feed and acidified (pH 2.8) boiled water.

Mastocytoma P815 (H-2^d) and L1210 (H-2^d) lymphoma cells were obtained from the Oncology Research Center and cultured *in vitro* 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 humid atmosphere with 5% CO_2.

Bone marrow cells were obtained by washing femoral and tibial bones in cooled culture medium [8].

Bone marrow cells (10^7/ml) with leucyl-methyl ether (Fluka) in a final concentration of 15 mM were incubated in buffered serum-free RPMI-1640 for 30 min. After incubation the cells were thoroughly washed in the culture medium and after assessing their viability by the Trypan Blue exclusion test were used in experiments.

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Fractionation of bone marrow cells based on binding to wheat rudiment agglutinin (WRA, Sigma) was carried out as described previously [4].

In the cytostatic test, bone marrow cells (3×10^5/well) were cultured with P815 or L1210 cells (10^4/well) in round-bottom 96-well plates (BDSL) for 48 h. In the control, tumor cells were cultured without other cells or with thymocytes incapable of suppressing leukemic cell growth *in vitro* [1,8]. Cellular proliferative activity was assessed by ^{3}H-thymidine incorporation, which was 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:

$$\%\ \text{suppression}=\left(\frac{\text{1-counts in experiment}}{\text{counts in control}}\right)\times100$$

Each sample was represented by 3 parallel cultures.

For assessing the activity of BNSC, normal mouse splenocytes (3×10^5/well) were stimulated with conA (5 μg/ml, Pharmacia) for 48 h in the presence of bone marrow cells (3×10^5/well) or without them in the control in a round-bottom 96-well plate (BDSL). The level of cell proliferation and percentage of its suppression were assessed as described above.

The production of nitric oxide (NO) was estimated by accumulation of nitrite in culture supernatant measured using Gries' reagent [5].

Tests were repeated at least 3 times. Results were statistically processed using Student's *t* test. All the differences are significant ($p<0.05$).

RESULTS

All BNSC types are sensitive to toxic effect of leucyl-methyl ether and are characterized by membrane expression of WRA-binding receptors [3,7]. After treatment with leucyl-methyl ether, bone marrow cells lost natural suppressor activity tested by inhibition of conA-induced lymphoblastogenesis and even cytostatic activity preventing the growth of P815 and L1210 cells *in vitro* (Fig. 1). On the other hand, removal of cells agglutinated by WRA from bone marrow population, which was associated with a drop of immunosuppressor activity (as was expected), did not attenuate the cytostatic activity. Moreover, in 3 out of 5 experiments bone marrow cells not agglutinated by WRA showed a higher capacity to inhibit the growth of leukemic cells than cells agglutinated by WRA.

Functional activity of BNSC depends on the presence of γ-interferon in the medium [3,6]. γ-Interferon produced by activated lymphocytes induces

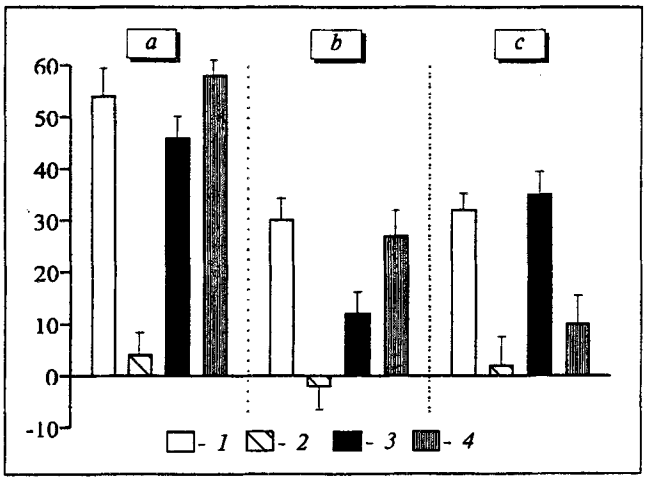

Fig. 1. Natural antitumor and immunosuppressor activities of intact (*1*), leucyl-methyl ether treated (*2*), agglutinated (*3*) and not agglutinated by wheat rudiment agglutinin (*4*) bone marrow cells. Here and in Fig. 2: ordinate: % growth suppression for P815 (*a*), L1210 (*b*) cells, and conA-induced lymphoblastogenesis (*c*). Control incorporation of ^{3}H-thymidine in P815 and L1210 cells and in conA-stimulated splenocytes was 210,000, 350,000, and 132,000 cpm, respectively.

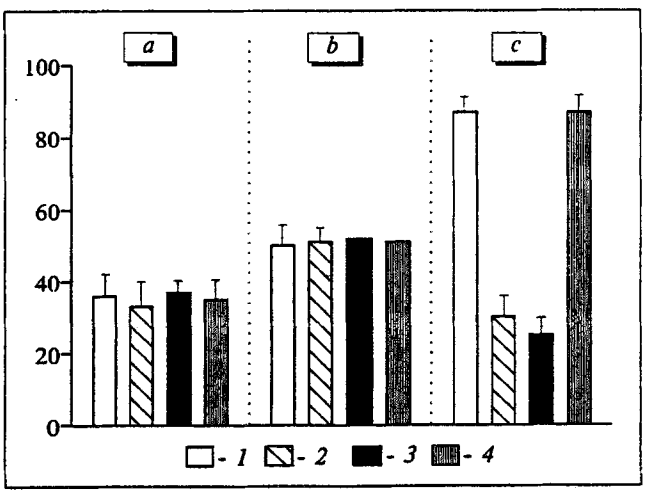

Fig. 2. Natural antitumor and immunosuppressor activity of bone marrow cells after neutralization of γ-interferon activity. Addition to cultures of: *1*) medium; *2*) antibodies to γ-interferon (4 μg/ml); *3*) monomethyl;-L-arginine (0.5 mM); *4*) monomethyl-D-arginine (0.5 mM). Control proliferation of P815 and L1210 cells and conA-stimulated splenocytes was 184,000, 191,000, and 98,000 cpm, respectively. None of the above soluble additives affected cell proliferation in the absence of bone marrow cells (data not presented).

the production of NO in BNSC, which can be responsible for suppression of cell proliferation by BNSC [3]. However, neutralization of γ-interferon by specific monoclonal antibodies (Genzyme) and blocking of NO production by adding monomethyl-L-arginine (Fluka) to the medium virtually did not affect the cytostatic activity of BACE (Fig. 2). In complete agreement with previous reports [3], antibodies specific to γ-interferon sharply decreased the capacity of BACE to suppress conA-induced lym-

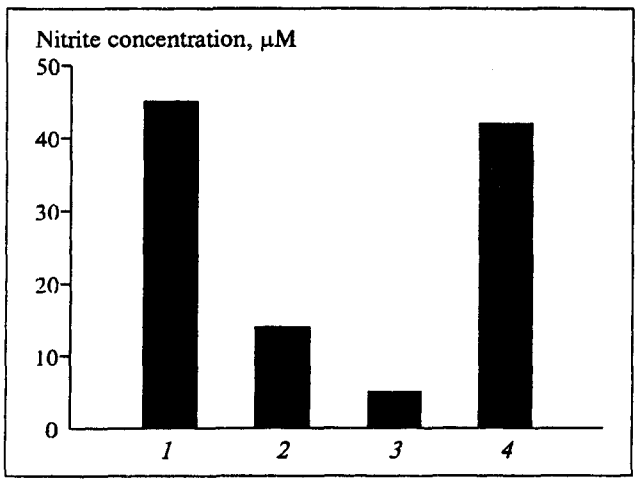

Fig. 3. Role of γ-interferon produced by conA-stimulated lymphocytes in induction of NO production by bone marrow cells. Addition to cultures of: *1*) medium; *2*) antibodies to γ-interferon (4 μg/ml); *3*) monomethyl-L-arginine (0.5 mM); *4*) monomethyl-D-arginine (0.5 mM). Concentration of nitrite in samples with bone marrow cells and splenocytes cultured separately was no higher than 8 μM. Mean arithmetic deviations were no higher than 10% of the corresponding absolute values.

phoblastogenesis. Drop of BACE activity was observed after addition of monomethyl-L-arginine to the medium, but not of monomethyl-D-arginine (Sigma), an agent incapable of inhibiting the production of NO. Therefore, our data suggest that NO may play an important role in mediating the natural suppressor, but not antitumor activity, of bone marrow cells. We revealed no significant production of NO by bone marrow cells exposed with tumor cells. NO production was observed during culturing of bone marrow cells with conA-activated splenocytes, as was expected. This production depended on the presence of γ-interferon and was almost leveled by monomethyl-L-arginine, but not monomethyl-D-arginine (Fig. 3).

These data indicate that BACE are not equivalent to BNSC by cellular composition and by mechanism of antiproliferative effect.

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