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NATURAL SUPPRESSORS OF HUMAN BONE MARROW TISSUE

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Suppressor cells play an essential role in the regulation of reactions of both humoral and cellular immunity. It has now been established that some of the suppressor effects are due to active production of soluble factors by the cells.

Research on experimental models [3-7] has shown that the suppressor effects of bone marrow tissue (BMT) may be determined by cells of different phenotypes [8].

Much attention is now being paid to natural suppressors (NS) of BMT [3, 11]. Their enormous role and practical importance in transplantation of BMT have been demonstrated [8, 10]. We described a preliminary densotometric analysis of bone marrow suppressor cells isolated on a stepwise Percoll density gradient [1]. As the investigation showed, the natural suppressor activity (NSA) of human BMT is a quite complex phenomenon, and it is difficult at the present time to identify the type of cells with which it is connected. It can be only tentatively suggested that these cells are suppressor cells of erythroid nature (Er-suppressors) [3].

To characterize NS further we studied the NSA of bone marrow cells from healthy blood donors, patients with solid tumors, and patients with acute leukemia in the clinical-hematologic remission (CHR) stage. The aim of the present investigation was to undertake a comparative densitometric analysis of bone marrow cells (BMC), possessing NSA, in these patients and in healthy subjects.

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TABLE 1. Dependence of Suppression (in % of BMC concentration) in LBTR Test System

Test system	Number of cells added		
	10^4	$3 \cdot 10^4$	10^5
	cpm·10^3		
LBTR (PHA):			
control	14,3±0,9	17,3±4,2	22,6±3,1
experiment	13,7±0,6 (4)	15,2±5,1 (12)	15,6±2,8 (31)

Legend. Here and in Table 2, figures in parentheses are percentages.

TABLE 2. Characteristics of NSA Among BMC Fractions from Healthy Subjects and Patients with Solid Tumors, and Acute Leukemia in the CHR Phase

Fraction of BMC	Density, g/ml	Degree of suppression, %		
		healthy blood donors (n = 4)	patients with solid tumors (n = 4)	patients with acute leukemia (n = 4)
1	1,050—1,060		cpm·10^3	
control		3,8±1,1	4,3±0,1	8,3±0,3
experiment		0,5±0,1 (87)	3,8±0,8 (11)	10,1±0,2
2	1,060—1,070	2,3±0,6 (41)	2,5±0,2 (41)	8,7±0,2
3	1,070—1,080	4,5±0,2	3,5±0,3 (16)	3,5±0,3 (58)

EXPERIMENTAL METHOD

Bone marrow was obtained from the Blood Transfusion Department of the All-Union Oncologic Scientific Center (Moscow). A suspension of mononuclear cells was obtained from a suspension of BMC by centrifugation on a Ficoll—Hypaque gradient, and the resulting cell suspension was fractionated on a stepwise Percoll gradient ("Pharmacia," Sweden) with a density of 1.050 to 1.080 g/ml. Each of the isolated fractions, and also unfractionated BMC, were tested for their ability to suppress lymphocyte proliferation in a concentration of $1 \cdot 10^5$ cells in each of a triplet of wells, in response to mitogens and in mixed lymphocyte culture (MLC). As mitogens we used PHA and con A ("Pharmacia," Sweden) in concentrations of 10 and 25 μg/ml, respectively. In each case the test model consisted of peripheral blood lymphocytes from healthy blood donors. Lymphocytes were isolated by the usual method [9]. Proliferation of the cells was estimated by counting the incorporation of ^{3}H-thymidine (in cpm).

To identify the cell composition of the isolated BMC on the Percoll gradient, specific markers of T and B lymphocyte populations (T4, T8, T3, 2H4, B1) and also markers of the myeloid series (Mol, My4, My9) ("Coulter Corporation Inc.," USA) were used.

Fractions exhibiting NSA were additionally purified from macrophages and also from T and B lymphocytes. To free the BMC from macrophages the suspension of BMC was passed through a column with Sephadex G_{10}. To purify the BMC from T lymphocytes, monoclonal anti-SD4 and anti-SD8 antibodies + complement were used. To remove B cells the BMC were adsorbed on a column with activated anti-IgM-BCN-sepharose 4B.

EXPERIMENTAL RESULTS

To study suppressor activity of the BMC the following experiments were carried out: investigation of suppressor activity of unfractionated and fractionated BMC from healthy blood donors, investigation of suppressor activity of unfrac-

tionated and fractionated BMC from patients with solid forms of tumors, and from patients with acute leukemia in the CHR phase.

In both the 1st and 2nd groups of experiments, BMC were added to proliferating lymphocytes in the lymphocyte blast transformation reaction (LBTR) and MLC. The concentration of BMC was $3 \cdot 10^3$, 10^4, $3 \cdot 10^4$, and 10^5. The results of the experiments are given in Table 1. As Table 1 shows, LBTR of healthy human peripheral blood in response to PHA varied from $14.3 \cdot 10^3$ to $22.6 \cdot 10^3$ cpm. Addition of BMC to the lymphocytes in a concentration of $3 \cdot 10^4$ led to inhibition of proliferation (12%), whereas in a dose of 10^5 BMC, the inhibitory effect rose to 31%.

By fractionation of bone marrow mononuclear cells on a stepwise Percoll density gradient, three cell fractions were isolated. Each fraction was tested on test models of inhibition of cell proliferation.

Analysis of the tests showed that NSA was present in all the different Percoll fractions (Table 2).

In the healthy blood donors a suppressor effect was found on the addition of cells of fraction 1 (density from 1.050 to 1.060 g/ml) to the proliferating lymphocytes. The suppressor effect amounted to 87%. Meanwhile, in patients with solid forms of tumors suppression was observed in all fractions of BMC with a maximum in fraction 2 (density from 1.060 to 1.070 g/ml). The degree of this suppression was 41%. In patients with acute leukemia in the CHR phase marked suppression by cells of fraction 3 (density from 1.070 to 1.080 g/ml) was observed. The degree of the suppressor effect was 58%.

Identification of the cell composition of the separate fractions by EPICS analysis showed that fraction 1 of BMC was enriched with macrophages, whereas fractions 2 and 3 were rich in B and T cells, respectively.

Cells of the fraction with the strongest suppressor activity were further purified from T and B cells and macrophages. To remove macrophages the cells of these fractions were adsorbed on a column with Sephadex G_{10}. To remove T cells the BMC were treated with monoclonal anti-SD4 and anti-SD8 antibodies + complement. To remove B cells the BMC were adsorbed on a column with anti-IgM-BCN-sepharose 4B. The BMC remaining after purification were studied in LBTR and MLC test systems. Analysis of the results showed that NSA was not significantly changed after removal of the T and B lymphocytes and macrophages.

The experiments thus demonstrate that human BMC can exhibit NSA of varied magnitude. NSA differs depending on the degree of the suppressor effect in the fractions from healthy donors and also from patients with tumors.

The question of the nature of the cells with NSA remains unanswered. Our preliminary data [1] suggest that in some cases they may be cells not carrying markers characteristic of macrophages or T and B lymphocytes.

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