BONE MARROW SUPPRESSOR CELLS IN NORMAL SUBJECTS AND PATIENTS WITH CIRRHOSIS OF THE LIVER

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It was discovered in 1976 that mouse bone marrow contains suppressor cells of the B type, which nonspecifically inhibit antibody formation [4, 9]. The mechanisms of B-cell suppression of antibody formation are linked with suppression of proliferation of target cells which are precursors of antibody-forming cells [3]. Bone marrow suppressor B Cells have been shown to inhibit proliferation of antigen- and mitogen-stimulated syngeneic, allogeneic, and xenogeneic T and B lymphocytes [6]. Human bone marrow also contains suppressor cells which inhibit spontaneous and mitogen-induced proliferation of xenogeneic target lymphocytes in culture *in vitro* [5] and suppressing the primary immune response of syngeneic and allogeneic lymphocytes [7]. It was therefore interesting to investigate the properties of human bone marrow suppressors in various pathological states.

In the investigation described below B suppressors from healthy individuals and patients with various types of cirrhosis of the liver were studied.

EXPERIMENTAL METHOD

Suspensions of lymphocytes from the spleen and bone marrow of (CBA × C57BL/6)F_1 mice were prepared under sterile conditions by the usual method. Human bone marrow obtained by sternal puncture, and peripheral blood taken from the cubital vein, were mixed in culture medium 199 containing 0.01 M HEPES (from Dynatech, USA), 150 μg/ml of heparin (from Reanal, Hungary), 250 U/ml penicillin and 250 μg/ml of streptomycin (from Serva, West Germany). Human bone marrow lymphoid cells and peripheral blood mononuclears were isolated on a Ficoll–Hypaque Gradient (Pharmacia, Sweden) at a density of 1.077 g/ml [8]. In a sterile test tube 4 volumes of puncture material, diluted twice with medium of the above-mentioned composition, was layered accurately above 3 volumes of Ficoll–Hypaque. The tube and its contents were centrifuged for 45 min at 400 g, after which the layer of lymphocytes (yellow in color, opalescent, located on the boundary between the Ficoll–Hypaque/medium phase) was withdrawn by means of a sterile Pasteur pipet. The lymphocytes were washed three times in fresh medium 199 at 700 g, for 10 min each time, and transferred into enriched culture medium. The enriched medium was prepared on the basis of medium RPMI-1640 with bicarbonate (from Serva, West Germany), with the addition of 5% embryonic calf serum, 20 mM L-glutamine, and 10^{-5} M 2-mercapto-ethanol. The cells were cultured in microwells in special "Cooke Microtiter" plastic plates in a humid atmosphere with 5% CO_2.

The method of estimating activity of bone marrow B suppressor cells based on determination of total incorporation of ^{3}H-thymidine into cells actively synthesizing DNA [1] was used. Into cultures of 10^6–2.5×10^6 mouse spleen cells (target cells) were placed 0.25×10^6–2.5×10^6 mouse bone marrow cells (control of suppression of proliferation) or human bone marrow or peripheral blood cells. To activate proliferation of the target cells, lipopolysaccharide (LPS) from *Escherichia coli* strain 0127 B8 (from Sigma, USA), was used. The suppressive properties of each cell population to be studied were assessed with the aid of the coefficient of suppression:

$$K_s = \frac{I_{cms} + I_x}{I_{(cms+x)}},$$

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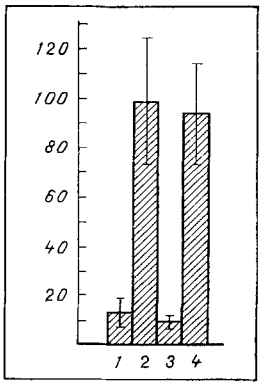

Fig. 1. Comparison of spontaneous proliferative activity of bone marrow cells from healthy blood donors and patients with cirrhosis of the liver. Ordinate, incorporation of ^{3}H-thymidine per 10^6 living cells (in cpm/10^3). 1) Mouse spleen cells; 2) mouse spleen cells activated by LPS; 3) healthy human bone marrow cells from patients with cirrohosis of the liver.

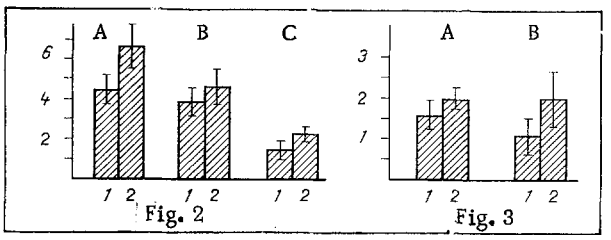

Fig. 2. Comparison of suppressive activity of bone marrow cells of different origin. Ordinate, coefficient of suppression of proliferation of target cells. A) mouse bone marrow cells; B) healthy human bone marrow cells; C) bone marrow cells from patients with cirrhosis of the liver. 1) Without stimulation of target cells by LPS; 2) with stimulation of target cells by LPS.

Fig. 3. Comparison of suppressive activity of peripheral blood lymphocytes from healthy subjects and patients with cirrhosis of the liver. Ordinate, coefficient of suppression of proliferation of target cells by peripheral blood cells. A) Normal blood donors; B) patients with cirrhosis of the liver. 1) Without stimulation of target cells by LPS; 2) with stimulation of target cells by LPS.

where I_{cms} indicates incorporation of ^{3}H-thymidine in a monoculture of mouse splenocytes (target cells), I_x incorporation of ^{3}H-thymidine in a monoculture of the test cell population (suppressor cells), and $I_{(cms+x)}$ denotes incorporation of ^{3}H-thymidine in a mixed culture

of mouse spleen cells + the test cell population. Incorporation of ^{3}H-thymidine to DNA was estimated on the 3rd day. The labeled nucleotide was added to the cultures 24 h before the end of culture in a concentration of 1 μCi/ml. Radioactivity of the acid-insoluble material, deposited on "Synpor" filters (pore diameter 0.6-2.5), was measured on an Intertechnique SL-30 scintillation counter. The viability of the cells was determined by the use of a 0.05% solution of trypan blue and a 0.05% solution of eosin.

EXPERIMENTAL RESULTS

Comparison of suppression of cell proliferation induced by bone marrow lymphocyted on normal blood donors and patients with cirrhosis of the liver must be preceded by examination of the character of "behavior" of these cell populations during culture *in vitro*. Levels of spontaneous proliferative activity of bone marrow cells from healthy blood donors and patients with cirrhosis of the liver are given in Fig. 1.

It will be clear from Fig. 1 that the intensity of DNA synthesis by bone marrow lymphocytes from normal human subjects was low and close to the corresponding parameter for a monoculture of mouse spleen cells, and was significantly lower than the intensity of DNA synthesis by splenocytes activated by the optimal dose of LPS (100 μg/ml). Cultures of bone marrow cells from patients with cirrhosis of the liver differed sharply in their level of spontaneous proliferation *in vitro* from healthy human bone marrow cells. Bone marrow lymphocytes from patients with cirrhosis of the liver, isolated on a Ficoll-Hypaque density gradient and cultured *in vitro* under standard conditions for all cell populations tested, began to divide intensively. Addition of LPS to the cultures had virtually no effect on incorporation of ^{3}H-thymidine into the cell DNA: the level of spontaneous proliferation was so high.

Consequently the study of suppression by bone marrow cells from patients with cirrhosis of the liver showed certain distinguishing features. Usually levels of proliferation of monocultures of spleen cells (target cells) and bone marrow cells (suppressors) of the same density are comparable in value. In the present case, however, on the addition of cells of the suppressor population (bone marrow from patients with cirrhosis of the liver) to cultures of mouse spleen cells in the ratio of 1:1 a high level of proliferation of the mixed culture was observed on account of spontaneous division of the bone marrow cells. Bone marrow cells containing suppressors proliferated 5-10 times more intensively than the target cells (Fig. 1). It was shown previously that removal of B suppressors from normal mouse bone marrow stimulates proliferation, whereas addition of the suppressor humoral factor, on the contrary, inhibits it [2]. This suggested that activity of suppressor cells in the bone marrow of patient with cirrhosis of the liver is depressed. However, because of great differences in levels of proliferation of target cells and bone narrow cells from patients with cirrhosis of the liver it was impossible to prove this fact strictly. In order to bring the system into line with that existing previously, and to estimate activity of bone-marrow suppressors in patients with cirrhosis of the liver, the number of suppressors in culture *in vitro* was reduced.

We know that bone-marrow suppressors are active if present in the numerical ratio of between 1:1 and 1:5 with target cells [2]. We found that incorporation of ^{3}H-thymidine per 10^6 spleen cells is approximately equivalent to incorporation of the label by 0.25×10^6 cells of the actively proliferating population. Thus when the suppressive properties of bone marrow cells from patients with cirrhosis of the liver were investigated, target cells and carriers of suppressors, i.e., bone marrow, were used in the ratio of 4:1.

Figure 2 demonstrated differences in the suppressive activity of bone marrow cells from patients with cirrhosis of the liver and normal subjects. Bone-marrow suppressors of healthy donors had a marked action on proliferation of target cells (K_S = 3.5-5.5), which was comparable with the inhibitory effect of mouse B suppressors (K_S = 3.5-6.5). Conversely, bone marrow cells from patients with different forms of cirrhosis of the liver had no such action. Coefficients of suppression of proliferation of mouses spleen cells not activated by LPS were close to 1, and on activation of the targets by the mitogen they did not exceed 2. The suppressive activity of bone marrow cells from patients with cirrhosis of the liver was evidently significantly lower than activity of bone-marrow lymphocytes from normal donors.

Peripheral blood lymphocytes from these patients, in culture *in vitro*, like healthy human lymphocytes, had virtually no suppressive action (Fig. 3).

It can be concluded from these results that in the presence of marked liver pathology

(cirrhosis of the liver) activity of bone-marrow B suppressors is depressed. It is valid hypothesis that the marked increase in the intensity of spontaneous proliferation of bone-marrow cells from patients with cirrhosis of the liver in vitro is the result of a decrease in number or depression of the function of B suppressors. The study of the causes of depression of activity of bone-marrow B suppressors will be a task for future research.

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STATE OF THE NATURAL KILLER CELL SYSTEM IN THE HUMAN FETUS

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Natural killer cells (NKC) are components of the hematopoietic system which play an important role in the maintenance of internal homeostasis and of immunologic surveillance in the body [4, 11, 13]. Phylogenetically the NKC system is the oldest component of the immune system, but its ontogenetic aspect has not been adequately studied [10, 12, 15]. Considering the role of NKC it can be postulated that the appearance of these cells and the beginning of their functioning coincide in ontogeny with the times of formation of hematopoiesis. No such investigations have been undertaken in the USSR, and the few papers published abroad do not give a complete picture of the problem.

The aim of this investigation was to study the state of the NKC system in human prenatal ontogeny, with consideration for the morphological structure of hematopoietic and lymphopoietic organs at certain stages of embryogenesis.

EXPERIMENTAL METHOD

The investigation was conducted on eight human fetuses from the 14th through the 26th weeks of prenatal development. Cells were isolated from bone marrow, spleen, liver, appendix, and tonsils on a Ficoll—Hypaque gradient (d = 1.077 g/ml) and used as effectors of natural cytotoxicity. Cells of human erythromyoleukemic strain K-562 were used as targets. Medium RPMI-1640, containing 10% bovine fetal serum, 2 mM glutamine, and monomycin (100 U/ml) were used for cell culture and the cytotoxic test.

The conditions for labeling the target cells and conducting the cytotoxic test were described by the writers previously [1, 2]. Cytotoxic activity of NKC was determined with effectors and targets in different ratios. Cells were fractionated (2×10^6/ml in a volume of 2 ml) on plastic Petri dishes in medium with 20% bovine fetal serum for 1 h at 37°C in a CO_2 incubator. Cells not adherent to the plastic were used in the cytotoxic test.

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