REGULATORY EFFECT OF BONE MARROW CELLS ON HUMAN T LYMPHOCYTE FUNCTION

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UDC 612.112.94.017.1-06:612.419.014.2

KEY WORDS: bone marrow; suppressor cells; macrophage migration inhibition factor.

Effects of bone marrow cells on functions of immunocompetent cells have recently been described in the literature [3, 6, 8]. For instance, B lymphocytes of bone-marrow origin, depending on the conditions of culture, can have twofold effects: They can inhibit or stimulate antibody production in experimental animals [3, 4, 9]. The nature and properties of bone-marrow suppressors have now been characterized sufficiently completely, and the target cells have been found [1, 5, 7]. It has been shown that bone-marrow cells can also regulate reactions of cellular immunity [2]. However, all investigations so far conducted have been done on experimental animals.

This paper describes a study of the effect of human bone marrow cells on production of macrophage migration inhibition factor (MIF) and on the proliferative response of T lymphocytes to phytohemagglutinin (PHA).

EXPERIMENTAL METHOD

Bone marrow cells were obtained by diagnostic trephine biopsy in patients with hereditary microspherocytosis. The bone marrow of these patients is characterized by complete integrity of all branches of hematopoiesis except for mild hyperplasia of cells of the erythroid series. Marrow cells were centrifuged in a Ficoll—Isopaque gradient (d = 1.077). The isolated cells were cultured in medium RPMI-1640 in a concentration of 5×10^6 cells/ml medium. Activity of the bone marrow cells was estimated in two model systems *in vitro* based on their effect on proliferative activity of allgeneic lymphocytes and on their spontaneous and induced production of a lymphokine (MIF).

Peripheral blood monocytes from healthy blood donors were isolated by the usual method in a one-step Ficoll-Isopaque gradient. Lymphocytes were stimulated by the use of a polyclonal stimulator, namely PHA-P (from Difco, USA) in a dose of 10 μg/ml. Proliferative activity of the lymphocytes was determined by measuring incorporation of [^{3}H]thymidine (1 μCi) on an Intertechnique SL-30 scintillation counter on the 3rd day of culture. Suppression of proliferative activity was determined in per cent by the equation:

$$K_{\text{supp}} = \left(1 - \frac{A}{B + C}\right) \cdot 100\ \%,$$

where A denotes the number of incorporations of [^{3}H]thymidine in a mixed culture of bone-marrow cells and lymphocytes activated by PHA; B the number of inclusions in a monoculture of bone-marrow cells; C the number of incorporations in a monoculture of stimulated lymphocytes. To estimate the effect of bone-marrow cells on MIF production, they were added to cultures of allogeneic lymphocytes stimulated for 3 h with PHA (experiment) or of unstimulated cells (control). After culture for 20 h activity of MIF was determined in cell-free supernatants by the capillary method [11]. Spontaneous and induced production of MIF was determined quantitatively as MI_1 and MI_2 (migration indices), calculated by the following equations, where P_1 is the weight of the zone of migration of cells in the medium; P_2 the weight of the migration zone of cells in the control supernatant; P_3 the weight of the migration zone of cells

Department of Immunology, N. I. Pirogov Second Moscow Medical Institute. Department of Hematology, Central Postgraduate Medical Institute, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 4, pp. 462-463, April, 1985. Original article submitted August 8, 1984.

0007-4888/85/9904-0489$09.50

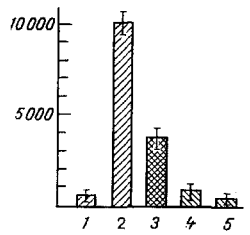

Fig. 1

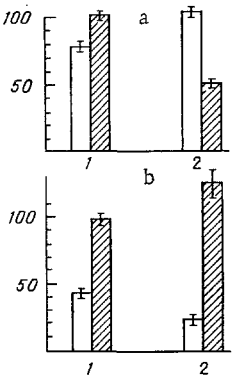

Fig. 2

Fig. 1. Suppressive action of human bone-marrow cells on proliferative response of peripheral blood lymphocytes. Ordinate, number of incorporations of [^{3}H]thymidine (in cpm). 1) Monoculture of 10^5 unactivated peripheral blood lymphocytes; 2) monoculture of PHA-activated lymphocytes; 3) mixed culture of 10^5 activated lymphocytes and 10^5 bone-marrow cells; 4) monoculture of 10^5 bone-marrow cells activated by PHA; 5) monoculture of 10^5 unstimulated bone-marrow cells.

Fig. 2. Effect of human bone-marrow cells on spontaneous (a) and PHA-induced (b) MIF production by peripheral blood lymphocytes. Ordinate, migration index (in %). 1, 2) Groups of subjects; unshaded columns — control, shaded — experiment.

in the experimental supernatant:

$$MI_1 = \left(\frac{P_2}{P_1}\right) \cdot 100\ \%,\ MI = \left(\frac{P_3}{P_2}\right) \cdot 100\ \%.$$

EXPERIMENTAL RESULTS

Addition of 10^5 bone-marrow cells to a culture of allogeneic peripheral blood lymphocytes (10^5) led to a reduction of the proliferative response in mixed culture compared with that in a monoculture of activated lymphocytes from 10,215 ± 1744 to 3887 ± 835 cpm (Fig. 1). If the proliferative activity of the bone-marrow cells in monoculture is taken into account, the percentage of suppression was 64. If the cell concentration in monoculture was doubled, no decrease in the number of incorporations of [^{3}H]thymidine was observed (2×10^5 stimulated lymphocytes — 13,711 ± 1529 cpm and 2×10^5 bone marrow cells — 967 ± 122 cpm).

Analysis of spontaneous and mitogen-induced MIF production enabled the blood donors tested to be divided into groups: Group 1 had a very low level of spontaneous MIF production by their lymphocytes (MI = 78.4 ± 3.2), and group 2 by the total absence of MIF production (MI = 114.2 ± 4.6) (Fig. 2). According to data obtained by several workers, lymphocytes of blood donors belonging to group 2, it can be tentatively suggested, synthesize a factor with different activity that stimulates cell migration [10]. Bone marrow cells do not affect spontaneous lymphokine production equally: They completely abolish it in subjects of group 1 but stimulate it in those of group 2. Combined culture of PHA-stimulated lymphocytes and bone-marrow cells leads to complete culture of PHA-stimulated lymphocytes and bone-marrow cells leads to complete abolition of MIF production by lymphocytes of donors of both groups (Fig.2).

It can thus be postulated that bone-marrow suppressors present in the human bone-marrow population can evidently exert a regulatory action not only on the proliferative activity of stimulated peripheral blood lymphocytes, but also on the function of a special T-cell population producing MIF without cell division. The action of bone-marrow cells on populations of immunocompetent cells, discovered in experiments on animals, probably takes place in man also, and reflects their regulatory function in immunogenesis.

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CHANGES IN PARAMETERS OF IMMUNITY IN INTACT AND PARTIALLY HEPATECTOMIZED MICE AFTER TRANSPLANATION OF SPLEEN CELLS FROM HYPOKINETIC DONORS

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UDC 616.45-001.1/3 + 612.917.11/12

KEY WORDS: stress; immunity; hypokinesia.

Spleen cells are known to transfer "regeneration information" from animals undergoing operations to intact recipients [1-3].

The writers have studied the state of the immunocompetent system in hypokinetic mice and have studied whether features of a stress state can be transferred from an animal exposed to hypokinesia [5] to recipients by the aid of splenic lymphocytes.

EXPERIMENTAL METHOD

Male (CBA × C57BL/6)F_1 hybrid mice weighing 20-22 g were used. The donors of spleen cells were kept in hypokinetic cages [4] for 17 h. A suspension of lymphocytes was prepared from the spleen in medium 199 and injected intravenously in a dose of 5×10^7 cells per mouse. Intact mice and animals from which two-thirds of the liver was resected under ether anesthesia [9] served as recipients. Splenocytes were injected into the hepatectomized mica 1 h after the operation. Intact and partially hepatectomized mice (PHM) receiving splenocytes from intact donors (SID), and intact and hepatectomized mice not subjected to any other procedure were used as the control. The animals were killed 1 and 7 days after transplantation of splenocytes at 8-10 a.m. by cervical dislocation. Altogether 120 donors were used and 158 recipients tested. In each group, consisting of 6-9 animals, the thymus and spleen were weighed. The state of the immunocompetent system was assessed by the number of spontaneous rosettes in the animals' blood in the E- and EAC-rosette-formation (E-RFC and EAC-RFC) tests [8]. The results were subjected to statistical analysis by the Fisher—Student method.

EXPERIMENTAL RESULTS

Hypokinesia for 17 h caused a decrease in weight of the spleen compared with the control by 1.6 times and of the thymus by 2.5 times. A tendency for the weight of the spleen to recover was observed 24 h after hypokinesia, but the weight of the thymus remained smaller as before. The weight of both organs returned to normal at the 7th day of the recovery period.

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