EFFECT OF BONE MARROW CELLS OF B AND NUDE MICE ON ANTIBODY PRODUCTION in vitro

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UDC 612.419.017.1-085

The thymus-dependence of the ability of bone marrow cells to suppress the primary immune response to sheep's red blood cells in a culture of spleen cells in vitro was studied. Bone marrow cells of mice with artificial (B mice) and inborn (nude mice) T-lymphocyte deficiency, as well as bone marrow cells from normal mice, were shownto suppress the production of antibody-forming cells in a culture of spleen cells.

KEY WORDS: antibody production in culture; B suppressors.

It is well known that subpopulations of thymus-dependent lymphocytes – T helpers and T suppressors [11-13] – are regular cells in the immune system. Meanwhile it has recently been clearly shown that cells of bone marrow origin can also act as regulators in processes of immunopoiesis [3, 9, 10, 14]. Depending on the concrete conditions, bone-marrow cells can either stimulate or suppress the immune response [4, 6, 15]. There is reason [4, 7, 14] to suppose that cells of the B-lymphocyte series may be the bone marrow suppressors.

The object of this investigation was to study the immunosuppressive function of the bone marrow in T-deficient animals.

EXPERIMENTAL METHODS

Inbred CBA mice and (CBA $\times$ C57BL)F_1 hybrids from the Stolbovaya nursery, Academy of Medical Sciences of the USSR, and athymic nude mice from the Nursery of Pure-Line Animals, Institute of Biophysics, Ministry of Health of the USSR, were used in the experiments. Induction of the primary immune response in the culture in vitro was carried out by a modified method [5]. Spleen cells in a concentration of $2.5 \cdot 10^6$ and $5 \cdot 10^6$ cells/ml were cultured in GIBCO Serumless medium with 10% fetal calf serum, 200 mM glutamine, and $2 \cdot 10^{-5}$ M 2-mercaptoethanol. Sheep's red blood cells preserved in Alsever's solution were used as the antigen. The viability of the cell was determined by staining with trypan blue and eosin. The number of antibody-forming cells (AFC) was determined by a modified Jerne's method [5]. Thymectomy and lethal irradiation followed by transplantation of syngeneic bone marrow were used to obtain B mice. Nude mice, obtained by crossing parents heterozygous for the nu gene, were used as model of an inborn deficiency of T cells. The nu/+ mice were obtained by crossing an nu/nu male with a C57BL/10 ScSn female. The numerical data were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The suppressive action of bone marrow cells on antibody production in the primary immune response was determined by addition of bone marrow cells in the ratio of 1:1 to spleen cells of intact CBA mice or (CBA $\times$ C57BL)F_1 hybrids, stimulated by sheep's red blood cells in culture. CBA and (CBA $\times$ C57BL)F_1 mice in an in vitro system are highly reactive to sheep's red blood cells and their immune response is about equal [5]. It will be clear from Table 1 that addition both of syngeneic bone marrow cells from normal donors and of bone marrow cells from athymic nude mice to cultures of spleen cells of (CBA $\times$ C57BL)F_1 mice led to about equal suppression of the immune response. AFC production under these circumstances was reduced in different experiments by 82-99%. Spleen cells of nude mice, which contained B cells but no T cells, had no suppressive action on AFC production. The small decrease in the number of AFC observed to accumulate in

Institute of Biophysics, Ministry of Health of the USSR, 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. 88, No. 8, pp. 171-173, August, 1979. Original article submitted September 1, 1978.

0007-4888/79/8808-0865 $07.50

TABLE 1. Effect of Cells of Lymphoid Organs of Nuce Mice on Antibody Production in Culture in vitro

Experiment No.	Number of cells in culture · 10^6 (per ml)				Survival rate at end of incubation, %	Number of AFC on fourth day of culture, per 10^6 living cells)	Percent suppression of AFC
	spleen cells of (CBA × C57BL)F1 mice	bone marrow cells of (CBA × C57BL)F1 mice	bone marrow cells of nude mice	spleen cells of nude mice			
1	2,5	—	—	—	38	544,3±200,2	—
	5	—	—	—	42	824,3±74,0	—
	2,5	2,5	—	—	50	17,3±9,6	97
	2,5	—	2,5	—	58	19,0±9,5	97
	2,5	—	—	2,5	51	691,7±139,3	0
2	2,5	—	—	—	30	133,8±26,1	—
	5	—	—	—	30	201,6±50,8	—
	2,5	2,5	—	—	59	24,0±6,3	82
	2,5	—	2,5	—	50	9,0±2,9	93
	2,5	—	—	2,5	37	445,2±158,2	0
3	2,5	—	—	—	50	329,7±74,3	—
	5	—	—	—	80	130,0±15,0	—
	2,5	2,5	—	—	42	38,3±0,9	88
	2,5	—	2,5	—	70	10,0±7,6	97
4	2,5	—	—	—	31	147,5±2,5	—
	5	—	—	—	43	332,0±162,4	—
	2,5	2,5	—	—	47	8,5±2,9	99
	2,5	—	2,5	—	29	16,5±10,5	89
	2,5	—	—	2,5	37	73,0±45,5	50
	—	—	—	2,5	34	0	—

TABLE 2. Effect of Cells of Lymphoid Organs of B-Mice on AFC Production in Cultures of Spleen Cells

Experiment No.	Number of cells in culture · 10^6 (per ml)			Survival rate after culture for 96 h, %	Number of AFC at end of incubation (per 10^6 living cells)	Percent suppression of antibody production
	spleen cells of CBA mice	bone marrow cells of CBA B mice	spleen cells of CBA B mice			
1	2,5	—	—	65	532,3±98,5	—
	5	—	—	70	327,3±50,7	—
	5	2,5	—	40	13,7±2,2	97
	2,5	—	2,5	60	308,0±32,6	42
2	2,5	—	—	32	83,2±35,0	—
	5	—	—	38	104,5±19,9	—
	2,5	2,5	—	20	15,8±8,92	82
	2,5	—	2,5	40	104,5±24,8	0

some experiments on the addition of spleen cells evidently took place as a result of an increase in the density of the cells in culture [1]. This is also clear if the number of AFC accumulating in monocultures of spleen cells containing 2.5 and 5 million cells is compared. The absence of allogeneic restriction in the ability of the bone marrow cells to suppress antibody formation in vitro must be noted [9].

Table 2 shows that bone marrow cells of B mice also suppressed AFC production by 82-97%, whereas the spleen cells of these mice did not possess this property.

Bone marrow cells of T-deficient animals thus have the same suppressive action on antibody production in vitro as bone marrow cells of normal mice. Consequently, the effect of suppression of antibody production, mediated through bone marrow cells is a thymus-independent process. The results also confirm the role of cells of the B-lymphocyte series in suppression of the immune response in vitro, in agreement with results obtained previously [6, 7] by the use of antisera against T and B lymphocytes.

The absence of a suppressive effect of spleen cells of B mice and nude mice can be explained by the affinity of the B suppressors of the immune response for bone marrow. It is also possible that their activity is realized through the participation of stromal cells or other bone marrow cells [2, 8].

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APPEARANCE OF AN ADJUVANT-LIKE FACTOR IN THE SERUM OF RATS AFTER UNILATERAL AND BILATERAL NEPHRECTOMY

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UDC 612.46-089.64:612.017.1.014.46

A factor capable of stimulating the immune response to heterologous red blood cells in syngeneic recipients was shown to be present in the serum of rats after unilateral and bilateral nephrectomy. This factor is similar in its adjuvant activity to the factor appearing in the serum after partial hepatectomy.

KEY WORDS: nephrectomy; immune response; adjuvants.

It was shown previously that after partial hepatectomy a factor stimulating the immune response to thymus-dependent antigens appears in the blood serum of rabbits [1]. An active factor was isolated from the serum by ion-exchange chromatography and a technique of immunoadsorption. In its immunochemical properties it is identical with the $F(ab')_2$ fragment of IgG and is formed by hydrolysis of this protein in vivo under the influence of neutral serine proteinases [2, 9]. It has also been shown that the adjuvant properties of the Fab fragments of homologous IgG are determined by the structure of the regions of the molecule located outside the combining sites of the antibodies [8].

Since the walls of large blood vessels contain considerable quantities of various proteinases [7], it might be supposed that during operations associated with ligation of the large arteries, breakdown products of IgG possessing adjuvant properties may appear in the blood stream.

In this investigation the appearance of an adjuvant-like factor in the serum of rats after nephrectomy was studied.

EXPERIMENTAL METHODS

Experiments were carried out on Wistar rats weighing 120-140 g. Unilateral and bilateral nephrectomy were performed under ether anesthesia by the usual method, and partial hepatectomy by the method of Higgins and Anderson [5]. The rats were exsanguinated 4 h after the operation. The serum obtained was injected intraperitoneally into syngeneic recipients, in a volume of 1 ml simultaneously with sheep's red blood cells

Kursk Medical Institute. Laboratory for Transplantation of Organs and Tissues, Academy of Medical Sciences of the USSR. N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kovanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 8, pp. 173-174, August, 1979. Original article submitted September 6, 1978.