EFFECT OF STIMULATION OF ANTIBODY FORMATION BY BONE MARROW CELLS in vivo

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If intact bone marrow is injected into mice at the peak of the secondary immune response, the number of antibody-forming cells in a regional lymph node is increased by 2.4 times. Preliminary injection of bone marrow into donors of cells of immune lymph nodes reduces the effect of stimulation of antibody formation during their subsequent combined culture with intact bone marrow cells. The results demonstrate interaction between cells at the level of mature antibody producers in vivo.

KEY WORDS: mature antibody producers; bone marrow; lymph nodes.

An effect of cooperation between cells at the level of mature antibody producers, discovered previously, is manifested as follows: During combined culture of cells of lymph nodes obtained from mice at the peak of the secondary immune response with cells of intact bone marrow the intensity of antibody formation is doubled or trebled [1]. This effect has been shown to be connected with the appearance of new antibody-forming cells (AFC) in the population of immune lymph nodes under the influence of a humoral factor produced by intact bone marrow cells [2, 7]. A regulatory role of bone marrow in the immune response has been postulated [7].

The object of this investigation was to study whether the phenomenon described above is observed only in an in vitro system or whether it is also found in the intact organism. Accordingly an in vivo system was devised for assessment of the effect of stimulation of antibody formation in the productive phase of the immune response.

EXPERIMENTAL METHOD

The model developed for the study of reactions of transplantation immunity in regional lymph nodes in response to local injection of allogeneic cells [5] was modified.

Experiments were carried out on (CBA × C57BL)F_1 mice immunized twice at an interval of 30 days in the footpads of the hind limbs with a 5 % suspension of sheep's red cells (0.1 mg in each limb). On the fourth day of the secondary response cells of intact bone marrow or of intact lymph nodes or medium 199 were injected subcutaneously into the footpads of the hind limbs. The dose of cells injected was $15 \cdot 10^6$ per mouse. The number of plaque-forming cells in the popliteal lymph nodes was determined 24 h after the injections by a modified Jerne's method for estimation of IgG-forming cells [4].

RPM_1-1640 medium with the addition of glutamine, embryonic serum, HEPES, sodium bicarbonate, and mercaptoethanol [3] was used in the cell culture experiments. The method of preparing the cell suspensions for culture was described previously [6].

EXPERIMENTAL RESULTS

In the experiments of series I the effect of injection of bone marrow cells or lymph node cells on the number of antibody producers in the popliteal lymph node of one of the animal's hind limbs was studied. This value was compared with the number of AFC in the popliteal lymph node of the contralateral limb of the same animal. Medium 199 was injected into the contralateral limb as the control. The results are shown in Table 1.

As Table 1 shows, injection of bone marrow cells increased by 2.4 times the number of AFC in the regional lymph node compared with their number in the control lymph node draining the limb which medium 199 had been injected.

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TABLE 1. Increase in Number of AFC after Injection of Bone Marrow Cells

Components injected	Number of experiments	Number of AFC per 10^6 nucleated cells	K	P
Bone marrow cells	30	195±43	2,4	<0,05
Medium 199		82±17		
Lymph node cells	10	202±56	1,1	>0,05
Medium 199		184±44		

Legend. K) Ratio of number of AFC in experimental lymph node to that in control.

TABLE 2. Decrease in Coefficient of Stimulation of Antibody Formation in Mixed Culture after Preliminary Injection of Bone Marrow Cells in vivo

Cells cultured	Number of cultures	Number of AFC per 10^6 nucleated cells	K	P
Immune lymph nodes from donors receiving in vivo:				
bone marrow	24	85±12,2	2,3	<0,05
bone marrow + intact bone marrow	24	198±23,0		
intact lymph nodes	24	49±6,1		
intact lymph nodes + intact bone marrow	24	259±23,8	5,3	<0,01

Legend. K) Ratio of number of AFC in mixed culture to number in monoculture.

After injection of lymph node cells or the medium alone into the different limbs, no significant differences in plaque formation were found.

After injection of bone marrow cells antibody formation was thus considerably intensified in the regional lymph node, confirming previous observations made with an in vitro system [1, 2, 6, 7].

In the experiments of series II the effect of bone marrow, injected previously in vivo, on stimulation of antibody formation was studied in a mixed culture of immune lymph nodes and intact bone marrow. The scheme of the experiments was as follows. At the peak of the secondary response in the mice bone marrow cells were injected into one hind limb. Cells from intact lymph nodes were injected into the contralateral limb as the control. The mice were killed 18-20 h later, cells of the popliteal lymph nodes were isolated, and these were then cultured both separately and mixed with intact bone marrow cells. The number of AFC in the monocultures and mixed cultures was determined 18-20 h after the beginning of culture. The results of these experiments are given in Table 2.

The coefficient of increase of the number of AFC in the mixed culture after preliminary injection of bone marrow into the donors of the immune lymph nodes were injected instead of bone marrow cells, it was 5.3.

The decrease in the coefficient of stimulation of antibody formation after preliminary injection of bone marrow cells is evidence that some of the "reserve" cells took part in antibody synthesis under the influence of bone marrow cells in the in vivo system, and thereby reduced their number in the mixed culture.

It follows from these results that the increase in the number of mature AFC under the influence of bone marrow cells is observed not only in a system in vitro, but also in vivo. This confirms the presence of cooperation between the cells at the level of mature antibody producers.

The suggested model is simple and convenient for studying processes of cellular interaction in a system in vivo at different stages of development of the immune response, including in its productive phase.

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CORRECTION OF T-IMMUNODEFICIENCY IN MICE BY BONE MARROW TRANSPLANTATION FROM HYDROCORTISONE-TREATED DONORS

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B mice were obtained by thymectomy, by lethal irradiation, and by protection of adult CBA mice with syngeneic bone marrow. In some experiments syngeneic bone marrow from donors treated with hydrocortisone in a dose of 125 mg/kg for 3 days was used. Cells carrying the Q marker were determined in the bone marrow of these donors. Thymectomized and lethally irradiated animals injected with bone marrow from donors treated with hydrocortisone rejected skin allografts and lymph node cells from these mice inhibited endogenous colony formation in sublethally irradiated (CBA × C57BL/6)F_1 hybrids.

KEY WORDS: B mice; cortisone-resistant T lymphocytes; endogenous colony formation.

The search for methods of correction of the immune reactivity of the organism in immunodeficient states when the function of the T or B system of immunity is disturbed is an important task in modern immunology. B mice, obtained by thymectomy followed by lethal irradiation and protection with syngeneic bone marrow or temporary blocking of the function of the thymus by lethal irradiation, provide an experimental model of T-cell immunodeficiency [4, 8, 10]. The possibility of increasing the immunologic response of B mice to sheep's red blood cells during treatment of these mice with poly-4-vinylpyridine has been demonstrated [4]. In this investigation the possibility of abolishing T-cell immunodeficiency in B mice by the use of syngeneic bone marrow from donors treated with hydrocortisone was studied, for T lymphocytes have been shown to enter the bone marrow of mice treated with hydrocortisone [9].

EXPERIMENTAL METHOD

To obtain B mice, CBA mice aged 4 months were used. Ten days after thymectomy the mice were irradiated in a dose of 900 R and protected with syngeneic bone marrow. Animals of the control group underwent a mock thymectomy. In two experiments syngeneic bone marrow from donors of the same age, treated with hydrocortisone in a dose of 125 mg/kg intraperitoneally daily for 3 days, were used. In one experiment the bone marrow was treated before injection with anti-Q serum, obtained by immunization of AKR mice by six injections of thymocytes from CBA mice. The anti-Q serum thus obtained was used to determine the presence of the Q marker on lymphocytes of the bone marrow of mice treated with hydrocortisone and of normal CBA mice in the cytotoxic test in Brondz's modification [2] of Gorer and O'Gorman's method. This serum caused death of 98% of thymocytes of CBA mice in a dilution of 1:5 but did not act on normal bone marrow. Skin allografts from C57BL/6 mice were transplanted on to B mice 14 days after irradiation and injection of bone marrow by the method described previously [7]. Activity of lymph node cells of B mice in the graft versus host reaction was studied on the basis of ability to inhibit endogenous colony formation in sublethally ir-

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