LITERATURE CITED

1. L. A. Zakharova and N. S. Galkina, Byull, Éksp. Biol. Med., No. 9, 67 (1974).
2. A. A. Mikhailova, R. V. Petrov, and L. A. Zakharov, Dokl. Akad. Nauk SSSR, 197, 209 (1971).
3. J. N. Corriere and K. A. Jacobson, J. Urol. (Baltimore), 116, 616 (1976).
4. D. W. Dresser and M. H. Wortis, Nature, 228, 859 (1968).
5. V. Haskova, L. Korcakova, M. Holub, et al., Lymphology, 8, 61 (1975).
6. R. V. Petrov et al., J. Immunol., 103, 679 (1969).
7. R. V. Petrov, A. A. Mikhailova, R. N. Stepanenko, et al., Cell. Immunol., 17, 342 (1975).

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

V. F. Semenkov

UDC 612.018.02.017.1

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-

Laboratory of Clinical Immunology, Department of Immunology, Research Center, N. I. Pirogov Second Moscow Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 4, pp. 449-451, April, 1978. Original article submitted July 28, 1977.

0007-4888/78/8504-0489$07.50

TABLE 1. Immunologic Reactivity of B Mice in Transplantation Immunity Reaction and Phenomenon of Inhibition of Endogenous Colony Formation

Group of animals	Thymectomy	Lethal irradiation	Injection of BMC in dose of 10×10^6 per mouse			Duration of survival of skin grafts		Endogenous colonies in (CBA × C57BL/6)F_1 hybrids after injection of LNC into mice of groups 1, 2, and 3 in dose of 1×10^6 per mouse	
			hydrocortisone	anti-Q-serum	untreated BMC	Days	$M \pm m$	$M \pm m$	index of inactivation %
1	+	+	+	—	—	12, 12, 20, 18, 17, 19, 23, 28, 18, 17, 18, 19, 20	18,6±3.6	7±0,5 (10)	53.9
2	+	+	—	—	+	31*, 37, 37, 37, 43*, 55, 41, 60, 62, 60, 30*, 43*	44,6±3.3	15.2±1,3 (10)	0
3	—	+	—	—	+	11, 12, 12, 12, 13, 15	12,5±0.6	1±0,8 (9)	92.3
4	+	+	—	+	—	24*, 31*, 34, 53*, 55*, 55, 52	43,6±6.0	—	—
5	—	—	—	—	—	—	—	12.9±1,2 (10)	—

Legend: 1. + and − denote presence or absence of action; BMC) bone marrow cells; LNC) lymph node cells. 2. The * indicates that skin allografts were not rejected by the mice at the time of the recipient's death. 3. In groups 1, 2, 3, and 5 the (CBA × C57BL/6)F_1 hybrids received sublethal irradiation. 4. Number of animals in parentheses.

radiated (CBA × C57BL/6)F_1 hybrids by the method of Petrov et al. [5]. The number of colony-forming cells in the spleens of the mice of the experimental and control groups was determined by the exogenous cloning method. For this purpose, 1 million nucleated spleen cells was injected intravenously into (CBA × C57BL/6)F_1 hybrids irradiated in a dose of 800 R. The spleens of the hybrids on the ninth day after injection were fixed in Bouin's fluid and the colonies were counted macroscopically. Inhibition of endogenous colony formation and the number of colony-forming cells in the B mice were studied 40 days after these mice had been obtained. The conditions of irradiation and preparation of the spleen and lymph node cells were the same as those used previously [6]. Altogether 140 animals of the above-mentioned inbred lines were used. The mice were obtained from the nursery of inbred animals of the Academy of Medical Sciences of the USSR (Stolbovaya). The experimental results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Injection of hydrocortisone for 3 days into 20 CBA mice, used as the donors of the bone marrow, caused a sharp reduction in size of the thymus, lymph nodes, and spleen and an increase in the number of nucleated cells in the bone marrow compared with untreated donors. The number of cells carrying Q marker in the bone marrow of the treated donors was 20%, but in normal bone marrow no such cells were found. As Table 1 shows, injection of bone marrow of donors treated with hydrocortisone into thymectomized and lethally irradiated CBA mice led to rejection of the skin allografts on most of the recipients before the 20th day (group 1). In recipients receiving bone marrow from untreated donors, no evidence of rejection of the grafts was present for 1 month, 4 recipients died with no sign of rejection of the grafts, and in 3 recipients rejection of the grafts of the chronic type took place on the 37th day, in 2 on the 41st and 55th days, and in 3 mice permanent survival of the skin allografts was observed (group 2). If the bone marrow before injection into the recipient was treated with anti-Q serum, the mean survival time of the grafts was indistinguishable from that of grafts on recipients injected with normal bone marrow (groups 2 and 4).

In the next series of experiments the ability of the lymph node cells of the experimental and control mice to inhibit endogenous colony formation was studied in sublethally irradiated hybrids (Table 1). Lymph node cells of the recipients of group 1 inhibited endogenous colony formation in the hybrids, whereas lymph node cells of the mice of group 2 did not inhibit endogenous colony formation (groups 1, 2, and 5). The index of inactivation was higher in recipients undergoing mock thymectomy (92.3%; group 3) than in recipients receiving injections of bone marrow from hydrocortisone-treated donors (53.9%: group 1). The number of exogenous colony-forming cells was greatest in the recipients of group 2 (18.4 ± 2.2), whereas in the recipients of groups 1 and 3 it was 4.4 ± 1.2 and 5 ± 0.7, respectively (20 lethally irradiated hybrids were used in this experiment).

The writers showed previously that spleen cells of B mice did not induce a graft versus host reaction in sublethally irradiated first-generation hybrids [8]. The Q antigen is a marker of T lymphocytes [3, 9]. In

the present experiments, 20% of cells with the Q marker were found in the bone marrow of hydrocortisone-treated CBA mice, whereas no such cells were present in the bone marrow of normal CBA mice, in agreement with data obtained by other workers [3]. T Lymphocytes can enter the bone marrow as a result of a stress reaction. For instance, 12% of cells with the Q marker were discovered in the bone marrow of CBA mice 6-9 h after the beginning of a stress reaction [3]. In the present investigation the immunologic reactivity of recipients protected with bone marrow from hydrocrotisone-treated donors was lower than in recipients undergoing mock thymectomy and protected with the bone marrow of normal donors. This difference in the immunologic reactivity of the two groups of recipients can be explained by the presence of the thymus in the mice undergoing the mock thymectomy and its effect on T-cell activity. Lymph node cells from B mice into which normal bone marrow was injected did not inhibit endogenous colony formation and the mean number of endogenous colonies in the irradiated hybrids after injection of lymph node cells from mice of this group was greater than the number of colonies in irradiated hybrids without injection of lymph node cells. The number of colony-forming cells in the spleen of recipients undergoing mock thymectomy and of B mice injected with bone marrow of hydrocortisone-treated donors was much smaller than in B mice protected with normal bone marrow. This result can evidently be explained by the presence of a certain number of T lymphocytes in the spleen of these mice.

LITERATURE CITED

1. I. P. Ashmarin and A. A. Vorob'ev, Statistical Methods in Microbiological Research [in Russian], Leningrad (1962).
2. B. D. Brondz, Vopr. Onkol., No. 8, 64 (1964).
3. Yu. I. Zimin and R. M. Khaitov, Byull, Éksp. Biol. Med., No. 12, 68 (1975).
4. R. V. Petrov, R. M. Khaitov, E. V. Kozhinova, et al., Tsitologiya, 18, 321 (1975).
5. R. V. Petrov, R. M. Khaitov, and L. S. Seslavina, Radiobiologiya, 10, 532 (1970).
6. V. F. Semenkov, Dokl. Akad. Nauk SSSR, 181, 1514 (1968).
7. V. F. Semenkov, Éksp. Khir., No. 1, 43 (1969).
8. V. F. Semenkov and A. P. Bogdanov, Dokl. Akad. Nauk SSSR, 225, 704 (1975).
9. A. C. Fanci, Immunology, 28, 669 (1975).
10. J. F. A. P. Miller, Nature, 195, 1318 (1962).